

5. RESULTS

5.1 Diversity and traditional knowledge of Unani medicinal plants from Northwest Gujarat.

A survey was conducted to explore the diversity and traditional knowledge of Unani medicinal plant species in Northwest Gujarat during 2020-2021. A total of 137 medicinal plant species were documented, with 84 of these plants identified as referenced in the Unani system of medicine. These plants are classified into 73 genera and 49 families, providing a comprehensive overview of the region's phytodiversity. The study aimed to create a preliminary checklist of Unani medicinal plants by comparing them with other traditional medicinal systems. Field visits were carried out in various villages, where interactions with local Hakims and Vaidyas provided valuable insights into the traditional use of these plants. The collected data are summarized in Table 8, which includes each plant's botanical name, family, Folk/Local name, Unani name, Ayurveda name, Siddha name and plant part uses. Among the documented species, Fabaceae and Solanaceae are the most dominant families, each with 5 species, followed by Asclepiadaceae, Apocynaceae, Asteraceae, Caesalpiniaceae, and Moraceae, each with 4 species. The Euphorbiaceae family includes 3 species, while Sterculiaceae, Combretaceae, Menispermaceae, Bombacaceae, Rutaceae, and Rhamnaceae each have 2 species. The remaining 35 families are represented by a single species each (Fig. 6). Of the 49 families identified, 44 (68 genera) belong to Dicotyledonae and 5 (5 genera) to Monocotyledonae. All recorded plant species possess medicinal value and are referenced in Ayurveda, Unani, and Siddha systems of medicine. This study offers a holistic perspective on the traditional knowledge associated with these plants, highlighting their significance in the region's traditional medicine practices.

Table 8. Enumeration of Unani Medicinal plants of Northwest Gujarat

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
1	<i>Abrus precatorius</i> L.	Fabaceae	Chirmiti, Ratti	Ghongchi	Gunjaa, Gunjaka	Kunri	Seeds
2	<i>Acacia catechu</i> (L.f) Willd.	Mimosaceae	Khair	Katthaa	Khadira	Karunkaali, Kalippakku, Kadiram. Katthakkaambu, Kaasukkatti	Wood and seeds
3	<i>Acacia leucophloea</i> Willd.	Mimosaceae	Safed Babuul, Safed Kikar, Renvaa	Kath Safed	Arimedaka, Vitakhadir	Valval, Velvayalam	Bark, seeds, leaves and Gum
4	<i>Acacia nilotica</i> (L.) Del.	Mimosaceae	Baval	Aqaaqia, Babuul, Kikar, Mughilaan, Samur	Babbuula	Karu-velamaram, Karuvelei. Velampisin	Stem bark, pods, seed oil, Gum
5	<i>Achyranthes aspera</i> L. var. <i>aspera</i>	Amaranthaceae	Puthkanda	Chirchitta, Latjeeraa	Apaamaarga, Chirchitaa	Naayuruvi	Whole Plant
6	<i>Adansonia digitata</i> L.	Bombacaceae	Gorakh Imli; Gorakh Chinchaa	Gorakh Imli	Ravanaamlikaa, Gorakshi	Papparapuli	Leaves, Flowers & Fruits
7	<i>Aegel marmelos</i> (L.) Corr.	Rutaceae	Bili	Belgiri (Bael)	Bilva, Kapitha	Vilvam, Koovilam	Root & Bark
8	<i>Ailanthus excelsa</i> Roxb.	Simaroubaceae	Araduso, Rukhdo	Neem Mitha	Aralu, Katvanga	Perru, Perumaruttu, Peruppi	Bark & Leaves

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
9	<i>Aloe vera</i> (L.) Burm. F.	Asphodelaceae	Aloe vera, Kuvarpaanthu	Gheekwaar, Sibr, Elva	Ghritkumari, Kumari	Kathalai	Leaves
10	<i>Annona squamosa</i> L.	Annonaceae	Anduri	Sharifaa	Gandagaatra	Sitaaphalam, Atta	Leaves seeds, ripe fruits & Unripe fruits
11	<i>Anogeissus latifolia</i> Wall. ex Bedd.	Combretaceae	Dhavado	Dhaawaa	Dhava	Namai, Vel-naga-maram, Vakkali	Gum
12	<i>Argemone Mexicana</i> L.	Papaveraceae	Darudi, Pilo dhaturo, Satyanashi	Satyaanaashi	Katuparni, Svarnkshiri	Piramathandu, Kudiyotti	Seed, Leaf juice & Root
13	<i>Asparagus racemosus</i> Willd.	Asparagaceae	Satawari	Satawar	Shataavari, Shatmuuli	Thanneervittan kizhangu, Sataavari Kizhangu	Tuberous root
14	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Limdo	Azaad-Darakht-e-Hindi, Neem	Nimba, Nimbaka	Vemmu, Veppu, Veppan, Arulundi	Leaves, Bark & Seed Oil
15	<i>Bacopa monneri</i> (L.) Wettst	Scrophulariaceae	Jalaneem, Safed-Chammi	Ulasimang-aso	Braahmi, Aindri	Piramivazhukkai, Neerbrami	Whole Plant
16	<i>Bahunia racemosa</i> Lam.	Caesalpiniaceae	Aapataa Kachnaala	Kachnal	Ashmantaka, Kanchini	Kokku mandarai	Bark, Leaves, Flowers & Seeds

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
17	<i>Balanites egyptica</i> (L.) Del.	Balanitaceae	Ingoro	Hingan, Hanguul	Ingudi, Angaar Vriksha	Nanjunda	Bark, Fruit, Seed & Seed Oil
18	<i>Bambusa arundinacea</i> (Retz.) Wild.	Poaceae	Baambu, Vaans	Tabasheer	Vansha, Kichaka	Moongil; Moongiluppu, (Bambo-manna.)	Leaves, Stem, Root & Bark
19	<i>Blumia eriantha</i> DC.	Asteraceae	Nirmudi	Kakarondaa	Kukundara (var.).	Kattumullangi, Narakkarandai	Leaves, oil
20	<i>Boerhavia diffusa</i> L.	Nyctaginaceae	Gadaha-purnaa	Itsit	Rakta-punarnavaa, Punarnavaa	Mookkirattai	Whole Plant
21	<i>Bombax ceiba</i> L.	Bombacaceae	Semar	Senbhal, Mochras, Tukhm-e-Sambhalu	Shaalmali, Raktapushpa	Mul Ilavam. Ielavampisin (gum)	Bark, Young root, gum, shoots, leaves
22	<i>Boswellia serrata</i> Roxb.	Burseraceae	Salai Guggul	Kundur	Shallaki, Susravaa	Parangisambirani, Kungli	Stem Fruit, Gum-Resin, and Oil
23	<i>Bryophyllum pinnatum</i> (Lam.) Oken.	Crassulaceae	Amar poi	Zakhm-e-Hayaat	Parnabija, Paashaanabheda	Runakkalli	Leaves
24	<i>Butea monosperma</i> (Lam.) Taub.	Fabaceae	Tesu, Keshudo	Dhak (Pilas Papra), Samagh, Chunnia Gond, Kamarkas	Paalasha, Raktapushpaka	Palasam, Purasus	Bark, Leaves, Flowers, Seeds

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
25	<i>Caesalpinia crista</i> L.	Caesalpinaceae	Kachka	Karanjawa	Lataakaranja	Kazharchikkaai	Root, Bark, leaves, Seeds, Seeds oil
26	<i>Cajanus cajan</i> (L.) Millsp.	Fabaceae	Tuver	Arhar	Aadhaki, Shanapushpikaa	Thuvarai	Root & Leaves
27	<i>Calotropis procera</i> (Ait.) R. Br.	Asclepiadaceae	Akado, Akad	Aakh, Madaar	Alarka, Arkaparna	Vellerukku, Erukku	Root, Leaves, Bark oil, Flowers, Seed oil
28	<i>Carica Papaya</i> L.	Caricaceae	Papaya	Papeeta	Erاند-karkati, Papitaa	Pappaali, Pappayi	Leaves, Fruits & Seeds
29	<i>Carissa carandas</i> L.	Apocynaceae	Karamdan	Karondaa	Karamarda	Kalakke	Stem Bark & Root
30	<i>Cassia auriculata</i> L.	Caesalpinaceae	Tarwar	Tarwar	Aavartaki, Aadaari	Aavaarai	Whole Plant
31	<i>Celastrus paniculata</i> Willd.	Celastraceae	Malkangana	Malkangani	Jyotishmati, Paaraavatpadi	Vaaluluvai	Root & Seeds
32	<i>Cissampelos pareira</i> L.	Menispermaceae	Venivel	Akanadi	Paathaa, Ambashthaa	Paadakkizhangu, Appatta	Root
33	<i>Citrullus coloncynthis</i> L.	Cucurbitaceae	Badi indrayaan, Kadvi kakadi	Hanzal	Indravaaruni, Indravalli	Kumatti	Root, Leaves & Fruits

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
34	<i>Citrus limon</i> (L.) Burm. F	Rutaceae	Limboo	Aab Leemun	Jambira, Nimbu	Periya elumuchhai	Leaves, Stems & Fruits
35	<i>Cordia dichotoma</i> Forst.	Boraginaceae	Gunda	Sapistan	Shleshmaataka, Picchila	Naruvili	Bark, Leaves & Fruit
36	<i>Crateva nurvula</i> Buch-Ham	Capparidaceae	Varno, Vayavarno	Baranaa	Varuna, Varana	Maavilingam	Whole Plant
37	<i>Curcuma longa</i> L.	Zingiberaceae	Haldi, Haldar	Haldi, Zard Chob	Haridraa, Haridruma	Manjal	Rhizome
38	<i>Datura inoxia</i> Mill	Solanaceae	Dhaturo	Dhaturaa	Dhattuura	Vellum mattai, Kara umattai	Whole Plant
39	<i>Datura metel</i> L.	Solanaceae	Dhaturo	Dhaturaa	Dhattuura, Harapriya	Oomatthai, Karuvoomatthai	Whole Plant
40	<i>Derris indica</i> (Lam.) Bennet.	Fabaceae	Karanja	Karanj	Guchpushpak Karanja	Pungu, Pongum	Bark, Leaves, Flowers & Root
41	<i>Diospyros melanoxylon</i> Roxb.	Ebanaceae	Timaru	Tinduka	Tinduka	Karum Dumbi, Thumbi, Beedi-elai	Leaves, Flowers & Fruits
42	<i>Echinops echinatus</i> L.	Asteraceae	Uunta-Kateraa, Utkanto, Shuliyo	Untkatara	Utkantaka, Uttundaka	Brahmadandi, Mullabanti	Whole Plant

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
43	<i>Eclipta prostrata</i> L.	Asteraceae	Bhangaraa	Bhangraa	Bhringaraaja, Maarkava	Karisalaankanni	Whole Plant
44	<i>Emblica officinalis</i> Gaertn.	Euphorbiaceae	Aamalaa	Amla	Aaamalaki, Aaamalaka, Dhaatri	Nellikkaai, Nelli	Bark, Leaves & Fruits
45	<i>Enicostema hyssopifolium</i> Verdoon	Gentianaceae	Chhotaa Chirayataa	Naai, Naahi	Naagjhvaa, Maamajjaka	Vellargu	Whole Plant
46	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Rati-dudheli. Moti-dudheli	Dudhi Khurd	Dudhi, Dudhikaa	Amman pachharisi.	Whole Plant.
47	<i>Ficus benghalensis</i> L.	Moraceae	Vad, Vadlo	Reesh Barged	Vata, Nyagrodha	Aalamaram.	Whole Plants.
48	<i>Ficus racemosa</i> L.	Moraceae	Gular, Umardo	Goolar, Anjir-e-Aadam, Anjir-e Ahmak	Udumbara, Sadaaphala, Jantuphala	Atthi	Bark, Root & Fruits
49	<i>Ficus religiosa</i> L.	Moraceae	Pipro/Peepal	Peepal	Ashvattha, Bodhi, Peeppal	Arasu, Ashvatham	Bark, Leaves & Fruits
50	<i>Gymnema sylvestre</i> (Retz.) Schult.	Asclepiadaceae	Madhunashini	Gurmaar Booti	Meshashringi, Meshavishaanikaa	Kannu Minnayamkodi, Passaam, Shirukurinja	Root & Leaves
51	<i>Helicteres isora</i> L.	Sterculiaceae	Maradashingh	Marorphali	Aavartani, Aavartphalaa, Aavartaki	Valampiri	Bark, Leaves, Pods & Seeds

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
52	<i>Hemidesmus indicus</i> (L.) Schult.	Asclepiadaceae	Sariva, Anantmul	Ushbaa Hindi	Anantmuula, Gopavalli	Nannaari, Suganthipala	Root
53	<i>Hibiscus rosasinensis</i> L.	Malvaceae	Jasood	Gul-e-Gurhal	Japa	Cembarutti	Roots, Leaves & Flowers
54	<i>Holarhena antidysenterica</i> Wall.	Apocynaceae	Kalokado	Inderjeo Talkh (Lisan-ul-asafee)	Kutaja, Indravriksha	Kudasappaalai-pattai, -vidai	Root, Bark & Seeds
55	<i>Hygrophila auriculata</i> Heine	Acanthaceae	Aekhro	Taalmakhaanaa.	Kokilaaksha, Ikshura	Neermulli	Roots, Leaves & Seeds
56	<i>Justicia adhatoda</i> L.	Acanthaceae	Aradusi	Arusa	Vasaka, Vasaghrita	Adhatoda, Acalai, Cimme-muki	Roots, Bark, Leaves, Flowers
57	<i>Madhuca indica</i> J. F. Gmel.	Sapotaceae	Mahudo	Mahuaa.	Madhuuka, Madhupushpa	Ieluppai	Bark, Flowers & Seed oil & Gum
58	<i>Mangifera indica</i> L.	Anacardiaceae	Aambo	Aam	Aamra, Amb	Manga, Mau, Mamaram, Mangottai Paruppu	Stem Bark, Leaves & Fruits
59	<i>Moringa oleifera</i> Lam.	Moringaceae	Saragavo	Sahajana	Shigru, Sahijanin	Moringa, Murungai	Whole Plant

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
60	<i>Morus alba</i> L.	Moraceae	Shahtoot	Shahtuut, Tuut	Tuta	Kambli chedi	Root, Bark, Leaves & Fruits
61	<i>Mucuna pruriens</i> (L.) DC.	Fabaceae	Bhair	Konch	Aatmaguptaa, Kapikacchuu	Poonaikkaali	Leaves, Fruits & Seeds
62	<i>Nicotiana tabacum</i> L.	Solanaceae	Tambaku	Tambaakhu	Taamraparna, Dhuumrapatraa	Pukaiyilai, Nattuppukaiyilai	Leaves
63	<i>Ocimum sanctum</i> L.	Lamiaceae	Tulasi	Raihan	Tulasi	Tulasi, Nalla-Tulasi	Whole Plant
64	<i>Pergularia damia</i> (Forsk.) Chiov.	Asclepiadaceae	Nagala dudhi	Mendhasingi	Visanika, Kakaangha	Uttamani, Seendhal kodi, Veliparuthi	Whole Plant
65	<i>Phyllanthus frateruns</i> Webst	Phyllanthaceae	Bhui amla	Bhui Aaamalaa	Tamalaki, Bhumyamalaki	Kila-nelli	Whole Plant
66	<i>Prosopis juliflora</i> (Sw.) DC.	Mimosaceae	Khejaraa, Vilaayati Kikar, Kaabuli Kikar	Ghaff	Shami	Velikathan	Leaves, Pods, Gum
67	<i>Punica grantum</i> L.	Punicaceae	Dadam	Anaar	Daadima, Daadimba	Maathulai	Whole Plant
68	<i>Ricinus communis</i> L.	Euphorbiaceae	Diveligo, Diveli, Erandi	Bed anjeer (Arand)	Eranda, Chitrabija	Ammanakku	Fesh sap, Palm Jaggery, Ash of Dry, Spadix, Leaf

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
							stalk & Young root
69	<i>Salvadora persica</i> L.	Salvadoraceae	Piludi	Miswaak, Araak	Pilu (smaller var.), Pilukaa, Sransi, Angaahva, Tikshnavrksha	Perungoli	Whole Plant
70	<i>Sapindus laurifolius</i> Vahl.	Sapindaceae	Aritha	Reethaa	Arishtaka, Reethaakaranja	Puvamkottai, Mani pungu	Root & Fruit
71	<i>Solanum surattense</i> Burm. F.	Solanaceae	Bhiya ringani	Kataai Khurd Hadaq	Kantakaari, Vyaaghri	Kandankatthiri	Whole Plant
72	<i>Sterculia urens</i> Roxb.	Sterculiaceae	Karai, Kandol	Kateera (Gond)	Karaya gum	Kavalam	Leaves & Gum
73	<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae	Jambu	Jamun	Jambu, Mahaaphalaa	Naaval	Bark, Leaves, Fruit & Seeds
74	<i>Tamarindus indica</i> L.	Caesalpiniaceae	Aamli	Tamar Hindi	Amli, Amlikaa, Chinchaa	Puli, Aanvilam	Bark, Leaves & Fruits
75	<i>Terminalia arjuna</i> (Roxb.) W. & A.	Combretaceae	Arjun, Sadado	Arjun	Arjuna	Poomarudhu, Neelamarudhu	Stem Bark & Fruits
76	<i>Tinospora cordifolia</i> (Willd.) Miers.	Menispermaceae	Giloya, Guduchi, Galo	Gilo, Sat-e-Gilo	Guduuchi, Amrita, Amritaa	Seenil, Amrida-valli	Root & Stem
77	<i>Tribulus terrestris</i> L.	Zygophyllaceae	Gokhru	Khar-e-khasak	Gokshura,) Gokharu	Sirunenunji, Nerinjil, Nerunjil	Root, Leaves & Fruits

Sr. No.	Plant Name	Family	Folk/local name	Unani name	Ayurveda name	Siddha name	Plant Part Used
78	<i>Urginea indica</i> L.	Liliaceae	Jangli pyaj	Isqeel (Jangali Piyaaz)	Vana-palaandu, Kolakanda, Vajrakanda	Narivengayam	Bulbs
79	<i>Vitex negundo</i> L.	Verbenaceae	Nagoda	Sambhalu	Nirgundi, Nilanirgundi	Nochi, Nalla Nochi, Vellai Nochchi, Nirkundi	Leaves, Flowers & Seeds
80	<i>Withania somnifera</i> (L.) Dunal	Solanaceae	Asvagandha	Asgand	Ashwagandhaa, Kshirakaakoli	Amukkuramkizha ngu	Root, Leaves & Seeds
81	<i>Wrightia tinctoria</i> R.Br.	Apocynceae	Dudhalo	Inderjao Shireen	Kutaja, Indrayava	Pala	Whole Plant
82	<i>Xanthium strumarium</i> L.	Asteraceae	Bana-okraa	Kutta Jhad	Shankheshwara, Arishta, Aartagala	Maruloomatham, Marlumutta	Whole Plant
83	<i>Zizyphus mauritiana</i> Lam.	Rhamnaceae	Bore	Ber	Badar, Kola	Handai	Whole Plant
84	<i>Zizyphus nummularia</i> (Burm. F.) W. & A.	Rhamnaceae	Jhar ber, Bordi	Jharber, Sadarber	Balakapriya	Korgodi, Parpalli-gidda, Narielandai	Root, Leaves and Fruits

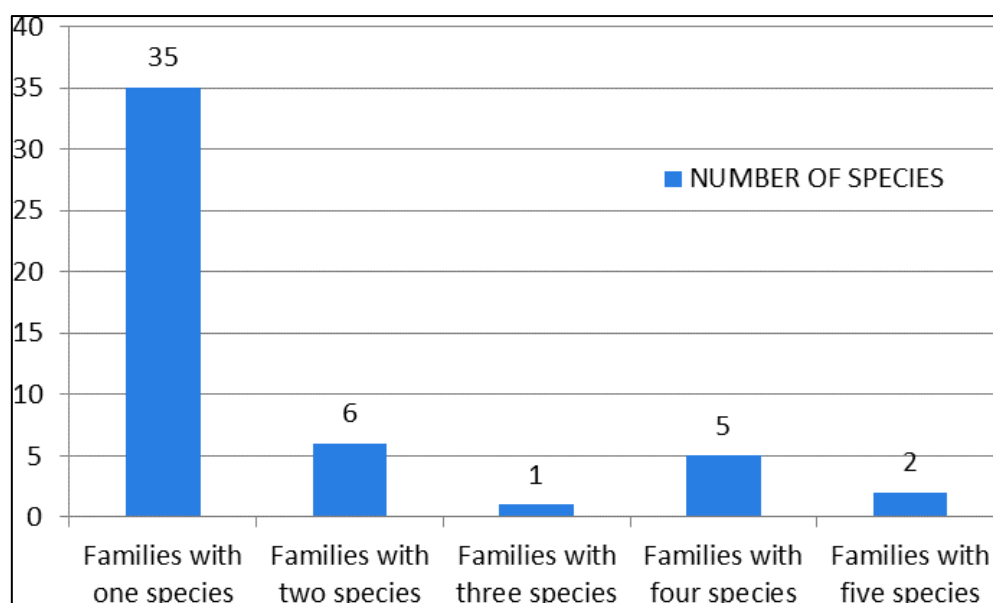


Fig. 6. Distribution of families based on number of species

5.2 Batrisu Vasanu: A Polyherbal Formulation from Central Gujarat.

In parallel with the primary survey, an additional study was conducted in Central Gujarat from 2020 to 2021, focusing on the popular polyherbal formulation known as Batrisu Vasanu or Katlu, which is traditionally used for post-pregnancy care. Although this survey was outside the specified Northwest Gujarat region, its relevance lies in its role as an example of the practical application of Unani medicinal plants in a widely recognized formulation.

The contemporary study, conducted in the central Gujarat region from 2020 to 2021, aimed to analyze the composition and authenticity of Unani medicinal products, specifically focusing on the local popular formulations known as Batrisu Vasanu or Katlu. To achieve this, we have selected as well as visited herbal and Unani medicinal raw material suppliers across three major districts: Vadodara, Ahmadabad and Panchmahal. A total of nine shops, randomly chosen from each district, were surveyed to gather samples of these products. Five products, each with ingredient labels, were included in the study, and their components were cross-verified with Unani practitioners and standard reference books. The ingredients listed on the product labels were validated against vernacular and scientific literature, and the data collected were

analyzed using Microsoft Excel. The Relative Frequency Citation (RFC) value was calculated to assess the prevalence of each plant species in the samples.

To overcome the physical & physiological challenges after childbirth our society has suggested one polyherbal formulation term as a Batrisu Vasanu. We have initiated this particular survey as the ingredients mentioned on the packet of commercial production houses vary in their composition. Botanically this study reports a total of 53 species belonging to 34 different families comprising of 49 genera (Table 9). The major families for these plant species were Zingiberaceae, Apiaceae, and Fabaceae. The relative frequency of citation (RFC) of these herbs ranged from 0.2 to 1.00 (Table 10). Nine plant taxa were commonly seen as in all the samples investigated which also had highest RFC value, supporting its fame for post pregnancy care (Table 11).

Table 9. Enumeration of plant species listed on five different samples

Sr. No.	Scientific name	Family	Unani name	Local name	Plant parts & exudates used
1	<i>Abutilon glaucum</i> (Cav.) Sweet	Malvaceae	Kanghi	Balbij, Baldana	Sd
2	<i>Alpinia galanga</i> (L.) Willd.	Zingiberaceae	Khoolanjan	Panjad, Khulinjan	Rh
3	<i>Anethum graveolens</i> Ucria	Apiaceae	Tukhm Soya	Suva	Sd
4	<i>Asparagus recemosus</i> (Wild)	Asparagaceae	Satawar	Shatavari	Ro
5	<i>Bambusa arundinacea</i> (Retz.) Willd.	Poaceae	Tabasheer	Vaskapoor, Vanslochan	Pe
6	<i>Bombax ceiba</i> L.	Malvaceae	Mochras, Sainbhal	Shimaro	Pe
7	<i>Butea monosperma</i> (Lam.) Kuntze	Fabaceae	Kamarkash	Keshudo	Pe
8	<i>Centaurea behen</i> Lam.	Asteraceae	Behman Safed	Behman Safed	Ro
9	<i>Chlorophytum borivilianum</i> Santapau & R.R. Fern.	Asparagaceae	Musli safaid	Safed Musli	Ro
10	<i>Cinnamomum camphora</i> (L.) J. Presl	Lauraceae	Kapoor	Kapoor	Pe
11	<i>Cinnamomum tamala</i> (Buch.Ham.) T. Nees & C.H. Eberm.	Lauraceae	Taj	Taj	Sb

Sr. No.	Scientific name	Family	Unani name	Local name	Plant parts & exudates used
			Tejpatta	Tamal patra, Tajpatra	Lf
12	<i>Cocos nucifera</i> L.	Arecaceae	Nariyal	Nariyal	Fr
13	<i>Colchicum luteum</i> Baker	Colchicaceae	Sooranjan	Suranjan	C & Sd
14	<i>Coriandrum sativum</i> L.	Apiaceae	Kashneez Khushk	Dhana	Fr
15	<i>Cuminum cyminum</i> L.	Apiaceae	Zeera safaid	Jeera	Sd
16	<i>Curculigo orchoides</i> Gaertn.	Amaryllidaceae	Musli siyah	Kali Musli	Rh
17	<i>Curcuma longa</i> L.	Zingiberaceae	Zardchob, Haldi	Haldar	Rh
18	<i>Dactylorhiza hatagirea</i> (D. Don) Soo	Orchidaceae	Salab misri	Salampanja, Panjabi Salam	Ro
19	<i>Elettaria cardamomum</i> (L.) Maton	Zingiberaceae	Heel Khurd	Elaichi	Fr
20	<i>Foeniculum vulgare</i> Mill.	Apiaceae	Saunf	Variyali	Fr
21	<i>Hygrophila auriculata</i> (Schumach.) Heine	Acanthaceae	Talmakhana	Ekharo	Sd
22	<i>Ipomoea hederacea</i> Jacq.	Convolvulaceae	Habbunneel	Mughalai	Sd

Sr. No.	Scientific name	Family	Unani name	Local name	Plant parts & exudates used
23	<i>Lepidium sativum</i> L.	Cruciferae	Tukhme Hilyun	Asaliyo, Aseriya	Sd
24	<i>Mesua ferrea</i> L.	Guttifereae	Zafran	Nagkesar	Fl
25	<i>Mucuna prurita</i> Wight	Fabaceae	Kauch	Safed Kaucha	Sd
26	<i>Myristica fragrans</i> Houtt.	Myristicaceae	Jayphal	Javintri	Ma
				Jayphal	Nu
27	<i>Orchis latifolia</i> L.	Orchidaceae	Sualab misri	Salabmisri	Ro
28	<i>Paeonia officinalis</i> L.	Paeoniaceae	Ood Saleeb	Peony	Ro
29	<i>Phoenix dactylifera</i> L.	Arecaceae	Khurma	Khajoor	Fr
30	<i>Pinus gerardiana</i> Wall. Ex D.Don	Pinaceae	Maghz Chilgoza	Chilgoza	Sk
31	<i>Piper longum</i> L.	Piperaceae	Filfil Daraz	Ganthoda, Pipalimool	Ro
				Lindipeepar, Pipali	Fr
32	<i>Piper nigrum</i> L.	Piperaceae	Filfil Siyah	Kala Mari	Sd

Sr. No.	Scientific name	Family	Unani name	Local name	Plant parts & exudates used
33	<i>Pistacia vera</i> L.	Anacardiaceae	Maghaze pista	Pista	Sk
34	<i>Plantago ovata</i> Forssk.	Plantaginaceae	Luab Aspghol	Isabgol dana, Othamijiru	Sd
35	<i>Prunus amygdalus</i> Batsch	Rosaceae	Maghaze badam	Badamgir	Sk
36	<i>Pueraria tuberosa</i> (Roxb. Ex Willd.) DC.	Fabaceae	Badari kund	Vidarikand,	Tu
37	<i>Quercus infectoria</i> G. Olivier	Fagaceae	Mazoo	Mayafal, Maujoophal	Ga
38	<i>Rubia cordifolia</i> L.	Rubiaceae	Majeeth	Manjistha	Ro
39	<i>Salvia haematodes</i> L.	Lamiaceae	Behman surkh	Behman Lal	Ro
40	<i>Sida acuta</i> Burm.f.	Malvaceae		Bala	Ro
41	<i>Symplocos racemosa</i> Roxb.	Symplocaceae	Lodh Pathani	Lodhar	Sb
42	<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	Myrtaceae	Qaranfal	Laving	Fl
43	<i>Terminalia arjuna</i> (Roxb. Ex DC.) Wight & Arn.	Combretaceae	Arjun	Arjun	Sb
44	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	Balela	Baheda	Sb

Sr. No.	Scientific name	Family	Unani name	Local name	Plant parts & exudates used
45	<i>Terminalia tomentosa</i> Wight & Arn.	Combretaceae		Sadad, Asana	Sb
46	<i>Trachyspermum ammi</i> (L.) Sprague	Apiaceae	Ajwain	Ajwain, Ajmo	Fr
47	<i>Trapa bispinosa</i> Roxb.	Lytheraceae	Singhara Khushk	Singhara nut	Fr
48	<i>Tribulus terrestris</i> L.	Zygophyllaceae	Kharkhasak	Gokhru	Fr
49	<i>Trigonella foenum-graecum</i> L.	Fabaceae	Hulba	Methi	Sd
50	<i>Vitex negundo</i> L.	Verbenaceae	Sambhaloo	Nirgundi, Harenu, Renuka	Fr
51	<i>Withania somnifera</i> (L.) Dunal	Solanaceae	Asgandh	Ashwagandha	Ro
52	<i>Zanthoxylum armatum</i> DC. (Fruit)	Rutaceae	Kababkhanda	Tejbal	Fr
				Tejovati	Sb
53	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Zanjabil	Sunth	Rh

Abbreviations: Rhizome (Rh); Root (Ro); Tuberous root (Tu); Stem bark (Sb); Corm (C); Leaf (Lf); Flower bud (Fl); Fruit (Fr); Seed (Sd); Seed Kernel (Sk); Gall (Ga); Nutmeg (Nu); Mace (Ma); Plant Exudates (Pe).

Table 10. Ingredients in five different samples (PH01-PH05) and relative frequency of citation (RFC) value

Sr. No.	Botanical name	Plant parts & exudates used	PH01	PH02	PH03	PH04	PH05	RFC value	Approximate Amount of plant parts & exudates used (grams)
1	<i>Abutilon glaucum</i> (Cav.) Sweet	Sd.	1	0	0	0	0	0.2	10
2	<i>Alpinia galanga</i> (L.) Willd.	Rh.	0	1	1	0	0	0.4	10
3	<i>Anethum graveolens</i> L.	Sd.	0	1	0	0	1	0.4	10
4	<i>Asparagus recemosus</i> Willd.	Ro.	1	1	1	1	1	1	20
5	<i>Bambusa arundinacea</i> (Retz.) Willd.	Pe.	0	0	0	1	0	0.2	10
6	<i>Bombax ceiba</i> L.	Pe.	1	0	0	0	1	0.4	10
7	<i>Butea monosperma</i> (Lam.) Kuntze	Pe.	1	0	1	0	1	0.6	10
8	<i>Centaurea behen</i> Lam.	Ro.	1	1	1	1	0	0.8	15
9	<i>Chlorophytum borivillianum</i> Santapau & R.R.Fern.	Ro.	1	0	1	0	1	0.6	25
10	<i>Cinnamomum camphora</i> (L.) J. Presl	Pe.	1	0	0	0	1	0.4	10
11	<i>Cinnamomum tamala</i> (Buch.Ham.) T. Nees & C.H. Eberm.	Sb.	1	0	0	1	1	0.6	10
		Lf.	1	0	0	1	1	0.6	25
12	<i>Cocos nucifera</i> L.	Fr.	0	1	0	0	0	0.2	10
13	<i>Colchicum luteum</i> Baker	C & Sd.	0	0	1	0	0	0.2	25

Sr. No.	Botanical name	Plant parts & exudates used	PH01	PH02	PH03	PH04	PH05	RFC value	Approximate Amount of plant parts & exudates used (grams)
14	<i>Coriandrum sativum</i> L.	Fr.	0	1	1	0	0	0.4	10
15	<i>Cuminum cyminum</i> L.	Sd.	0	0	0	1	0	0.2	10
16	<i>Curculigo orchiodes</i> Gaertn.	Rh.	1	0	1	0	1	0.6	30
17	<i>Curcuma longa</i> L.	Rh.	1	1	1	0	1	0.8	10
18	<i>Dactylorhiza hatagirea</i> (D. Don) Soo	Ro.	1	0	1	0	0	0.4	15
19	<i>Elettaria cardamomum</i> (L.) Maton	Fr.	1	1	1	1	1	1	10
20	<i>Foeniculum vulgare</i> Mill.	Fr.	0	0	0	1	0	0.2	10
21	<i>Hygrophila auriculata</i> (Schumach.) Heine	Sd.	1	1	1	0	0	0.6	10
22	<i>Ipomoea hederacea</i> Anon.	Sd.	1	0	0	0	0	0.2	10
23	<i>Lepidium sativum</i> L.	Sd.	0	1	1	0	0	0.4	10
24	<i>Mesua ferrea</i> L.	Fl.	1	0	0	1	1	0.6	15
25	<i>Mucuna prurita</i> Wight	Sd.	1	1	1	1	1	1	50
26	<i>Myristica fragrans</i> Houtt.	Ma.	1	1	1	1	1	1	20
		Nu.	1	1	1	1	1	1	10
27	<i>Orchis latifolia</i> L.	Ro.	1	1	0	1	0	0.6	20
28	<i>Paeonia officinalis</i> Thunb.	Ro.	0	1	1	0	0	0.4	10

Sr. No.	Botanical name	Plant parts & exudates used	PH01	PH02	PH03	PH04	PH05	RFC value	Approximate Amount of plant parts & exudates used (grams)
29	<i>Phoenix dactylifera</i> L.	Fr.	0	1	1	0	0	0.4	50
30	<i>Pinus gerardiana</i> Wall. ex D.Don	Sk.	0	1	0	0	0	0.2	10
31	<i>Piper longum</i> L.	Ro.	1	1	1	1	1	1	50
		Fr.	1	0	0	0	1	0.4	30
32	<i>Piper nigrum</i> L.	Sd.	1	1	1	1	1	1	15
33	<i>Pistacia vera</i> L.	Sk.	0	0	0	0	1	0.2	10
34	<i>Plantago ovata</i> Forssk.	Sd.	0	0	0	0	1	0.2	10
35	<i>Prunus amygdalus</i> Batsch	Sk.	0	0	0	0	1	0.2	10
36	<i>Pueraria tuberosa</i> (Roxb. ex Willd.) DC.	Tu.	1	0	0	0	0	0.2	10
37	<i>Quercus infectoria</i> G. Olivier	Ga.	0	0	1	0	0	0.2	25
38	<i>Rubia cordifolia</i> L.	Ro.	1	0	0	0	0	0.2	10
39	<i>Salvia haematodes</i> L.	Ro.	1	1	1	1	0	0.8	10
40	<i>Sida acuta</i> Burm.f.	Ro.	1	0	1	0	0	0.4	10
41	<i>Symplocos macrophylla</i> subsp. Rosea (Bedd.) Noot.	Sb.	1	0	0	0	1	0.4	10
42	<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	Fl.	0	0	0	1	0	0.2	10

Sr. No.	Botanical name	Plant parts & exudates used	PH01	PH02	PH03	PH04	PH05	RFC value	Approximate Amount of plant parts & exudates used (grams)
43	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Sb.	0	1	1	1	0	0.6	10
44	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Sb.	0	0	0	1	0	0.2	10
45	<i>Terminalia tomentosa</i> Wight & Arn.	Sb.	0	0	1	0	0	0.2	10
46	<i>Trachyspermum ammi</i> (L.) Sprague	Fr.	0	0	0	1	0	0.2	10
47	<i>Trapa bispinosa</i> Roxb.	Fr.	0	1	1	0	0	0.4	15
48	<i>Tribulus terrestris</i> L.	Fr.	1	0	0	0	0	0.2	30
49	<i>Trigonella foenum-graecum</i> L.	Sd.	0	1	1	1	0	0.6	15
50	<i>Vitex negundo</i> L.	Fr.	1	0	0	0	0	0.2	10
51	<i>Withania somnifera</i> (L.) Dunal	Ro.	1	1	1	1	1	1	50
52	<i>Zanthoxylum armatum</i> DC.	Fr.	1	1	1	1	1	1	10
		Sb.	0	1	0	1	1	0.6	20
53	<i>Zingiber officinale</i> Roscoe	Rh.	1	1	1	1	1	1	50

Abbreviations: Rhizome (Rh); Root (Ro); Tuberous root (Tu); Stem bark (Sb); Corm (C); Leaf (Lf); Flower bud (Fl); Fruit (Fr); Seed (Sd); Seed Kernel (Sk); Gall (Ga); Nutmeg (Nu); Mace (Ma); Plant Exudates (Pe).

Table 11. Nine common plants found in all five samples

Sr. No.	Scientific name	Plant part	Therapeutic actions
1	<i>Asparagus racemosus</i> Willd.	Root	To treat Lactation Problems (Yadav et al., 2006), Increased Milk production concurrent with increased growth of mammary glands (Farzana et. al., 2016), Female tonic, in threatened abortion, preeclampsia associated with pregnancy, Galactagogue (Karemore and Avari, 2017).
2	<i>Elettaria cardamomum</i> (L.) Maton	Fruit	Aids in digestion (Korikanthimath et. al., 2001), antiemetic & antispasmodic (Goyal, 2017). Useful in general weakness, diuretic, useful in abdominal pain & piles (Gogte, 2000).
3	<i>Mucuna prurita</i> Wight	Seed	Aphrodisiac, Anti galactagogue, Fertility promotion effect, Anti-inflammatory, promotes muscle mass & body weight, high nutritional property, useful in impotence & oligospermia (Gogte, 2000).
4	<i>Myristica fragrans</i> Houtt.	Mace, Nutmeg	Stimulant of menstruation, dysmenorrhoea, used in impotency & premature ejaculation (Gogte, 2000). Anti-oxidant, anti-inflammatory & anti-collagenolytic activity (Jangid et. al., 2014).
5	<i>Piper longum</i> L.	Root	Galactagogue, digestive & haematinic (Goyal, 2017). brain tonic, rejuvenator Increased breast milk secretion (Sonitha & Kumar, 2021).
6	<i>Piper nigrum</i> L.	Seed	Galactagogue (Luecha & Umehara, 2013).
7	<i>Withania somnifera</i> (L.) Dunal	Root	Antianxiety & neuroprotective (Kulkarni & Dhir, 2008). General tonic (Umadevi et.al., 2012). Galactagogue (Roqaiya et al., 2015).
8	<i>Zanthoxylum armatum</i> DC. (Bark)	Bark	Improves speech and increases saliva secretion, abortifacient, antifertility & antipyretic (Alam & Saqib, 2015).
9	<i>Zingiber officinale</i> Roscoe	Rhizome	Anti-hyperglycaemic (Kulkarni et al., 2008). Antiemetic, antispasmodic & anti-inflammatory (Goyal, 2017). Galactagogue (Kwan & Abdul-Rahman, 2021).

During the study period, a total of five samples of Batrisu Vasanu from Unani and herbal raw material suppliers' products were collected. The samples were coded as PH01 to PH05 for further study. Only two samples have mentioned the manufacturing date and a detailed nutrition chart on the packet. The names of the herbal constituents were generally written in vernacular Gujarati. The number of plants per sample was highest in PH01 and lowest in PH04 (Fig. 7). The comparative account of herbal plants shows the presence of 58 plant parts and exudates in all samples. A total of 14 different plant parts like seed, stem bark, corm, floral bud, fruit, gall, kernel, leaf, mace, nutmeg, rhizome, root, tuberous root, plant exudates (gum & resin) were reported in the study. The major plant parts used in products were roots, fruits & seeds (18.96%) followed by stem bark (17.24%) (Fig. 8).

Out of 53 plants 12 plants were used in Unani System of Medicines in curing gynaecological disorders of which one plant species is suggested to be for abortifacient, six for emmenagogues, two species having stimulant action on uterine muscles and three species having depressant action on uterine muscles (Kumar, 2014).

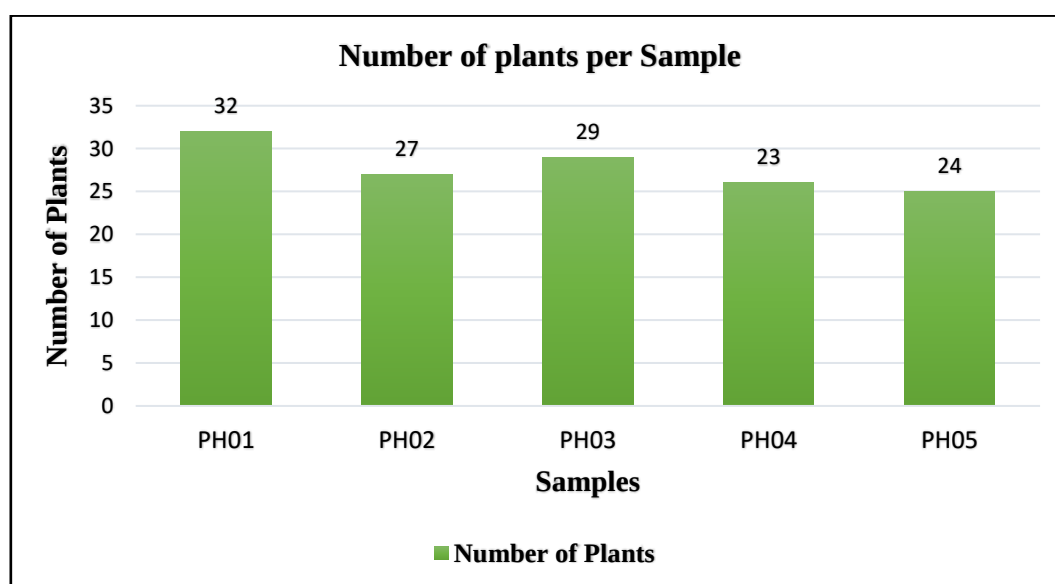


Fig. 7. Number of various plant parts and exudates in five samples

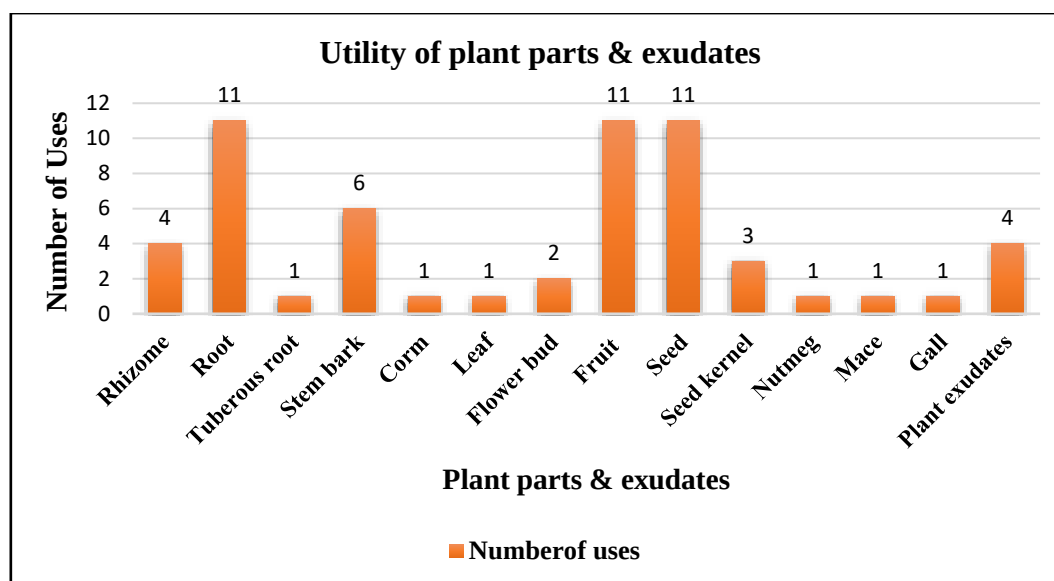


Fig. 8. Number of uses per plant parts and exudates

Together, these surveys provide a robust foundation for understanding both the diversity of Unani medicinal plants in Northwest Gujarat and their practical applications, as exemplified by the Batrisu Vasanu formulation. This dual approach enriches the documentation of traditional knowledge and meets the first objective by offering both a broad overview and a specific case study of Unani medicinal plant use.

5.3 Selection and Authentication of Unani Polyherbal Formulations

5.3.1 Selection of Unani Polyherbal Formulations

The five Unani polyherbal formulations selected for this study provide a balanced representation of both traditional and innovative Unani medicine. The three traditional formulations—Majoon-E-Najah (MN), Sufoof-E-Najah (SN), and Sufoof-E-Chobchini (SC)—were prepared according to the formulations detailed in the Unani Pharmacopoeia (UPI) and the National Formulary of Unani Medicine (NFUM), ensuring their historical significance and therapeutic relevance. The two novel formulations, labeled Formulation 1 (F1) and Formulation 2 (F2), reflect the dynamic and evolving nature of Unani medicine, incorporating contemporary modifications introduced by Hakims while retaining core principles.

5.3.2 Collection and Morphological Authentication of Unani Drugs

The majority of the ingredients for the five formulations were sourced from their natural habitats, with the remainder procured from reputable Unani commercial suppliers. Identification and authentication of the raw materials were carried out using recognized taxonomic literature and floras (Cooke, 1908; Shah, 1978). Herbarium specimens of all the Unani drugs used in this study were prepared and submitted to the BARO Herbarium at The Maharaja Sayajirao University of Baroda, Vadodara, for verification under accession numbers BAROKS01–BAROKS15. These specimens are available for future reference, ensuring the study's reproducibility and traceability.

5.4 Preparation and Extraction of Selected Unani Polyherbal Formulations

5.4.1 Preparation of Unani Polyherbal Formulations

The preparation of the five selected Unani polyherbal formulations was successfully executed, adhering to the standards established by the National Formulary of Unani Medicine (NFUM) and the Unani Pharmacopoeia of India (UPI). Traditional formulations such as Majoon-E-Najah (MN), Safoof-Najah (SN), and Safoof-E-Chobchini (SC) were prepared according to official guidelines, while the two novel formulations, Formulation 1 (F1) and Formulation 2 (F2), were developed based on methodologies recommended by practicing Hakims. This rigorous adherence to standardized preparation methods ensured consistency, facilitating the subsequent pharmacological evaluations.

5.4.2 Organoleptic Characteristics

The organoleptic evaluation of the five selected Unani polyherbal formulations revealed distinct and consistent sensory attributes, which are vital for their therapeutic efficacy and patient acceptance (Mukharjee, 2019). These characteristics align with traditional expectations and are essential for maintaining the quality and consistency of indigenous medicines, which play a significant role in primary health care systems (Kumar and Maneem, 2012; Ekor, 2014).

5.4.2.1 Majoon-E-Najah (MN):

Dark brown, semi-solid consistency with a sweet and slightly bitter taste, accompanied by a pleasant aroma (Fig. 9).



Fig. 9. Majoon-E-Najah (MN) Formulation

5.4.2.2 Sufoof-E-Najah (SN):

Yellowish-brown color, distinctly bitter taste, and a characteristic odor (Fig. 10).



Fig. 10. Powder of Sufoof-E-Najah (SN) Formulation

5.4.2.3 Sufoof-E-Chobchini (SC):

Yellowish-green powder with a slightly bitter taste and an aromatic fragrance (Fig.11).



Fig. 11. Powder of Sufoof-E-Chobchini (SC) Formulation

5.4.2.4 Formulation 1 (F1):

Brown color with a bitter taste and a strong odor (Fig. 12).



Fig. 12. Powder of Formulation 1 (F1)

5.4.2.5 Formulation 2 (F2):

Yellowish Colour with A Slightly Bitter Taste and A Characteristic Odor (Fig.13).

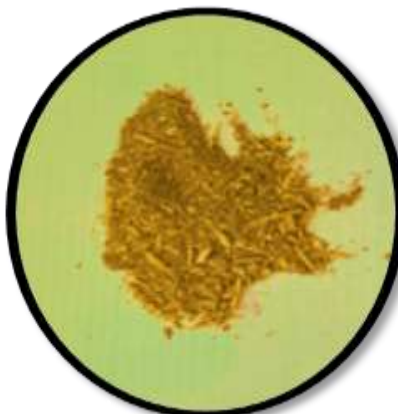


Fig. 13. Powder of Formulation 2 (F2)

5.4.3 Determination of Percentage Yield of Extracts

The efficiency of the extraction process is a crucial factor in the overall effectiveness of an herbal formulation, as it directly influences the concentration of bioactive compounds present in the final product. In this study, the extraction yields of five different formulations—Majoon-E-Najah (MN), Sufoof-E-Najah (SN), Sufoof-E-Chobchini (SC), Formulation 1 (F1), and Formulation 2 (F2)—were determined using various solvent systems (Aqueous, Hydroalcoholic, and Ethanol) and extraction methods (Reflux, Sonication, and Soxhlet) (Fig. 14 and Fig. 15).

The results, summarized in Table 12, reveal considerable variability in extraction efficiencies across different formulations and solvent systems, highlighting the importance of optimizing extraction conditions for each formulation. The following sections provide a detailed formulation-wise analysis of the extraction yields obtained.

5.4.3.1 Majoon-E-Najah (MN)

The extraction yield of Majoon-E-Najah (MN) was evaluated using three solvent systems: aqueous, hydroalcoholic, and ethanolic, with varying extraction methods. The highest yield was achieved using the aqueous solvent with sonication (SN (AQ)), resulting in an extractive value of 55%. This was followed by the hydroalcoholic sonication extract (SN (HA)) with a yield of 28.5%. The ethanolic Soxhlet extraction (SX (ET)) yielded 40%, while sonication in ethanol (SN (ET)) resulted in a 31% yield. The lowest extraction yield was recorded with the aqueous reflux method (RF (AQ)) at 20%, highlighting the efficiency of sonication, especially with aqueous solvents, for maximizing extractive values (Table 5).

5.4.3.2 Sufoof-E-Najah (SN)

The extraction yield of Sufoof-E-Najah (SN) was assessed using aqueous, hydroalcoholic, and ethanolic solvents were determined through sonication. The hydroalcoholic extract (SN (HA)) demonstrated the highest yield at 36.058%, indicating superior solvent efficiency for extracting bioactive constituents from SN. The aqueous extract (SN (AQ)) followed with a yield of 27.862%, while the ethanolic extract (SN (ET)) had the lowest yield of 25.105% (Table 5). These results suggest that the hydroalcoholic solvent system is most effective in this formulation.

5.4.3.3 Sufoof-E-Chobchini (SC)

The extraction yield of Sufoof-E-Chobchini (SC) was also assessed using aqueous, hydroalcoholic, and ethanolic solvents via sonication. The highest yield was observed in the hydroalcoholic extract (SC (HA)) at 14.638%, followed closely by the aqueous extract (SC (AQ)) with a yield of 13.758%. The ethanolic extract (SC (ET)) had the lowest yield at 8.495% (Table 5). This indicates a consistent pattern where hydroalcoholic solvents generally provide higher yields compared to other solvents.

5.4.3.4 Formulation 1 (F1)

In the case of Formulation 1 (F1), the sonication method was employed using aqueous, hydroalcoholic, and ethanolic solvents. The highest extraction yield was recorded with the hydroalcoholic solvent (F1 (HA)) at 16.138%, suggesting this solvent system's efficiency in extracting active compounds. The aqueous extract (F1 (AQ)) produced a yield of 12.795%, while the ethanolic extract (F1 (ET)) had a comparatively lower yield of 7.818% (Table 5).

5.4.3.5 Formulation 2 (F2)

The extraction yields for Formulation 2 (F2) using the sonication method with aqueous, hydroalcoholic, and ethanolic solvents were evaluated. The aqueous extract (F2 (AQ)) yielded to the highest at 11.98%, followed by the hydroalcoholic extract (F2 (HA)) with 11.522%. The lowest yield was observed in the ethanolic extract (F2 (ET)) at 8.403% (Table 12). These findings suggest that both aqueous and hydroalcoholic solvents are comparatively more efficient than ethanolic solvents for this formulation.

Table 12. Extraction Methods for Selected Unani Polyherbal Formulations using Different Solvents and Techniques

SR. NO.	Formulation	Solvent	Extraction method	% Extractive Yield Value (gm)	Code given
1	Majoon-E-Najah (MN)	Aqueous	Reflux	20	RF (AQ)
		Aqueous	Sonication	55	SN (AQ)
		Hydro alcoholic	Reflux	19.25	RF (HA)
		Hydro alcoholic	Sonication	28.5	SN (HA)
		Ethanol	Soxhlet	40	SX (ET)
		Ethanol	Sonication	31	SN (ET)
2	Safoof-E-Najah (SN)	Aqueous	Sonication	27.862	SN (AQ)
		Hydro alcoholic	Sonication	36.058	SN (HA)
		Ethanol	Sonication	25.105	SN (ET)
3	Safoof-E-Chobchini (SC)	Aqueous	Sonication	13.758	SC (AQ)
		Hydro alcoholic	Sonication	14.638	SC (HA)
		Ethanol	Sonication	8.495	SC (ET)
4	Formulation 1 (F1)	Aqueous	Sonication	7.818	F1 (AQ)
		Hydro alcoholic	Sonication	16.138	F1 (HA)
		Ethanol	Sonication	12.795	F1 (ET)
5	Formulation 2 (F2)	Aqueous	Sonication	11.98	F2 (AQ)
		Hydro alcoholic	Sonication	11.522	F2 (HA)
		Ethanol	Sonication	8.403	F2 (ET)

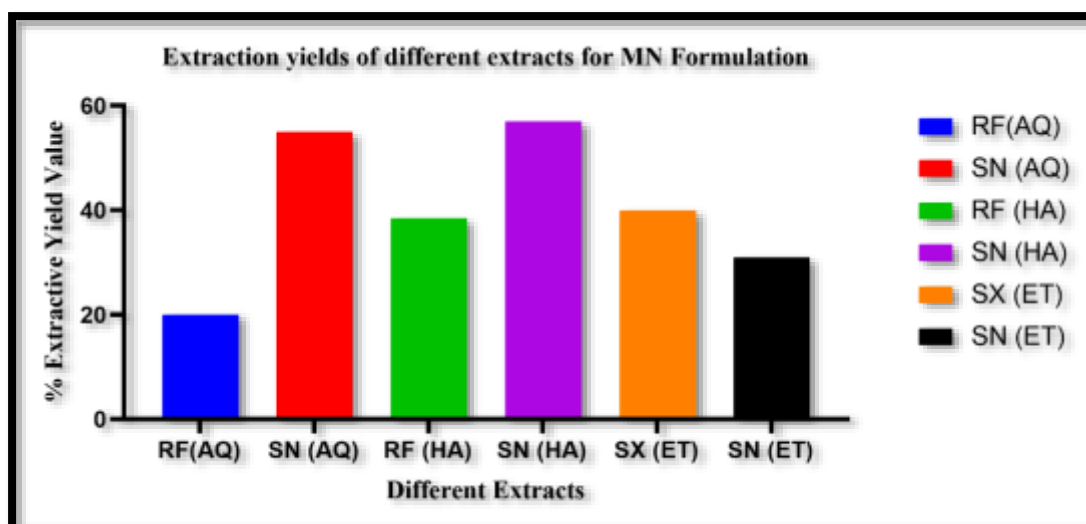


Fig. 14. Extraction yields of different extracts for Majoon-E-Najah (MN) Formulation

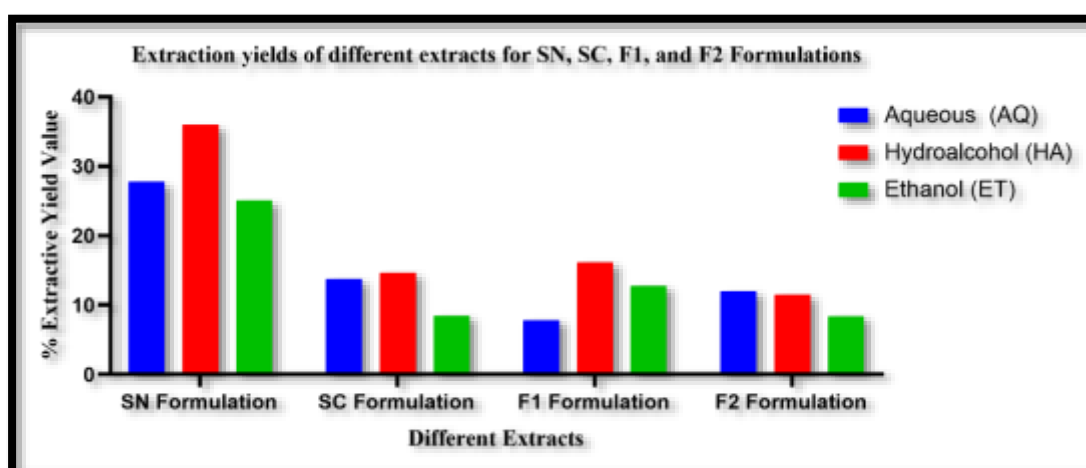


Fig. 15. Extraction yields of different extracts for SN, SC, F1, F2 Formulations

5.5 *In vitro* Pharmacological Evaluation of Selected Unani Polyherbal Formulations

The *In vitro* pharmacological evaluation of five selected Unani polyherbal formulations, using three extracts (Aqueous, Hydroalcoholic, and Ethanolic), was conducted to assess their therapeutic potential. Antibacterial activity was tested against *Escherichia coli* and *Bacillus megaterium*, while antifungal activity was evaluated

against *Candida albicans* and *Aspergillus niger*. In addition, the antioxidant activity of these extracts was determined using the DPPH free radical scavenging assay.

5.5.1 Evaluation of Antibacterial activity

In the investigation of antibacterial activity, selected five distinct Unani polyherbal formulations were tested against two bacterial strains, gram-negative (*Escherichia coli*) and gram-positive (*Bacillus megaterium*). The Agar Well diffusion assay was employed to assess the antibacterial activity of these formulations. Using different extracts, the formulations exhibited substantial antibacterial efficacy, as evidenced by the formation of clear inhibition zones in the agar well diffusion assay. The antibacterial activity demonstrated variability among the formulations, a phenomenon notably influenced by the choice of the extraction solvent. Concentration-dependent effects were observed, with higher concentrations of the formulations resulting in larger inhibition zones. Standard antibiotics, Ciprofloxacin, and Gentamicin were employed as positive controls, while the negative control was prepared using the respective solvents. Clear zones of inhibition (ZOI) were observed around the wells impregnated with the formulations, indicating potential antimicrobial efficacy. The diameter of the inhibition zones was measured, and the data is presented as mean $\pm$ SD of the three replicates.

5.5.1.1 Majoon-E-Najah (MN)

The antibacterial efficacy of Majoon-E-Najah (MN) was evaluated against *Escherichia coli* and *Bacillus megaterium* using aqueous (RF (AQ)), hydroalcoholic (RF (HA)), and ethanolic (SX (ET) and SN (ET)) extracts at concentrations ranging from 0.2 to 10.0 mg/ml (Table 13).

In the case of *Escherichia coli*, the RF (AQ) extract exhibited limited antibacterial activity, with inhibition zones observed only at higher concentrations, measuring 9.2 mm and 6 mm at 5 mg/ml and 10 mg/ml, respectively. The RF (HA) extract demonstrated slightly improved activity, achieving a maximum inhibition zone of 6.9 mm at 10 mg/ml; however, its overall performance remained lower compared to the ethanolic extracts. The ethanolic extracts demonstrated superior and consistent

antibacterial activity across all concentrations tested. The SX (ET) extract exhibited inhibition zones ranging from 5.1 mm to a maximum of 8.5 mm at 10 mg/ml, while the SN (ET) extract achieved zones from 5 mm to a maximum of 10 mm at 10 mg/ml (Fig. 16). These findings underscore the enhanced antibacterial efficacy of the ethanolic extracts, which significantly outperformed their aqueous and hydroalcoholic counterparts.

Similarly, against *Bacillus megaterium*, RF (AQ) displayed minimal antibacterial activity, with inhibition zones of 6 mm and 6.5 mm at 5 mg/ml and 10 mg/ml, respectively. The RF (HA) extract showed moderate activity, producing a 9 mm inhibition zone at 10 mg/ml, but it was less effective than the ethanolic extracts. The ethanolic extracts demonstrated remarkable antibacterial potency. SX (ET) exhibited inhibition zones ranging from 1 mm at 0.6 mg/ml to 11 mm at 10 mg/ml, while SN (ET) achieved zones from 1 mm to a maximum of 13 mm at 10 mg/ml (Fig. 17). These findings highlight the exceptional antibacterial potential of the ethanolic extracts, which consistently surpassed the activity of both aqueous and hydroalcoholic extracts, emphasizing the efficacy of ethanolic extraction in isolating bioactive antibacterial compounds (Photoplates 1 and 2).

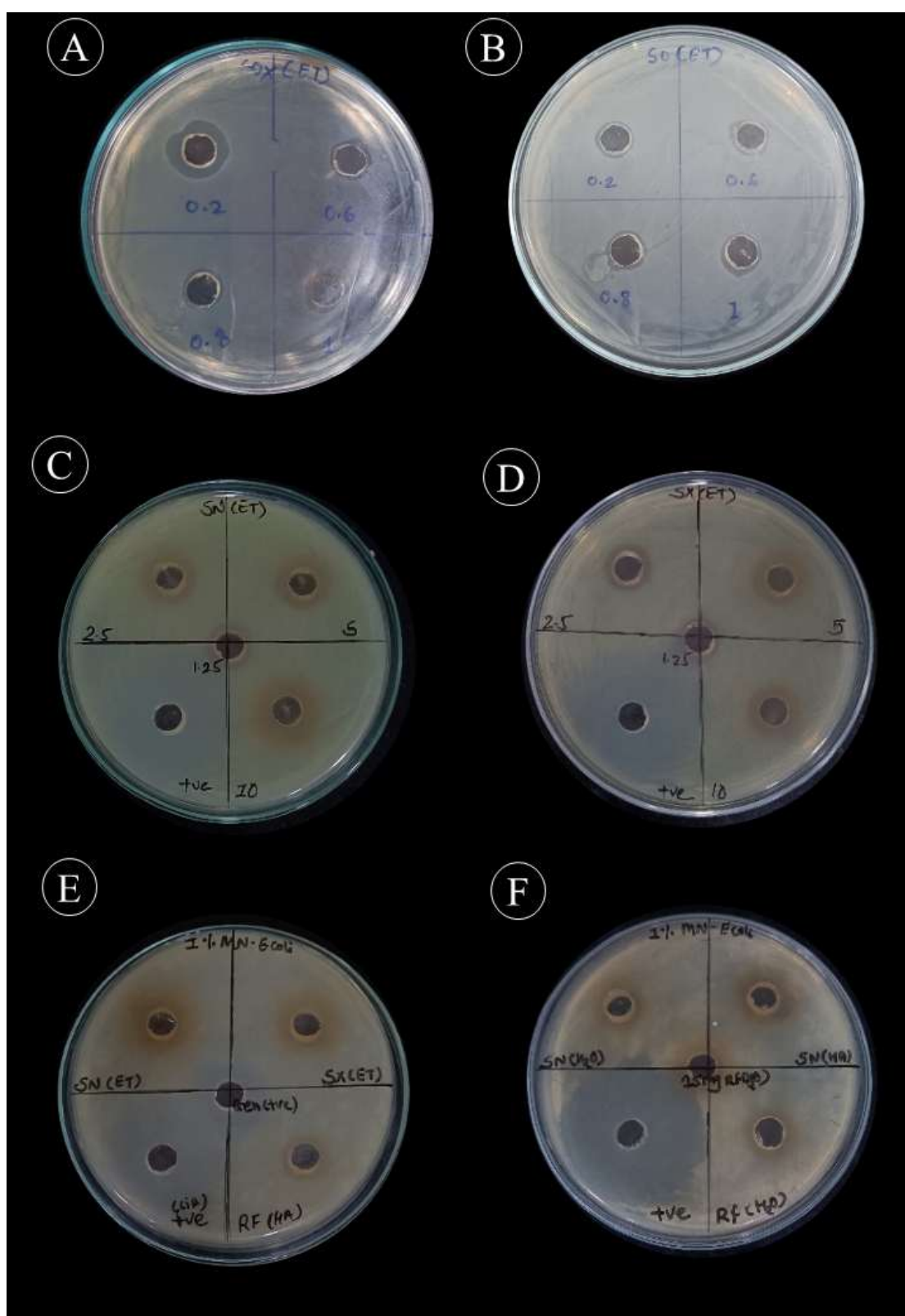
Table 13. Antibacterial activity of Majoon-E-Najah (MN) polyherbal extracts.

Micro-organism Bacteria	Different extracti on methods	Different Concentrations (mg/ml) (Mean Inhibition Zone in mm)								Ciprofloxacin (5 µg/ml)	(Gentamicin (5 µg/ml)
		0.2 mg/ml	0.6 mg/ml	0.8 mg/ml	1.0 mg/ml	1.25mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml (1%)		
<i>Escherichia coli</i>	RF (AQ)	5±0.57 ^a	0±00	5.5±0.15 _a	0±00	0±00	0±00	9.2±0.5 ^a	6±0.3 ^a	18.6±0.66 ^a	9±0.57 ^a
	RF (HA)	0±00	0±00	0±00	0±00	0±00	0±00	0±00	6.9±0.12 _b	18.8±0.33 ^b	9.2±0.13 ^b
	SN (AQ)	0±00	0±00	0±00	0±00	8.5±0.57 _c	0±00	0±00	6±0.5 ^c	19±0.44 ^c	9±0.39 ^c
	SN (HA)	0±00 ^b	0±00	0±00	0±00	0±00	0±00	0±00	8.2±0.57 _b	18.9±0.25 ^b	9.1±0.13 ^b
	SX (ET)	8.5±0.2 ^d	6.5±0.1 _{21^d}	5.5±0.57 _d	6.5±0.1 ^d	5.1±0.5 ^d	7±0.22 ^d	8.2±0.15 _d	8.5±0.20 _d	19±0.76 ^d	9.4±0.60 ^d
	SN (ET)	6±0.27 ^d	5.5±0.5 _{7^d}	5±0.2 ^d	5.5±0.1 _{5^d}	6.2±0.2 ^d	7.2±0.5 _d	8±0.34 ^d	10±0.57 ^d	19.1±0.25 ^d	9.3±0.36 ^d

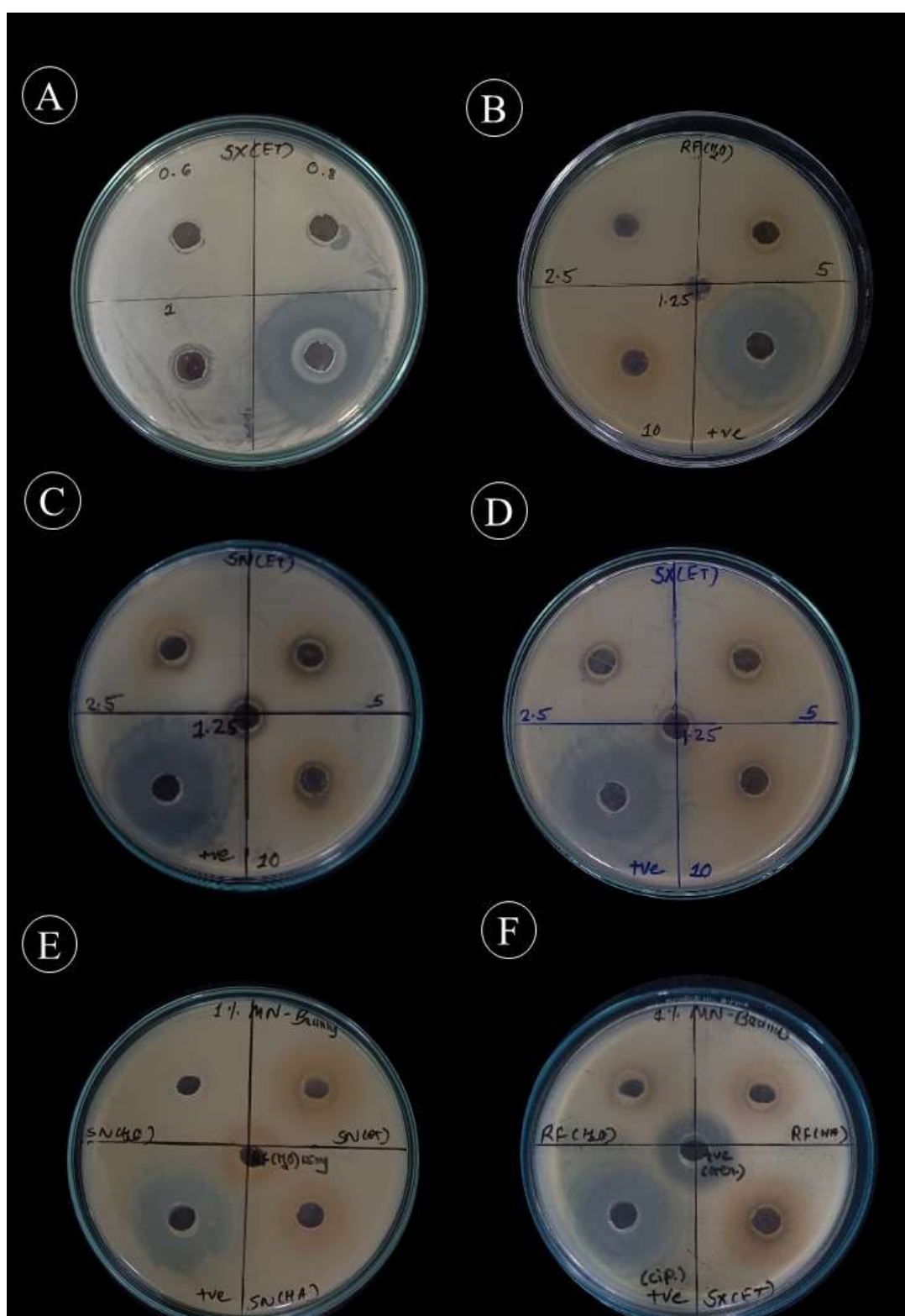
Micro-organism Bacteria	Different t extracti on methods	Different Concentrations (mg/ml) (Mean Inhibition Zone in mm)								Ciprofloxacin (5 µg/ml)	(Gentamycin (5 µg/ml)
		0.2 mg/ml	0.6 mg/ml	0.8 mg/ml	1.0 mg/ml	1.25mg/ ml	2.5 mg/ml	5 mg/ml	10 mg/ml (1%)		
<i>Bacillus megaterium</i>	RF (AQ)	0±00	0±00	0±00	0±00	0±00	0±00	6±0.07 ^a	6.5±0.14 _a	19±0.33 ^a	10±0.29 ^a
	RF (HA)	0±00	0±00	0±00	0±00	0±00	0±00	0±00	9±0.12 ^b	21±0.57 ^b	10.1±0.66 ^b
	SN (AQ)	0±00	0±00	0±00	0±00	0±00	0±00	0±00	6.5±0.10 _c	20.9±0.56 ^c	10.3±0.78 ^c
	SN (HA)	0±00	0±00	0±00	0±00	0±00	0±00	0±00	8.5±0.22 _d	19.8±1.2 ^d	10.2±0.33 ^d
	SX (ET)	0±00	1±0.07 ^e	1±0.23 ^e	2±0.16 ^e	6±0.14 ^e	6.3±0.3 _{3e}	8±0.39 ^e	11±0.24 ^e	20±0.66 ^e	10.37±0.5 _{6e}
	SN (ET)	0±00 ^f	1±0.15 ^f	1±0.12 ^f	1.2±0.1 _{7f}	6.2±0.37 _f	8.3±0.5 _{5f}	8.5±0.35 _f	13±0.49 ^f	21.2±0.57 ^f	10.3±0.78 ^f

Each value is mean ± SD (n = 3). Different letters (a, b, c, d) indicate significant differences between groups at $p < 0.0001$ for *E. coli*. Same letters denote no significant difference. For *B. megaterium*, different letters (a, b, c, d, e, f) denote significant differences at various significant levels, with some pairs showing differences as strong as $p < 0.0001$.

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Photoplate 1. Antibacterial activity of Majoon-E-Najah (MN)**Antibacterial activity of MN formulation: A-F: MN (AQ, HA, ET) against *Escherichia coli*.**

Photoplate 2. Antibacterial activity of Majoon-E-Najah (MN)



Antibacterial activity of MN formulation: A-F: MN (AQ, HA, ET) *Bacillus megaterium*.

5.5.1.2 Sufoof-E-Najah (SN)

The antibacterial activity of Sufoof-E-Najah (SN) extracts were tested at concentrations ranging from 1.5 to 10 mg/ml (Table 14; Photoplate 3 and 4). The Hydroalcoholic (HA) extract demonstrated the highest efficacy against *Escherichia coli*, with zones of inhibition (ZOI) ranging from 7.4 mm to 13 mm, followed closely by the Ethanolic (ET) extract with ZOI from 7 mm to 8.7 mm. The Aqueous (AQ) extract was less effective, showing ZOI from 7.5 mm to 8 mm (Fig. 18). Against *Bacillus megaterium*, the Ethanolic extract was the most potent, with ZOI ranging from 6 mm to 10 mm, while the Hydroalcoholic extract showed ZOI from 5 mm to 8 mm. The Aqueous extract was the least effective, with ZOI from 5 mm to 7 mm (Fig. 19).

Table 14. Antibacterial activity of Suffof-E-Najah (SN) Formulation

Micro-organism Bacteria	Different extraction methods	Different Concentrations (mg/ml) (Mean Inhibition Zone in mm)								
		1.5 mg/ml	2 mg/ml	2.5 mg/ml	3 mg/ml	4 mg/ml	5 mg/ml	10 mg/ml	Ciprofloxacin (5 µg/ml)	Gentamicin (5 µg/ml)
<i>Escherichia coli</i>	Su.MN (AQ)	7.5±0.57 ^a	8±0.26 ^a	0±00	0±00	0±00	0±00	0±00	21.66±0.79 ^a	9±0.66 ^a
	Su. MN (HA)	7.4±0.78 ^b	7.6±0.2 ^b ₂	8±0.44	8.1±0.14 ^b	11±0.57 ^b	13±0.5 ^b	0±00	21.33±0.54 ^b	9.1±0.33 ^b
	Su.MN (ET)	7.2±0.66 ^c	8±0.35 ^c	8.3±0.4 ^c ₅	8.5±0.57 ^c	7±0.48 ^c	8.7±0.7 ^c ₈	0±00	22±0.60 ^c	9.30±0.41 ^c
<i>Bacillus megaterium</i>	Su.MN (AQ)	7±0.33 ^a	6±0.22 ^a	6.1±0.6 ^a ₆	0±00	5±0.46 ^a	7±0.35 ^a	6.5±0.78 ^a	18.7±0.14 ^a	9±0.25 ^a
	Su. MN (HA)	6±0.13 ^a	6.1±0.1 ^a ₇	8±0.39 ^a	0±00	5±0.27 ^a	6.5±0.6 ^a ₉	7±0.38 ^a	19.8±0.67 ^a	9.2±0.58 ^a
	Su.MN (ET)	10±0.38 ^b	7±0.20 ^b	6.5±0.6 ^b ₆	10±0.33 ^b	6±0.15 ^b	8±0.22 ^b	7.8±0.60 ^b	20.6±0.57 ^b	9.5±0.47 ^b

Each value is presented as mean ± SD (n = 3). Different letters (a, b, c) denote statistically highly significant differences between groups at $p < 0.0001$ (****) against *Escherichia coli*. In the case of *Bacillus megaterium*, the same letter "a" indicates no statistically significant difference (ns) between Aqueous (AQ) and Hydroalcoholic (HA) extracts. Different letters indicate significant differences between the pairs: "a" and "b" between AQ and Ethanolic (ET) extracts (****, $p < 0.0001$), and "a" and "b" between HA and ET extracts (****, $p < 0.0001$).

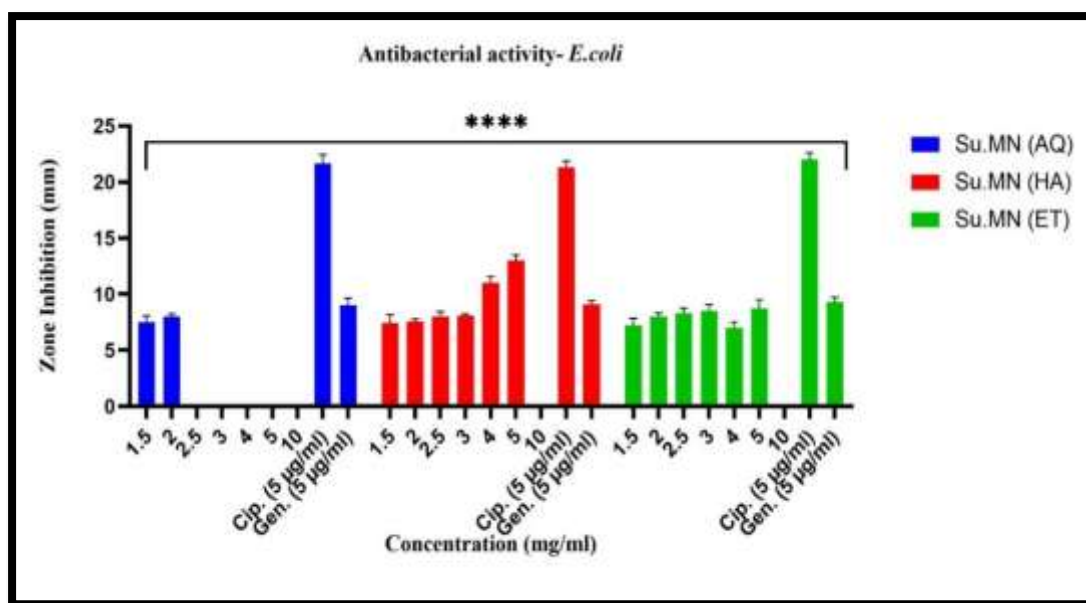


Fig. 18. Antibacterial activity of Su.MN Polyherbal extracts against *Escherichia coli*

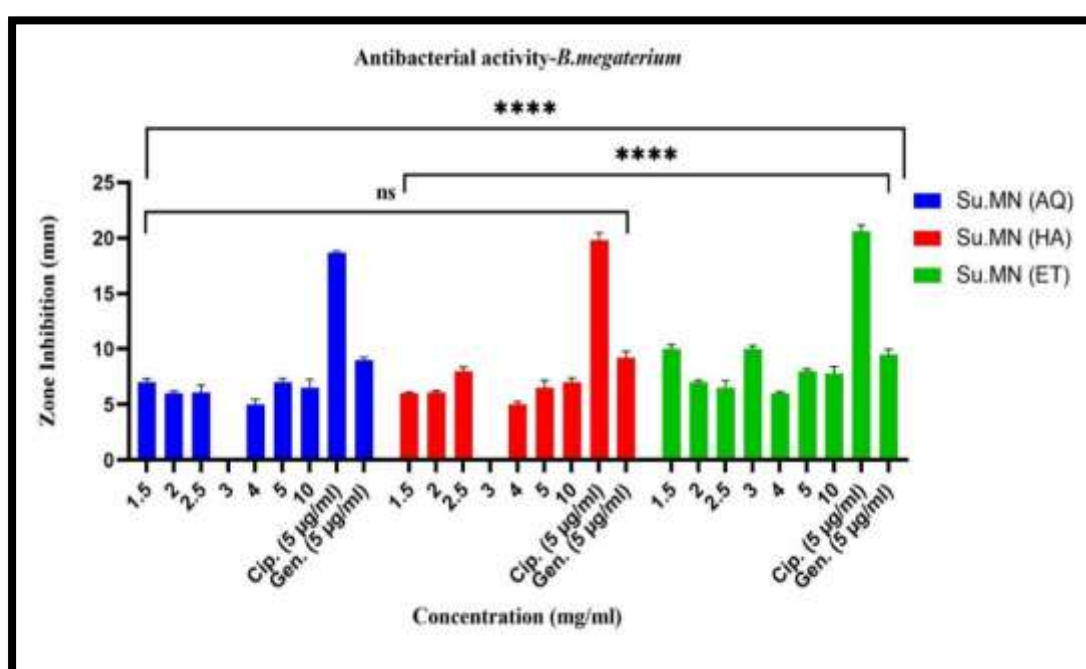
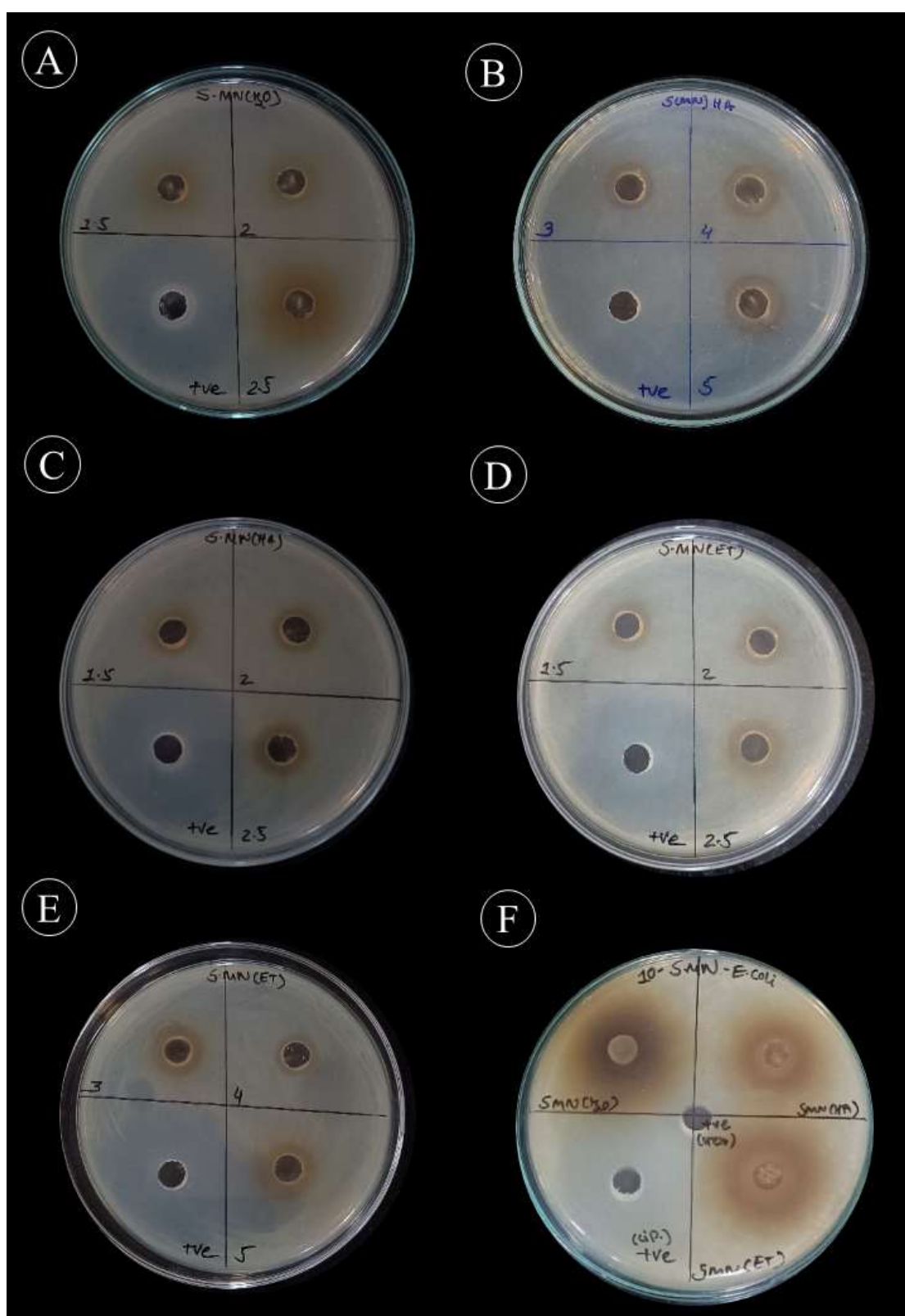
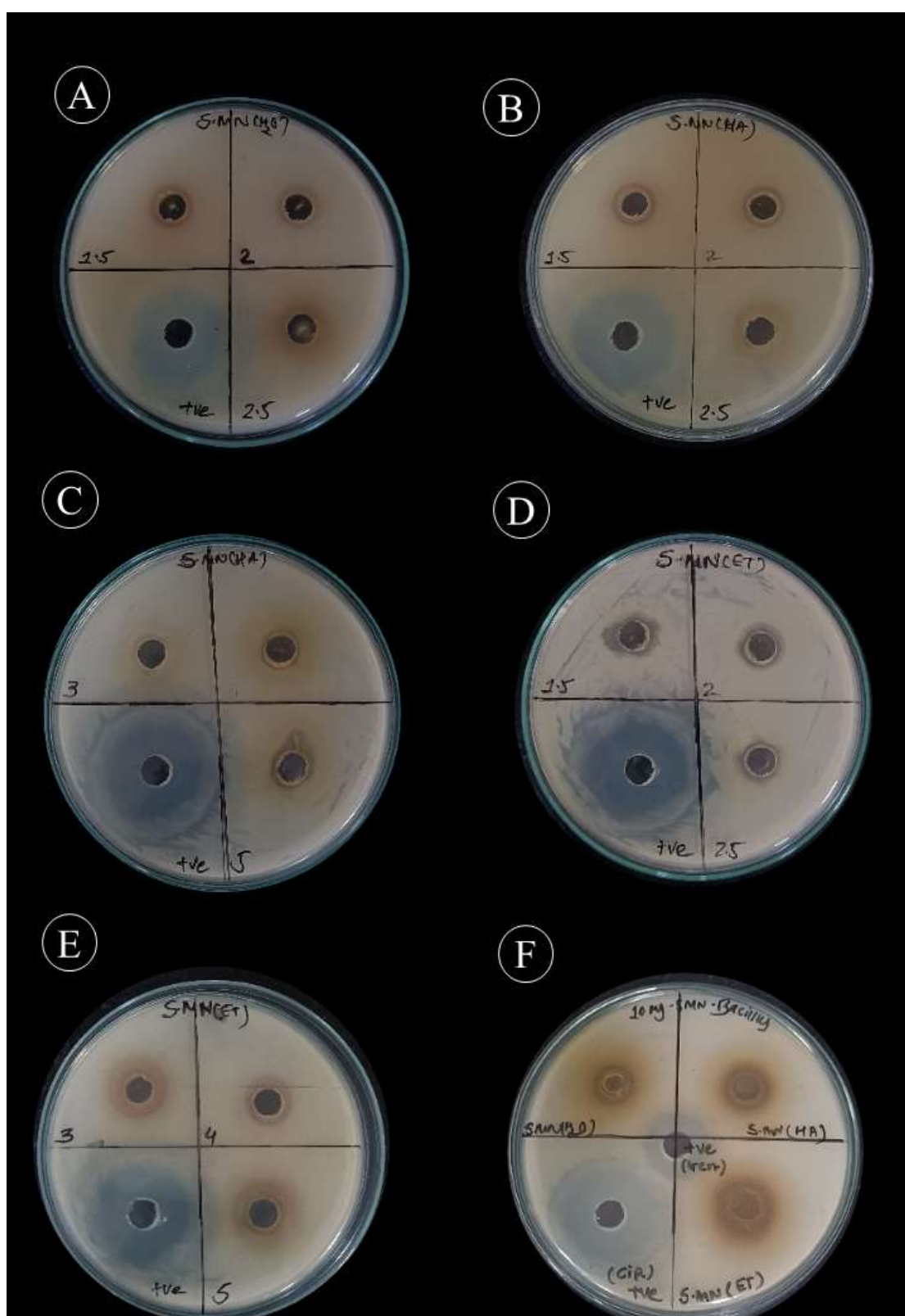


Fig. 19. Antibacterial activity of Su.MN Polyherbal extracts against *Bacillus megaterium*

Photoplate 3. Antibacterial activity of Suffof-E-Najah (SN)**Antibacterial activity of SN formulation: A-F: SN (AQ, HA, ET) against *Escherichia coli*.**

Photoplate 4. Antibacterial activity of Suffof-E-Najah (SN)



Antibacterial activity of SN formulation: A–F: SN (AQ, HA, ET) against *Bacillus megaterium*.

5.5.1.3 Sufoof-E-Chobchini (SC)

The antibacterial activity of Sufoof-E-Chobchini (SC) extracts were tested at concentrations ranging from 1.25 to 10 mg/ml (Table 15; Photoplate 5). In the case of *Escherichia coli*, the Hydroalcoholic (HA) extract exhibited the highest antibacterial activity, with ZOI from 6.2 mm to 8 mm, followed by the Aqueous (AQ) extract with ZOI from 5.5 mm to 7 mm. The Ethanolic (ET) extract showed minimal activity, with ZOI from 0 mm to 7.2 mm (Fig. 20). In contrast, against *Bacillus megaterium*, both the Hydroalcoholic (HA) and Ethanolic (ET) extracts showed significant activity, with ZOI ranging from 7.1 mm to 14 mm for HA and 6.3 mm to 8.5 mm for ET, while the Aqueous (AQ) extract exhibited ZOI from 0 mm to 8 mm (Fig. 21).

Table 15. Antibacterial activity of Suffof-E-Chobchini (SC) Formulation

Microorganism Bacteria	Different Concentrations (mg/ml)	Different extraction methods (Mean Inhibition Zone in mm)		
		SC (AQ) extract	SC (HA) extract	SC (ET) extract
<i>Escherichia coli</i>	1.25	0±00	6.2±0.233 ^b	0±00
	2.5	5.5±0.121 ^a	6.5±0.151 ^b	0±00
	5	6±0.33 ^a	7±0.152 ^b	0±00
	10	7±0.57 ^a	8±0.5 ^b	7.2±0.66 ^c
Standard - Ciprofloxacin	5 µg/ml	17±0.21 ^a	17.2±0.125 ^b	17.3±0.28 ^c
Standard- Gentamicin	5 µg/ml	15±0.57 ^a	15.1±0.45 ^b	15±0.78 ^c
<i>Bacillus megaterium</i>	1.25	0±00	7.1±1.1 ^b	6.3±0.54 ^c
	2.5	0±00	10.7±0.57 ^b	7±0.56 ^c
	5	0±00	11±0.33 ^b	6.7±0.144 ^c
	10	8±0.33 ^a	14±0.58 ^b	8.5±0.480 ^c
Standard - Ciprofloxacin	5 µg/ml	17±0.25 ^a	16.7±0.134 ^b	17.1±0.630 ^c
Standard - Gentamicin	5 µg/ml	12.5±0.66 ^a	12.6±0.89 ^b	12.4±0.57 ^c

Values are presented as mean ± SD (n = 3). Different letters (a, b, c) indicate statistically highly significant differences between groups, with a significance level of $p < 0.0001$ (****) for both *E. coli* and *B. megaterium* strains.

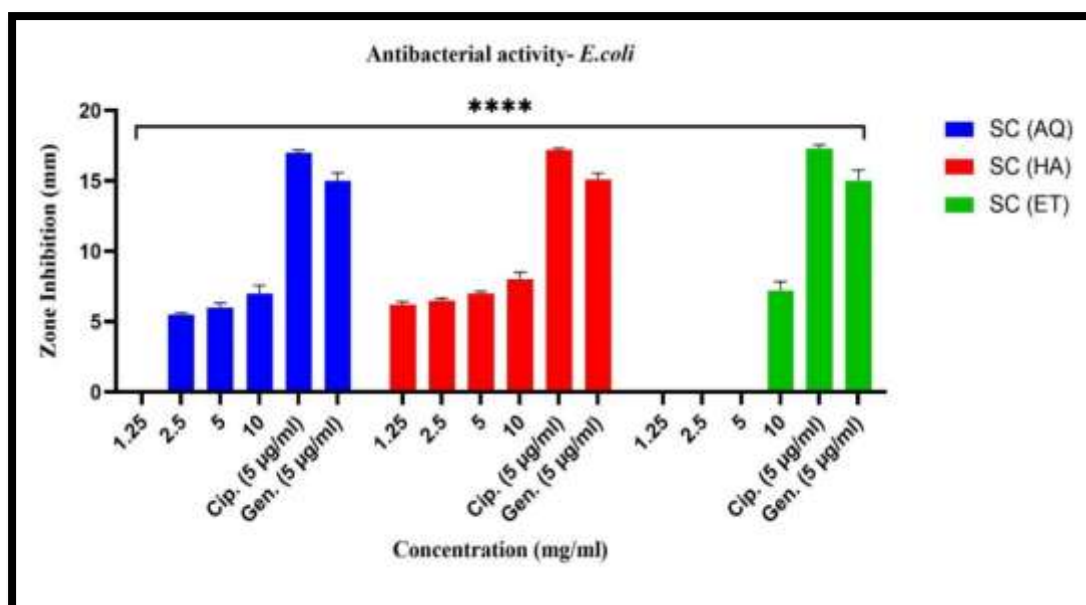


Fig. 20. Antibacterial activity of SC Polyherbal extracts against *Escherichia coli*

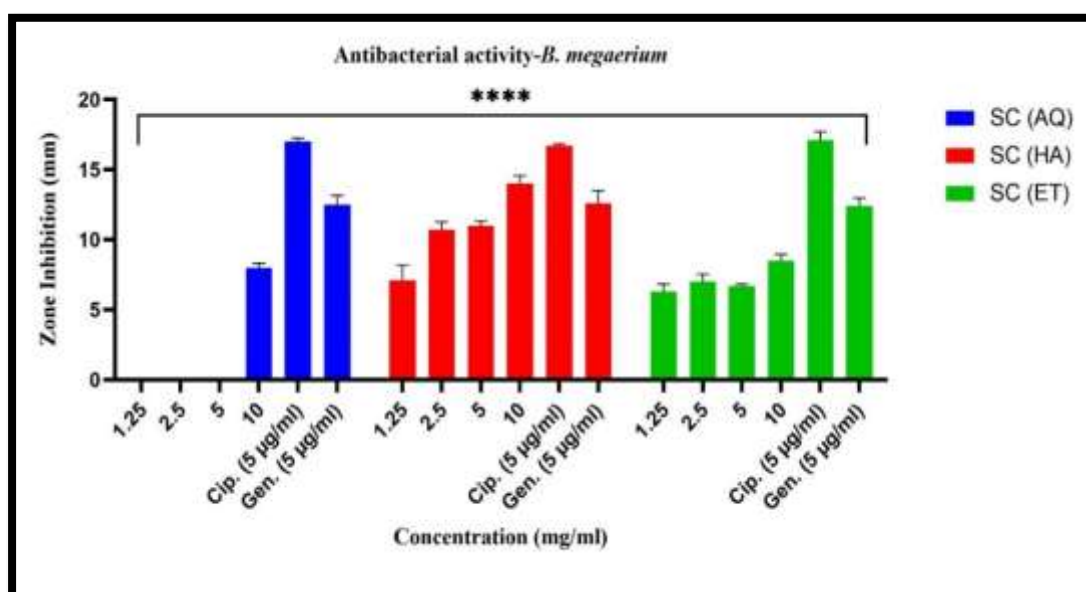
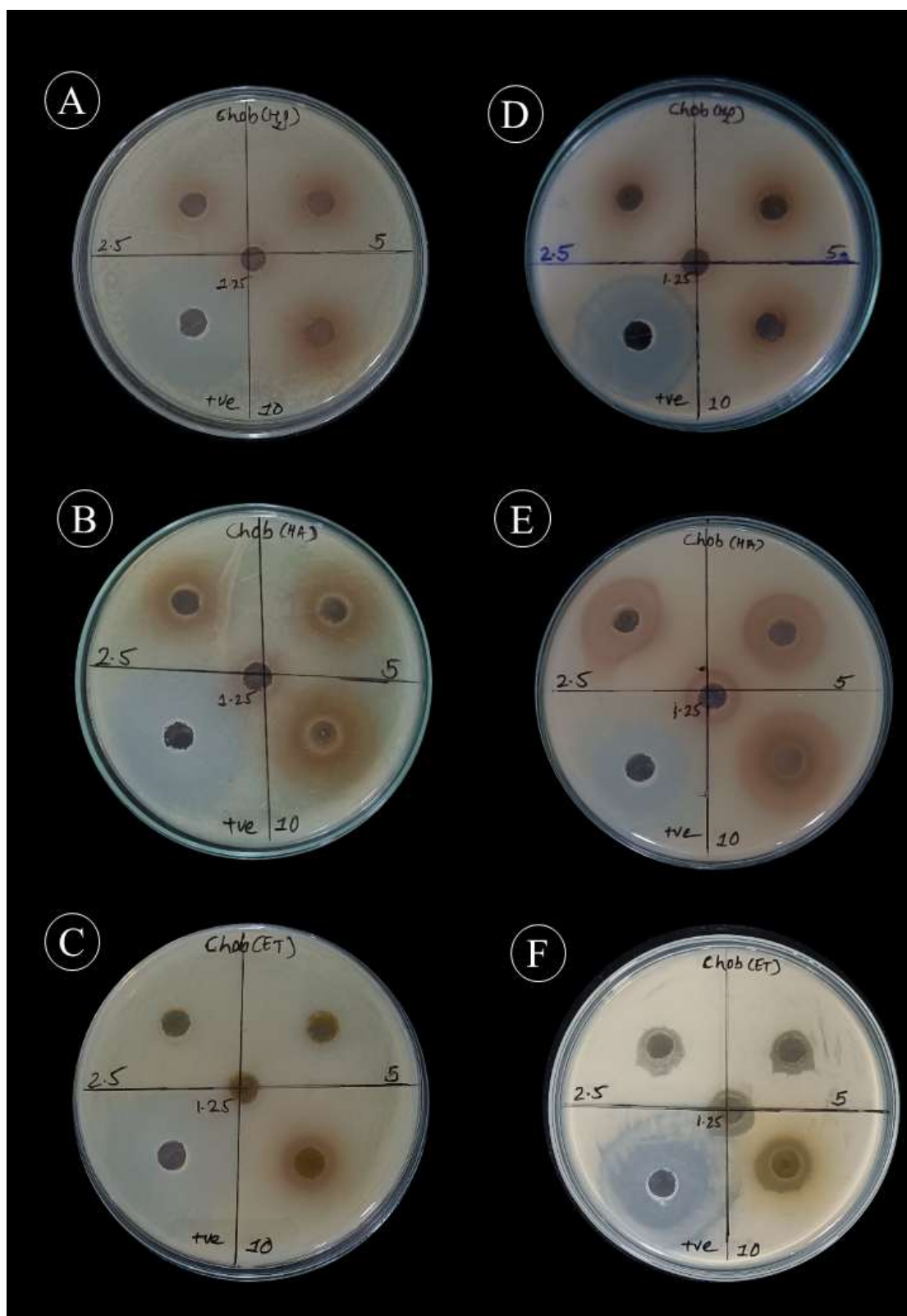


Fig. 21. Antibacterial activity of SC Polyherbal extracts against *Bacillus megaterium*

Photoplate 5. Antibacterial activity of Suffof-E-Chobchini (SC)



Antibacterial activity of SC formulation: A–C: SC (AQ, HA, ET) against *Escherichia coli*; D–F: SC (AQ, HA, ET) against *Bacillus megaterium*.

5.5.1.4 Formulation 1 (F1)

The antibacterial activity of Formulation 1 (F1) was evaluated using extracts at concentrations between 1.25 and 10 mg/mL (Table 16; Photoplate 6). Both the Hydroalcoholic (HA) and Ethanolic (ET) extracts demonstrated significant antibacterial effects against *Escherichia coli*, with zones of inhibition (ZOI) ranging from 5.5 mm to 8.3 mm for HA and 5.0 mm to 8.5 mm for ET. In contrast, the Aqueous (AQ) extract exhibited no antibacterial activity (Fig. 22). In the case of *Bacillus megaterium*, the HA and ET extracts again showed superior activity compared to AQ, with ZOI measurements from 6.5 mm to 9.8 mm for HA and 6.3 mm to 7.7 mm for ET, while AQ displayed a ZOI ranging from 6.0 mm to 7.5 mm (Fig. 23).

Table 16. Antibacterial activity of Formulation 1 (F1)

Microorganism Bacteria	Different Concentrations (mg/ml)	Different extraction methods (Mean Inhibition zone in mm)		
		F1 (AQ) extract	F1 (HA) extract	F1 (ET) extract
<i>Escherichia coli</i>	1.25	0±00	5.5±0.33 ^a	5±0.36 ^a
	2.5	0±00	6±0.66 ^a	7±0.57 ^a
	5	0±00	7.2±0.57 ^a	8±0.66 ^a
	10	0±00	8.3±0.78 ^a	8.5±0.47 ^a
Standard- Ciprofloxacin	5 µg/ml	17±1 ^b	17.2±1.14 ^a	17.5±1.01 ^a
Standard- Gentamicin	5 µg/ml	15±1 ^b	15.3±1.23 ^a	15.6±1.20 ^a
<i>Bacillus megaterium</i>	1.25	0±00	7±1 ^b	6.3±0.56 ^c
	2.5	0±00	6.5±0.57 ^b	7.1±0.76 ^c
	5	6±0.53 ^a	9±0.39 ^b	7.5±0.71 ^c
	10	7.5±0.76 ^a	9.8±0.47 ^b	7.7±0.49 ^c
Standard- Ciprofloxacin	5 µg/ml	19±00 ^a	18±1 ^b	18±1 ^c
Standard- Gentamicin	5 µg/ml	12.5±0.57 ^a	12.6±0.78 ^b	12.5±0.57 ^c

Each value is mean ± SD (n = 3). For *E. coli*, the letter b indicates a highly significant difference (****, $p < 0.0001$), while the same letter a denotes no significant difference (ns). For *B. megaterium*, the letters a and b indicate highly significant differences (****, $p < 0.0001$), and the letter c represents a significant difference (*, $p < 0.05$).

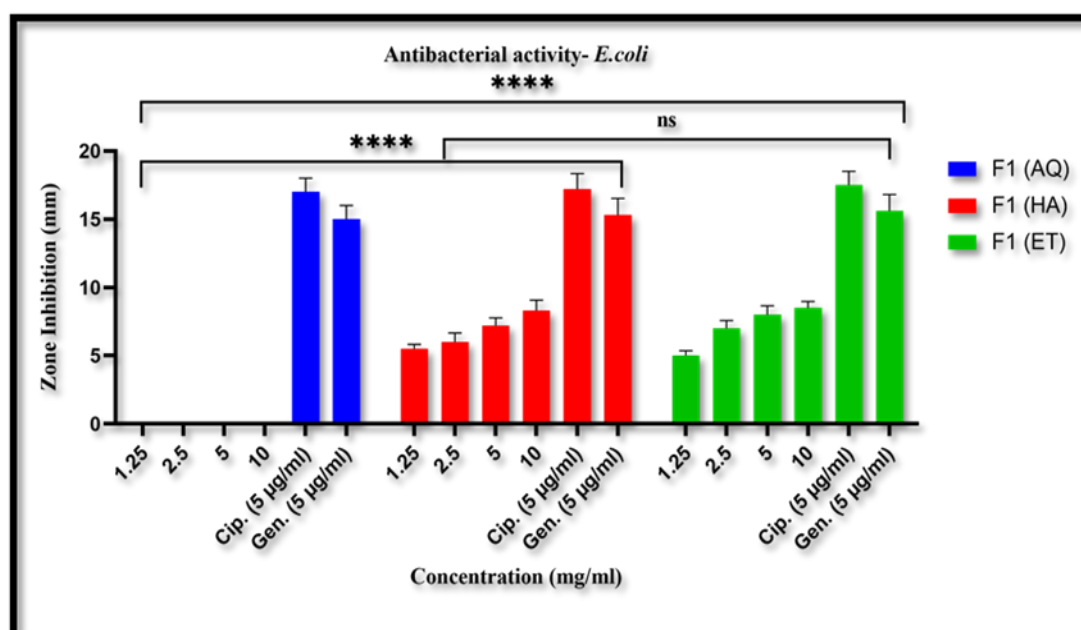


Fig. 22. Antibacterial activity of F1 Polyherbal extracts against *Escherichia coli*

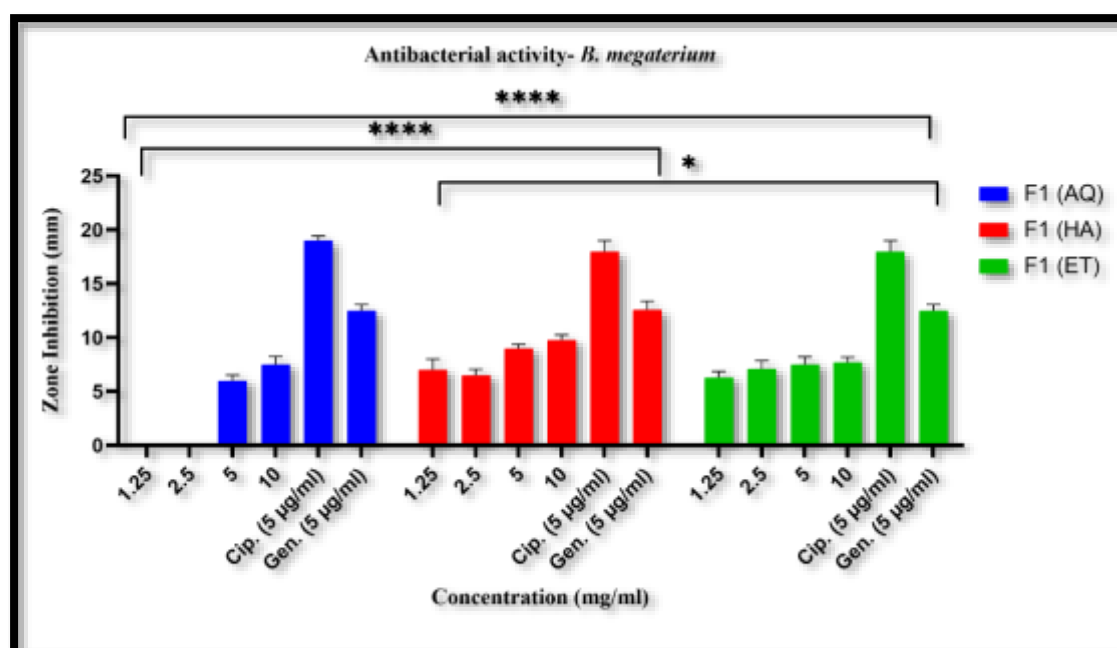
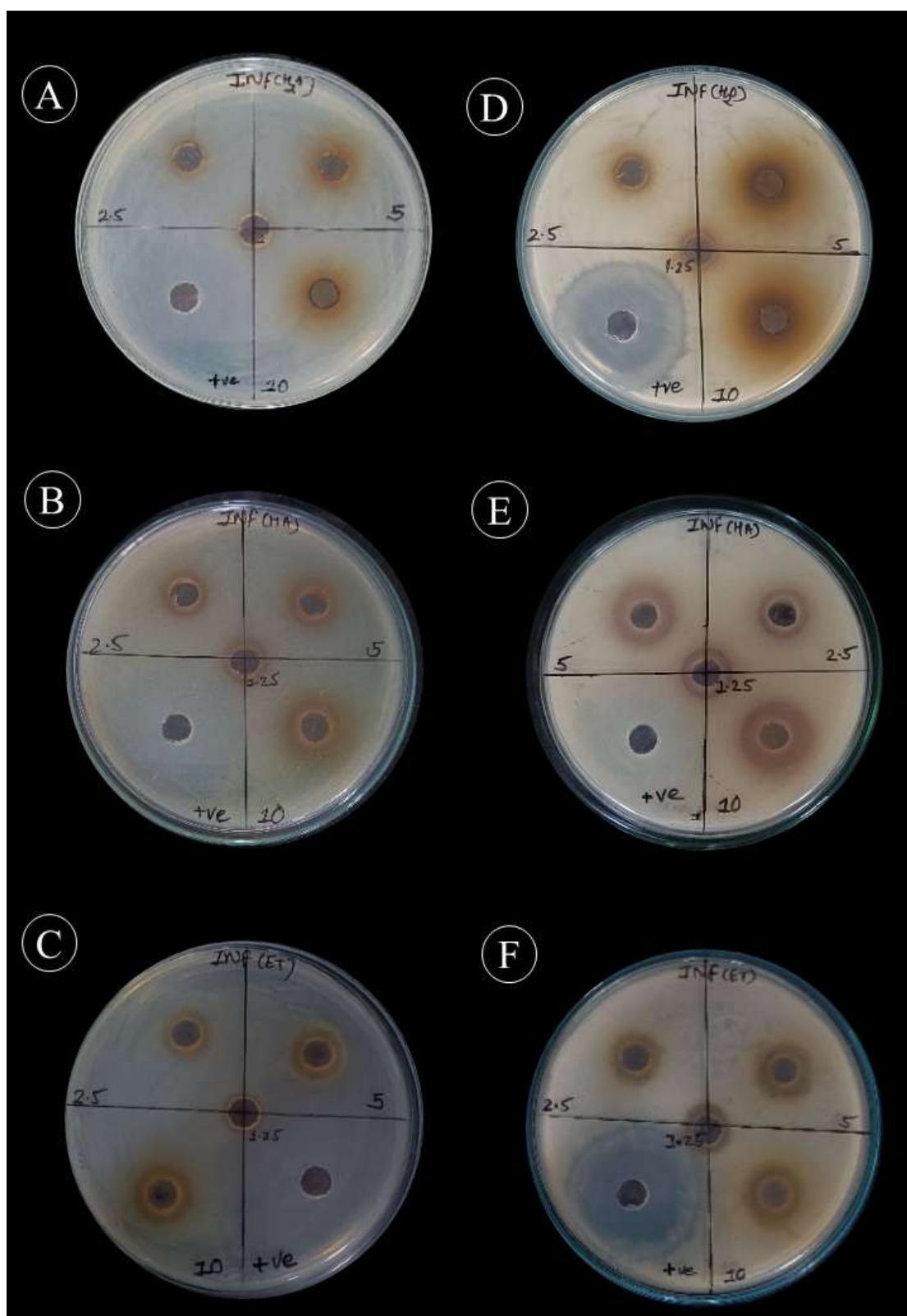


Fig. 23. Antibacterial activity of F1 Polyherbal extracts against *Bacillus megaterium*

Photoplate 6. Antibacterial activity of Formulation 1(F1)



Antibacterial activity of F1 formulation: A–C: F2 (AQ, HA, ET) against *Escherichia coli*; D–F: F2 (AQ, HA, ET) against *Bacillus megaterium*.

5.5.1.5 Formulation 2 (F2)

The antibacterial activity of Formulation 2 (F2) was evaluated using three extracts—Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET)—at concentrations ranging from 1.5 to 10 mg/ml (Table 17; Photoplate 7). Against *Escherichia coli*, the Hydroalcoholic (HA) extract demonstrated the strongest antibacterial activity, with zones of inhibition (ZOI) ranging from 6 mm to a maximum of 7.2 mm. The Ethanolic (ET) extract also exhibited notable activity, with ZOI ranging from 5 mm to a maximum of 6.3 mm. In contrast, the Aqueous (AQ) extract did not exhibit any antibacterial activity against *E. coli* (Fig. 24). For *Bacillus megaterium*, the Ethanolic (ET) extract proved most effective, producing ZOI ranging from 5 mm to 8 mm. The Hydroalcoholic (HA) extract showed moderate efficacy, with a ZOI of up to 7 mm only, while the Aqueous (AQ) extract exhibited relatively limited activity, with ZOI ranging from 5.1 mm to 6.1 mm. These findings highlight the superior antibacterial efficacy of the Ethanolic and Hydroalcoholic extracts compared to the Aqueous extract for both bacterial strains (Fig. 25).

Table 17. Antibacterial activity of Formulation 2 (F2)

Microorganism Bacteria	Different Concentrations (mg/ml)	Different extraction methods (Mean Inhibition zone in mm)		
		F2 (AQ) extract	F2 (HA) extract	F2 (ET) extract
<i>Escherichia coli</i>	1.5	0±00	7±0.33 ^b	5±0.54 ^c
	2	0±00	7.2±0.121 ^b	6±0.33 ^c
	2.5	0±00	6±0.251 ^b	6.3±0.41 ^c
	3	0±00	0±00	6±0.57 ^c
	4	7±0.35	00±00	6.1±0.76 ^c
	5	0±00	0±00	6.2±0.36 ^c
	10	0±00	0±00	0±00
Standard - Ciprofloxacin	5 µg/ml	22±0.14 ^a	22±0.33 ^b	21.7±0.66 ^c
Standard- Gentamicin	5 µg/ml	14.9±0.57 ^a	14.8±0.66 ^b	15±0.33 ^c

Microorganism Bacteria	Different Concentrations (mg/ml)	Different extraction methods (Mean Inhibition zone in mm)		
		F2 (AQ) extract	F2 (HA) extract	F2 (ET) extract
<i>Bacillus megaterium</i>	1.5	0±00	7±1 ^b	5±0.23 ^c
	2	5.1±0.57 ^a	0±00	8±0.12 ^c
	2.5	0±00	0±00	7±0.39 ^c
	3	0±00	0±00	5±0.33 ^c
	4	6±0.35	0±00	6±0.51 ^c
	5	6.1±0.51 ^a	0±00	6.5±0.57 ^c
	10	0±00	0±00	6.6±0.76 ^c
Standard - Ciprofloxacin	5 µg/ml	18±0.15	19±0.33 ^b	20±0.60 ^c
Standard - Gentamicin	5 µg/ml	12.5±0.68 ^a	12.5±0.68 ^b	12.4±1.14 ^c

Values are presented as mean ± SD (n = 3). Different letters (a, b, c) indicate statistically highly significant differences between groups, with a significance level of $p < 0.0001$ (****) for both *E. coli* and *B. megaterium* strains.

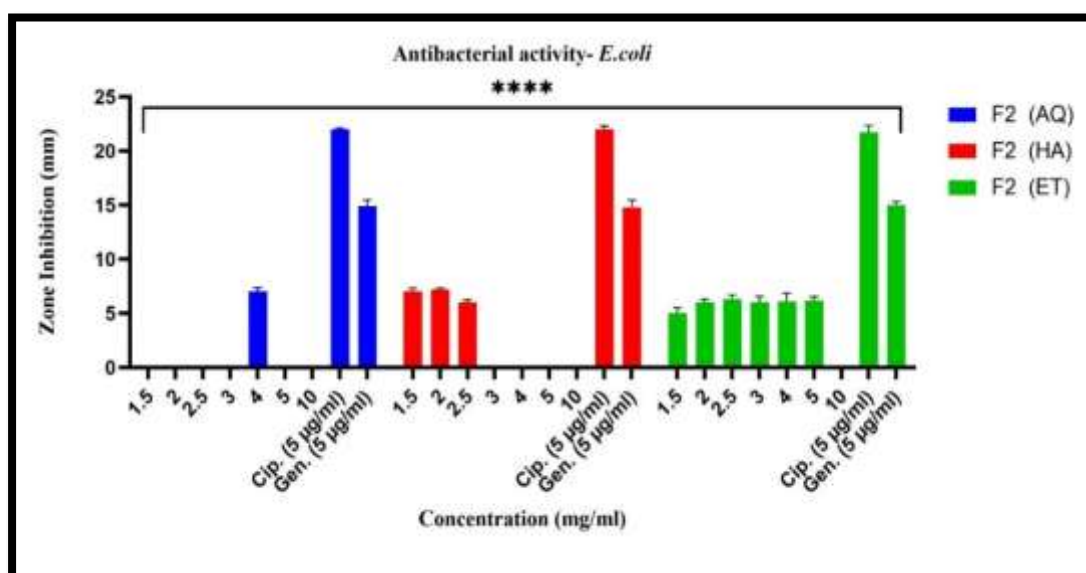


Fig. 24. Antibacterial activity of F2 Polyherbal extracts against *Escherichia coli*

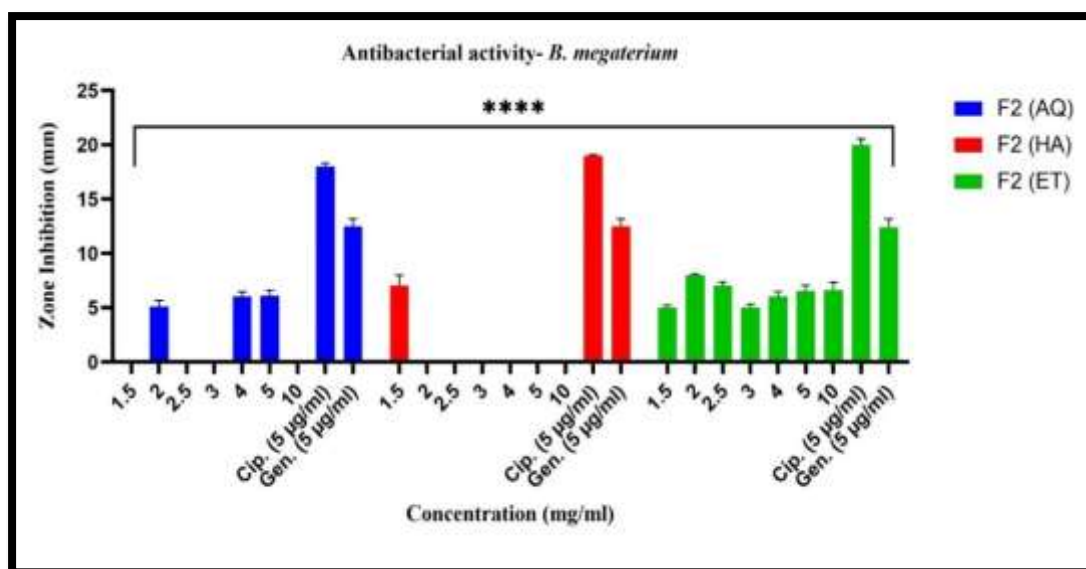
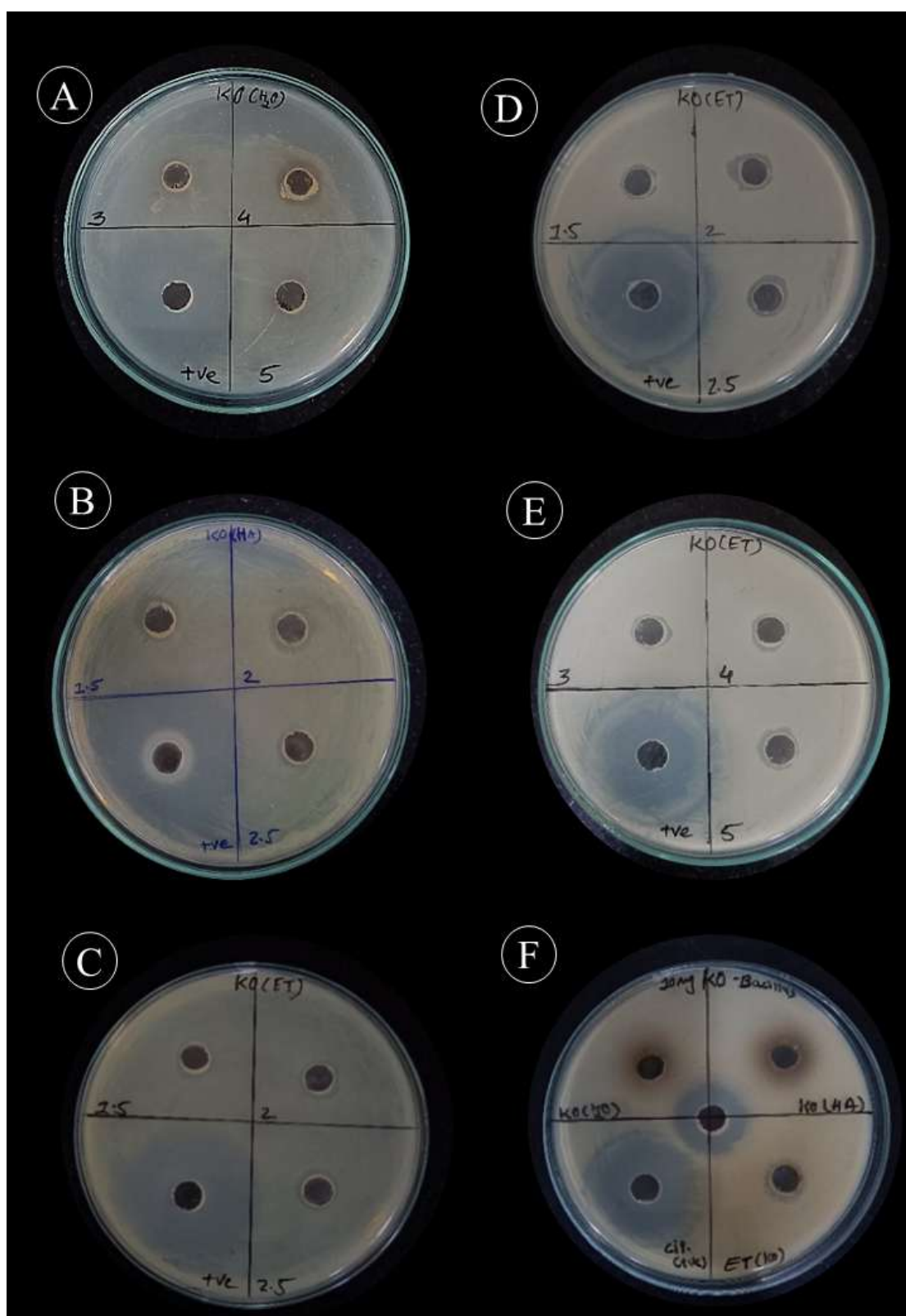


Fig. 25. Antibacterial activity of F2 Polyherbal extracts against *Bacillus megaterium*

Photoplate 7. Antibacterial activity of Formulation 2 (F2)



Antibacterial activity of F2 formulation: A–C: F2 (AQ, HA, ET) against *Escherichia coli*; D–F: F2 (AQ, HA, ET) against *Bacillus megaterium*.

5.5.2 Evaluation of Antifungal activity

In the agar well diffusion assay evaluating antifungal activity on five distinct Unani polyherbal formulations demonstrated substantial efficacy against *Candida albicans* and *Aspergillus niger*. Utilizing three distinct solvents (Aqueous (AQ), Hydro alcoholic (HA), and Ethanolic (ET)) for extraction and testing various concentrations, the formulations consistently produced clear inhibition zones around the wells. The observed antifungal activity displayed a concentration-dependent pattern, with increasing concentrations correlating with larger inhibition zones. Notably, there was variability in antifungal activity among formulations extracted with different solvents, indicating a potential influence of the extraction medium on bioactivity. Standard antibiotics, Amphotericin was employed as positive controls, while the negative control was prepared using the respective solvents. Clear zones of inhibition were observed around the wells impregnated with the formulations, indicating potential antimicrobial efficacy. The diameter of the zones of inhibition (ZOI) was measured, and the data is presented as mean $\pm$ SD of the three replicates. These findings highlight the significant antifungal potential of the Unani polyherbal formulations and warrant further exploration to identify specific bioactive compounds responsible for these observed effects.

5.5.2.1 Majoon-E-Najah (MN)

The antifungal activity of Majoon-E-Najah (MN) was assessed using three different solvent extracts—Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET)—at various concentrations (Table 18; Photoplate 8). The HA extract demonstrated significant antifungal activity, with zones of inhibition (ZOI) ranging from 8 to 11.33 mm across concentrations of 62.5, 125, 250, 500, and 1000 $\mu\text{g/ml}$. However, the AQ and ET extracts did not exhibit significant antifungal effects against *Candida albicans* (Fig. 26). On the other hand, the ET extract showed a pronounced antifungal effect, with ZOI ranging from 8 to 17.33 mm at concentrations of 312.5, 625, 1250, 2500, and 5000 $\mu\text{g/ml}$. The HA and AQ extracts did not display significant antifungal activity against *A. niger* (Fig. 27).

Table 18. Antifungal activity of Majoon-E-Najah (MN) polyherbal extracts

Microorganism Fungi	Different Concentrations ($\mu\text{g/ml}$)	Different extracts		
		MN (AQ) extract	MN (HA) extract	MN(ET) extract
<i>Candida albicans</i>	62.5	0 $\pm$ 00	8 $\pm$ 0.33 ^b	0 $\pm$ 00
	125	0 $\pm$ 00	9 $\pm$ 0.56 ^b	0 $\pm$ 00
	250	0 $\pm$ 00	9 $\pm$ 0.53 ^b	0 $\pm$ 00
	500	0 $\pm$ 00	10.33 $\pm$ 0.57 ^b	0 $\pm$ 00
	1000	0 $\pm$ 00	11.33 $\pm$ 0.57 ^b	0 $\pm$ 00
Amphotericin	25 $\mu\text{g/ml}$	16 $\pm$ 0.66 ^a	17 $\pm$ 0.35 ^b	15.66 $\pm$ 0.57 ^a
<i>Aspergillus niger</i>	312.5	0 $\pm$ 00	0 $\pm$ 00	17.33 $\pm$ 1.15 ^b
	625	0 $\pm$ 00	0 $\pm$ 00	15.33 $\pm$ 1.15 ^b
	1250	0 $\pm$ 00	0 $\pm$ 00	13.66 $\pm$ 0.57 ^b
	2500	0 $\pm$ 00	0 $\pm$ 00	8 $\pm$ 0.33 ^b
	5000	0 $\pm$ 00	0 $\pm$ 00	0 $\pm$ 00
Amphotericin	25 $\mu\text{g/ml}$	23 $\pm$ 0.43 ^a	20 $\pm$ 0.45 ^a	22.66 $\pm$ 0.60 ^b

Each value is presented as mean $\pm$ SD (n = 3). Different letters (a, b) denote statistically significant differences between groups. For *C. albicans*, 'b' indicates a highly significant difference ($p < 0.0001$) and 'a' indicates no significant difference. For *A. niger*, 'b' indicates a highly significant difference ($p < 0.0001$) and 'a' indicates a significant difference ($p < 0.01$).

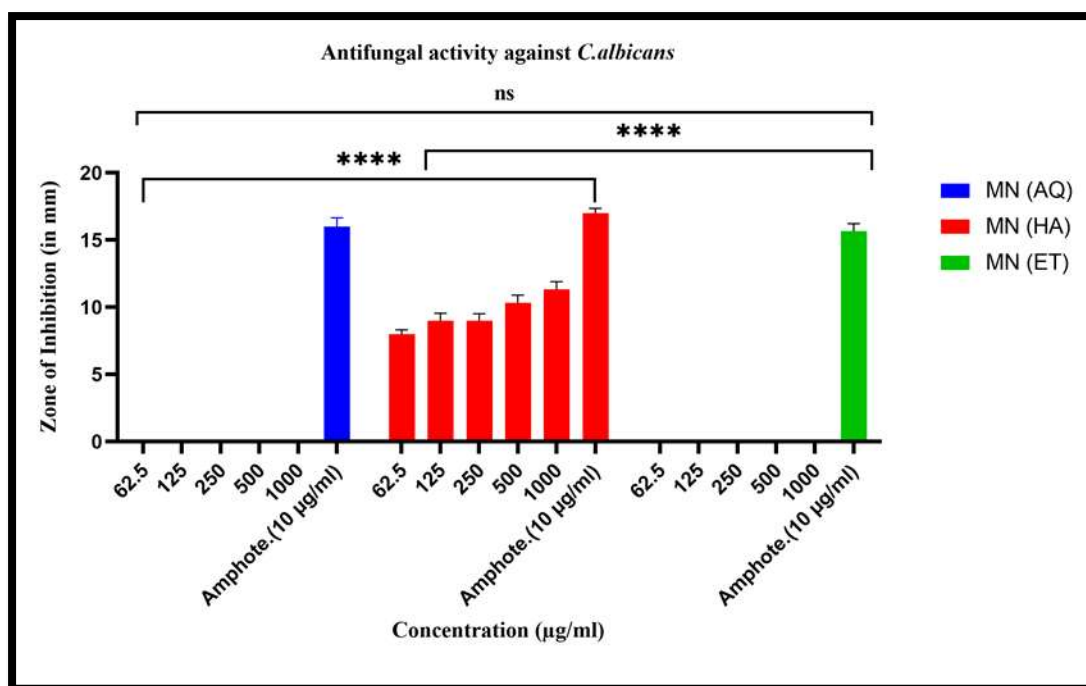


Fig. 26. Antifungal activity of MN polyherbal extracts against *Candida albicans*

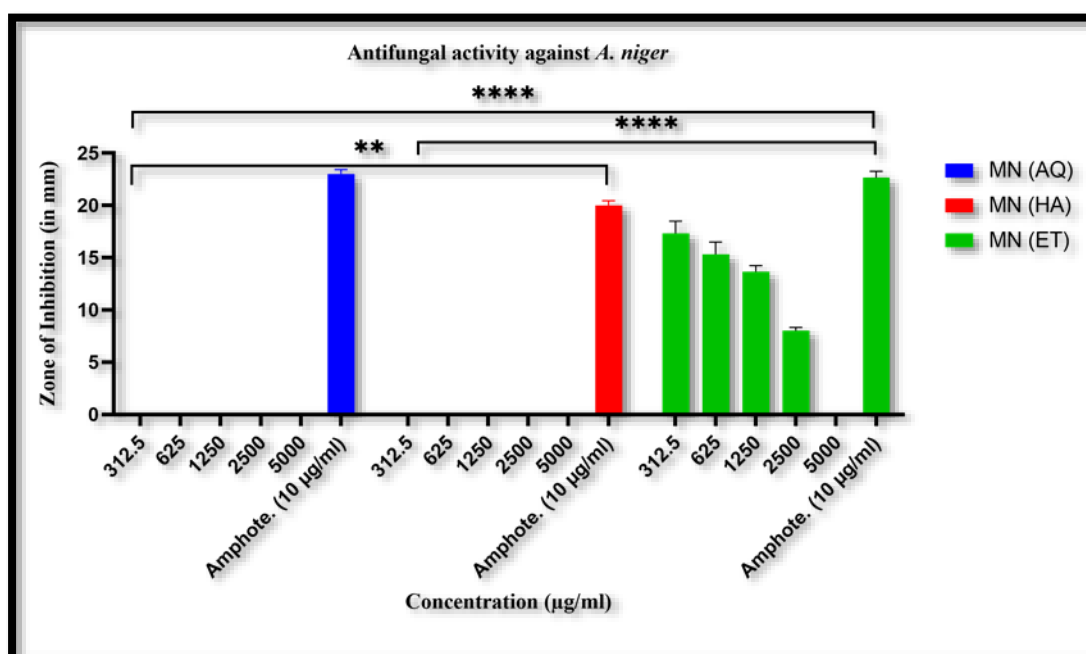
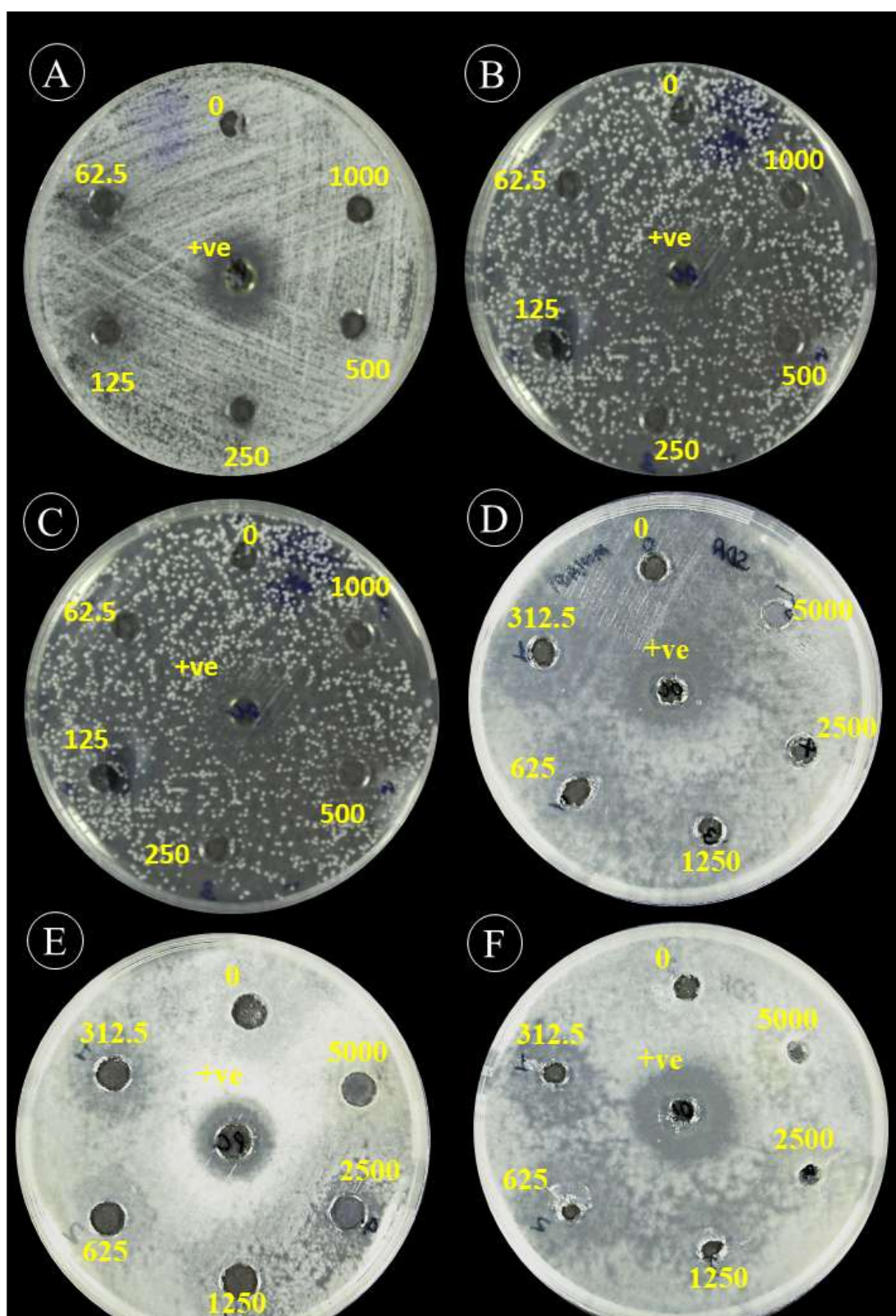


Fig. 27. Antifungal activity of MN polyherbal extracts against *Aspergillus niger*

Photoplate 8. Antifungal activity of Majoon-E-Najah (MN) formulation



Antifungal activity of MN formulation: A–C: MN (AQ, HA, ET) against *C. albicans*; D–F: MN (AQ, HA, ET) against *A. niger*.

5.5.2.2 Suffof-E-Najah (SN)

In the case of Suffof-E-Najah (SN), the antifungal activity was also evaluated using the Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET) extracts (Table 19; Photoplate 9). In the case of *Candida albicans*, all three extracts exhibited robust and consistent antifungal activity across concentrations of 62.5, 125, 250, 500, and 1000 µg/ml. The AQ and HA extracts, in particular, displayed strong effects, with ZOI ranging from 8 to 13 mm and 9 to 13 mm, respectively (Fig. 28). However, for *Aspergillus niger*, only the AQ and HA extracts showed significant antifungal activity, with ZOI ranging from 10.66 to 15.66 mm and 9 to 15.33 mm, respectively, across concentrations of 312.5, 625, 1250, 2500, and 5000 µg/ml. The ET extract did not show significant efficacy. (Fig. 29).

Table 19. Antifungal activity of Suffof-E-Najah (SN) polyherbal extracts

Microorganism Fungi	Different Concentrations (µg/ml)	Different extraction methods (Mean Inhibition zone in mm)		
		SN (AQ) extract	SN (HA) extract	SN(ET) extract
<i>Candida albicans</i>	62.5	13±0.34 ^a	9±0.27 ^b	8±0.45 ^a
	125	9.33±0.57 ^a	9.33±0.78 ^b	8.33±1.01 ^a
	250	9±0.50 ^a	11±0.56 ^b	9.33±0.67 ^a
	500	9±0.24 ^a	12±0.68 ^b	11±0.65 ^a
	1000	8±0.28 ^a	13±0.65 ^b	12±0.33 ^a
Standard - Amphotericin	25 µg/ml	15.66±0.58 ^a	16.33±0.66 ^b	17.33±0.57 ^a
<i>Aspergillus niger</i>	312.5	15.66±1.15 ^a	9±1 ^b	0
	625	14.33±1.14 ^a	9.66±0.57 ^b	0
	1250	12.66±0.66 ^a	11±1 ^b	0
	2500	11.66±0.89 ^a	14±0.37 ^b	0
	5000	10.66±0.57 ^a	15.33±1.15 ^b	0
Standard - Amphotericin	25 µg/ml	21±0.54 ^a	21±0.66 ^b	22.1±0.66 ^c

Values are expressed as mean ± SD (n = 3). For *Candida albicans*, the same letter "a" indicates no statistically significant difference (ns) between Aqueous (AQ) and Ethanolic (ET) extracts. Different letters indicate significant differences between the

pairs: "a" and "b" between AQ and HA (****, $p < 0.0001$), and "b" and "a" between HA and ET (****, $p < 0.0001$). For *Aspergillus niger*, different letters indicate significant differences between the pairs: "a" and "b" between AQ and HA (***, $p < 0.001$), "a" and "c" between AQ and ET (****, $p < 0.0001$), and "b" and "c" between HA and ET (****, $p < 0.0001$).

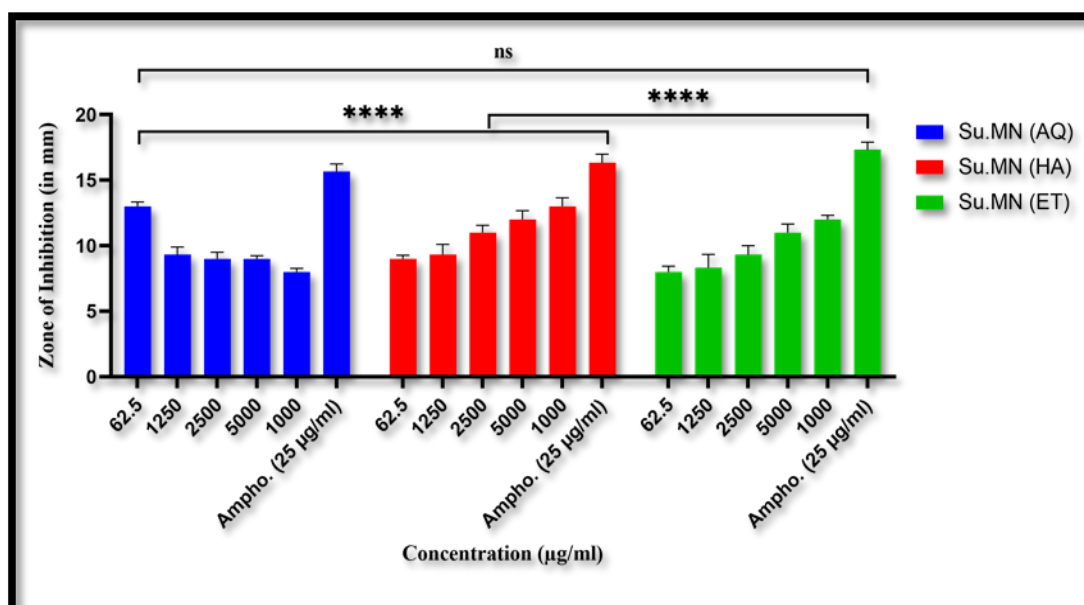


Fig. 28. Antifungal activity of Su.MN polyherbal extracts against *Candida albicans*

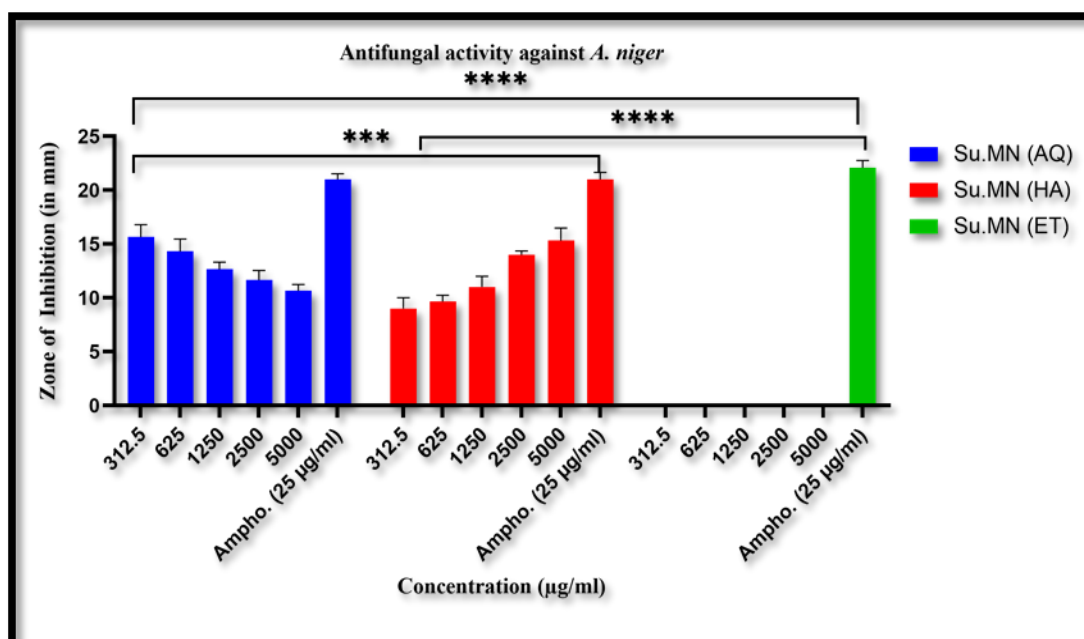
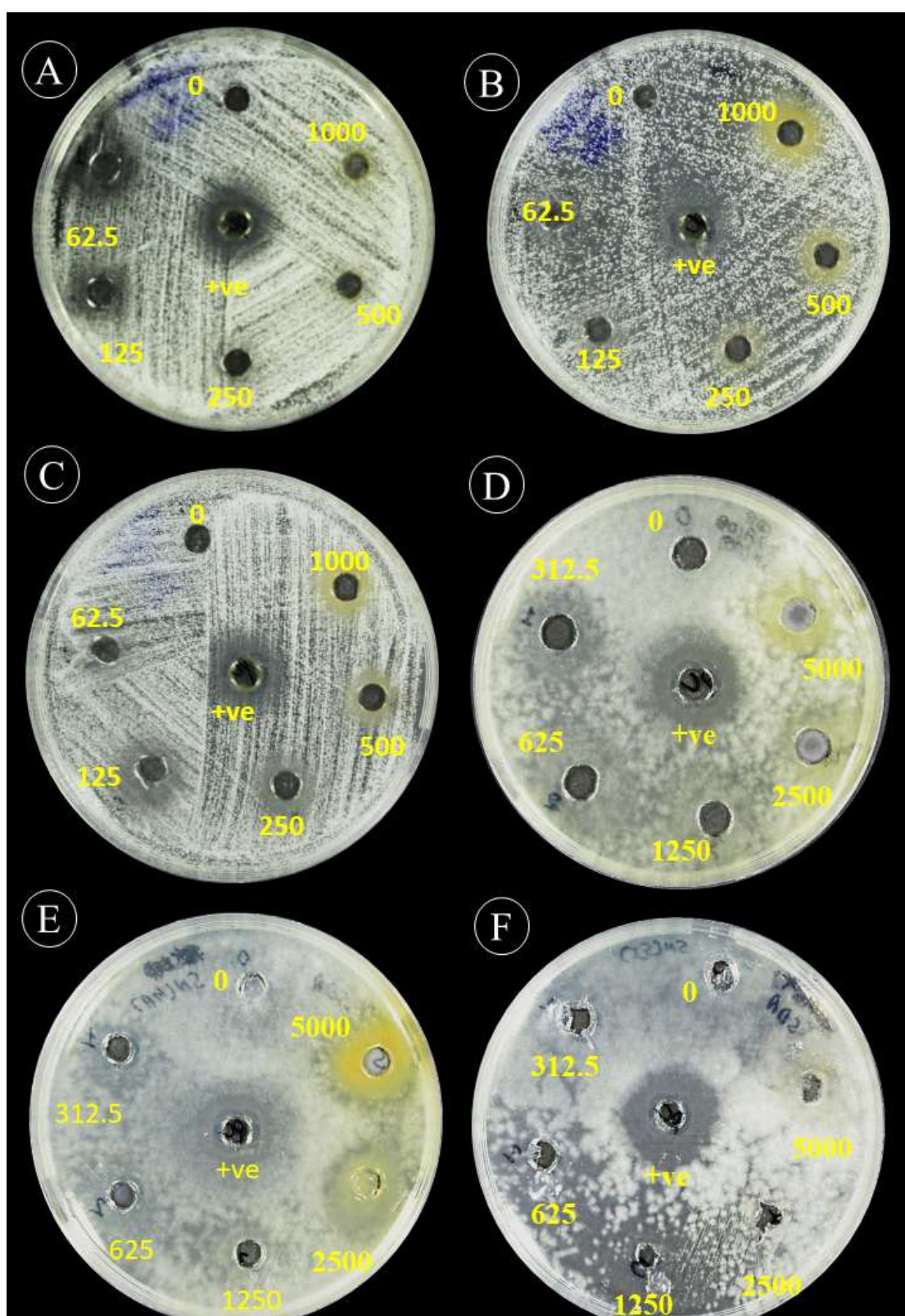


Fig. 29. Antifungal activity of Su.MN polyherbal extracts against *Aspergillus niger*

Photoplate 9. Antifungal activity of Suffof-E-Najah (SN) formulation



Antifungal activity of SN formulation: A–C: F2 (AQ, HA, ET) against *C. albicans*; D–F: SN (AQ, HA, ET) against *A. niger*.

5.5.2.3 Suffof-E-Chobchini (SC)

The antifungal activity of Suffof-E-Chobchini (SC) was tested with the Aqueous (AQ), Hydroalcoholic (HA) and Ethanolic (ET) extracts (Table 20; Photoplate 10). In the case of *Candida albicans*, the ET extract demonstrated significant activity, with ZOI ranging from 9 to 11 mm across concentrations of 62.5, 125, 250, 500, and 1000 µg/ml. In contrast, the HA extract showed weaker activity, with ZOI ranging from 5 to 6 mm, and the AQ extract did not exhibit significant antifungal effects (Fig. 30). Regarding *Aspergillus niger*, all three extracts were effective across all tested concentrations at 312.5, 625, 1250, 2500, 5000 µg/ml. The ET extract was particularly potent, with ZOI ranging from 9 to 22.33 mm, followed by the AQ extract with ZOI from 8.66 to 14 mm, and the HA extract with ZOI from 14 to 18 mm (and Fig. 31).

Table 20. Antifungal activity of Suffof-E-Chobchini (SC) polyherbal extracts

Microorganism Fungi	Different Concentrations (µg/ml)	Different extraction methods (Mean Inhibition zone in mm)		
		SC (AQ) extract	SC (HA) extract	SC (ET) extract
<i>Candida albicans</i>	62.5	0±00	6±0.23 ^b	9±0.33 ^c
	125	0±00	5±0.33 ^b	9±0.38 ^c
	250	0±00	5±0.38 ^b	9±0.35 ^c
	500	0±00	0±00	10±0.46 ^c
	1000	0±00	0±00	11±0.49 ^c
Standard - Amphotericin	25 µg/ml	16.67±0.57 ^a	15±0.66 ^b	16.67±0.57 ^c
<i>Aspergillus niger</i>	312.5	8.67±1.15 ^a	14±0.33 ^b	9±1 ^c
	625	9±1.01 ^a	15±0.56 ^b	10.33±1.52 ^c
	1250	10.67±1.13 ^a	15.1±0.11 ^b	15±1 ^c
	2500	12±0.66 ^a	16±0.60 ^b	18±0.54 ^c
	5000	14±0.67 ^a	18±0.27 ^b	22.33±0.57 ^c
Standard - Amphotericin	25 µg/ml	21.33±0.57 ^a	20±0.58 ^b	16±0.45 ^c

Values are presented as mean ± SD (n = 3). Different letters (a, b, c) indicate statistically highly significant differences between groups, with a significance level of $p < 0.0001$ (****) for both *C. albicans* and *A. niger* strains.

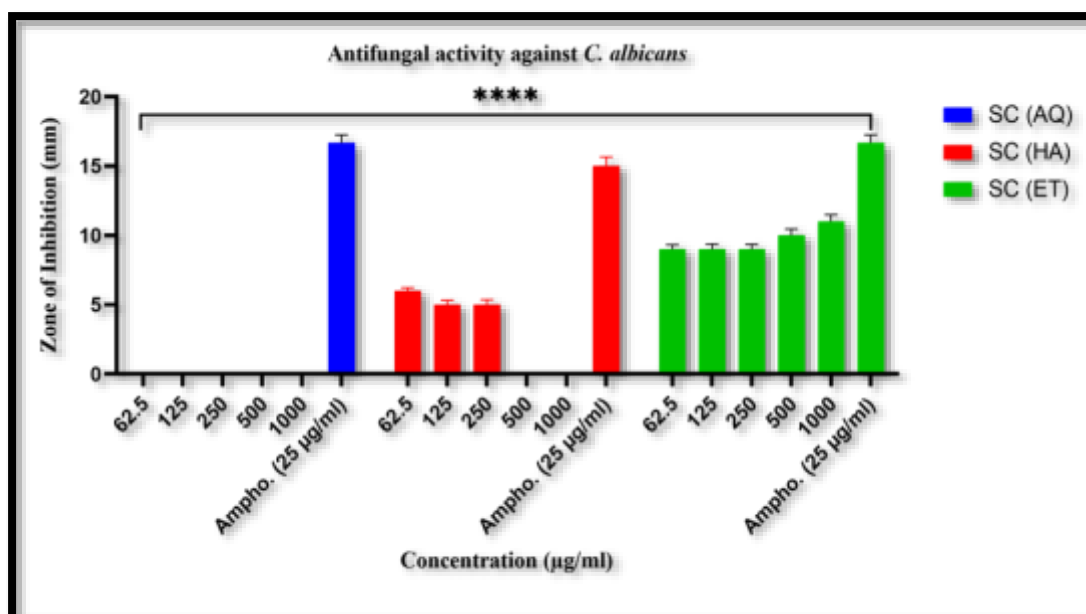


Fig. 30. Antifungal activity of SC polyherbal extracts against *Candida albicans*

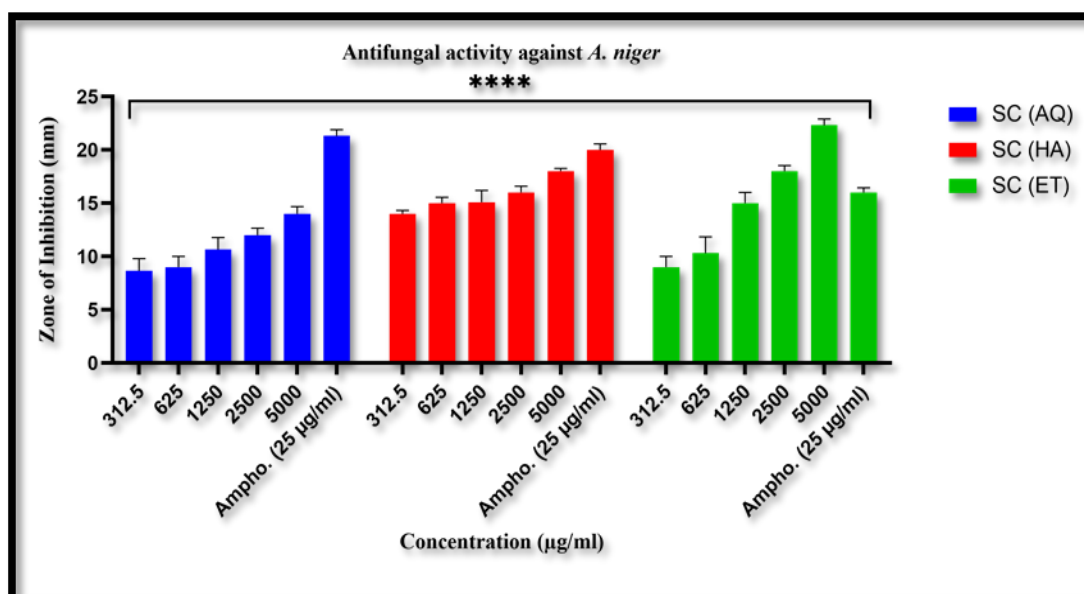
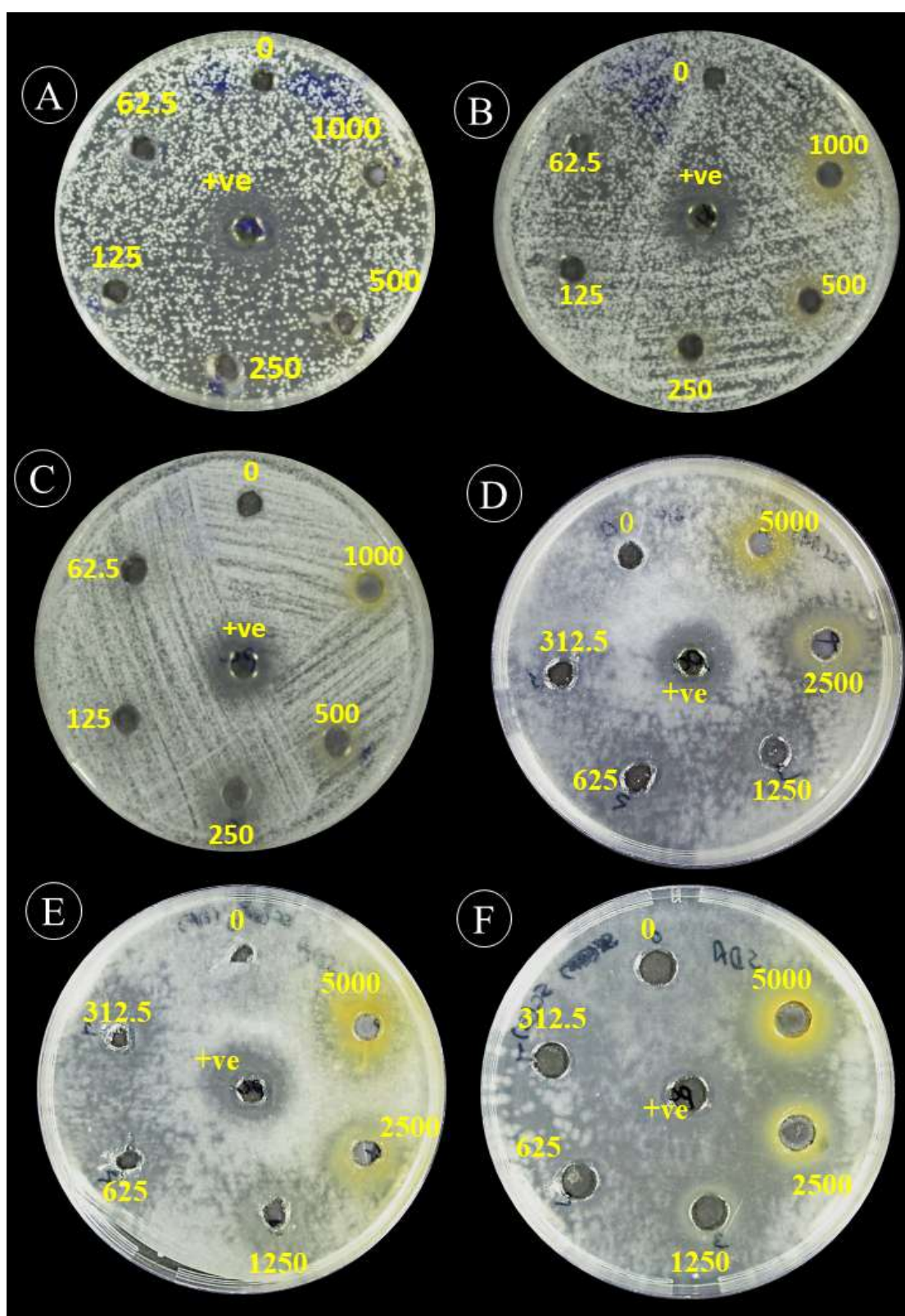


Fig. 31. Antifungal activity of SC polyherbal extracts against *Aspergillus niger*

Photoplate 10. Antifungal activity of Suffof-E-Chobchini (SC) formulation



Antifungal activity of SC formulation: A–C: SC (AQ, HA, ET) against *C. albicans*; D–F: SC (AQ, HA, ET) against *A. niger*

Formulation 1 (F1)

The antifungal activity of Formulation 1 (F1) was tested using the Aqueous (AQ), Hydroalcoholic (HA) and Ethanolic (ET) extracts (Table 21; Photoplate 11). The HA extract exhibited strong antifungal activity against *Candida albicans*, with ZOI ranging from 10 to 12.6 mm at concentrations of 62.5, 125, 250, 500, and 1000 µg/ml. The ET extract showed moderate activity, with ZOI ranging from 5 to 7.6 mm, while the AQ extract exhibited less antifungal activity, with ZOI ranging from 5 to 6.3 mm (Fig. 32). In the case of *Aspergillus niger*, all three extracts were effective across all tested concentrations (312.5, 625, 1250, 2500, and 5000 µg/ml). The HA extract was the most potent, with ZOI ranging from 13 to 17.33 mm, followed by the ET extract with ZOI from 10 to 17.66 mm, and the AQ extract with ZOI from 8.3 to 16.2 mm (Fig. 33).

Table 21. Antifungal activity of Formulation 1 (F1) polyherbal extracts

Microorganism Fungi	Different Concentrations (µg/ml)	Different extraction methods (Mean Inhibition zone in mm)		
		F1 (AQ) extract	F1 (HA) extract	F1 (ET) extract
<i>Candida albicans</i>	62.5	0±00	10±0.34 ^b	5±0.39 ^c
	125	0±00	10±0.45 ^b	5±0.41 ^c
	250	0±00	11±0.37 ^b	5.6±0.58 ^c
	500	5±0.33 ^a	11±0.56 ^b	6.5±0.87 ^c
	1000	6.3±0.57 ^a	12.6±0.78 ^b	7.6±0.57 ^c
Standard - Amphotericin	25 µg/ml	14.3±0.66 ^a	15.6±0.57 ^b	15±0.38 ^c
<i>Aspergillus niger</i>	312.5	8.3±1.03 ^a	13±0.28 ^b	10±0.44 ^c
	625	9.2±0.89 ^a	14±0.33 ^b	12±0.61 ^c
	1250	10.6±0.66 ^a	15.66±1.21 ^b	12±0.42 ^c
	2500	11.3±1.15 ^a	16±0.45 ^b	12.3±1.14 ^c
	5000	16.2±0.51 ^a	17.33±1.04 ^b	17.66±1.23 ^c
Standard- Amphotericin	25 µg/ml	23±0.56 ^a	21±1 ^b	24.6±0.57 ^c

Values are presented as mean ± SD (n = 3). Different letters (a, b, c) indicate statistically highly significant differences between groups, with a significance level of $p < 0.0001$ (****) for both *C. albicans* and *A. niger* strains.

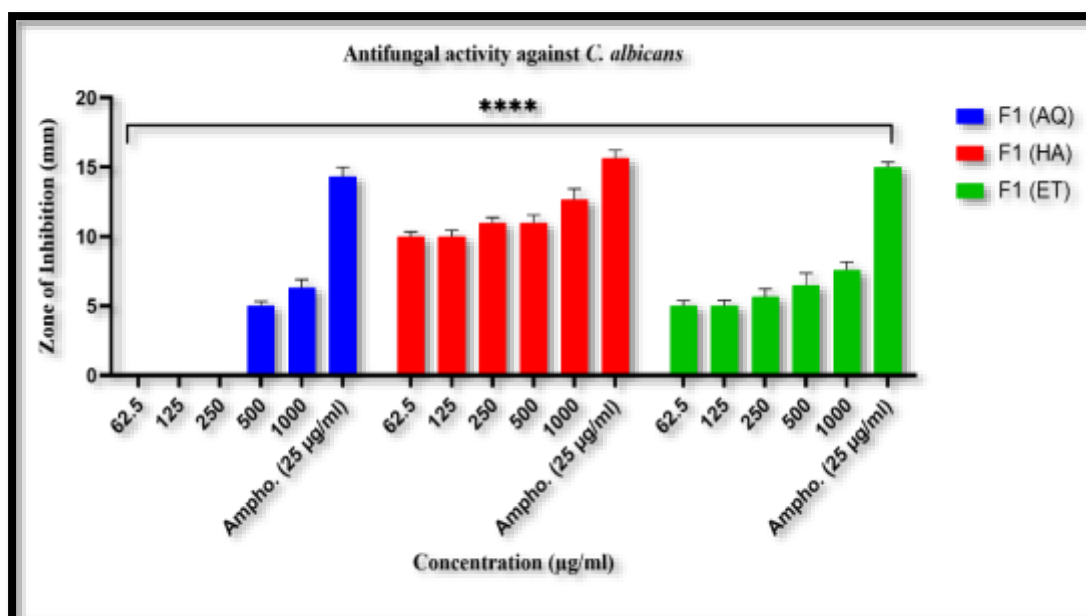


Fig. 32. Antifungal activity of F1 polyherbal extracts against *Candida albicans*

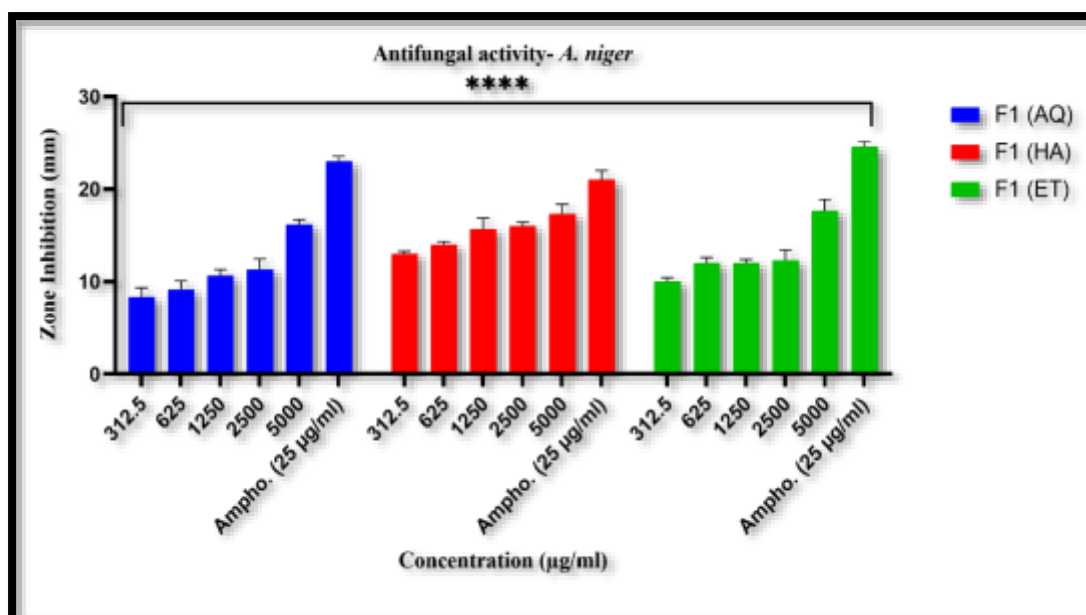
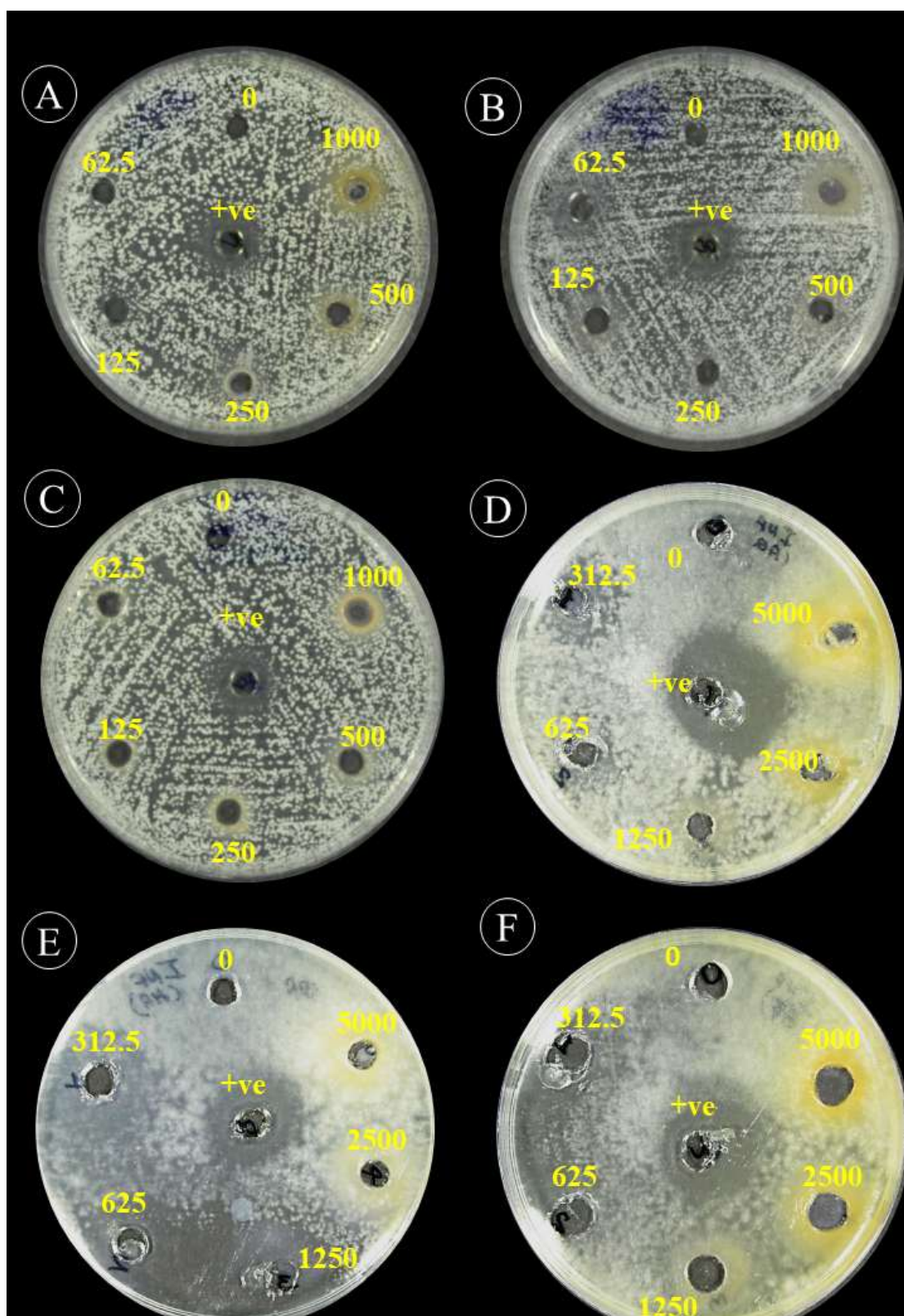


Fig. 33. Antifungal activity of F1 polyherbal extracts against *Aspergillus niger*

Photoplate 11. Antifungal activity of Formulation 1 (F1)



Antifungal activity of F1 formulation: A–C: F1 (AQ, HA, ET) against *C. albicans*;
D–F: F1 (AQ, HA, ET) against *A. niger*.

5.5.2.4 Formulation 2 (F2)

The antifungal activity of Formulation 2 (F2) was tested using the Aqueous (AQ), Hydroalcoholic (HA) and Ethanolic (ET) extracts (Table 22; Photoplate 12). In the case of *Candida albicans*, the AQ extract exhibited significant antifungal activity, with ZOI ranging from 8.66 to 11 mm across concentrations of 62.5, 125, 250, 500, and 1000 µg/ml. The HA and ET extracts did not show significant antifungal effects against *C. albicans* (Fig. 34). For *Aspergillus niger*, the HA extract exhibited strong antifungal activity, with ZOI ranging from 14 to 20 mm across concentrations of 312.5, 625, 1250, 2500, and 5000 µg/ml. The ET and AQ extracts also showed moderate antifungal activity, with ZOI ranging from 10 to 17 mm and 12 to 13 mm, respectively (Fig. 35).

Table 22. Antifungal activity of Formulation 2 (F2) polyherbal extracts

Microorganism Fungi	Different Concentrations (µg/ml)	Different extraction methods (Mean Inhibition zone in mm)		
		F2 (AQ) extract	F2 (HA) extract	F2 (ET) extract
<i>Candida albicans</i>	62.5	9±0.33 ^b	0±00	0±00
	125	9±0.45 ^b	0±00	0±00
	250	8.66±0.51 ^b	0±00	0±00
	500	10±0.54 ^b	0±00	0±00
	1000	11±0.41 ^b	0±00	0±00
Standard-Amphotericin	25 µg/ml	16±0.60 ^b	16.66±0.57 ^a	16.66±0.57 ^a
<i>Aspergillus niger</i>	312.5	12±0.26 ^a	14±0.36 ^b	17±0.34 ^c
	625	13±0.45 ^a	15±0.44 ^b	15±0.51 ^c
	1250	13±0.51 ^a	16±0.51 ^b	13.3±0.57 ^c
	2500	13±0.47 ^a	18±0.39 ^b	11.3±0.78 ^c
	5000	13±0.33 ^a	20±0.45 ^b	10±0.66 ^c
Standard-Amphotericin	25 µg/ml	21±0.60 ^a	20±1. ^b	21±0.63 ^c

Each value is presented as mean ± SD (n = 3). For *E. coli*, the letter "b" indicates a highly significant difference (****, p < 0.0001), while the letter "a" denotes no significant difference (ns). For *B. megaterium*, the letter "a" is assigned to the Aqueous (AQ) extract, "b" to the Hydroalcoholic (HA) extract, and "c" to the Ethanolic (ET)

extract. A highly significant difference was observed between AQ and HA (****, $p < 0.0001$), HA and ET (****, $p < 0.0001$), and a significant difference between AQ and ET (*** $p < 0.001$).

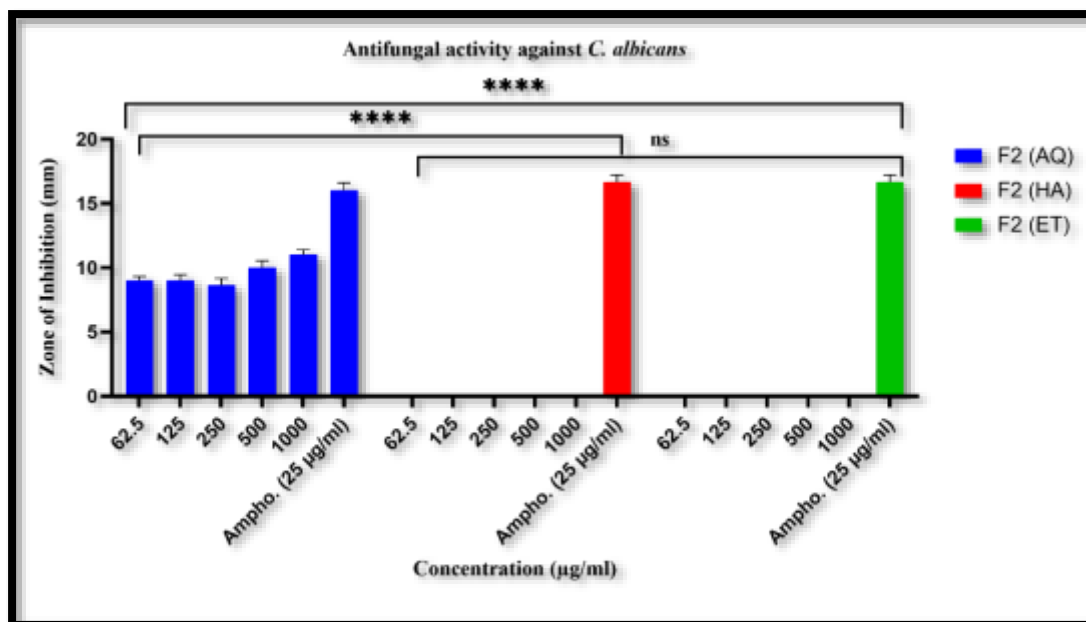


Fig. 34. Antifungal activity of F2 polyherbal extracts against *Candida albicans*

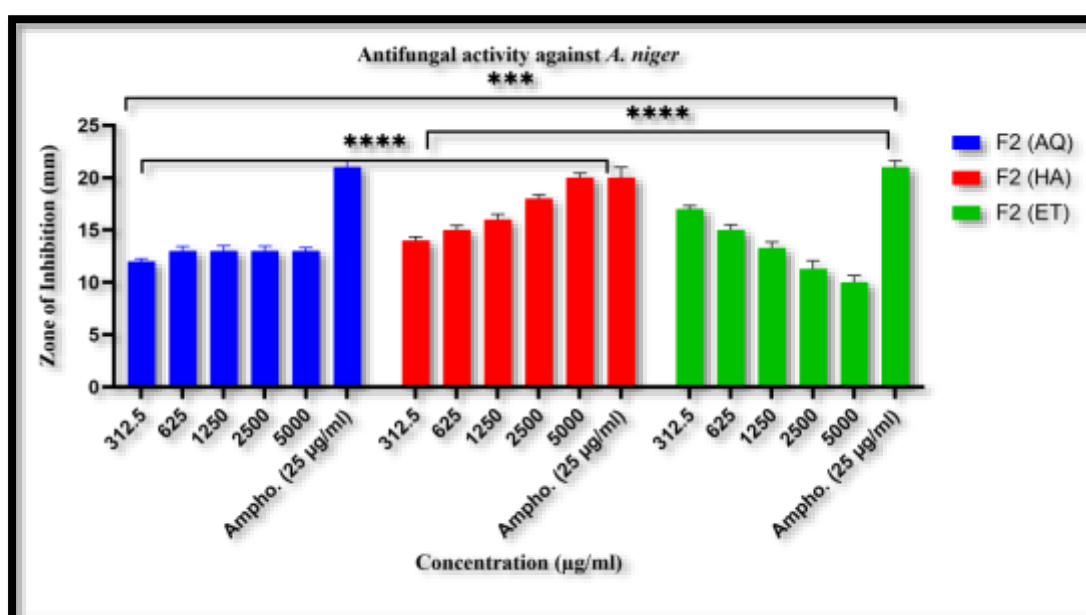
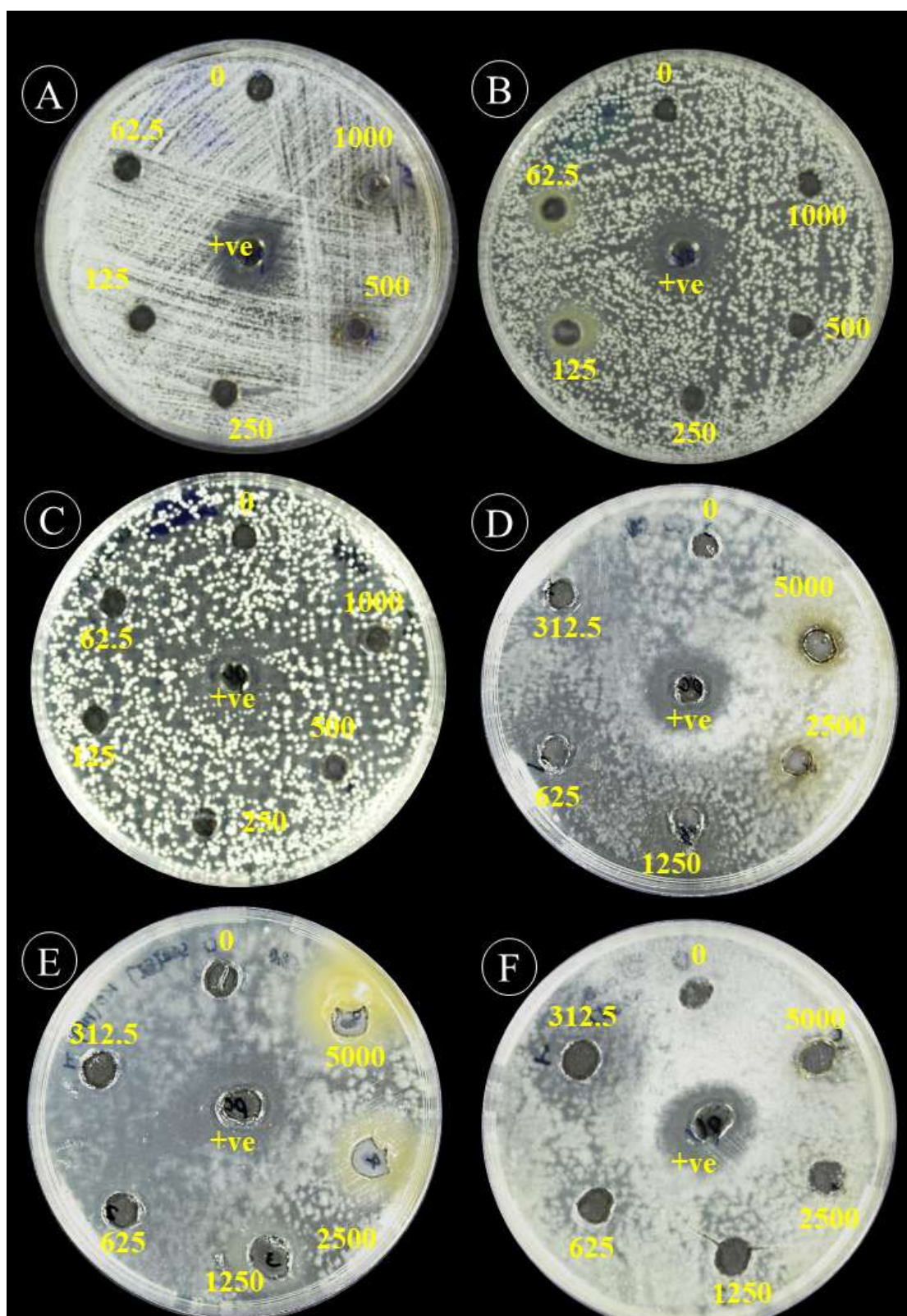


Fig. 35. Antifungal activity of F2 polyherbal extracts against *Aspergillus niger*

Photoplate 12. Antifungal activity of Formulation 2 (F2)



Antifungal activity of F2 formulation: A–C: F2 (AQ, HA, ET) against *C. albicans*;
 D–F: F2 (AQ, HA, ET) against *A. niger*.

5.5.3 Evaluation of Antioxidant activity by DPPH assay

This study marks the first time that the antioxidant activity of five Unani polyherbal formulations has been evaluated using the DPPH assay. No earlier studies have reported on these formulations. Three different extracts—Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET)—were analyzed, with ascorbic acid as the standard. The antioxidant potential was assessed using IC₅₀ values, % inhibition, and non-linear regression curve analysis. Across all formulations, a concentration-dependent increase in radical scavenging activity was observed, with the ethanolic extracts generally displaying the highest antioxidant activity.

5.5.3.1 Majoon-E-Najah (MN)

In the case of Majoon-E-Najah (MN), the ethanolic (ET) extract showed the highest antioxidant activity, with the lowest IC₅₀ value (32.94±1.21) and % inhibition ranging from 35.5±0.97% to 93.63±1.14 at concentrations between 20 and 120 µg/ml. The aqueous (AQ) extract exhibited moderate antioxidant activity, while the hydroalcoholic (HA) extract demonstrated the lowest scavenging potential, indicated by a higher IC₅₀ value. These findings suggest that the ethanolic extraction method is more effective at isolating potent antioxidant compounds from MN (Table 23 and Figures 36, 37).

Table 23. DPPH radical scavenging activity (% inhibition) of MN polyherbal extracts

Concentration (µg/ml)	% RSA AQ	% RSA HA	% RSA ET	% RSA AS
20	23.89±1.01 ^a	21.4±1.03 ^b	35.5±0.97 ^c	65.41±0.93 ^d
40	63.48±0.57 ^a	23.17±0.82 ^b	75.6±1.00 ^c	83.56±1.06 ^d
60	78.32±1.52 ^a	40.1±0.83 ^b	93.36±1.07 ^c	88.86±1.10 ^d
80	88.56±1.52 ^a	49.32±0.88 ^b	93.49±0.97 ^c	91.09±1.56 ^d
100	93.85±0.96 ^a	55.14±0.66 ^b	93.63±1.14 ^c	95.03±0.85 ^d
120	94.36±0.77 ^a	54.74±0.93 ^b	93.63±1.14 ^c	95.71±1.16 ^d
IC ₅₀ VALUE	62.15±0.57	109.9±0.36	32.94±1.21	11.79±0.20

DPPH radical scavenging activity (RSA) is compared across MN extracts (AQ, HA, ET) and Ascorbic Acid (AS). Each value is presented as mean ± SD (n = 3). Different letters (a, b, c, d) denote statistically significant differences between groups (p < 0.0001).

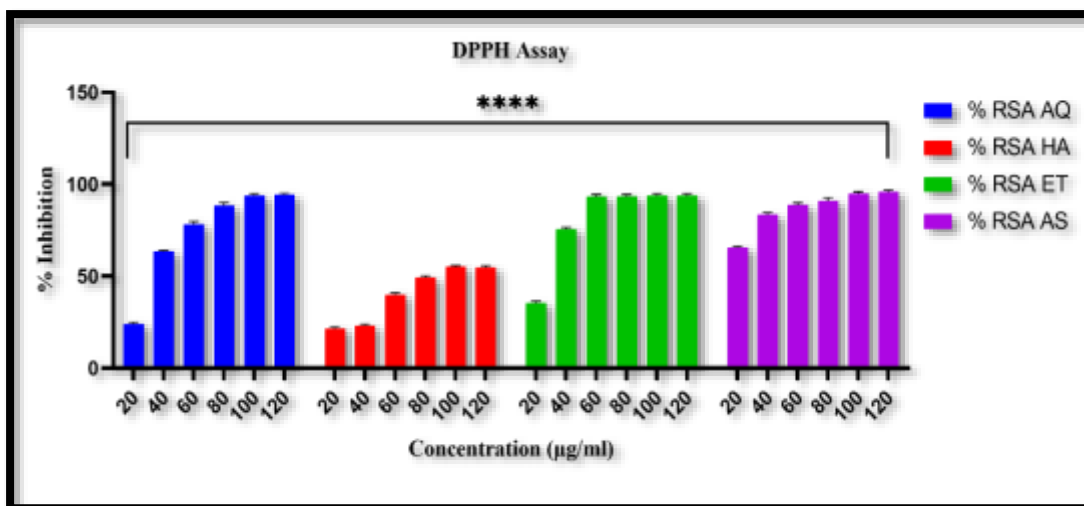


Fig. 36. DPPH radical scavenging activity (RSA), represented by percentage of inhibition, of the three different extracts of MN formulation: Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET) compared to ascorbic acid (AS).

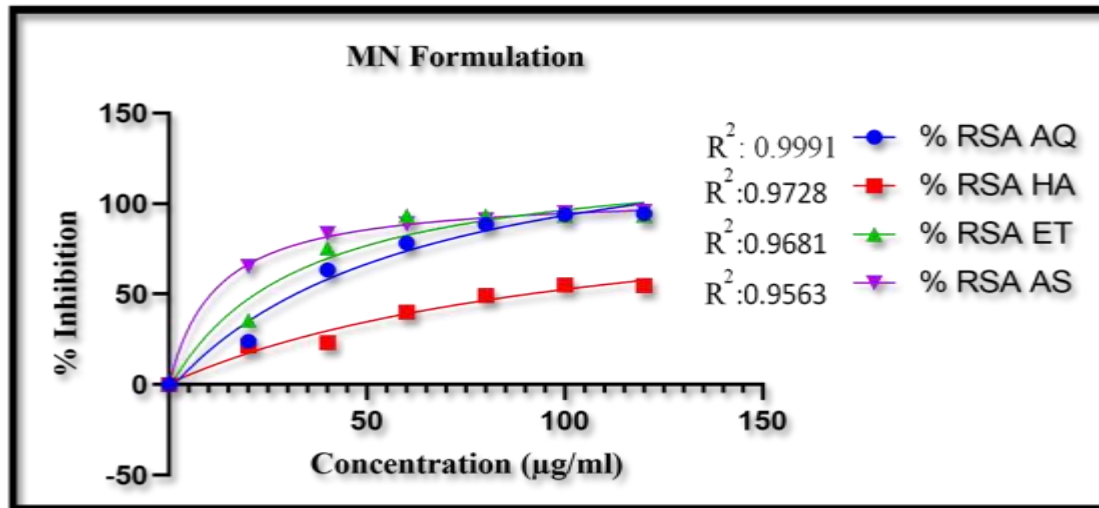


Fig. 37. Non-linear regression curves of MN formulation for three extracts (Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET)), compared with standard Ascorbic acid (AS).

5.5.3.2 Suffof-E-Najah (SN)

In the case of Suffof-E-Najah (SN), the ethanolic (ET) extract exhibited the highest antioxidant activity, with an IC₅₀ value of 3.79 ± 0.28 µg/ml and a percentage inhibition ranging from $59.45 \pm 0.36\%$ to $95.48 \pm 1.10\%$ at concentrations between 5 and 50 µg/ml. This outperformed both the aqueous (AQ) and hydroalcoholic (HA) extracts. The aqueous extract demonstrated moderate antioxidant potential, while the hydroalcoholic extract showed the lowest scavenging activity, reflected by a higher IC₅₀ value. Notably, the ethanolic extract displayed an IC₅₀ value lower than that of ascorbic acid (6.286 ± 0.47 µg/ml), the standard used in this assay. This indicates that the ethanolic extract of SN may contain highly potent antioxidant compounds (Table 24 and Figures 38, 39).

Table 24. DPPH radical scavenging activity (% inhibition) of SN polyherbal extracts

Concentration (µg/ml)	% RSA AQ	% RSA HA	% RSA ET	% RSA AS
5	50.41 ± 0.57^a	49.34 ± 1.11^b	59.45 ± 0.36^c	33.65 ± 0.67^b
10	71.10 ± 0.66^a	69.56 ± 0.78^b	72.88 ± 0.89^c	79.54 ± 0.45^b
20	91.08 ± 0.33^a	82.87 ± 0.56^b	92.62 ± 1.17^c	90.48 ± 1.15^b
30	95.24 ± 1.01^a	90.84 ± 1.14^b	92.86 ± 1.20^c	91.43 ± 0.44^b
40	96.43 ± 0.89^a	93.10 ± 1.23^b	93.81 ± 0.57^c	91.91 ± 1.25^b
50	97.14 ± 0.49^a	96.90 ± 1.07^b	95.48 ± 1.10^c	92.62 ± 1.14^b
IC ₅₀ VALUE	5.55 ± 0.66	5.72 ± 0.33	3.79 ± 0.28	6.28 ± 0.47

DPPH radical scavenging activity (RSA) was compared across Su.MN extracts (AQ, HA, ET) and Ascorbic Acid (AS). Each value is presented as mean $\pm$ SD (n = 3). The same letter "b" indicates no statistically significant difference (ns) between Hydroalcoholic (HA) and Aqueous (AS) extracts. Different letters indicate significant differences between the pairs: "a" and "b" between AQ and HA (****, $p < 0.0001$), "a" and "c" between AQ and ET (*, $p < 0.05$), "a" and "b" between AQ and AS (****, $p < 0.0001$), "b" and "c" between HA and ET (****, $p < 0.0001$), and "c" and "b" between ET and AS (****, $p < 0.0001$).

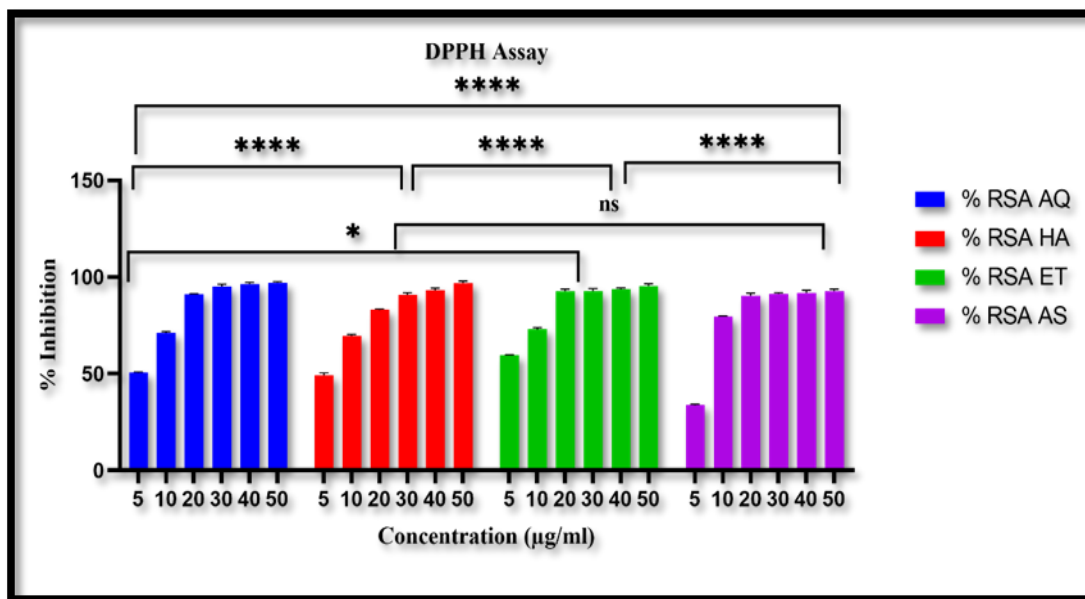


Fig. 38. DPPH radical scavenging activity (RSA), represented by percentage of inhibition, of the three different extracts of Su. MN formulation: Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET) compared to ascorbic acid (AS).

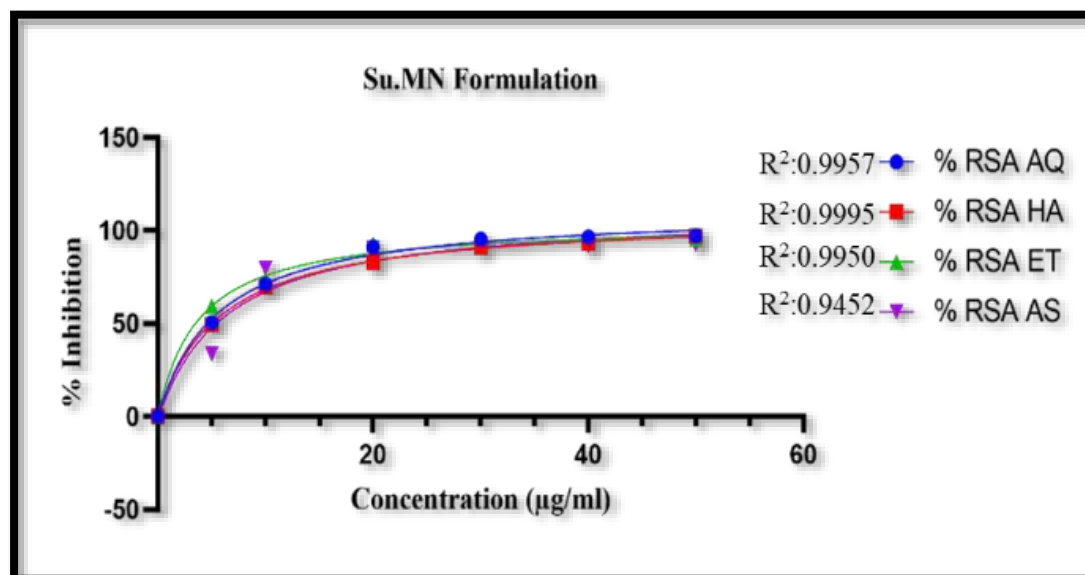


Fig. 39. Non-linear regression curves of Su.MN formulation for three extracts (Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET)), compared with standard Ascorbic acid (AS).

5.5.3.3 Suffof-E-Chobchini (SC)

In the case of Suffof-E-Chobchini (SC), the ethanolic (ET) extract consistently exhibited the highest antioxidant activity, with an IC₅₀ value of 22.72±0.53 and a percentage inhibition ranging from 32.093±0.45% to 51.93±1.17% at concentrations between 20 and 120 µg/ml. This trend highlights the effectiveness of the ethanolic solvent in extracting potent antioxidant compounds from SC. In comparison, the hydroalcoholic (HA) and aqueous (AQ) extracts demonstrated lower scavenging potential, as indicated by their higher IC₅₀ values. These results suggest that the ethanolic extraction method is particularly advantageous for isolating bioactive components with significant antioxidant properties from Suffof-E-Chobchini (Table 25 and Figures 40, 41).

Table 25. DPPH radical scavenging activity (% inhibition) of SC polyherbal extracts

Concentration (µg/ml)	% RSA AQ	% RSA HA	% RSA ET	% RSA AS
20	11.38± 0.57 ^a	39.84±0.47 ^b	32.093±0.45 ^c	65.41±0.59 ^d
40	23.03±0.66 ^a	59.53±0.39 ^b	36.43±0.57 ^c	83.56±0.39 ^d
60	31.3±0.47 ^a	64.8±0.87 ^b	43.72±0.66 ^c	88.86±0.86 ^d
80	34.28±0.78 ^a	86.66±0.81 ^b	42.79±0.49 ^c	91.09±0.66 ^d
100	43.49±0.64 ^a	91.62±0.57 ^b	55.5±1.04 ^c	95.03±1.05 ^d
120	47.42±1.02 ^a	89.92±1.14 ^b	51.93±1.17 ^c	95.71±1.16 ^d
IC ₅₀ VALUE	157.6±0.66	49.30±0.47	22.72±0.53	11.79±0.39

DPPH radical scavenging activity (RSA) is compared across SC extracts (AQ, HA, ET) and Ascorbic Acid (AS). Each value is presented as mean ± SD (n = 3). Different letters (a, b, c, d) denote statistically highly significant differences between groups at $p < 0.0001$ (****).

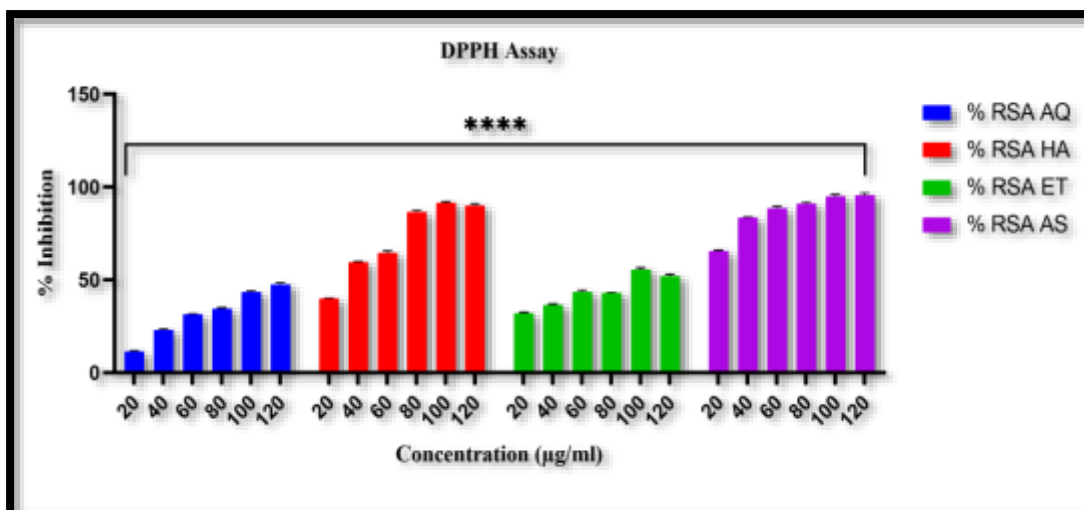


Fig. 40. DPPH radical scavenging activity (RSA), represented by percentage of inhibition, of the three different extracts of SC formulation: Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET) compared to ascorbic acid (AS).

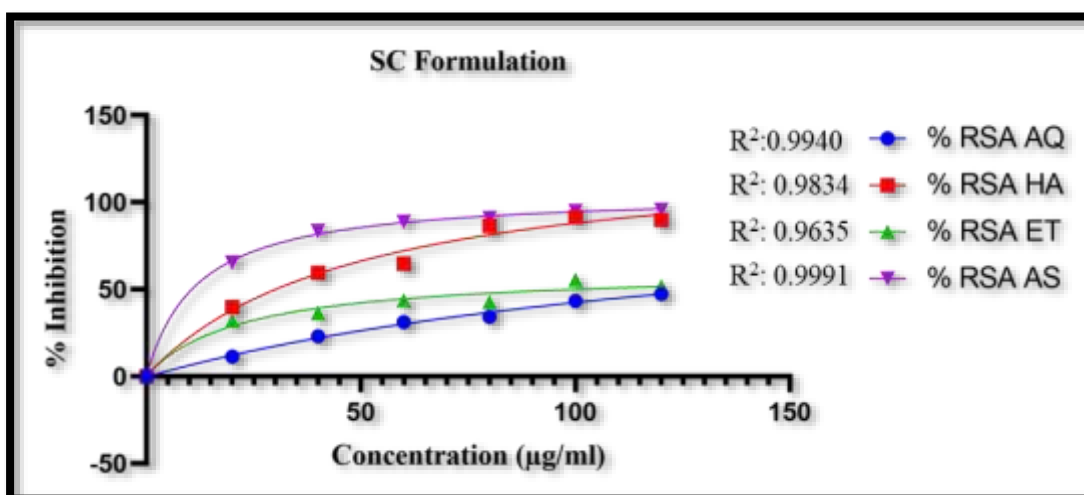


Fig. 41. Non-linear regression curves of SC formulation for three extracts (Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET)), compared with standard Ascorbic acid (AS).

5.5.3.4 Formulation 1 (F1)

In the case of Formulation 1 (F1), the aqueous (AQ) extract exhibited the highest antioxidant activity, with an IC₅₀ value of 73.58±0.33. This was followed by the hydroalcoholic (HA) extract, which demonstrated moderate antioxidant activity with an IC₅₀ value of 79.86±0.66. In contrast, the ethanolic (ET) extract showed the lowest

antioxidant activity, indicated by a significantly higher IC₅₀ value of 1834±1.07. The results were consistent across the concentration range of 10 to 60 µg/ml, suggesting that the aqueous extraction method is particularly effective at isolating potent antioxidant compounds from Formulation 1 (Table 26 and Figures 42, 43).

Table 26. DPPH radical scavenging activity (% inhibition) of F1 polyherbal extracts

Concentration (µg/ml)	% RSA AQ	% RSA HA	% RSA ET	% RSA AS
10	12.10±0.46 ^a	16.48±0.57 ^b	17.49±0.56 ^c	35.91±0.67 ^d
20	17.90±0.33 ^a	28.11±0.66 ^b	26.04±0.43 ^c	63.68±0.46 ^d
30	21.05±0.66 ^a	35.91±0.89 ^b	34.48±0.78 ^c	91.75±0.78 ^d
40	34.89±0.51 ^a	43.57±0.78 ^b	51.78±0.57 ^c	92.77±0.57 ^d
50	37.33±0.57 ^a	52.59±0.68 ^b	62.05±0.66 ^c	93.28±1.02 ^d
60	36.72±1.20 ^a	58.087±1.14 ^b	73.24±1.01 ^c	95.93±1.15 ^d
IC ₅₀ VALUE	73.58±0.33	79.86±0.66	1834±1.07	21.28±0.51

DPPH radical scavenging activity (RSA) is compared across F1 extracts (AQ, HA, ET) and Ascorbic Acid (AS). Each value is presented as mean ± SD (n = 3). Different letters (a, b, c, d) denote statistically highly significant differences between groups at $p < 0.0001$ (****).

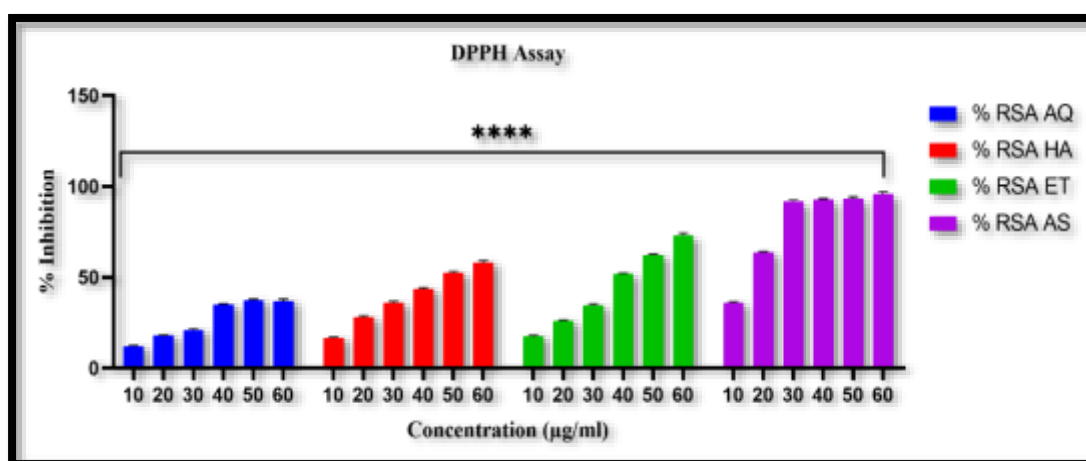


Fig. 42. DPPH radical scavenging activity (RSA), represented by percentage of inhibition, of the three different extracts of F1 formulation: Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET) compared to ascorbic acid (AS).

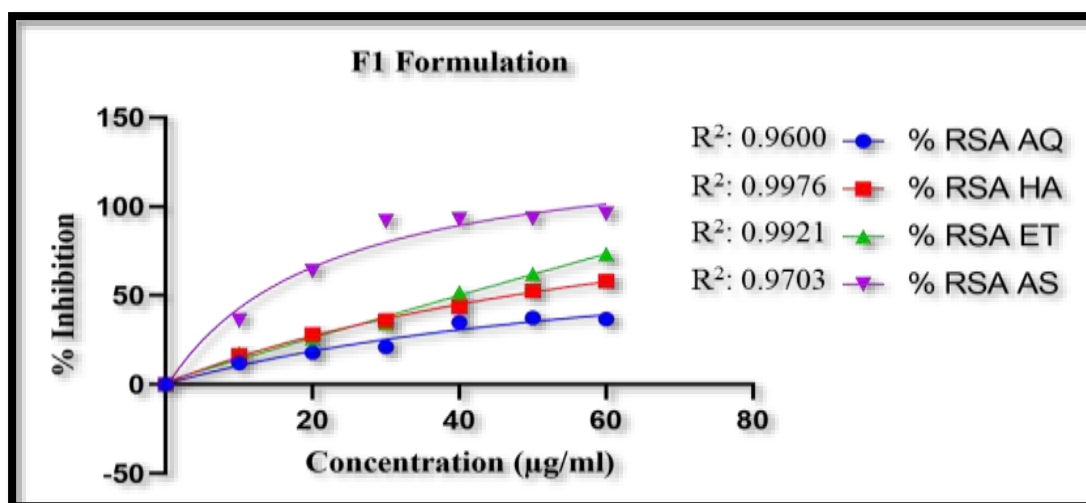


Fig. 43. Non-linear regression curves of F1 formulation for three extracts (Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET)), compared with standard Ascorbic acid (AS).

5.5.3.5 Formulation 2 (F2)

In the case of Formulation 2 (F2), the hydroalcoholic (HA) extract exhibited the highest antioxidant activity, with an IC₅₀ value of 91.56±0.51. This was followed by the ethanolic (ET) extract, which demonstrated moderate antioxidant activity with an IC₅₀ value of 144.2±0.66. The aqueous (AQ) extract showed the least antioxidant activity, indicated by a higher IC₅₀ value of 241.7±0.56. These findings were consistent across the concentration range of 20 to 120 µg/ml, underscoring the effectiveness of hydroalcoholic extraction in preserving the antioxidant potency of Formulation 2 (Table 27 and Figures 44, 45).

Table 27. DPPH radical scavenging activity (% inhibition) of F2 polyherbal extracts

Concentration (µg/ml)	% RSA AQ	% RSA HA	% RSA ET	% RSA AS
20	1.67±0.66 ^a	14.06±0.45 ^b	3.35±0.65 ^c	65.41±0.78 ^d
40	4.19±0.57 ^a	20.88±0.76 ^b	8.18±0.45 ^c	83.56±0.66 ^d
60	11.33±0.33 ^a	33.99±0.57 ^b	14.27±0.66 ^c	88.86±0.57 ^d
80	15.42±0.78 ^a	39.97±0.36 ^b	22.56±0.57 ^c	91.09±1.02 ^d
100	19.3±1.02 ^a	57.39±0.87 ^b	31.16±1.14 ^c	95.03±1.14 ^d
120	22.45±1.15 ^a	65.16±1.20 ^b	43.54±1.01 ^c	95.71±1.23 ^d
IC ₅₀ VALUE	241.7±0.56	91.56±0.51	144.2±0.66	11.79±0.33

DPPH radical scavenging activity (RSA) is compared across F2 extracts (AQ, HA, ET) and Ascorbic Acid (AS). Each value is presented as mean $\pm$ SD ($n = 3$). Different letters (a, b, c, d) denote statistically significant differences between groups at $p < 0.0001$ (****).

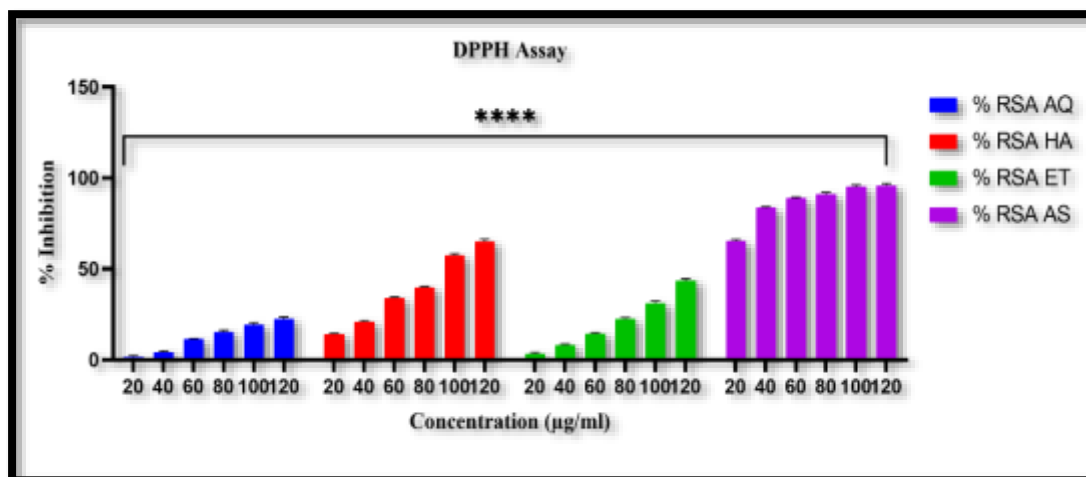


Fig. 44. DPPH radical scavenging activity (RSA), represented by percentage of inhibition, of the three different extracts of F2 formulation: Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET) compared to ascorbic acid (AS).

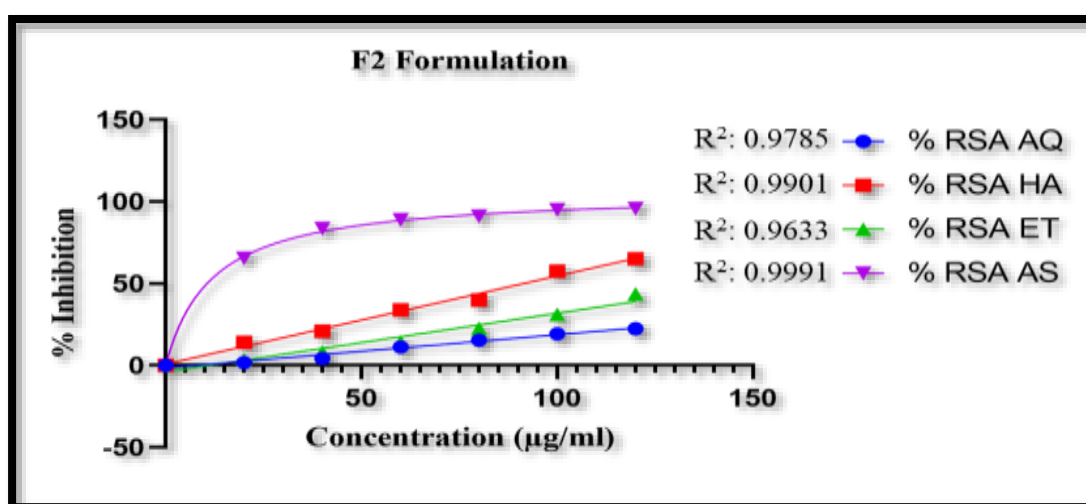


Fig. 45. Non-linear regression curves of F2 formulation for three extracts (Aqueous (AQ), Hydroalcoholic (HA), Ethanolic (ET)), compared with standard Ascorbic acid (AS).

5.6 Phytochemical characterization

For an in-depth phytochemical analysis, the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of five Unani polyherbal formulations were quantified. Among these, two formulations—Formulation 1 and Formulation 2—were selected for further study. Extracts were prepared using three different solvents: aqueous, hydroalcoholic, and ethanolic, facilitating a comprehensive examination of their chemical composition. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) fingerprinting were performed on all three extracts, as well as on the individual ingredients of the formulations, allowing for comparative analysis. These techniques provided valuable insights into the diversity of secondary metabolites present in the formulations. Notably, the ethanolic extracts of both novel Formulation 1 and Formulation 2 exhibited the highest profiles in HPTLC fingerprinting compared to the other two extracts and were subsequently selected for advanced High-Resolution Liquid Chromatography-Mass Spectrometry Quadrupole Time-of-Flight (HRLC-MS-QTOF) analysis.

5.6.1 Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

5.6.1.1 Total Phenolic Content (TPC)

The total phenolic content of extracts from five distinct Unani polyherbal formulations was assessed using the Folin-Ciocalteu method, with results expressed as gallic acid equivalents (GAE). The evaluation involved three types of extracts—aqueous, hydroalcoholic, and ethanolic. The calibration curve for gallic acid, showing maximum absorbance at 765 nm, was represented by the equation $y=0.0299x+0.2257$ with an R^2 value of 0.9738, ensuring precise quantification of phenolic compounds (Fig. 46). The results revealed notable variations in phenolic content across the extracts, reflecting the effectiveness of different extraction methods in isolating phenolic compounds. This study is the first to report the total phenolic content of these five Unani polyherbal formulations, as no prior studies have documented these findings.

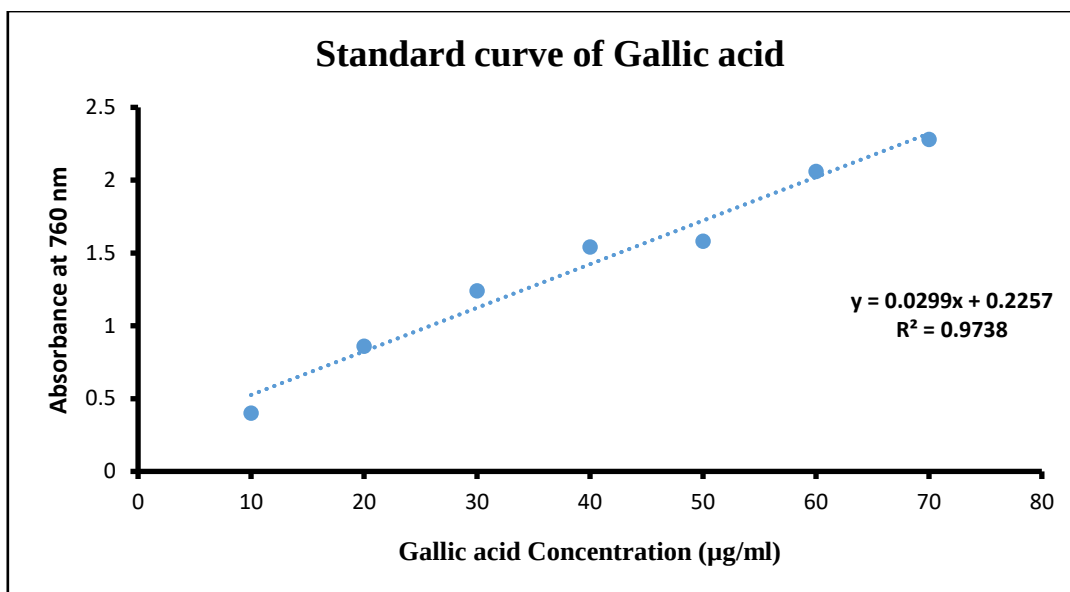


Fig. 46. Total phenolic content for standard gallic acid

5.6.1.2 Total Flavonoid Content (TFC)

The total flavonoid content of three different extracts (Aqueous, Hydroalcoholic, and Ethanolic) from five distinct Unani polyherbal formulations was evaluated using the colorimetric method with quercetin as the standard. The analysis was based on a calibration curve with the equation $y=0.0292x-0.1205$ and an R^2 value of 0.9941, ensuring precise measurement of flavonoid compounds (Fig. 47). This assessment highlighted substantial variation in flavonoid content across the extracts, reflecting the effectiveness of various extraction methods in isolating these valuable bioactive compounds. This is also the first time that the total flavonoid content of these Unani polyherbal formulations has been reported, as no prior research has reported these findings.

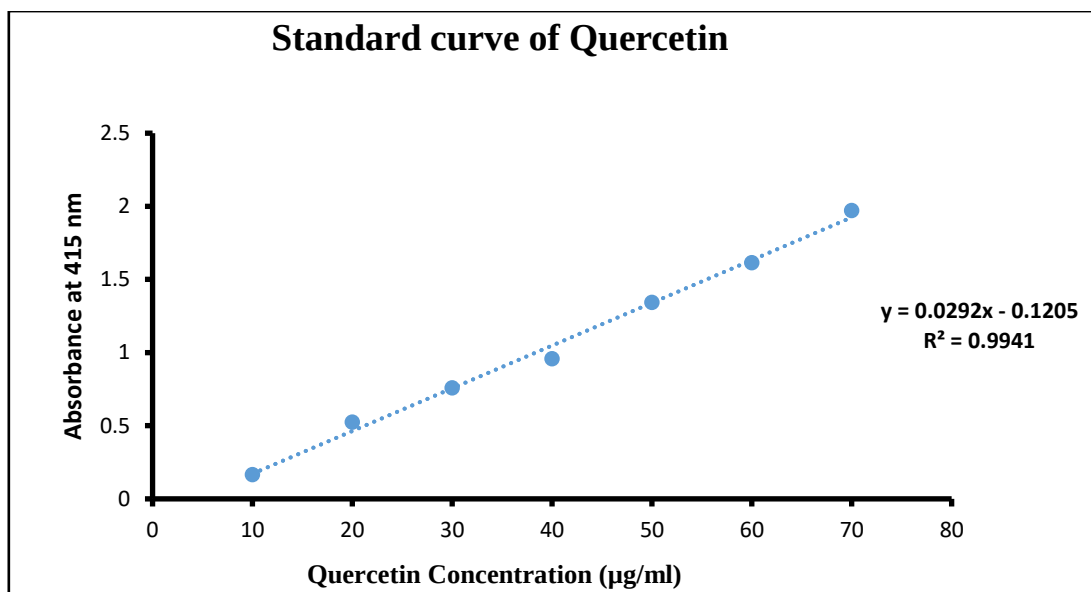


Fig. 47. Total flavonoid content for standard quercetin

5.6.1.3 Majoon-E-Najah (MN)

5.6.1.3.1 TPC:

In the Majoon-E-Najah (MN) extracts, the ethanolic (ET) extract had the highest total phenolic content, measuring 15.852 mg GAE/g. This was followed by the hydro alcoholic (HA) extract with 6.605 mg GAE/g and the aqueous (AQ) extract with 4.749 mg GAE/g (Table 28 and Fig. 48). These results indicate that the ethanolic extraction method is most effective for extracting phenolic compounds in this formulation.

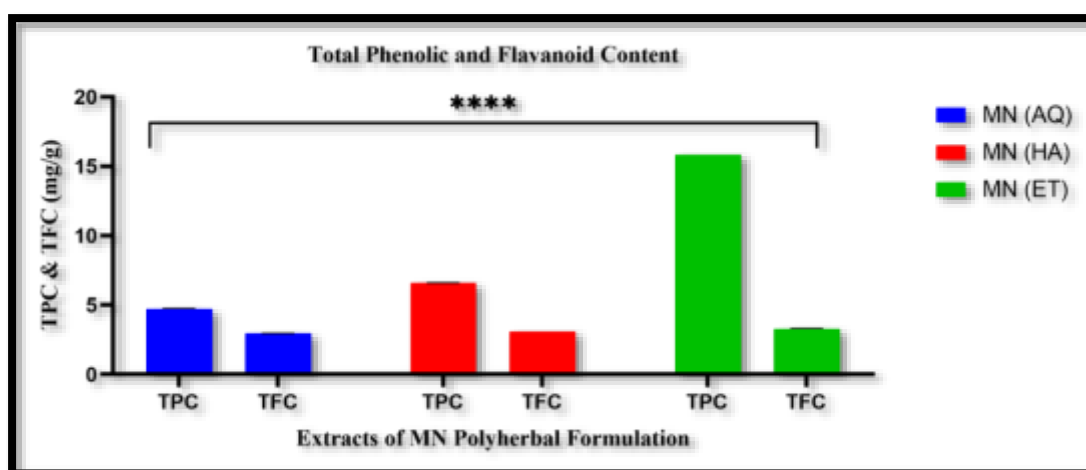
5.6.1.3.2 TFC:

The total flavonoid content in Majoon-E-Najah (MN) extracts was highest in the ethanolic (ET) extract, with a value of 3.313 mg QE/g. This was followed by the hydro alcoholic (HA) extract, which had a content of 3.091 mg QE/g, and the aqueous (AQ) extract, which showed a content of 2.962 mg QE/g (Table 28 and Fig. 48). These findings suggest that the ethanolic extraction method is more effective in extracting flavonoids from MN.

Table 28. Total Phenolic Content and Total Flavonoid Content of MN Polyherbal Extracts

MN extracts (1 mg/ml)	TPC (mg/g)	TFC (mg/g)
MN (AQ)	4.7486065 $\pm$ 0.012 ^a	2.962329 $\pm$ 0.010 ^a
MN (HA)	6.604795 $\pm$ 0.010 ^b	3.0907535 $\pm$ 0.001 ^b
MN (ET)	15.852285 $\pm$ 0.0011 ^c	3.313356 $\pm$ 0.009 ^c

Each value is presented as mean $\pm$ SD (n = 3). Different letters (a, b, c) denote statistically significant differences between the extracts for both Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) at $p < 0.0001$.

**Fig. 48. Total phenolic and total flavonoid content of MN polyherbal extracts**

5.6.1.4 Suffof-E-Najah (SN)

5.6.1.4.1 TPC:

The highest total phenolic content in Suffof-E-Najah (SN) was found in the aqueous (AQ) extract, with a value of 37.329 mg GAE/g. The hydro alcoholic (HA) extract followed closely with 37.196 mg GAE/g, while the ethanolic (ET) extract had a lower content of 32.263 mg GAE/g (Table 29 and Fig. 49). The aqueous extraction method appears to be particularly efficient for this formulation.

5.6.1.4.2 TFC:

In the Suffof-E-Najah (SN) extracts, the hydro alcoholic (HA) extract exhibited the highest flavonoid content at 10.634 mg QE/g. This was significantly higher compared to the aqueous (AQ) extract at 9.307 mg QE/g and the ethanolic (ET) extract at 8.587 mg QE/g (Table 29 and Fig. 49). The hydro alcoholic extraction appears to be particularly efficient for extracting flavonoids in this formulation.

Table 29. Total Phenolic Content and Total Flavonoid Content of SN Polyherbal Extracts

Su.MN extracts (1 mg/ml)	TPC (mg/g)	TFC (mg/g)
Su. MN (AQ)	37.32943±0.49 ^a	9.306505±0.87 ^a
Su. MN (HA)	37.19565±1.06 ^a	10.63356±0.57 ^a
Su. MN (ET)	32.26254±1.25 ^b	8.58733±1.03 ^b

Each value is presented as mean $\pm$ SD (n = 3). The same letter "a" indicates no statistically significant difference (ns) between Aqueous (AQ) and Hydroalcoholic (HA) extracts. The letter "a" is assigned to both AQ and HA. The letter "b" is assigned to the Ethanolic (ET) extract, indicating a significant difference between AQ and ET (***, $p < 0.001$) and a highly significant difference between HA and ET (****, $p < 0.0001$).

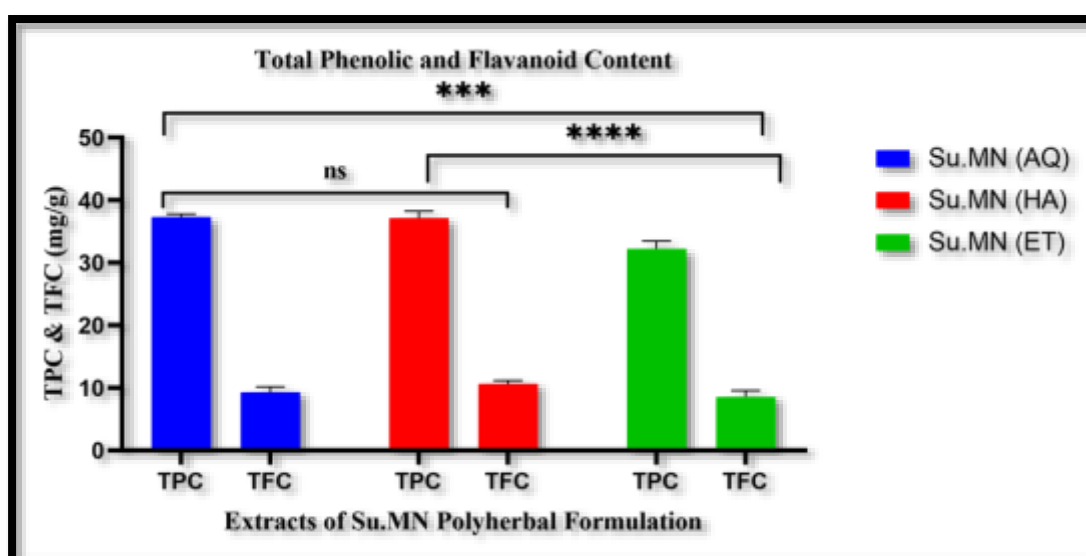


Fig. 49. Total phenolic and total flavonoid content of SN polyherbal extracts

5.6.1.5 Suffof-E-Chobchini (SC)

5.6.1.5.1 TPC:

In Suffof-E-Chobchini (SC), the hydro alcoholic (HA) extract exhibited the highest total phenolic content at 26.376 mg GAE/g. The aqueous (AQ) extract had a content of 22.959 mg GAE/g, and the ethanolic (ET) extract showed a lower content of 17.106 mg GAE/g (Table 30 and Fig. 50) The hydro alcoholic extraction method is most effective for this formulation.

5.6.1.5.2 TFC:

The ethanolic (ET) extract of Suffof-E-Chobchini (SC) displayed the highest total flavonoid content at 23.313 mg QE/g. The hydro alcoholic (HA) extract followed with a content of 17.851 mg QE/g, while the aqueous (AQ) extract had a considerably lower content of 6.430 mg QE/g (Table 30 and Fig. 50). This indicates a pronounced efficiency of the ethanolic extraction method for SC.

Table 30. Total Phenolic Content and Total Flavonoid Content of SC Polyherbal Extracts

SC extracts (1 mg/ml)	TPC (mg/g)	TFC (mg/g)
SC (AQ)	22.95931± 1.09 ^a	6.429795± 0.46 ^a
SC (HA)	26.376255± 1.23 ^b	17.851025± 0.97 ^b
SC (ET)	17.106465± 1.11 ^c	23.313355± 0.57 ^c

Values are expressed as mean ± SD (n = 3). Different letters (a, b, c) indicate statistically significant differences between the groups. The letter a is assigned to the Aqueous (AQ) extract, b to the Hydroalcoholic (HA) extract, and c to the Ethanolic (ET) extract. A significant difference was observed between AQ and HA (****, $p < 0.0001$), AQ and ET (****, $p < 0.0001$), and HA and ET (*, $p < 0.05$).

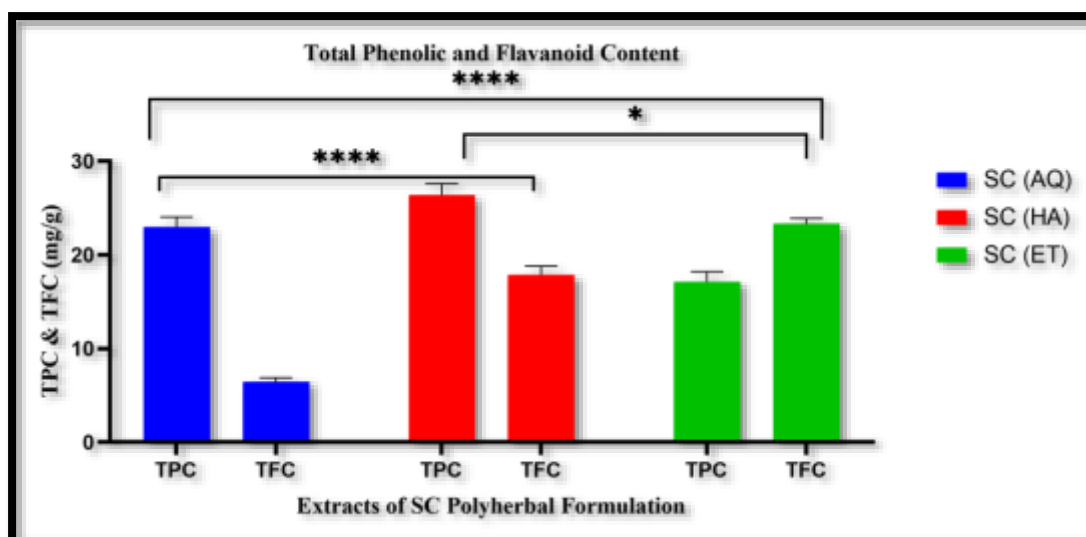


Fig. 50. Total Phenolic Content and Total Flavanoid Content of SC Polyherbal Extracts

5.6.1.6 Formulation 1 (F1)

5.6.1.6.1 TPC:

The total phenolic content of the Formulation 1 (F1) extracts was highest in the aqueous (AQ) extract, with 36.727 mg GAE/g. The hydro alcoholic (HA) extract followed with 35.044 mg GAE/g, and the ethanolic (ET) extract had a lower content of 30.468 mg GAE/g (Table 31 and Fig. 51). The aqueous extraction method proves to be more effective in this formulation.

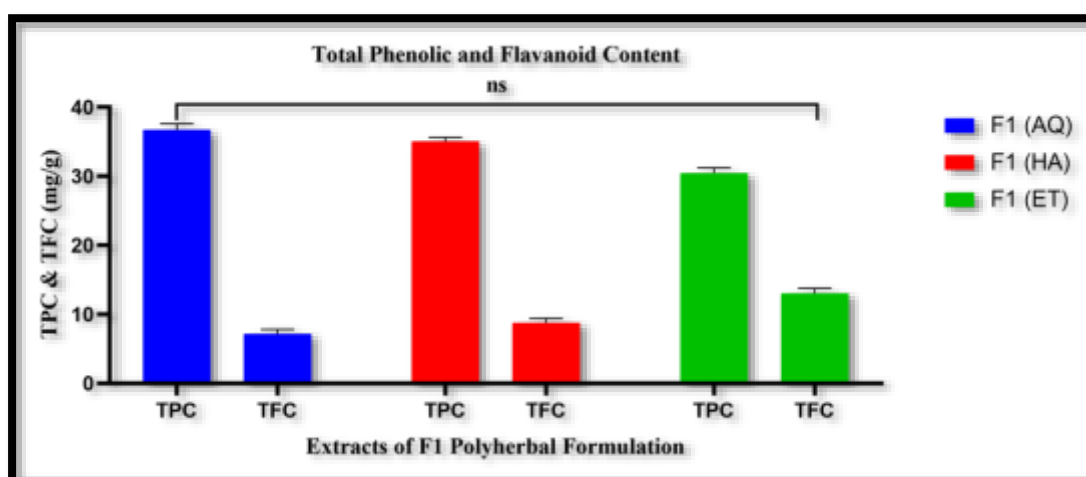
5.6.1.6.2 TFC:

In the formulation 1 (F1) extracts, the ethanolic (ET) extract also showed the highest total flavonoid content, measuring 13.031 mg QE/g. The hydro alcoholic (HA) extract had a content of 8.784 mg QE/g, and the aqueous (AQ) extract had the lowest content at 7.158 mg QE/g (Table 31 and Fig. 51). The ethanolic extraction method demonstrates a higher yield of flavonoids in this formulation as well.

Table 31. Total Phenolic Content and Total Flavonoid Content of F1 Polyherbal Extracts

F1 extracts (1 mg/ml)	TPC (mg/g)	TFC (mg/g)
F1 (AQ)	36.72742±0.89 ^a	7.157535±0.66 ^a
F1 (HA)	35.04403±0.57 ^a	8.784245±0.63 ^a
F1 (ET)	30.467665±0.76 ^a	13.03082±0.71 ^a

Each value is presented as mean ± SD (n = 3). The letter **a** denotes no statistically significant differences between the extracts (ns).

**Fig. 51. Total Phenolic Content and Total Flavonoid Content of F1 Polyherbal Extracts**

5.6.1.7 Formulation 2 (F2)

5.6.1.7.1 TPC:

In the Formulation 2 (F2) extracts, the aqueous (AQ) extract had the highest total phenolic content, measuring 21.770 mg GAE/g. The hydro alcoholic (HA) extract followed with 17.023 mg GAE/g, and the ethanolic (ET) extract had the lowest content at 7.101 mg GAE/g (Table 32 and Fig. 52). The aqueous extraction method is particularly effective for this formulation.

5.6.1.7.2 TFC:

The ethanolic (ET) extract of Formulation 2 (F2) exhibited the highest flavonoid content at 11.276 mg QE/g. This was followed by the hydro alcoholic (HA) extract with a content of 7.825 mg QE/g, and the aqueous (AQ) extract with a content of 5.779 mg QE/g (Table 32 and Fig. 52). The results highlight the superior flavonoid extraction capacity of the ethanolic method in F2.

Table 32. Total Phenolic Content and Total Flavonoid Content of F2 Polyherbal Extracts

F2 extracts (1 mg/ml)	TPC (mg/g)	TFC (mg/g)
F2 (AQ)	21.77±1.86 ^a	5.77911±0.77 ^a
F2 (HA)	17.02285±1.56 ^a	7.82534±0.52 ^a
F2 (ET)	7.100885±1.78 ^b	11.275685±0.66 ^b

Each value is presented as mean $\pm$ SD (n = 3). The same letter "a" denotes no statistically significant difference (ns) between Aqueous (AQ) and Hydroalcoholic (HA) extracts. The letter "a" is assigned to both AQ and HA, while the letter "b" is assigned to the Ethanolic (ET) extract, indicating a significant difference between AQ and ET (***, $p < 0.001$) and between HA and ET (**, $p < 0.01$).

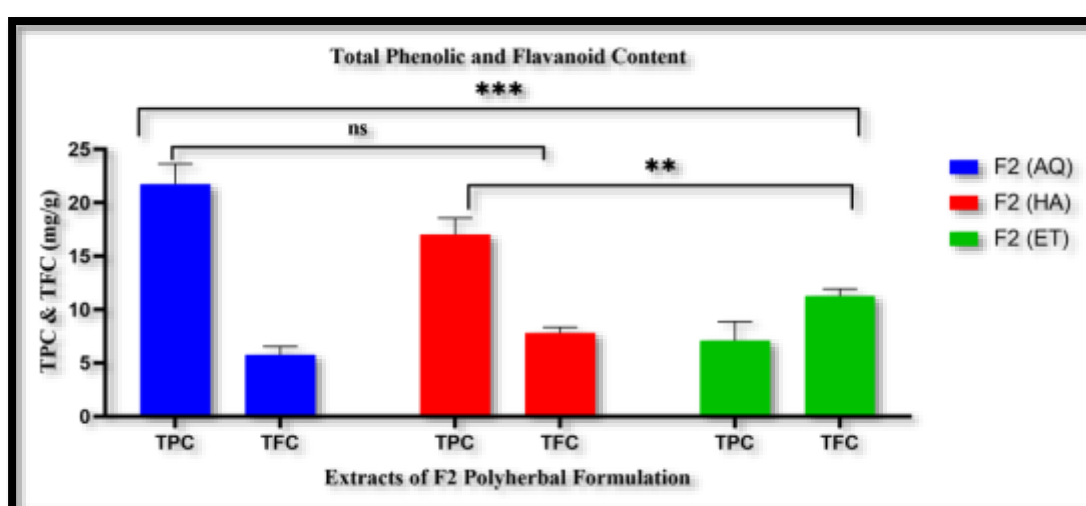


Fig. 52. Total Phenolic Content and Total Flavonoid Content of F2 Polyherbal Extracts

5.6.2 Pearson Correlation and Implications

Pearson correlation analysis was conducted on five Unani polyherbal formulations across three extracts—Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET). This analysis aimed to explore the relationships between various pharmacological activities, including antibacterial, antifungal, and antioxidant activities, and the phytochemical content (Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)) of each formulation. The specific parameters used in this analysis were MIC values for antibacterial and antifungal activities, IC₅₀ values for antioxidant activity, and concentrations in mg/g for TPC and TFC.

The correlation coefficient (*r*) and *p*-values (set at 0.05) were calculated for each formulation to assess the strength and significance of these relationships. The results are presented in both tabular format and correlogram to provide a comprehensive overview of the correlations for each formulation. These findings offer insights into the potential interdependence of the pharmacological activities and phytochemical composition, which may further elucidate the therapeutic efficacy of these Unani formulations.

5.6.2.1 Majoon-E-Najah (MN)

The Pearson correlation analysis of Majoon-E-Najah (MN) extracts highlighted several significant relationships among antibacterial and antifungal activities, DPPH assay, total phenolic content (TPC), and total flavonoid content (TFC) (Table 33). Antibacterial activity against *Escherichia coli* showed positive correlations with *Bacillus megaterium*, *Candida albicans*, and DPPH, while exhibiting negative correlations with *Aspergillus niger*, TPC, and a minor negative correlation with TFC. Similarly, antibacterial activity against *Bacillus megaterium* was positively correlated with *E. coli*, *C. albicans*, and DPPH, but negatively correlated with *A. niger*, TPC, and TFC. Antifungal activity against *C. albicans* was positively correlated with *E. coli*, *B. megaterium*, and DPPH, while negatively correlated with *A. niger*, TPC, and showed a minor negative correlation with TFC. Conversely, antifungal activity against *A. niger* demonstrated perfect negative correlations with *B. megaterium* and DPPH, and moderate negative correlations with *E. coli* and *C. albicans*. The DPPH assay results were positively correlated with *E. coli*, *B. megaterium*, and *C. albicans*, while

negatively correlated with *A. niger*, TPC, and moderately negatively with TFC. TPC exhibited positive correlations with *A. niger* and TFC, but was negatively correlated with *E. coli* and *C. albicans*, with perfect negative correlations with *B. megaterium* and DPPH. TFC displayed positive correlations with *A. niger* and TPC, while showing minor negative correlations with *E. coli* and *C. albicans*, and moderate negative correlations with *B. megaterium* and DPPH (Fig. 53). These findings suggest complex interactions between bioactive compounds and their antimicrobial and antioxidant effects (Cowan, 1999; Rice-Evans et al., 1996). The correlations highlight the nuanced role of phenolic and flavonoid compounds in mediating the antimicrobial and antioxidant properties of MN extracts.

Table 33. Pearson's Correlation Coefficient Matrix Between Phytochemical Content and Invitro Pharmacological Activities of MN Polyherbal Extracts

Attributes	<i>E. coli</i>	<i>B. megaterium</i>	<i>C. albicans</i>	<i>A. niger</i>	DPPH	TPC	TFC
<i>E. coli</i>	1*						
<i>B. megaterium</i>	0.862727222	1*					
<i>C. albicans</i>	0.999058601*	0.883851509	1*				
<i>A. niger</i>	-0.461960244	-0.847024363	-0.5	1*			
DPPH	0.909467915	0.994867055	0.926648	-0.78889	1*		
TPC	-0.31791	-0.753704128	-0.35874	0.98775	-0.68333	1*	
TFC	-0.110059523	-0.597548998	-0.15307	0.932356	-0.51334	0.977351	1*

Note: Values marked with an asterisk (*) indicate statistically significant differences at $P < 0.05$.

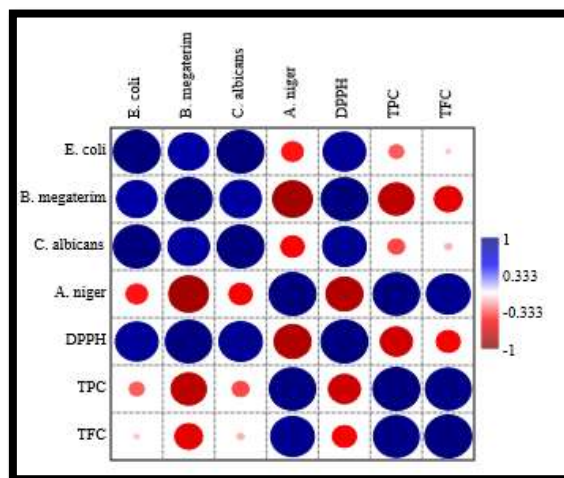


Fig. 53. Pearson's Correlation analysis between Phytochemical Content and Invitro Pharmacological activities of MN Polyherbal Extracts

5.6.2.2 Suffof-E-Najah (SN)

Pearson correlation analysis of Suffof-E-Najah (SN) extracts (aqueous, hydroalcoholic, and ethanolic) highlighted key relationships among antibacterial and antifungal activities, DPPH assay results, and total phenolic content (TPC) and total flavonoid content (TFC) (Table 34). Antibacterial activity against *Escherichia coli* was negatively correlated with antifungal activities against *Candida albicans* and *Aspergillus niger*, as well as with TPC and TFC. Conversely, antibacterial activity against *Bacillus megaterium* showed strong positive correlations with TPC, TFC, and antifungal activities against both fungal strains. Antifungal activity against *Candida albicans* was positively correlated with antifungal activity against *Aspergillus niger*, DPPH assay results, TPC, and TFC, suggesting that these compounds enhance antifungal efficacy. Similarly, antifungal activity against *Aspergillus niger* showed positive correlations with *Candida albicans* activity, DPPH assay results, TPC, and TFC. The DPPH assay results were positively correlated with antibacterial activity against *Bacillus megaterium* and antifungal activities against both fungal strains, but negatively correlated with antibacterial activity against *Escherichia coli* (Fig 54). Overall, these findings reveal complex interactions where TPC and TFC contribute to both antioxidant and antifungal properties, but their relationships with antibacterial activity vary depending on the target pathogen.

Table 34. Pearson's Correlation Coefficient Matrix Between Phytochemical Content and Invitro Pharmacological Activities of SN Polyherbal Extracts

Attributes	<i>E. coli</i>	<i>B. megaterium</i>	<i>C. albicans</i>	<i>A. niger</i>	DPPH	TPC	TFC
<i>E. coli</i>	1*						
<i>B. megaterium</i>	-1*	1*					
<i>C. albicans</i>	-0.5	0.5	1*				
<i>A. niger</i>	-0.5	0.5	1*	1*			
DPPH	-0.99706*	0.997063*	0.432204	0.432204	1*		
TPC	-0.99973*	0.999732*	0.519927	0.519927	0.995021	1*	
TFC	-0.76904	0.76904	-0.16904	-0.16904	0.815736	0.754026	1*

Note: Values marked with an asterisk (*) indicate statistically significant differences at $P < 0.05$.

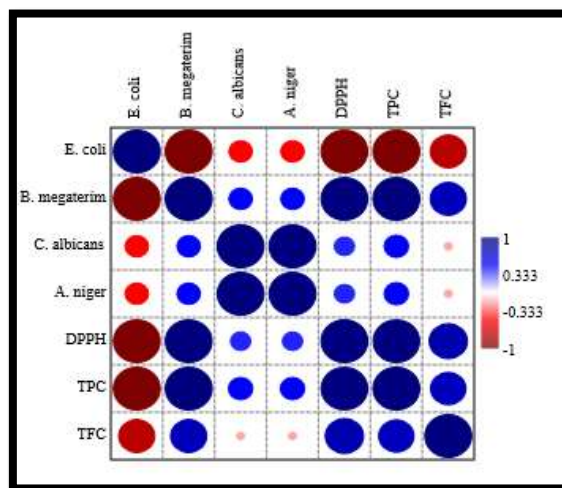


Fig. 54. Pearson's Correlation analysis between Phytochemical Content and Invitro Pharmacological activities of Su. MN Polyherbal Extracts

5.6.2.3 Suffof-E-Chobchini (SC)

Pearson correlation analysis of Suffof-E-Chobchini (SC) extracts revealed complex interactions among antibacterial and antifungal activities, DPPH assay, TPC, and TFC (Table 35). *E. coli* antibacterial activity was negatively correlated with *B. megaterium*, DPPH, and TPC, while positively correlated with TFC, with no correlation to fungal strains. *B. megaterium* antibacterial activity showed a perfect positive correlation with DPPH, a minor positive correlation with TFC, but was negatively correlated with TPC, and had no correlation with *A. niger*. Antifungal activity against *C. albicans* had positive correlations with TPC and TFC but was negatively correlated with DPPH and showed no correlation with *E. coli* and *A. niger*. *A. niger* antifungal activity had no significant correlations with any parameters. DPPH was positively correlated with *B. megaterium* and TPC, but negatively correlated with *E. coli*, *C. albicans*, and TFC. TPC showed minimal positive correlations with DPPH, *C. albicans*, and *B. megaterium*, but was negatively correlated with *E. coli* and TFC, with no correlation to *A. niger*. TFC was positively correlated with *E. coli* and *C. albicans*, but negatively correlated with *B. megaterium*, DPPH, and TPC (Fig. 55). These findings underscore the significant role of TFC in SC's antifungal activity.

Table 35. Pearson's Correlation Coefficient Matrix Between Phytochemical Content and Invitro Pharmacological Activities of SC Polyherbal Extracts

Attributes	<i>E. coli</i>	<i>B. megaterium</i>	<i>C. albicans</i>	<i>A. niger</i>	DPPH	TPC	TFC
<i>E. coli</i>	1*						
<i>B. megaterium</i>	-0.5	1*					
<i>C. albicans</i>	0	-0.86603	1*				
<i>A. niger</i>	0	0	0	1*			
DPPH	-0.65236	0.982547	-0.7579	0	1*		
TPC	-0.93123	0.149999	0.36444	0	0.331288	1*	
TFC	0.748753	-0.94842	0.662849	0	-0.99084	-0.45569	1*

Note: Values marked with an asterisk (*) indicate statistically significant differences at $P < 0.05$.

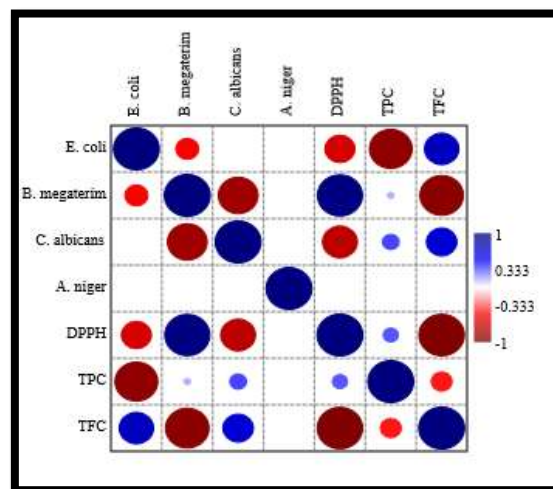


Fig. 55. Pearson's Correlation analysis between Phytochemical Content and Invitro Pharmacological Activities of SC Polyherbal Extracts

5.6.2.4 Formulation 1 (F1)

Pearson correlation analysis for Formulation 1 (F1) revealed intricate relationships among antibacterial and antifungal activities, DPPH assay, TPC, and TFC (Table 36). Antibacterial activity against *E. coli* was positively correlated with TFC and DPPH, but negatively correlated with *B. megaterium*, *C. albicans*, and TPC, with no correlation to *A. niger*. Antibacterial activity against *B. megaterium* was positively correlated with *C. albicans* and TPC, but negatively correlated with *E. coli*, DPPH, and TFC, and showed no correlation with *A. niger*. Antifungal activity against *C. albicans* was negatively correlated with *E. coli*, DPPH, and TFC, but positively correlated with *B. megaterium* and TPC, with no correlation to *A. niger*. No significant correlations were observed for antifungal activity against *A. niger*. DPPH assay results were negatively correlated with *B. megaterium*, *C. albicans*, and TPC, but positively correlated with *E. coli* and TFC, with no correlation to *A. niger*. TPC showed positive correlations with *B. megaterium* and *C. albicans*, but negative correlations with *E. coli*, DPPH, and TFC, with no correlation to *A. niger*. TFC showed positive correlations with *E. coli* and DPPH, but negative correlations with *B. megaterium*, *C. albicans*, and TPC, with no correlation to *A. niger* (Fig. 56). These findings underscore the complex interactions between the bioactivities and phytochemical components in F1.

Table 36. Pearson's Correlation Coefficient Matrix Between Phytochemical Content and Invitro Pharmacological Activities of F1 Polyherbal Extracts

Attributes	<i>E. coli</i>	<i>B. megaterium</i>	<i>C. albicans</i>	<i>A. niger</i>	DPPH	TPC	TFC
<i>E. coli</i>	1*						
<i>B. megaterium</i>	-0.80296	1*					
<i>C. albicans</i>	-1*	0.802955	1*				
<i>A. niger</i>	0	0	0	1*			
DPPH	0.502678	-0.91889	-0.50268	0	1*		
TPC	-0.70785	0.989392	0.707848	0	-0.96645331	1*	
TFC	0.713961	-0.99062	-0.71396	0	0.96418435	-0.9999622	1*

Note: Values marked with an asterisk (*) indicate statistically significant differences at $P < 0.05$.

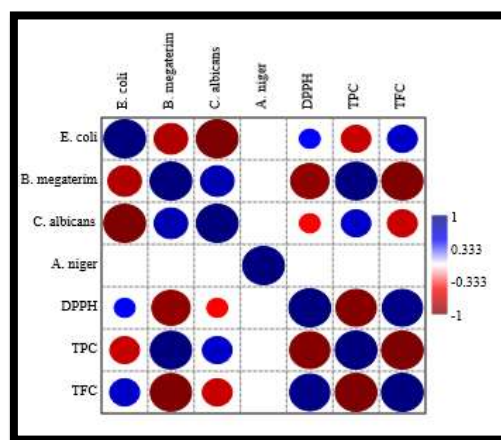


Fig. 56. Pearson's Correlation analysis between Phytochemical Content and Invitro Pharmacological Activities of F1 Polyherbal Extracts

5.6.2.5 Formulation 2 (F2)

Pearson correlation analysis for Formulation 2 (F2) revealed intricate interactions between antibacterial, antifungal activities, DPPH assay, TPC, and TFC (Table 37). Antibacterial activity against *E. coli* was negatively correlated with *B. megaterium*, *C. albicans*, and TPC, while positively correlated with *A. niger*, DPPH, and TFC. In contrast, antibacterial activity against *B. megaterium* showed negative correlations with *E. coli*, *A. niger*, and TFC, and positive correlations with *C. albicans* and TPC. Antifungal activity against *C. albicans* was negatively correlated with *E. coli*, *A. niger*, TFC, and DPPH, but positively correlated with *B. megaterium* and TPC. Activity against *A. niger* was negatively correlated with TPC and *B. megaterium*, yet positively correlated with *E. coli*, DPPH, and TFC. DPPH results were negatively correlated with TPC and *C. albicans*, but positively correlated with *E. coli*, *A. niger*, and TFC. TPC was negatively correlated with *E. coli*, *A. niger*, DPPH, and TFC, but positively correlated with *B. megaterium* and *C. albicans*. TFC showed positive correlations with *E. coli*, *A. niger*, DPPH, and itself, while negatively correlated with *B. megaterium* and *C. albicans* (Fig. 57). These correlations suggest a complex interplay between the bioactive components and the observed pharmacological effects in F2.

Table 37. Pearson's Correlation Coefficient Matrix Between Phytochemical Content and Invitro Pharmacological Activities of F2 Polyherbal Extracts

Attributes	<i>E. coli</i>	<i>B. megaterium</i>	<i>C. albicans</i>	<i>A. niger</i>	DPPH	TPC	TFC
<i>E. coli</i>	1*						
<i>B. megaterium</i>	-0.5	1*					
<i>C. albicans</i>	-0.5	1*	1*				
<i>A. niger</i>	1*	-0.5	-0.5	1*			
DPPH	0.91167	-0.09998	-0.09998	0.91167	1*		
TPC	-0.94839	0.74882	0.74882	-0.94839	-0.73432	1*	
TFC	0.92971	-0.7838	-0.7838	0.92971	0.69626	-0.99852	1*

Note: Values marked with an asterisk (*) indicate statistically significant differences at $P < 0.05$.

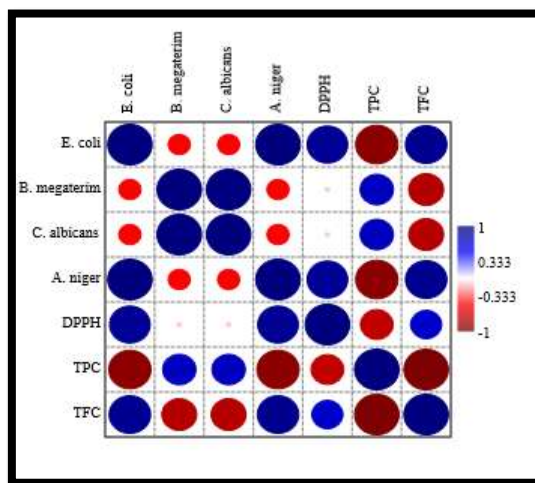


Fig. 57. Pearson's Correlation analysis between Phytochemical Content and Invitro Pharmacological Activities of F2 Polyherbal Extracts

5.6.3 Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) Fingerprinting

The ethanolic, hydroalcoholic, and aqueous extracts of Formulation 1 (F1) and Formulation 2 (F2) were subjected to Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) to establish their phytoconstituents profiles. TLC provided an initial qualitative assessment, while HPTLC, with its enhanced resolution capabilities, allowed for the generation of detailed and precise fingerprints. This dual analytical approach facilitated the comprehensive characterization of the formulations, yielding unique fingerprints for each formulation, which are critical for quality control, authentication, and the exploration of potential bioactive compounds.

5.6.3.1 HPTLC Analysis

The HPTLC analysis was conducted using silica gel 60 F254-coated aluminum plates. A total of 15 µL of each ethanolic extract was applied to the plates, which were then developed using optimized solvent systems tailored for each formulation. The plates were sprayed with a 10% methanolic sulphuric acid reagent and dried at room temperature to enhance the visualization of phytoconstituents. Subsequent scanning at multiple wavelengths (254 nm, 366 nm, and 540 nm) revealed distinct bands corresponding to separated compounds. Derivatization was performed using an Automated CAMAG Derivatizer at 105°C, which further enhanced the visibility of specific phytoconstituents, confirmed through additional scanning.

The resulting chromatograms, with calculated R_f values, displayed a diverse array of separated compounds for both formulations, highlighting the complexity and richness of their phytochemical profiles. The comprehensive HPTLC fingerprints generated at multiple wavelengths serve as robust tools for the characterization of these polyherbal formulations.

5.6.3.2 HPTLC Fingerprints for Formulation 1 (F1)

For Formulation 1 (F1), HPTLC fingerprinting was conducted by applying the aqueous (AQ), hydroalcoholic (HA), and ethanolic (ET) extracts to a single plate, labelled as tracks 1, 2, and 3, respectively. Additionally, the individual ingredients of Formulation 1 were applied as tracks 4 (Root of *Withania somnifera*), 5 (Root of *Barleria prionitis*),

6 (Flower of *Woodfordia fruticosa*), and 7 (Flower of *Nymphaea alba*). Several solvent systems like Toluene, chloroform, ethyl acetate, methanol, formic acid, glacial acetic acid, etc., in different ratios were tried and tested for development of HPTLC fingerprint. It was observed that mobile phase consisting of Toluene: Ethyl acetate: Formic acid: Methanol (9:9:2.4:0.6 v/v/v/v) provided the best separation (Figures 48 and 49).

The HPTLC plates, visualized at different wavelengths, revealed distinct patterns of bands, with the ethanolic extract showing the highest number of bands, followed by the hydroalcoholic and aqueous extracts in Formulation 1 (Fig. 58). The derivatization of the HPTLC plates with 10% methanolic sulphuric acid significantly enhanced the detection of specific phytoconstituents by inducing chemical changes that increased their visibility (Fig. 59). Densitometric scanning at 254 nm and 366 nm revealed a large number of bands across all samples, with distinct visual distinctions among the extracts and formulations. Furthermore, track-wise chromatograms and corresponding R_f values were generated for each of the seven tracks (Tables 38 - 51 and Figures 61- 63), providing a detailed fingerprint profile that highlights both the individual ingredient contributions and the composite phytoconstituents pattern of the entire formulation.

Fig. 58. HPTLC Fingerprinting of Formulation 1 (F1) Before Derivatization

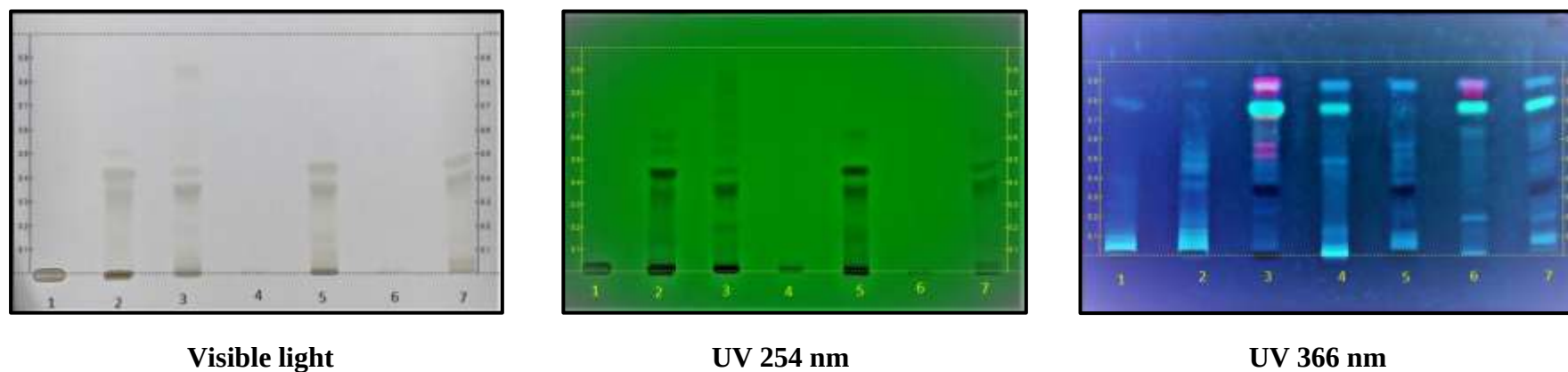
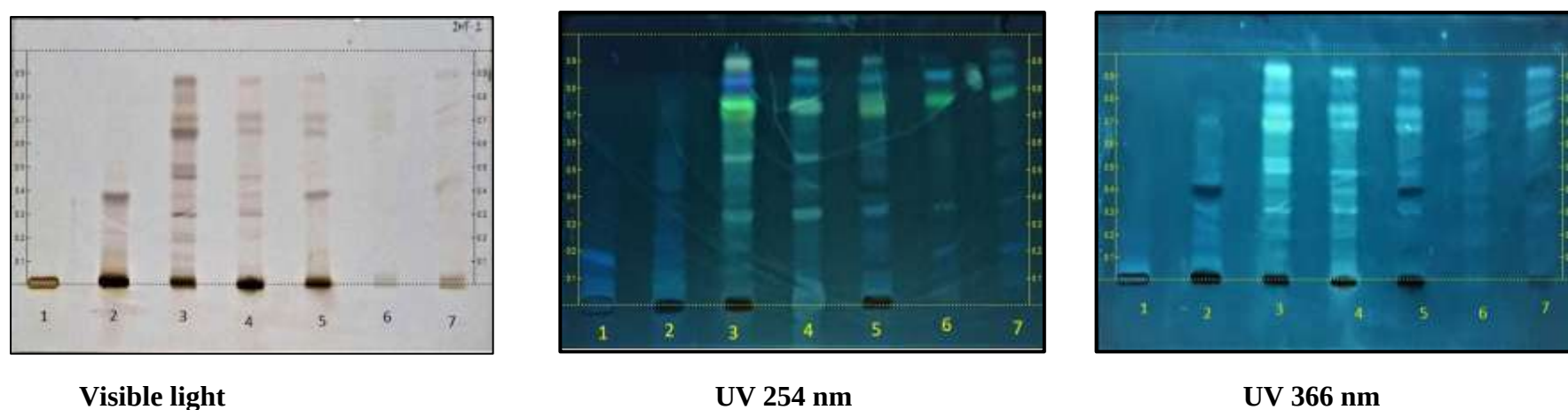


Fig. 59. HPTLC Fingerprinting of Formulation 1 (F1) After Derivatization



Track 1. Formulation 1 (Aqueous extract), **Track 2.** Formulation 1 (Hydroalcoholic extract), **Track 3.** Formulation 1 (Ethanolic extract), **Track 4.** Root of *Withania somnifera*, **Track 5.** Root of *Barleria prionitis*, **Track 6.** Flowers of *Woodfordia fruticosa*, **Track 7.** Flowers of *Nymphaea alba*

HPTLC Peak Values and Densitograms for Formulation 1 (F1) at 254 nm (Before Derivatization)

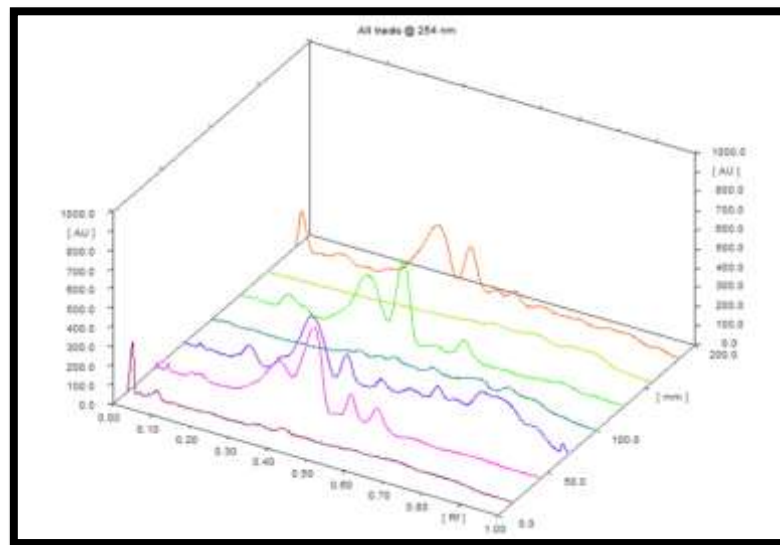


Fig. 60. 3D densitogram of all tracks at 254 nm (Before Derivatization).

Table 38. HPTLC Peak Values for Track 1 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.00 Rf	27.8 AU	0.01 Rf	261.4 AU	65.46%	0.03 Rf	0.5 AU	2014.8 AU	36.01%	Unknown *
1	2	0.05 Rf	0.1 AU	0.08 Rf	45.1 AU	11.28%	0.09 Rf	0.1 AU	744.2 AU	13.30%	Unknown *
1	3	0.30 Rf	1.6 AU	0.34 Rf	22.7 AU	5.69%	0.36 Rf	11.6 AU	650.5 AU	11.63%	Unknown *
1	4	0.37 Rf	11.8 AU	0.40 Rf	38.2 AU	9.57%	0.43 Rf	17.8 AU	1163.1 AU	20.79%	Unknown *
1	5	0.56 Rf	20.5 AU	0.59 Rf	32.0 AU	8.00%	0.61 Rf	27.0 AU	1022.5 AU	18.28%	Unknown *

Table 39. HPTLC Peak Values for Track 2 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
2	1	0.02 Rf	0.5 AU	0.03 Rf	37.5 AU	3.65%	0.05 Rf	0.2 AU	217.8 AU	0.53%	Unknown *
2	2	0.08 Rf	0.3 AU	0.09 Rf	25.0 AU	2.44%	0.11 Rf	21.8 AU	408.5 AU	0.99%	Unknown *
2	3	0.11 Rf	22.4 AU	0.11 Rf	31.5 AU	3.06%	0.15 Rf	0.2 AU	577.5 AU	1.40%	Unknown *
2	4	0.19 Rf	4.8 AU	0.32 Rf	215.9 AU	21.01%	0.35 Rf	152.6 AU	13074.4 AU	31.60%	Unknown *
2	5	0.36 Rf	153.1 AU	0.41 Rf	439.9 AU	42.81%	0.45 Rf	19.7 AU	19502.4 AU	47.14%	Unknown *
2	6	0.47 Rf	26.1 AU	0.51 Rf	149.3 AU	14.53%	0.54 Rf	24.4 AU	3644.1 AU	8.81%	Unknown *
2	7	0.54 Rf	25.8 AU	0.57 Rf	114.5 AU	11.15%	0.62 Rf	1.8 AU	3410.7 AU	8.24%	Unknown *
2	8	0.64 Rf	3.0 AU	0.67 Rf	13.8 AU	1.35%	0.71 Rf	4.5 AU	539.8 AU	1.30%	Unknown *

Table 40. HPTLC Peak Values for Track 3 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	1	0.03 Rf	0.0 AU	0.04 Rf	23.1 AU	1.46%	0.06 Rf	1.0 AU	235.3 AU	0.34%	Unknown *
3	2	0.14 Rf	18.1 AU	0.17 Rf	85.5 AU	5.41%	0.21 Rf	0.2 AU	2336.9 AU	3.39%	Unknown *
3	3	0.21 Rf	0.5 AU	0.33 Rf	330.4 AU	20.88%	0.39 Rf	50.4 AU	20995.3 AU	30.48%	Unknown *
3	4	0.39 Rf	50.9 AU	0.42 Rf	181.7 AU	11.48%	0.47 Rf	47.4 AU	5670.6 AU	8.23%	Unknown *
3	5	0.48 Rf	48.9 AU	0.51 Rf	106.9 AU	6.76%	0.54 Rf	64.6 AU	3828.8 AU	5.56%	Unknown *

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	6	0.55 Rf	63.6 AU	0.59 Rf	103.3 AU	6.53%	0.62 Rf	85.8 AU	4891.9 AU	7.10%	Unknown *
3	7	0.63 Rf	85.8 AU	0.66 Rf	155.4 AU	9.82%	0.69 Rf	103.0 AU	5190.8 AU	7.54%	Unknown *
3	8	0.69 Rf	105.2 AU	0.71 Rf	115.5 AU	7.30%	0.73 Rf	103.6 AU	3517.2 AU	5.11%	Unknown *
3	9	0.73 Rf	103.7 AU	0.78 Rf	194.8 AU	12.31%	0.80 Rf	185.3 AU	8321.0 AU	12.08%	Unknown *
3	10	0.80 Rf	185.4 AU	0.82 Rf	191.9 AU	12.13%	0.89 Rf	90.0 AU	10620.7 AU	15.42%	Unknown *
3	11	0.89 Rf	89.3 AU	0.91 Rf	93.8 AU	5.93%	0.97 Rf	5.3 AU	3272.0 AU	4.75%	Unknown *

Table 41. HPTLC Peak Values for Track 4 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
4	1	0.30 Rf	14.6 AU	0.35 Rf	43.9 AU	7.46%	0.36 Rf	38.4 AU	1296.1 AU	5.50%	Unknown *
4	2	0.36 Rf	38.6 AU	0.38 Rf	57.9 AU	9.82%	0.41 Rf	41.1 AU	1717.6 AU	7.29%	Unknown *
4	3	0.42 Rf	42.9 AU	0.44 Rf	62.5 AU	10.60%	0.45 Rf	53.6 AU	1596.7 AU	6.77%	Unknown *
4	4	0.45 Rf	53.8 AU	0.47 Rf	76.5 AU	12.98%	0.50 Rf	56.4 AU	2324.7 AU	9.86%	Unknown *
4	5	0.54 Rf	47.6 AU	0.58 Rf	79.3 AU	13.47%	0.61 Rf	57.9 AU	3233.5 AU	13.72%	Unknown *
4	6	0.63 Rf	55.3 AU	0.70 Rf	92.0 AU	15.61%	0.74 Rf	54.5 AU	6164.2 AU	26.15%	Unknown *
4	7	0.74 Rf	54.5 AU	0.78 Rf	91.1 AU	15.47%	0.81 Rf	63.4 AU	3868.5 AU	16.41%	Unknown *
4	8	0.82 Rf	62.3 AU	0.84 Rf	65.4 AU	11.10%	0.90 Rf	19.4 AU	2886.4 AU	12.25%	Unknown *
4	9	0.90 Rf	19.1 AU	0.91 Rf	20.5 AU	3.49%	0.95 Rf	3.3 AU	480.4 AU	2.04%	Unknown *

Table 42. HPTLC Peak Values for Track 5 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
5	1	0.03 Rf	0.2 AU	0.05 Rf	11.2 AU	1.11%	0.06 Rf	0.4 AU	118.0 AU	0.28%	Unknown *
5	2	0.09 Rf	0.0 AU	0.13 Rf	76.8 AU	7.59%	0.19 Rf	0.9 AU	3051.0 AU	7.21%	Unknown *
5	3	0.21 Rf	0.7 AU	0.33 Rf	301.5 AU	29.82%	0.39 Rf	86.6 AU	18739.5 AU	44.31%	Unknown *
5	4	0.39 Rf	86.6 AU	0.43 Rf	433.9 AU	42.91%	0.47 Rf	3.8 AU	14286.6 AU	33.78%	Unknown *
5	5	0.47 Rf	3.9 AU	0.52 Rf	34.4 AU	3.40%	0.54 Rf	12.5 AU	1148.5 AU	2.72%	Unknown *
5	6	0.54 Rf	12.8 AU	0.59 Rf	103.5 AU	10.23%	0.64 Rf	5.8 AU	3358.8 AU	7.94%	Unknown *
5	7	0.75 Rf	5.2 AU	0.79 Rf	22.8 AU	2.26%	0.80 Rf	12.5 AU	433.0 AU	1.02%	Unknown *
5	8	0.81 Rf	16.6 AU	0.83 Rf	27.1 AU	2.68%	0.89 Rf	0.8 AU	1152.9 AU	2.73%	Unknown *

Table 43. HPTLC Peak Values for Track 6 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
6	1	0.27 Rf	9.5 AU	0.33 Rf	18.5 AU	4.91%	0.33 Rf	18.0 AU	650.6 AU	4.56%	Unknown *
6	2	0.41 Rf	27.0 AU	0.45 Rf	35.0 AU	9.28%	0.46 Rf	32.8 AU	1119.1 AU	7.84%	Unknown *
6	3	0.48 Rf	36.8 AU	0.53 Rf	51.4 AU	13.61%	0.54 Rf	49.1 AU	1954.5 AU	13.68%	Unknown *
6	4	0.55 Rf	49.4 AU	0.60 Rf	66.8 AU	17.69%	0.61 Rf	63.4 AU	2710.0 AU	18.97%	Unknown *
6	5	0.67 Rf	64.5 AU	0.70 Rf	74.3 AU	19.70%	0.70 Rf	73.4 AU	2040.7 AU	14.29%	Unknown *
6	6	0.77 Rf	50.5 AU	0.80 Rf	65.7 AU	17.42%	0.82 Rf	62.6 AU	2682.6 AU	18.78%	Unknown *
6	7	0.82 Rf	63.4 AU	0.84 Rf	65.6 AU	17.38%	0.92 Rf	9.6 AU	3125.0 AU	21.88%	Unknown *

Table 44. HPTLC Peak Values for Track 7 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
7	1	0.00 Rf	5.7 AU	0.02 Rf	201.3 AU	15.90%	0.05 Rf	0.1 AU	3251.3 AU	6.38%	Unknown *
7	2	0.06 Rf	0.3 AU	0.07 Rf	10.9 AU	0.86%	0.08 Rf	6.4 AU	129.3 AU	0.25%	Unknown *
7	3	0.09 Rf	7.1 AU	0.13 Rf	37.3 AU	2.94%	0.16 Rf	0.2 AU	1346.6 AU	2.64%	Unknown *
7	4	0.26 Rf	35.7 AU	0.38 Rf	332.0 AU	26.23%	0.43 Rf	81.7 AU	22668.0 AU	44.45%	Unknown *
7	5	0.43 Rf	83.1 AU	0.46 Rf	269.8 AU	21.32%	0.50 Rf	54.5 AU	8966.5 AU	17.58%	Unknown *
7	6	0.50 Rf	55.1 AU	0.53 Rf	80.3 AU	6.34%	0.55 Rf	60.6 AU	2331.2 AU	4.57%	Unknown *
7	7	0.55 Rf	60.7 AU	0.57 Rf	100.5 AU	7.94%	0.61 Rf	39.3 AU	3257.8 AU	6.39%	Unknown *
7	8	0.61 Rf	39.3 AU	0.64 Rf	61.4 AU	4.85%	0.68 Rf	45.6 AU	2744.1 AU	5.38%	Unknown *
7	9	0.71 Rf	44.1 AU	0.74 Rf	51.2 AU	4.05%	0.77 Rf	35.7 AU	2111.4 AU	4.14%	Unknown *
7	10	0.79 Rf	35.5 AU	0.82 Rf	48.8 AU	3.85%	0.84 Rf	44.7 AU	1535.7 AU	3.01%	Unknown *
7	11	0.84 Rf	45.1 AU	0.86 Rf	49.8 AU	3.93%	0.92 Rf	11.7 AU	2132.9 AU	4.18%	Unknown *
7	12	0.93 Rf	11.5 AU	0.94 Rf	22.5 AU	1.78%	0.99 Rf	0.1 AU	522.3 AU	1.02%	Unknown *

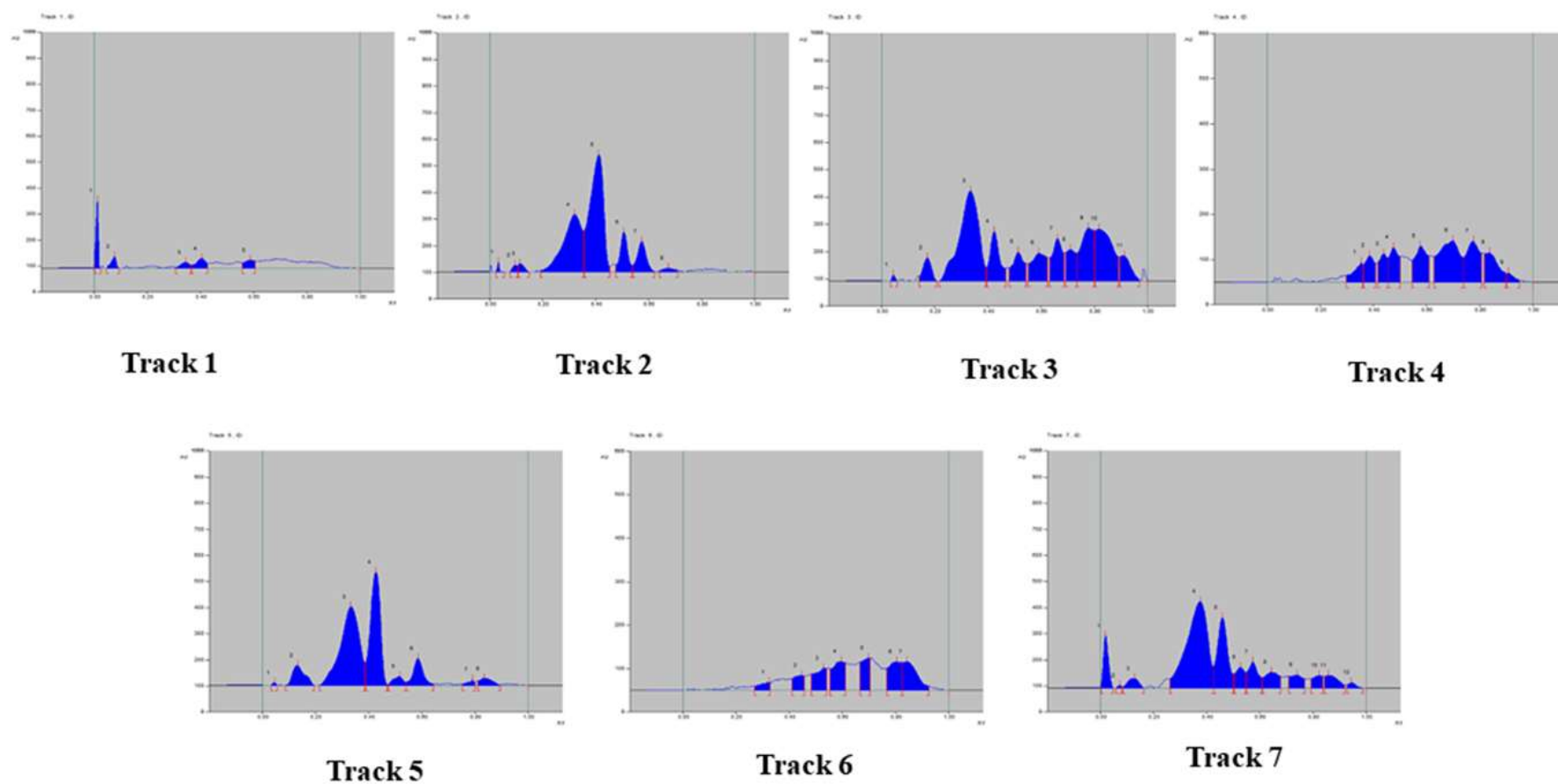


Fig. 61. Track-wise Densitograms of Formulation 1 (F1) at 254 nm (Before Derivatization).

HPTLC Peak Values and Densitograms for Formulation 1 (F1) at 366 nm (Before Derivatization).

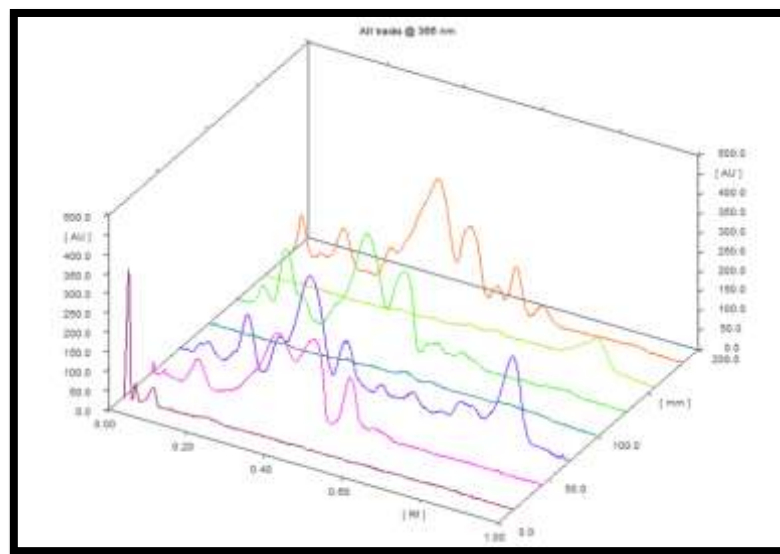


Fig. 62. 3D densitogram of all tracks at 254 nm (Before Derivatization).

Table 45. HPTLC Peak Values for Track 1 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.00 Rf	32.1 AU	0.01 Rf	331.2 AU	78.49%	0.02 Rf	6.3 AU	2424.7 AU	71.55%	Unknown *
1	2	0.03 Rf	14.6 AU	0.03 Rf	44.1 AU	10.46%	0.04 Rf	0.0 AU	253.0 AU	7.47%	Unknown *
1	3	0.05 Rf	0.4 AU	0.08 Rf	46.6 AU	11.05%	0.09 Rf	0.5 AU	711.0 AU	20.98%	Unknown *

Table 46. HPTLC Peak Values for Track 2 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
2	1	0.01 Rf	0.0 AU	0.03 Rf	16.5 AU	2.60%	0.05 Rf	0.0 AU	142.7 AU	0.47%	Unknown *
2	2	0.08 Rf	0.5 AU	0.12 Rf	68.8 AU	10.85%	0.15 Rf	0.3 AU	1899.8 AU	6.30%	Unknown *
2	3	0.18 Rf	4.5 AU	0.32 Rf	193.4 AU	30.50%	0.37 Rf	134.4 AU	13943.4 AU	46.26%	Unknown *
2	4	0.37 Rf	134.7 AU	0.42 Rf	203.6 AU	32.10%	0.45 Rf	0.0 AU	9645.6 AU	32.00%	Unknown *
2	5	0.46 Rf	0.1 AU	0.51 Rf	130.9 AU	20.64%	0.55 Rf	12.3 AU	3789.4 AU	12.57%	Unknown *
2	6	0.55 Rf	12.5 AU	0.57 Rf	21.1 AU	3.32%	0.63 Rf	0.1 AU	722.9 AU	2.40%	Unknown *

Table 47. HPTLC Peak Values for Track 3 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	1	0.03 Rf	0.5 AU	0.07 Rf	37.4 AU	3.26%	0.11 Rf	0.2 AU	1081.4 AU	2.17%	Unknown *
3	2	0.11 Rf	0.1 AU	0.17 Rf	134.8 AU	11.73%	0.21 Rf	0.4 AU	4007.1 AU	8.03%	Unknown *
3	3	0.21 Rf	0.2 AU	0.26 Rf	102.8 AU	8.95%	0.27 Rf	94.1 AU	2797.8 AU	5.61%	Unknown *
3	4	0.27 Rf	94.4 AU	0.33 Rf	282.0 AU	24.55%	0.39 Rf	43.7 AU	16300.4 AU	32.68%	Unknown *
3	5	0.39 Rf	43.9 AU	0.42 Rf	139.6 AU	12.15%	0.48 Rf	28.9 AU	5659.2 AU	11.35%	Unknown *
3	6	0.49 Rf	28.4 AU	0.51 Rf	53.4 AU	4.65%	0.54 Rf	31.1 AU	1490.1 AU	2.99%	Unknown *
3	7	0.56 Rf	32.0 AU	0.60 Rf	58.7 AU	5.11%	0.64 Rf	21.1 AU	2571.4 AU	5.16%	Unknown *
3	8	0.65 Rf	21.1 AU	0.67 Rf	31.6 AU	2.75%	0.69 Rf	28.1 AU	854.4 AU	1.71%	Unknown *
3	9	0.69 Rf	28.3 AU	0.72 Rf	70.3 AU	6.12%	0.78 Rf	44.3 AU	3847.7 AU	7.71%	Unknown *

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	10	0.78 Rf	44.4 AU	0.85 Rf	225.0 AU	19.59%	0.91 Rf	12.2 AU	10912.8 AU	21.88%	Unknown *
3	11	0.91 Rf	12.3 AU	0.91 Rf	13.2 AU	1.15%	0.95 Rf	3.6 AU	352.4 AU	0.71%	Unknown *

Table 48. HPTLC Peak Values for Track 4 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
4	1	0.47 Rf	11.6 AU	0.51 Rf	23.8 AU	100.00%	0.54 Rf	9.6 AU	797.2 AU	100.00%	Unknown *

Table 49. HPTLC Peak Values for Track 5 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
5	1	0.03 Rf	0.4 AU	0.07 Rf	56.0 AU	7.30%	0.09 Rf	22.8 AU	1337.8 AU	3.73%	Unknown *
5	2	0.09 Rf	23.3 AU	0.13 Rf	167.6 AU	21.86%	0.20 Rf	0.2 AU	6165.0 AU	17.18%	Unknown *
5	3	0.22 Rf	0.2 AU	0.33 Rf	265.6 AU	34.64%	0.39 Rf	84.6 AU	17159.1 AU	47.81%	Unknown *
5	4	0.39 Rf	84.7 AU	0.43 Rf	198.1 AU	25.85%	0.47 Rf	0.1 AU	8513.9 AU	23.72%	Unknown *
5	5	0.47 Rf	0.3 AU	0.52 Rf	36.2 AU	4.72%	0.54 Rf	16.1 AU	1245.2 AU	3.47%	Unknown *
5	6	0.56 Rf	16.7 AU	0.58 Rf	31.8 AU	4.14%	0.64 Rf	0.1 AU	1201.9 AU	3.35%	Unknown *
5	7	0.84 Rf	8.3 AU	0.85 Rf	11.4 AU	1.48%	0.89 Rf	0.1 AU	268.1 AU	0.75%	Unknown *

Table 50. HPTLC Peak Values for Track 6 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
6	1	0.49 Rf	13.2 AU	0.53 Rf	26.1 AU	23.51%	0.55 Rf	20.5 AU	1007.3 AU	14.48%	Unknown *
6	2	0.75 Rf	22.7 AU	0.85 Rf	85.1 AU	76.49%	0.92 Rf	8.2 AU	5948.2 AU	85.52%	Unknown *

Table 51. HPTLC Peak Values for Track 7 (Formulation 1, F1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
7	1	0.01 Rf	24.8 AU	0.02 Rf	97.4 AU	9.72%	0.05 Rf	0.1 AU	1586.2 AU	3.79%	Unknown *
7	2	0.06 Rf	0.4 AU	0.07 Rf	15.3 AU	1.53%	0.08 Rf	11.0 AU	186.4 AU	0.45%	Unknown *
7	3	0.09 Rf	10.8 AU	0.13 Rf	95.0 AU	9.49%	0.17 Rf	0.2 AU	2824.6 AU	6.74%	Unknown *
7	4	0.21 Rf	0.1 AU	0.25 Rf	79.7 AU	7.95%	0.26 Rf	72.2 AU	1995.8 AU	4.77%	Unknown *
7	5	0.27 Rf	72.3 AU	0.38 Rf	291.9 AU	29.13%	0.42 Rf	92.3 AU	21510.0 AU	51.36%	Unknown *
7	6	0.42 Rf	92.3 AU	0.45 Rf	194.7 AU	19.43%	0.50 Rf	25.2 AU	8055.5 AU	19.24%	Unknown *
7	7	0.50 Rf	25.3 AU	0.53 Rf	59.7 AU	5.95%	0.55 Rf	31.7 AU	1405.9 AU	3.36%	Unknown *
7	8	0.55 Rf	31.9 AU	0.57 Rf	125.0 AU	12.48%	0.61 Rf	7.3 AU	3049.8 AU	7.28%	Unknown *
7	9	0.61 Rf	7.5 AU	0.64 Rf	43.4 AU	4.33%	0.68 Rf	2.3 AU	1264.0 AU	3.02%	Unknown *

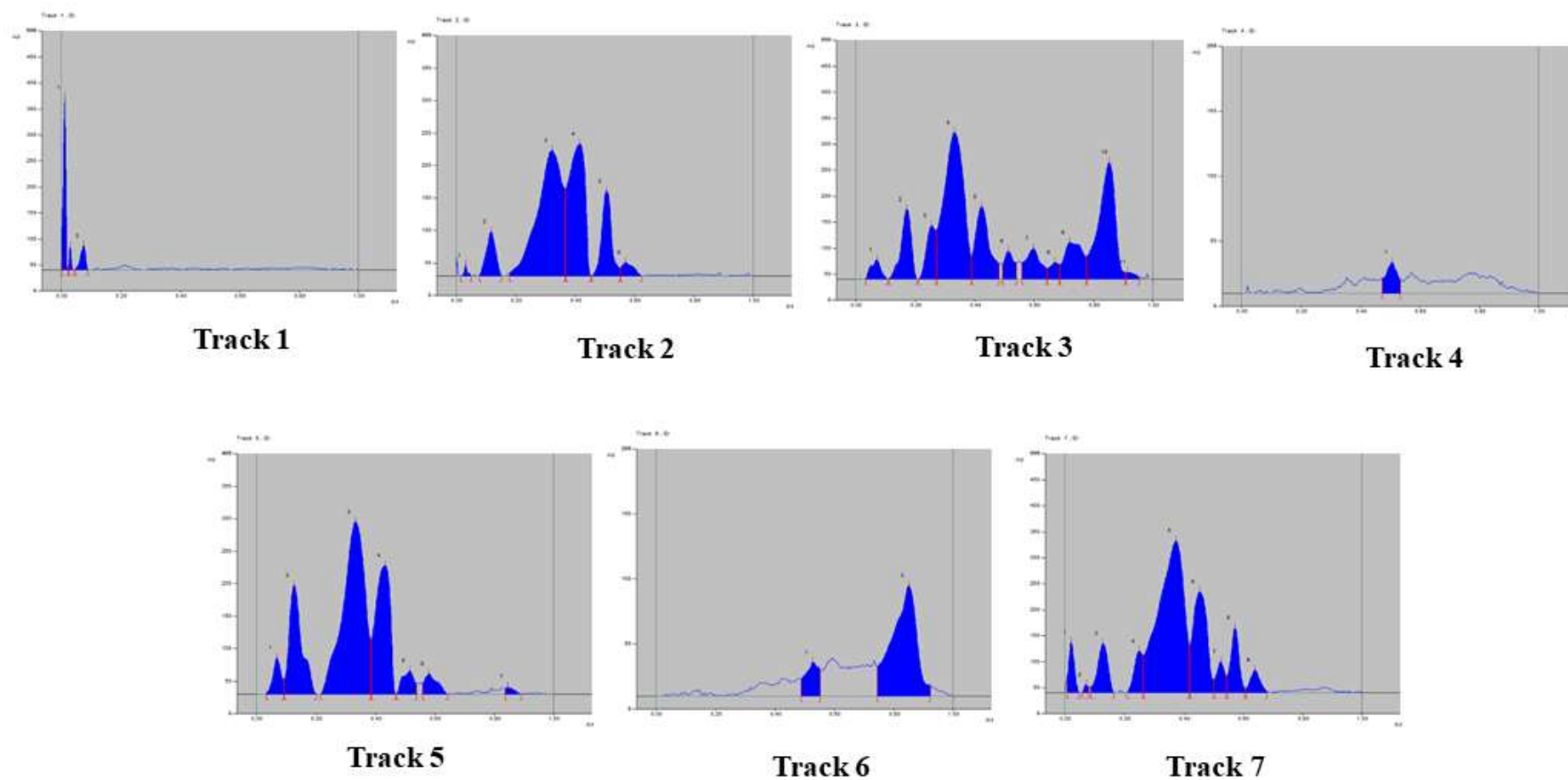


Fig. 63. Track-wise Densitograms of Formulation 1 (F1) at 366 nm (Before Derivatization).

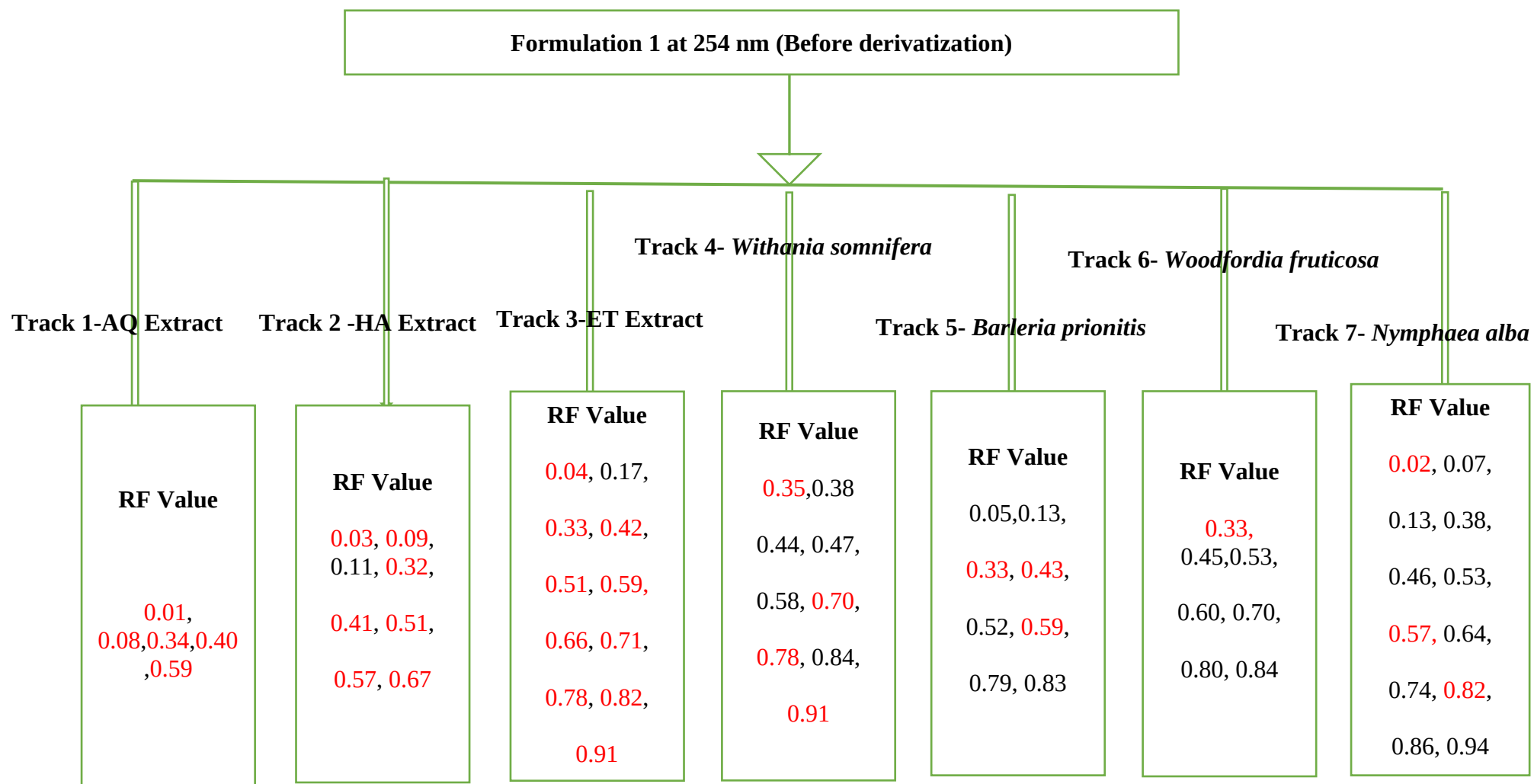


Fig. 64. Schematic Representation of Rf Values of All Tracks for Formulation 1 (F1) at 254 nm (Before Derivatization).

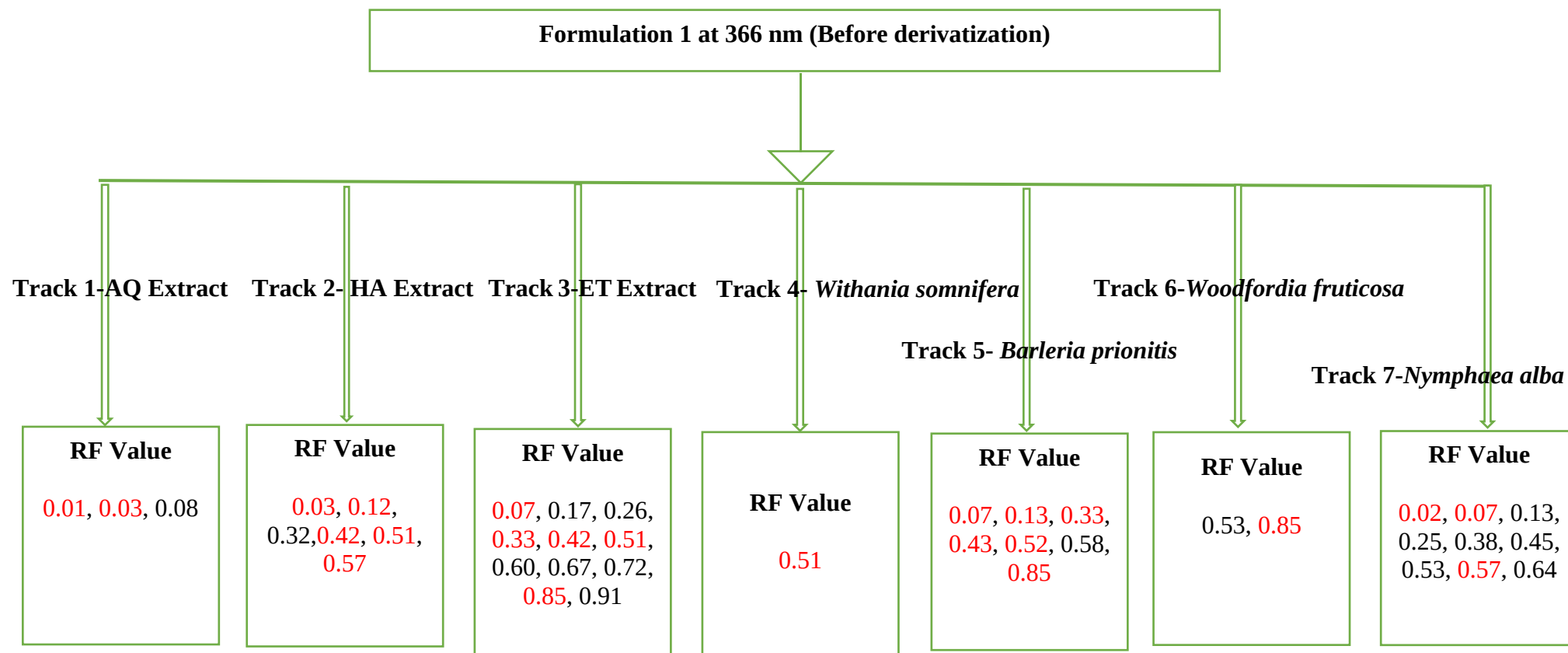


Fig. 65. Schematic Representation of Rf Values of All Tracks for Formulation 1 (F1) at 366 nm (Before Derivatization).

5.6.3.3 HPTLC Fingerprints for Formulation 2 (F2)

For Formulation 2 (F2), HPTLC fingerprinting followed a similar approach, with the aqueous (AQ), hydroalcoholic (HA), and ethanolic (ET) extracts applied to a single plate, labelled as tracks 1, 2, and 3, respectively. The individual ingredients of Formulation 2 were applied as tracks 4 (Root of *Withania somnifera*), 5 (Seeds of *Daucus carota*), 6 (Seeds of *Mucuna prurita*), 7 (Seeds of *Hygrophila auriculata*), and 8 (Root of *Asparagus racemosus*). Several solvent systems like Chloroform, n-hexane, ethyl acetate, methanol, formic acid, toluene, glacial acetic acid water, etc., in different ratios were tried and tested for development of HPTLC fingerprint. It was observed that mobile phase consisting of Toluene: Ethyl acetate: Formic acid (10:8:2 v/v/v) was found to be most effective for Formulation 2 (Figures 52 and 53).

The HPTLC plates, visualized under different wavelengths, revealed distinct patterns of bands, with the ethanolic extract showing the highest number of bands, followed by the aqueous and hydroalcoholic extracts in Formulation 2 (Fig. 62). Derivatization with 10% methanolic sulphuric acid further enhanced the detection of specific phytoconstituents (Fig. 63). Densitometric scanning at 254 nm and 366 nm revealed a large number of bands across all samples, with distinct visual differences among the extracts and formulations. Additionally, track-wise Densitograms and corresponding R_f values were generated for each of the eight tracks (Tables 52 - 67 and Figures 69-71), providing a detailed fingerprint profile that highlights both the individual ingredient contributions and the composite phytoconstituents pattern of the entire formulation.

Fig. 66. HPTLC Fingerprinting of Formulation 2 (F2) Before Derivatization

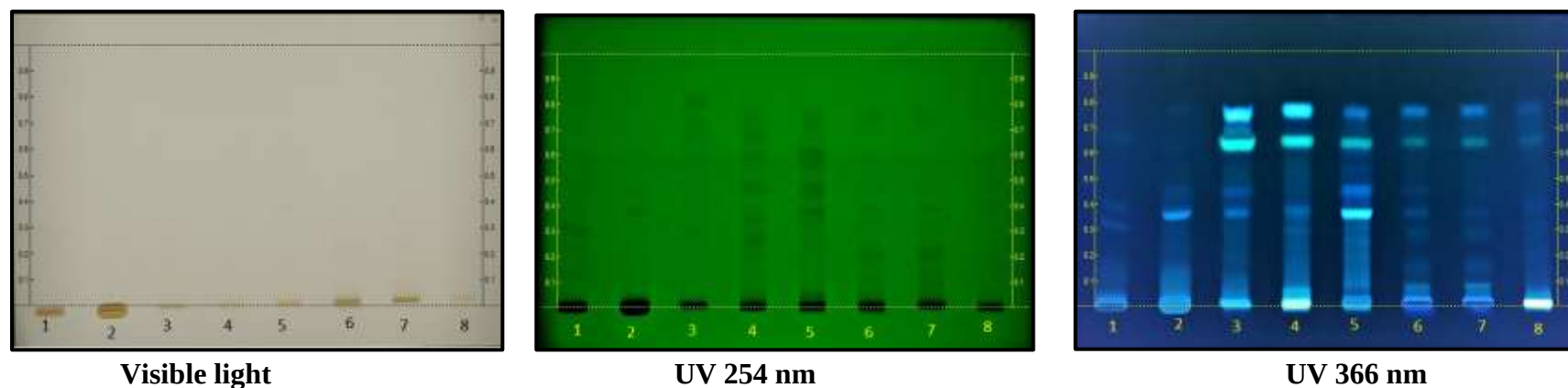
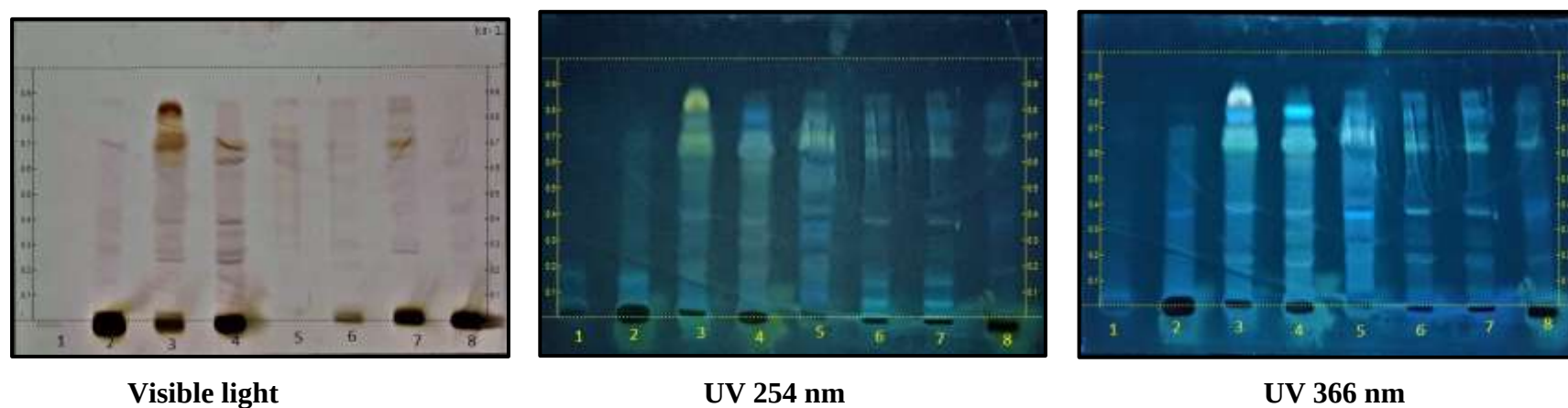


Fig. 67. HPTLC Fingerprinting of Formulation 2 (F2) After Derivatization



Track 1. Formulation 1 (Aqueous extract), **Track 2.** Formulation 1 (Hydroalcoholic extract), **Track 3.** Formulation 1 (Ethanollic extract), **Track 4.** Root of *Withania somnifera*, **Track 5.** Seed of *Daucus carota*, **Track 6.** Seed of *Mucuna pruriens*, **Track 7.** Seed of *Hygrophila auriculata*, **Track 8.** Root of *Asparagus racemosus*

HPTLC Peak Values and Densitograms for Formulation 2 (F2) at 254 nm (Before Derivatization).

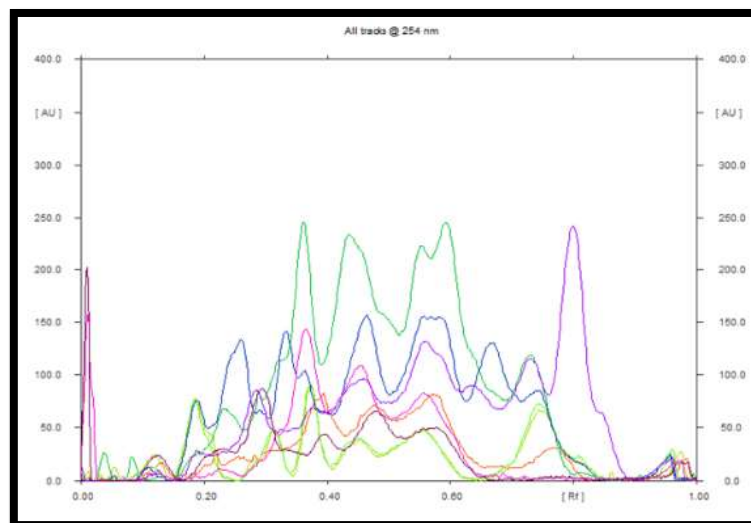


Fig. 68. Track-wise densitograms of Formulation 1 (F1) at 366 nm (Before Derivatization).

Table 52. HPTLC Peak Values for Track 1 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.00 Rf	22.8 AU	0.01 Rf	203.2 AU	39.02%	0.02 Rf	0.0 AU	1416.6 AU	10.43%	Unknown *
1	2	0.09 Rf	0.0 AU	0.13 Rf	23.9 AU	4.59%	0.16 Rf	0.4 AU	693.8 AU	5.11%	Unknown *

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	3	0.17 Rf	1.6 AU	0.23 Rf	30.1 AU	5.77%	0.24 Rf	27.9 AU	1109.3 AU	8.17%	Unknown *
1	4	0.25 Rf	26.3 AU	0.29 Rf	88.1 AU	16.91%	0.34 Rf	28.4 AU	3379.8 AU	24.90%	Unknown *
1	5	0.37 Rf	23.5 AU	0.40 Rf	44.2 AU	8.48%	0.42 Rf	29.0 AU	1542.5 AU	11.36%	Unknown *
1	6	0.43 Rf	28.3 AU	0.48 Rf	66.3 AU	12.72%	0.51 Rf	41.0 AU	3347.9 AU	24.66%	Unknown *
1	7	0.57 Rf	49.4 AU	0.58 Rf	50.3 AU	9.65%	0.65 Rf	3.5 AU	1841.9 AU	13.57%	Unknown *
1	8	0.95 Rf	1.7 AU	0.98 Rf	14.9 AU	2.86%	0.98 Rf	13.7 AU	243.9 AU	1.80%	Unknown *

Table 53. HPTLC Peak Values for Track 2 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
2	1	0.01 Rf	83.9 AU	0.01 Rf	157.9 AU	30.75%	0.03 Rf	7.5 AU	1526.8 AU	7.78%	Unknown *
2	2	0.30 Rf	18.7 AU	0.36 Rf	143.8 AU	28.00%	0.40 Rf	63.6 AU	6152.9 AU	31.37%	Unknown *
2	3	0.40 Rf	63.8 AU	0.45 Rf	109.4 AU	21.31%	0.50 Rf	58.4 AU	6125.1 AU	31.23%	Unknown *
2	4	0.51 Rf	59.0 AU	0.56 Rf	83.2 AU	16.21%	0.66 Rf	4.0 AU	5383.8 AU	27.45%	Unknown *
2	5	0.93 Rf	2.4 AU	0.97 Rf	19.1 AU	3.72%	0.99 Rf	0.1 AU	425.9 AU	2.17%	Unknown *

Table 54. HPTLC Peak Values for Track 3 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	1	0.16 Rf	1.1 AU	0.19 Rf	27.8 AU	3.15%	0.21 Rf	23.3 AU	670.5 AU	1.64%	Unknown *
3	2	0.24 Rf	26.3 AU	0.29 Rf	85.6 AU	9.71%	0.32 Rf	45.7 AU	3485.3 AU	8.53%	Unknown *
3	3	0.35 Rf	49.1 AU	0.38 Rf	69.2 AU	7.85%	0.40 Rf	64.3 AU	2342.8 AU	5.73%	Unknown *
3	4	0.40 Rf	64.5 AU	0.46 Rf	96.2 AU	10.91%	0.49 Rf	73.6 AU	5559.8 AU	13.61%	Unknown *
3	5	0.50 Rf	73.3 AU	0.56 Rf	132.0 AU	14.98%	0.62 Rf	84.1 AU	9007.7 AU	22.05%	Unknown *
3	6	0.62 Rf	84.2 AU	0.64 Rf	90.1 AU	10.22%	0.68 Rf	67.7 AU	3594.9 AU	8.80%	Unknown *
3	7	0.68 Rf	67.7 AU	0.73 Rf	115.8 AU	13.14%	0.76 Rf	81.4 AU	5459.2 AU	13.36%	Unknown *
3	8	0.76 Rf	81.5 AU	0.80 Rf	242.0 AU	27.46%	0.89 Rf	2.1 AU	10274.1 AU	25.15%	Unknown *
3	9	0.93 Rf	7.2 AU	0.96 Rf	22.6 AU	2.57%	0.97 Rf	1.6 AU	458.1 AU	1.12%	Unknown *

Table 55. HPTLC Peak Values for Track 4 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
4	1	0.09 Rf	0.4 AU	0.11 Rf	12.5 AU	1.06%	0.14 Rf	0.0 AU	239.4 AU	0.56%	Unknown *
4	2	0.15 Rf	1.9 AU	0.19 Rf	75.7 AU	6.45%	0.21 Rf	49.2 AU	1879.1 AU	4.36%	Unknown *
4	3	0.21 Rf	49.6 AU	0.26 Rf	133.6 AU	11.39%	0.28 Rf	63.5 AU	5365.4 AU	12.46%	Unknown *
4	4	0.30 Rf	59.3 AU	0.33 Rf	141.2 AU	12.03%	0.35 Rf	97.7 AU	3989.4 AU	9.27%	Unknown *
4	5	0.35 Rf	98.2 AU	0.36 Rf	103.9 AU	8.85%	0.39 Rf	64.0 AU	2457.1 AU	5.71%	Unknown *

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
4	6	0.41 Rf	62.4 AU	0.46 Rf	156.7 AU	13.35%	0.50 Rf	80.9 AU	7802.1 AU	18.12%	Unknown *
4	7	0.50 Rf	81.1 AU	0.56 Rf	155.6 AU	13.26%	0.56 Rf	153.0 AU	5502.7 AU	12.78%	Unknown *
4	8	0.58 Rf	152.8 AU	0.58 Rf	155.0 AU	13.21%	0.63 Rf	75.5 AU	4758.6 AU	11.05%	Unknown *
4	9	0.63 Rf	75.5 AU	0.67 Rf	130.4 AU	11.11%	0.71 Rf	74.9 AU	6715.8 AU	15.60%	Unknown *
4	10	0.72 Rf	75.0 AU	0.74 Rf	85.1 AU	7.25%	0.84 Rf	1.4 AU	3922.9 AU	9.11%	Unknown *
4	11	0.92 Rf	3.2 AU	0.96 Rf	24.0 AU	2.04%	0.97 Rf	0.0 AU	421.0 AU	0.98%	Unknown *

Table 56. HPTLC Peak Values for Track 5 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
5	1	0.02 Rf	0.3 AU	0.04 Rf	26.7 AU	2.02%	0.05 Rf	0.6 AU	301.6 AU	0.53%	Unknown *
5	2	0.07 Rf	1.2 AU	0.08 Rf	22.5 AU	1.70%	0.10 Rf	0.2 AU	221.2 AU	0.39%	Unknown *
5	3	0.15 Rf	0.1 AU	0.19 Rf	28.8 AU	2.18%	0.19 Rf	24.7 AU	515.9 AU	0.90%	Unknown *
5	4	0.20 Rf	25.6 AU	0.23 Rf	67.7 AU	5.12%	0.26 Rf	47.8 AU	2596.2 AU	4.53%	Unknown *
5	5	0.27 Rf	48.9 AU	0.29 Rf	83.9 AU	6.34%	0.30 Rf	77.0 AU	1389.3 AU	2.43%	Unknown *
5	6	0.32 Rf	113.3 AU	0.36 Rf	245.6 AU	18.57%	0.39 Rf	109.7 AU	8238.2 AU	14.39%	Unknown *
5	7	0.39 Rf	109.7 AU	0.43 Rf	234.0 AU	17.70%	0.52 Rf	137.6 AU	16938.3 AU	29.58%	Unknown *
5	8	0.52 Rf	138.0 AU	0.55 Rf	223.4 AU	16.90%	0.57 Rf	211.7 AU	7371.2 AU	12.87%	Unknown *
5	9	0.57 Rf	211.9 AU	0.59 Rf	245.4 AU	18.56%	0.69 Rf	76.7 AU	13244.4 AU	23.13%	Unknown *
5	10	0.69 Rf	76.7 AU	0.73 Rf	118.5 AU	8.97%	0.80 Rf	5.4 AU	5961.9 AU	10.41%	Unknown *
5	11	0.92 Rf	2.3 AU	0.95 Rf	25.5 AU	1.93%	0.99 Rf	0.0 AU	481.6 AU	0.84%	Unknown *

Table 57. HPTLC Peak Values for Track 6 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
6	1	0.07 Rf	0.0 AU	0.11 Rf	13.3 AU	3.03%	0.14 Rf	0.2 AU	364.3 AU	2.43%	Unknown *
6	2	0.14 Rf	0.2 AU	0.18 Rf	76.5 AU	17.38%	0.23 Rf	2.3 AU	2209.9 AU	14.76%	Unknown *
6	3	0.26 Rf	4.3 AU	0.31 Rf	47.0 AU	10.66%	0.34 Rf	7.7 AU	1409.1 AU	9.41%	Unknown *
6	4	0.34 Rf	7.8 AU	0.37 Rf	90.2 AU	20.47%	0.40 Rf	13.3 AU	2205.0 AU	14.73%	Unknown *
6	5	0.41 Rf	15.8 AU	0.45 Rf	39.8 AU	9.02%	0.48 Rf	24.9 AU	1745.6 AU	11.66%	Unknown *
6	6	0.52 Rf	33.3 AU	0.56 Rf	48.2 AU	10.93%	0.62 Rf	2.7 AU	2533.0 AU	16.92%	Unknown *
6	7	0.67 Rf	6.5 AU	0.74 Rf	72.9 AU	16.55%	0.80 Rf	21.9 AU	3624.8 AU	24.22%	Unknown *
6	8	0.80 Rf	20.7 AU	0.81 Rf	23.3 AU	5.30%	0.84 Rf	3.0 AU	438.8 AU	2.93%	Unknown *
6	9	0.93 Rf	3.0 AU	0.96 Rf	29.3 AU	6.66%	0.98 Rf	0.3 AU	438.5 AU	2.93%	Unknown *

Table 58. HPTLC Peak Values for Track 7 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
7	1	0.04 Rf	1.7 AU	0.05 Rf	12.1 AU	2.67%	0.07 Rf	0.5 AU	117.1 AU	0.78%	Unknown *
7	2	0.08 Rf	0.6 AU	0.12 Rf	24.5 AU	5.41%	0.14 Rf	2.8 AU	615.4 AU	4.08%	Unknown *
7	3	0.15 Rf	0.1 AU	0.19 Rf	78.3 AU	17.26%	0.23 Rf	2.9 AU	2344.2 AU	15.54%	Unknown *

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
7	4	0.26 Rf	0.0 AU	0.28 Rf	19.8 AU	4.36%	0.29 Rf	17.1 AU	262.6 AU	1.74%	Unknown *
7	5	0.29 Rf	18.1 AU	0.32 Rf	48.0 AU	10.58%	0.34 Rf	4.3 AU	1162.7 AU	7.71%	Unknown *
7	6	0.34 Rf	4.9 AU	0.37 Rf	90.9 AU	20.04%	0.41 Rf	10.7 AU	2080.5 AU	13.79%	Unknown *
7	7	0.41 Rf	10.9 AU	0.45 Rf	38.8 AU	8.56%	0.48 Rf	23.0 AU	1556.3 AU	10.32%	Unknown *
7	8	0.49 Rf	23.6 AU	0.56 Rf	47.2 AU	10.41%	0.63 Rf	1.2 AU	3058.8 AU	20.28%	Unknown *
7	9	0.69 Rf	8.2 AU	0.74 Rf	66.5 AU	14.65%	0.81 Rf	15.4 AU	3463.8 AU	22.97%	Unknown *
7	10	0.94 Rf	2.7 AU	0.97 Rf	27.6 AU	6.08%	0.99 Rf	1.6 AU	420.8 AU	2.79%	Unknown *

Table 59. HPTLC Peak Values for Track 8 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
8	1	0.09 Rf	0.6 AU	0.13 Rf	17.1 AU	5.10%	0.15 Rf	1.5 AU	418.9 AU	2.83%	Unknown *
8	2	0.19 Rf	5.8 AU	0.22 Rf	29.4 AU	8.78%	0.23 Rf	14.9 AU	534.4 AU	3.62%	Unknown *
8	3	0.34 Rf	30.6 AU	0.39 Rf	83.4 AU	24.89%	0.43 Rf	42.1 AU	3855.2 AU	26.09%	Unknown *
8	4	0.44 Rf	47.5 AU	0.48 Rf	71.3 AU	21.28%	0.51 Rf	57.3 AU	3073.1 AU	20.80%	Unknown *
8	5	0.52 Rf	58.3 AU	0.57 Rf	81.7 AU	24.40%	0.64 Rf	17.0 AU	5172.1 AU	35.00%	Unknown *
8	6	0.73 Rf	17.0 AU	0.77 Rf	31.5 AU	9.41%	0.83 Rf	0.2 AU	1360.3 AU	9.21%	Unknown *
8	7	0.95 Rf	4.0 AU	0.98 Rf	20.5 AU	6.13%	0.99 Rf	0.4 AU	363.0 AU	2.46%	Unknown *

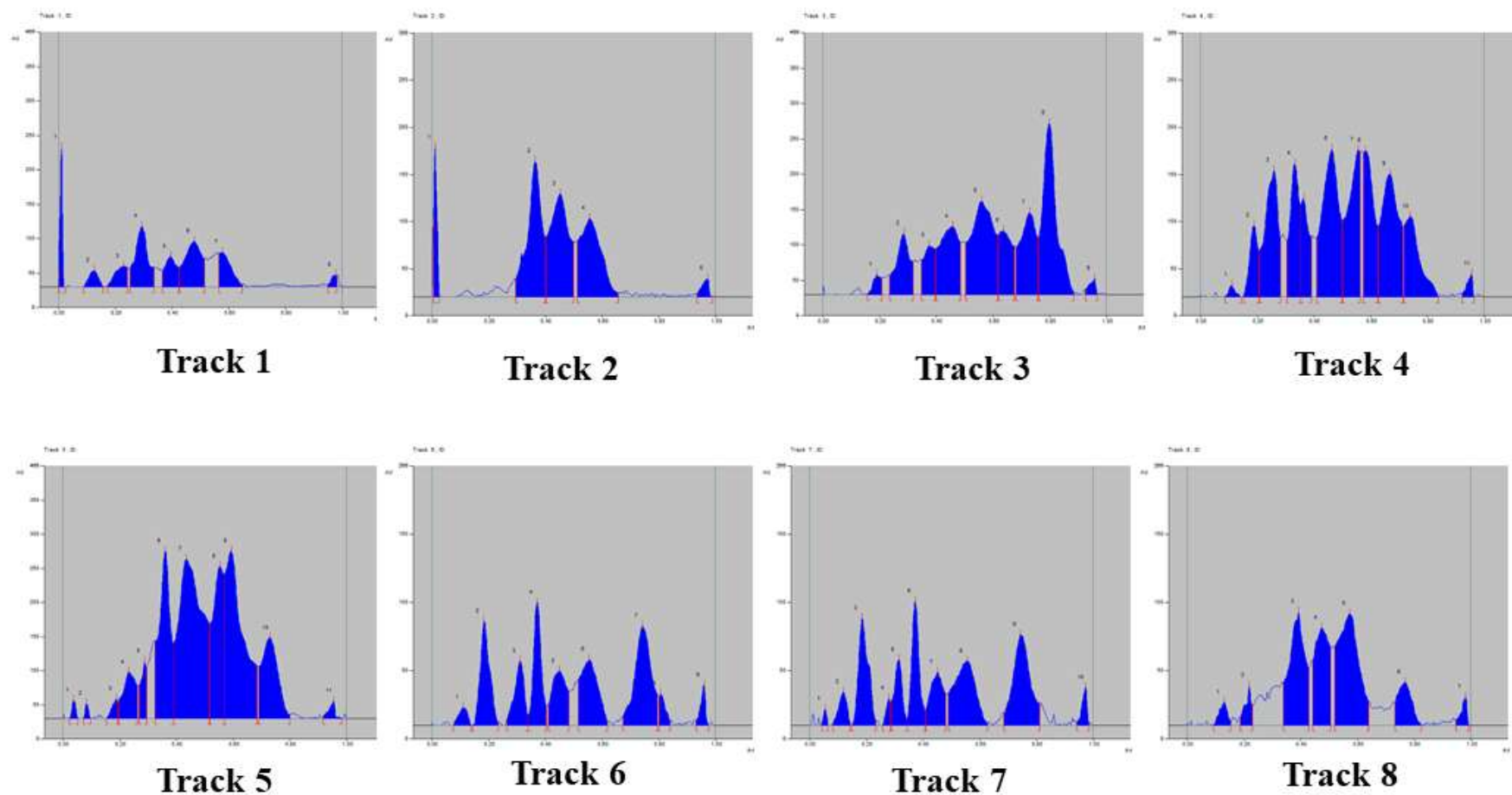


Fig. 69. Track-wise Densitograms of Formulation 2 (F2) at 254 nm (Before Derivatization).

HPTLC Peak Values and Densitograms for Formulation 2 (F2) at 366 nm (Before Derivatization)

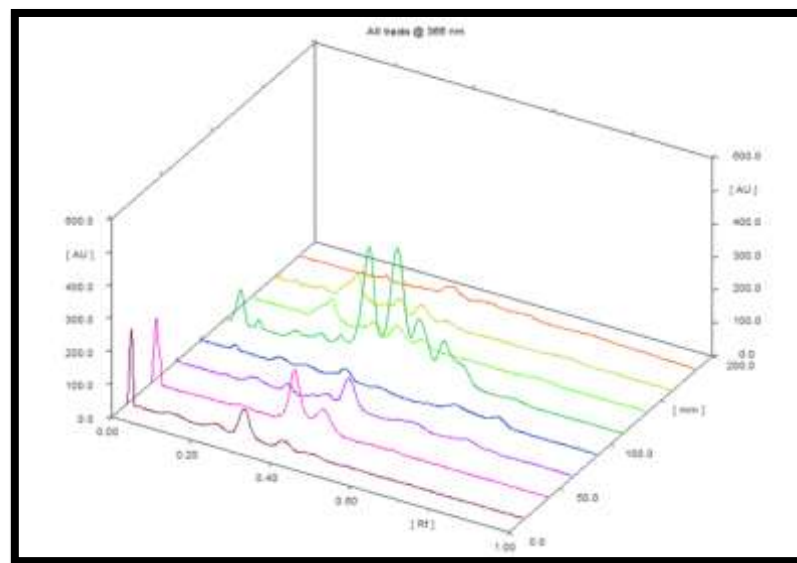


Fig. 70. 3D Densitogram of all tracks at 366 nm (Before Derivatization).

Table 60. HPTLC Peak Values for Track 1 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.00 Rf	19.4 AU	0.01 Rf	229.4 AU	60.50%	0.03 Rf	1.1 AU	1633.1 AU	28.42%	Unknown *
1	2	0.09 Rf	1.7 AU	0.11 Rf	10.5 AU	2.77%	0.16 Rf	1.4 AU	379.3 AU	6.60%	Unknown *

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	3	0.19 Rf	2.4 AU	0.23 Rf	17.8 AU	4.69%	0.25 Rf	3.1 AU	518.1 AU	9.02%	Unknown *
1	4	0.25 Rf	3.3 AU	0.30 Rf	85.7 AU	22.61%	0.35 Rf	0.3 AU	2203.9 AU	38.36%	Unknown *
1	5	0.36 Rf	0.1 AU	0.39 Rf	25.4 AU	6.69%	0.43 Rf	3.4 AU	679.0 AU	11.82%	Unknown *
1	6	0.44 Rf	3.6 AU	0.46 Rf	10.4 AU	2.73%	0.50 Rf	1.4 AU	332.3 AU	5.78%	Unknown *

Table 61. HPTLC Peak Values for Track 2 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
2	1	0.01 Rf	105.5 AU	0.01 Rf	198.5 AU	46.44%	0.03 Rf	1.6 AU	1959.6 AU	22.28%	Unknown*
2	2	0.32 Rf	11.2 AU	0.36 Rf	161.6 AU	37.79%	0.40 Rf	30.7 AU	4470.2 AU	50.81%	Unknown *
2	3	0.40 Rf	30.9 AU	0.43 Rf	67.4 AU	15.77%	0.49 Rf	1.5 AU	2367.3 AU	26.91%	Unknown *

Table 62. HPTLC Peak Values for Track 3 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	1	0.16 Rf	1.0 AU	0.20 Rf	21.0 AU	7.94%	0.24 Rf	6.9 AU	755.2 AU	9.54%	Unknown *
3	2	0.26 Rf	5.6 AU	0.28 Rf	29.6 AU	11.22%	0.31 Rf	4.2 AU	638.3 AU	8.07%	Unknown *

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
3	3	0.32 Rf	5.8 AU	0.35 Rf	19.3 AU	7.29%	0.35 Rf	18.6 AU	373.9 AU	4.73%	Unknown *
3	4	0.35 Rf	18.7 AU	0.38 Rf	33.3 AU	12.59%	0.40 Rf	21.1 AU	946.8 AU	11.97%	Unknown *
3	5	0.40 Rf	21.3 AU	0.44 Rf	99.4 AU	37.62%	0.48 Rf	9.8 AU	2996.6 AU	37.88%	Unknown *
3	6	0.53 Rf	6.9 AU	0.57 Rf	19.1 AU	7.23%	0.57 Rf	18.0 AU	505.1 AU	6.38%	Unknown *
3	7	0.57 Rf	18.0 AU	0.59 Rf	23.2 AU	8.78%	0.65 Rf	6.1 AU	969.8 AU	12.26%	Unknown *
3	8	0.70 Rf	10.6 AU	0.74 Rf	19.4 AU	7.34%	0.77 Rf	4.3 AU	725.9 AU	9.17%	Unknown *

Table 63. HPTLC Peak Values for Track 4 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
4	1	0.07 Rf	0.3 AU	0.09 Rf	15.1 AU	9.64%	0.11 Rf	1.0 AU	185.2 AU	3.48%	Unknown *
4	2	0.17 Rf	0.1 AU	0.20 Rf	17.1 AU	10.86%	0.23 Rf	6.6 AU	516.8 AU	9.71%	Unknown *
4	3	0.24 Rf	6.6 AU	0.26 Rf	17.2 AU	10.96%	0.30 Rf	8.2 AU	606.4 AU	11.39%	Unknown *
4	4	0.32 Rf	13.3 AU	0.36 Rf	39.9 AU	25.45%	0.39 Rf	7.3 AU	1224.7 AU	23.01%	Unknown *
4	5	0.42 Rf	6.4 AU	0.45 Rf	12.6 AU	8.00%	0.50 Rf	1.9 AU	470.9 AU	8.85%	Unknown *
4	6	0.61 Rf	10.9 AU	0.64 Rf	27.5 AU	17.52%	0.70 Rf	15.3 AU	1337.8 AU	25.13%	Unknown *
4	7	0.71 Rf	13.4 AU	0.75 Rf	27.6 AU	17.56%	0.79 Rf	2.4 AU	980.6 AU	18.42%	Unknown *

Table 64. HPTLC Peak Values for Track 5 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
5	1	0.01 Rf	1.4 AU	0.04 Rf	100.7 AU	8.03%	0.06 Rf	1.5 AU	1576.3 AU	4.00%	Unknown *
5	2	0.07 Rf	0.0 AU	0.08 Rf	24.8 AU	1.98%	0.10 Rf	0.1 AU	248.2 AU	0.63%	Unknown *
5	3	0.15 Rf	0.8 AU	0.18 Rf	26.3 AU	2.10%	0.20 Rf	17.8 AU	616.3 AU	1.56%	Unknown *
5	4	0.20 Rf	18.0 AU	0.24 Rf	38.3 AU	3.05%	0.26 Rf	22.6 AU	1354.4 AU	3.44%	Unknown *
5	5	0.26 Rf	22.7 AU	0.29 Rf	53.4 AU	4.26%	0.31 Rf	36.7 AU	1258.8 AU	3.19%	Unknown *
5	6	0.31 Rf	36.6 AU	0.36 Rf	344.4 AU	27.46%	0.39 Rf	81.3 AU	10190.7 AU	25.85%	Unknown *
5	7	0.39 Rf	81.5 AU	0.43 Rf	364.6 AU	29.07%	0.47 Rf	104.0 AU	12290.5 AU	31.18%	Unknown *
5	8	0.47 Rf	105.0 AU	0.49 Rf	167.0 AU	13.32%	0.53 Rf	57.6 AU	5643.6 AU	14.32%	Unknown *
5	9	0.53 Rf	58.2 AU	0.55 Rf	124.2 AU	9.91%	0.64 Rf	9.8 AU	5759.7 AU	14.61%	Unknown *
5	10	0.68 Rf	5.7 AU	0.73 Rf	10.4 AU	0.83%	0.77 Rf	2.4 AU	477.8 AU	1.21%	Unknown *

Table 65. HPTLC Peak Values for Track 6 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
6	1	0.14 Rf	0.2 AU	0.21 Rf	67.9 AU	38.02%	0.25 Rf	0.0 AU	2187.6 AU	45.34%	Unknown *
6	2	0.26 Rf	0.7 AU	0.28 Rf	10.5 AU	5.86%	0.28 Rf	9.0 AU	146.5 AU	3.04%	Unknown *
6	3	0.29 Rf	9.1 AU	0.31 Rf	33.8 AU	18.92%	0.34 Rf	0.0 AU	788.3 AU	16.34%	Unknown *
6	4	0.34 Rf	0.3 AU	0.37 Rf	43.2 AU	24.21%	0.40 Rf	5.9 AU	1097.3 AU	22.74%	Unknown *
6	5	0.41 Rf	7.5 AU	0.44 Rf	23.2 AU	13.00%	0.47 Rf	0.0 AU	604.8 AU	12.54%	Unknown *

Table 66. HPTLC Peak Values for Track 7 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
7	1	0.01 Rf	0.2 AU	0.01 Rf	14.0 AU	6.39%	0.03 Rf	0.0 AU	71.1 AU	1.31%	Unknown *
7	2	0.04 Rf	0.1 AU	0.05 Rf	11.5 AU	5.23%	0.07 Rf	0.1 AU	103.3 AU	1.91%	Unknown *
7	3	0.15 Rf	0.1 AU	0.21 Rf	83.1 AU	37.84%	0.25 Rf	0.2 AU	2640.3 AU	48.76%	Unknown *
7	4	0.25 Rf	0.2 AU	0.28 Rf	14.8 AU	6.75%	0.29 Rf	10.8 AU	220.2 AU	4.07%	Unknown *
7	5	0.29 Rf	11.0 AU	0.32 Rf	40.2 AU	18.29%	0.34 Rf	2.7 AU	882.7 AU	16.30%	Unknown *
7	6	0.34 Rf	0.7 AU	0.37 Rf	41.0 AU	18.67%	0.41 Rf	5.2 AU	1058.3 AU	19.54%	Unknown *
7	7	0.41 Rf	5.3 AU	0.43 Rf	15.0 AU	6.83%	0.47 Rf	1.1 AU	439.5 AU	8.12%	Unknown *

Table 67. HPTLC Peak Values for Track 8 (Formulation 2, F2).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
8	1	0.19 Rf	2.0 AU	0.22 Rf	19.2 AU	16.49%	0.23 Rf	4.6 AU	279.4 AU	10.62%	Unknown *
8	2	0.34 Rf	9.6 AU	0.38 Rf	38.5 AU	33.04%	0.39 Rf	37.7 AU	862.5 AU	32.79%	Unknown *
8	3	0.39 Rf	36.9 AU	0.39 Rf	40.0 AU	34.33%	0.43 Rf	11.9 AU	801.6 AU	30.47%	Unknown *
8	4	0.55 Rf	15.4 AU	0.58 Rf	18.8 AU	16.14%	0.61 Rf	9.4 AU	687.1 AU	26.12%	Unknown *

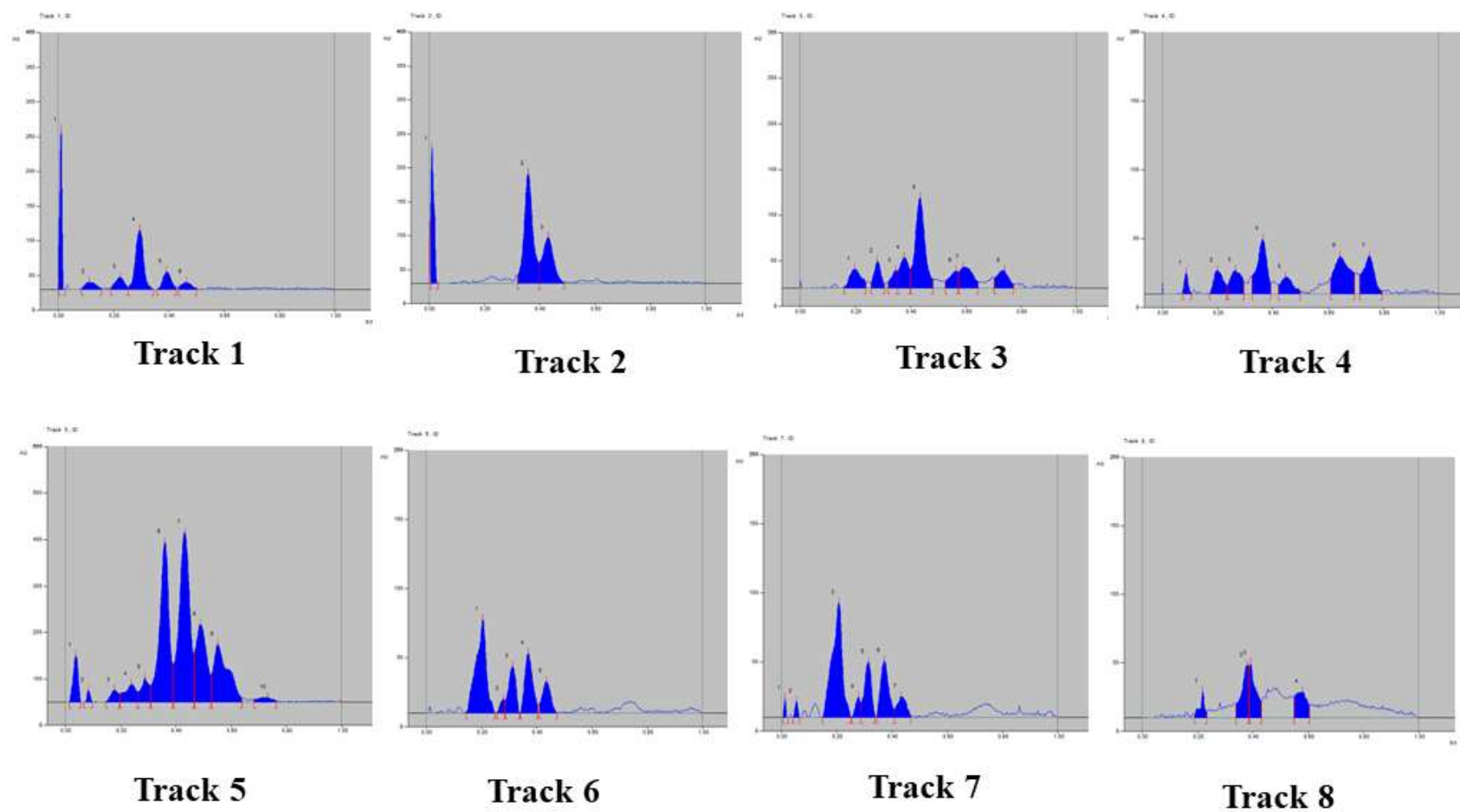


Fig. 71. Track-wise densitograms of Formulation 2 (F2) at 366 nm (Before Derivatization).

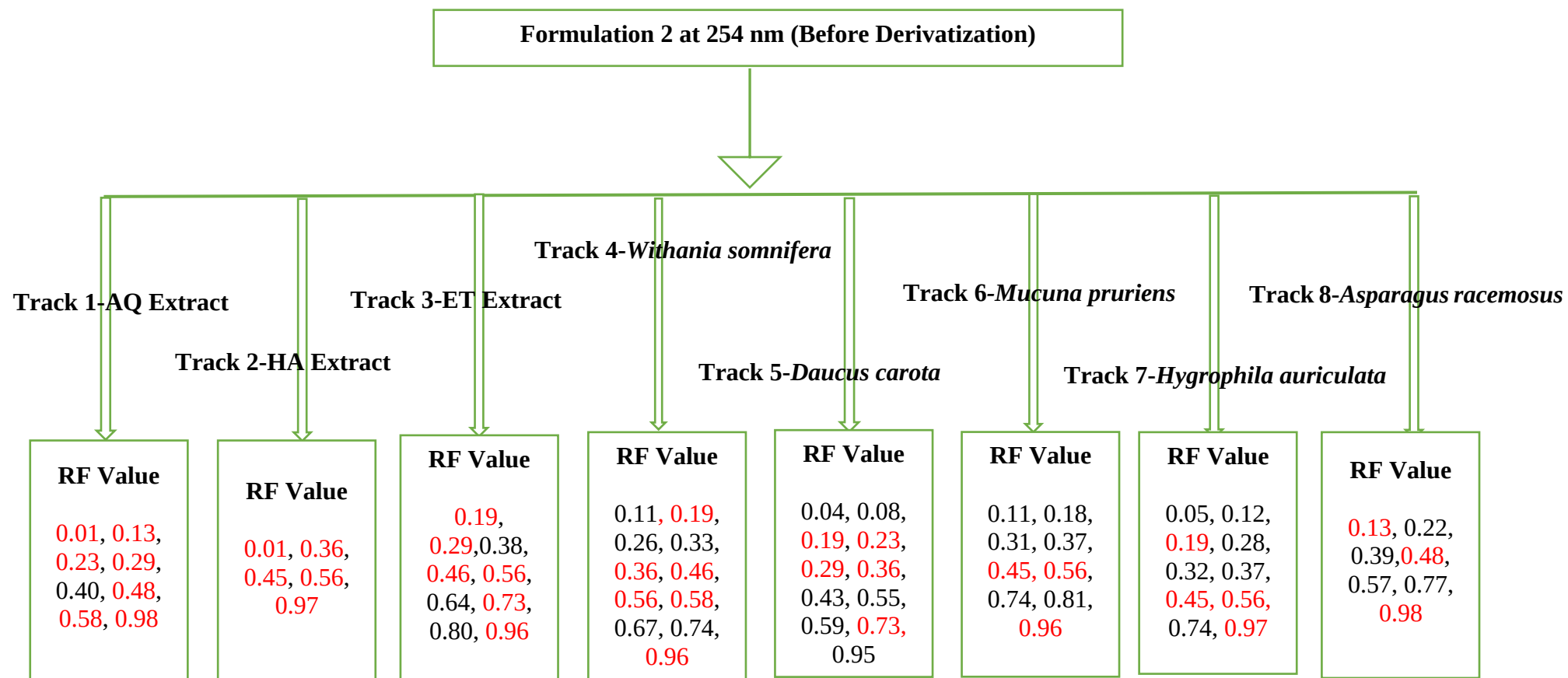


Fig. 72. Schematic Representation of Rf Values of All Tracks for Formulation 2 (F2) at 254 nm (Before Derivatization).

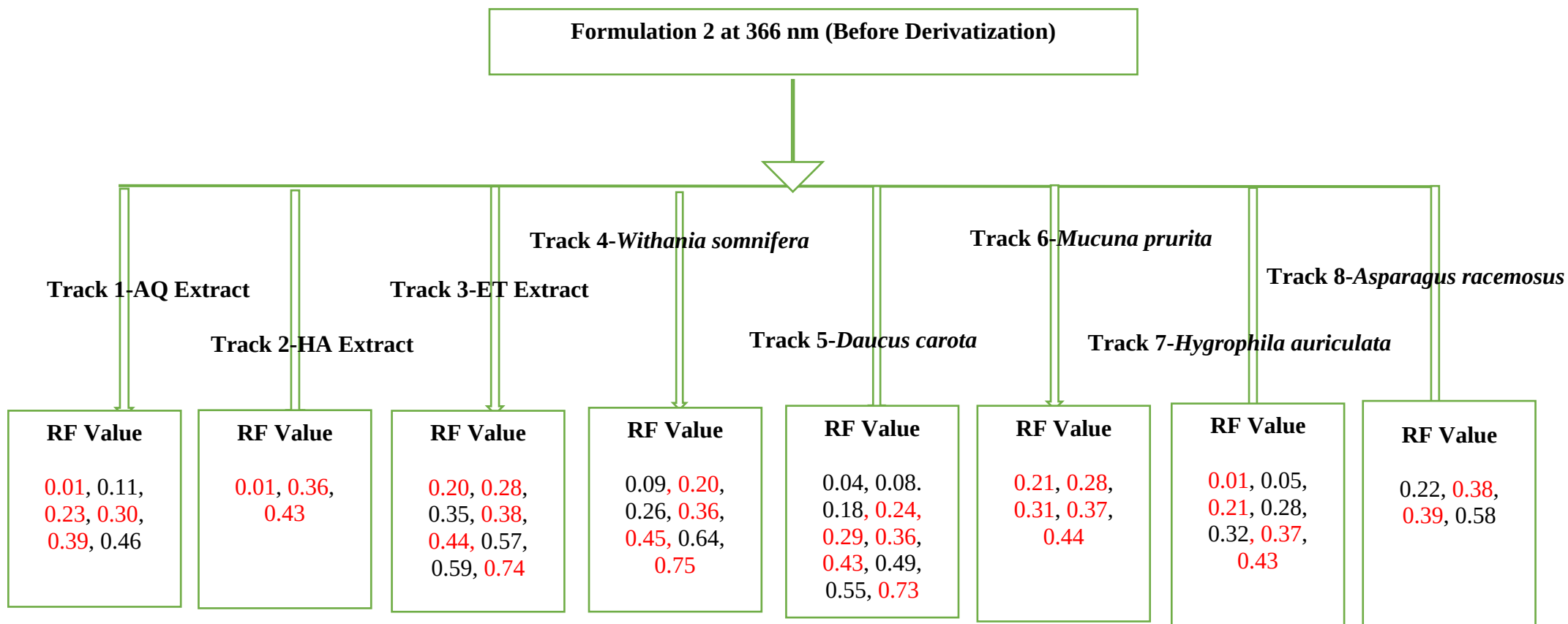


Fig. 73. Schematic Representation of Rf Values of All Tracks for Formulation 2 (F2) at 366 nm (Before Derivatization).

5.6.3.4 Interpretation of HPTLC Fingerprints for Formulations 1 and 2

The analysis of R_f values from the HPTLC Densitograms revealed both similarities and differences between the phytoconstituents profiles of Formulation 1 and Formulation 2. The apparent similarities can be attributed to the presence of common phytoconstituents as validated by matching R_f values and marked by red color in the corresponding tables (Fig. 60, 61, 64, 65). However, variations in the number and intensity of bands may reflect differences in the concentration of these phytoconstituents, potentially due to factors such as geographical variation, collection conditions, climate, and temperature (Mukherjee, 2007).

The fingerprints generated for both formulations highlight their unique phytochemical compositions and provide valuable insights into the potential influence of environmental factors on the plants' components. In the absence of pure marker compounds, several well-resolved phytoconstituents identified in both formulations may serve as marker peaks, offering critical information for the standardization and quality control of these polyherbal formulations.

5.6.4 HR-LCMS-QTOF Analysis

This study marks the first use of High-Resolution Liquid Chromatography-Mass Spectrometry Quadrupole Time-of-Flight (HR-LCMS-QTOF) for the metabolite profiling of Formulation 1 (F1) and Formulation 2 (F2), two traditional Unani polyherbal formulations. Prior to HR-LCMS-QTOF analysis, Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) fingerprinting were performed on the aqueous, hydroalcoholic, and ethanolic extracts of both formulations. Among these, the ethanolic extracts demonstrated superior separation and the highest number of bands, indicating a richer phytochemical composition. Based on these findings, the ethanolic extracts of both formulations were selected for detailed metabolite profiling using HR-LCMS-QTOF.

HR-LCMS-QTOF analysis was conducted at the Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, utilizing Electrospray Ionization (ESI) in both positive and negative modes to achieve a comprehensive metabolic profile. In Formulation 1 (F1), a total of 49 metabolites were tentatively identified in the positive ESI mode (Table 68) and 64 metabolites in the negative ESI mode (Table 69). For Formulation 2

(F2), 64 metabolites were tentatively identified in the positive ESI mode (Table 70) and 43 in the negative ESI mode (Table 71).

The identification of metabolites was based on HR-LCMS-QTOF data, which included parameters such as molecular formula, retention time (RT), observed mass, mass-to-charge ratio (m/z), score, peak height, peak area (abundance), and matches with reference databases (Tables 68 to 71). While the HR-LCMS-QTOF analysis provided a detailed tentative identification of metabolites, further confirmation through advanced techniques like Nuclear Magnetic Resonance (NMR) spectroscopy or targeted MS analysis is necessary. These findings mark a significant step forward in understanding the metabolite composition of these Unani formulations, showcasing their phytochemical richness and laying a foundation for future studies to validate and characterize their bioactive compounds.

Table 68. HRLCMS QTOF Profiling of Ethanol Extract (Formulation 1) for Metabolite Identification in Positive ESI Mode

SR. NO.	Name	Formula	Phyto group	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
1	Dihydrocaffeic acid 3-O-glucuronide	C ₁₅ H ₁₈ O ₁₀	Phenolic glycosides	62.86	1.124	358.0867	381.076	158012	4540255	9.31	2
2	(R)-Pelletierine	C ₈ H ₁₅ N O	Piperidines	80	1.356	141.1142	142.1215	974491	31930596	8.06	10
3	(-)-Hygroline	C ₈ H ₁₇ N O	Pyrrolidine alkaloids	79.11	1.498	143.1298	144.1371	1546369	30741332	8.37	3
4	Dambonitol	C ₈ H ₁₆ O ₆	Cyclohexanols	70.96	1.577	208.0927	231.0819	227057	7084446	9.65	2
5	Prolyl-Arginine	C ₁₁ H ₂₁ N ₅ O ₃	Proline Arginine dipeptide	90.41	1.588	271.1629	294.1521	355667	9342273	5.59	7
6	3-(1-Pyrrolidinyl)-2-pentanone	C ₉ H ₁₇ N O	N-alkylpyrrolidines	79.33	1.608	155.1297	156.137	284909	9180876	8.21	4
7	N(alpha)-t-Butoxycarbonyl-L-leucine	C ₁₁ H ₂₁ N O ₄	Carbamate ester and a L-leucine derivative.	71.53	1.75	231.145	232.1523	117310	4565994	8.9	1

SR. NO.	Name	Formula	Phyto group	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
8	Sarmentosin	C ₁₁ H ₁₇ N O ₇	Cyanogenic glycosides	84.41	1.95	275.1001	298.0893	141871	6622204	1.63	8
9	Apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)]	C ₇ H ₁₅ N ₃ O ₂	Carboximide acid.	75.59	2.571	173.1148	174.1221	124380	6149333	9.21	2
10	2,4,6-Trihydroxytoluene	C ₇ H ₈ O ₃	Trihydroxytoluene (Phenols)	76.59	3.457	140.0461	141.0534	189494	6747163	9.17	10
11	N-Methacrylylglycine methylester	C ₇ H ₁₁ N O ₃	Ester and amino acid derivative	93.74	3.526	157.0729	158.0802	777106	27494576	6.55	5
12	Ricinine	C ₈ H ₈ N ₂ O ₂	3-pyridinecarbonitriles (pyridine alkaloid).	93.07	3.527	164.0575	165.0648	746776	27828850	6.59	3
13	Diflubenzuron	C ₁₄ H ₉ Cl F ₂ N ₂ O ₂	N-benzoyl-N'-phenylureas (Benzonoids)	59.39	3.65	310.032	333.0212	199786	6758709	0.3	3
14	(R)-(+)-2-Pyrrolidone-5-carboxylic acid	C ₅ H ₇ N O ₃	Alpha amino acids and derivatives	79.83	4.023	129.0414	130.0487	177815	4996582	9.05	7

SR. NO.	Name	Formula	Phyto group	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
15	1-Isothiocyanato-8-(methylthio)octane	C ₁₀ H ₁₉ N S ₂	Isothiocyanates	41.66	4.03	217.093	218.1002	220801	7634108	13.17	1
16	LysoPE(0:0/16:1 (9Z))	C ₂₁ H ₄₂ N O ₇ P	2-acyl-sn-glycero-3-phosphoethanolamines (Glycerophospholipids)	80.66	4.713	451.2667	474.2561	199277	4747256	7.04	5
17	L-isoleucyl-L-proline	C ₁₁ H ₂₀ N ₂ O ₃	Dipeptides	72.62	6.515	228.1454	229.1527	125457	4401967	8.63	1
18	Quercetin	C ₁₅ H ₁₀ O ₇	Flavonols	81.13	6.593	302.0402	303.0475	434602	10053098	8.23	10
19	2,3-Dihydrowithanolide E	C ₂₈ H ₄₀ O ₇	Withanolides and derivatives	74.39	9.602	488.2734	489.2808	281733	7641072	8.1	5
20	3-Nonanone-1-yl acetate	C ₁₁ H ₂₀ O ₃	Fatty alcohol esters	85.78	11.103	200.1417	223.131	127375	2024645	-2.38	7
21	Gentamicin C1a	C ₁₉ H ₃₉ N ₅ O ₇	Aminocyclitol glycosides	79.8	11.215	449.2845	472.2737	162192	2536180	1.1	1

SR. NO.	Name	Formula	Phyto group	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
22	Glu-P-1	C ₁₁ H ₁₀ N ₄	Imidazopyridine s	86.15	11.241	198.09	221.0792	473011	7566736	2.62	10
23	Boviquinone 4	C ₂₆ H ₃₆ O ₄	Diterpenoids	96.54	11.475	412.2603	435.2496	228404	4328284	2.53	4
24	Dehydrofalcari one	C ₁₇ H ₂₀ O	Ynone (Oxygenated hydrocarbons) (Fatty acyls)	86.1	11.476	240.1517	263.141	246838	3916959	-1.18	10
25	Pholcodine	C ₂₃ H ₃₀ N ₂ O ₄	Morphinans (Alkaloids and derivatives)	80.79	11.478	398.2213	399.2287	220075	3513408	-1.85	3
26	2-(2-Methylbutanoyl)- -9-(3- methyl- 2E-pentenoyl)- 2b,9a- dihydroxy- 4Z,10(14)- oplopadien-3- one	C ₂₆ H ₃₈ O ₅	Sesquiterpenoid s	81.43	11.763	430.2709	453.2598	142773	2618050	2.28	5
27	Stanolone benzoate	C ₂₆ H ₃₄ O ₃	Steroid esters	81.03	11.766	394.2501	417.2394	458885	6899072	1.87	4

SR. NO.	Name	Formula	Phyto group	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
28	D-Linalool 3-(6"-malonylglucoside)	C ₁₉ H ₃₀ O ₉	Fatty acyl glycosides of mono- and disaccharides	72.55	11.926	402.1908	403.1982	219571	3645776	-4.42	2
29	C16 Sphinganine	C ₁₆ H ₃₅ N O ₂	Hexadecasphinganine (Sphingolipids)	83.82	12.278	273.2647	274.272	1192146	31691966	7.73	1
30	6-Methyl-2-methylene-6-octene-1,3,8-triol	C ₁₀ H ₁₈ O ₃	Fatty alcohols	85.85	12.953	186.126	209.1152	140910	2318548	-2.13	10
31	Valdiate	C ₁₇ H ₂₆ O ₅	iridoid monoterpenoid (Iridoids and derivatives).	79.41	12.96	310.1754	311.1827	453993	8689701	8.49	3
32	12-Methyltridecanal	C ₁₄ H ₂₈ O	Fatty aldehydes (Fatty acyls)	86.79	13.946	212.2143	235.2036	133647	2230117	-1.41	3
33	Ricinoleic acid	C ₁₈ H ₃₄ O ₃	Long-chain fatty acids (Fatty acyls)	67.74	13.947	298.2483	299.2556	279148	4689618	8.36	6
34	Sphinganine	C ₁₈ H ₃₉ N O ₂	1,2-aminoalcohols (Amines).	66.3	14.035	301.2954	302.3027	295299	8884370	9.01	1

SR. NO.	Name	Formula	Phyto group	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
35	Deoxycholic acid glycine conjugate	C ₂₆ H ₄₃ N O ₅	Glycinated bile acids and derivatives	94.05	14.184	449.3126	472.3019	246203	4248960	3.46	6
36	12-Methyltridecanal	C ₁₄ H ₂₈ O	Fatty aldehydes (Fatty acyls)	86.82	14.276	212.2142	235.2035	128834	1806342	-0.76	3
37	Phytosphingosine	C ₁₈ H ₃₉ N O ₃	1,3-aminoalcohols (Amines)	78.77	14.327	317.2903	318.2977	427393	7665052	8.54	1
38	MG(22:5(4Z,7Z,10Z,13Z,16Z)/0:0/0:0)	C ₂₅ H ₄₀ O ₄	1-monoacylglycerols (Glycerolipids)	90.67	14.476	404.2915	427.2804	112291	2015482	2.89	6
39	Adlupulone	C ₂₆ H ₃₈ O ₄	M-benzoquinones	95.42	15.03	414.2758	437.2651	266406	3933607	2.94	4
40	Cycloate	C ₁₁ H ₂₁ N O S	Thiocarbamic acid derivatives	19.61	15.452	215.1314	238.1206	1565361	23733698	14.03	1
41	Estradiol cypionate	C ₂₆ H ₃₆ O ₃	C18 steroids (estrogens) and derivatives (Steroids).	96.11	15.773	396.2653	419.2546	314403	7409417	2.89	3

SR. NO.	Name	Formula	Phyto group	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
42	Fesoterodine	C ₂₆ H ₃₇ N O ₃	Diphenylmethanes (Benzenoids)	36.24	16.671	411.2818	412.289	128530	2597862	-10.89	1
43	Schidigeragenin B	C ₂₇ H ₄₀ O ₄	Triterpenoid (Sterol Lipids).	74.73	16.672	428.289	429.2962	1876028	43938760	8.56	4
44	10-Oxo-11-octadecen-13-olide	C ₁₈ H ₃₀ O ₃	Macrolides and analogues	61.82	16.781	294.2165	295.2238	110154	4645970	10.24	10
45	Citronellyl hexanoate	C ₁₆ H ₃₀ O ₂	Fatty alcohol esters (Fatty Acyls)	83.45	16.795	254.2241	277.2133	148591	5339552	1.94	10
46	Methyl 2-furoate	C ₆ H ₆ O ₃	Furoic acid esters (Furans)	78.86	16.849	126.0328	149.022	133539	7045442	-8.48	9
47	Lyngbyatoxin	C ₂₇ H ₃₉ N ₃ O ₂	Alpha amino acid amides	93.79	17.6	437.3027	460.2919	182760	3463075	3.6	2
48	Austroinulin	C ₂₀ H ₃₄ O ₃	Diterpenoids	62.24	19.806	322.2477	323.2551	265530	12763738	9.54	3
49	Ganoderic acid F	C ₃₂ H ₄₂ O ₉	Triterpenoid	93.06	20.961	570.281	593.2704	112654	3305722	3.29	5

Table 69. HRLCMS QTOF Profiling of Ethanol Extract (Formulation 1) for Metabolite Identification in Negative ESI Mode

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
1	L-Iditol	C ₆ H ₁₄ O ₆	Sugar alcohols	45.89	1.004	182.0785	181.0712	130271	1133124	3.19	10
2	Brompheniramine	C ₁₆ H ₁₉ Br N ₂	Pheniramines (Pyridines and derivatives)	69.64	1.169	318.0725	377.0861	636915	17274964	2.09	10
3	L-Sorbose	C ₆ H ₁₂ O ₆	Ketoses (Monosaccharides)	87.47	1.178	180.0638	215.0326	196926	8598297	-2.42	10
4	Quinic acid	C ₇ H ₁₂ O ₆	Quinic acids and derivatives	99.38	1.184	192.0634	191.0561	4731212	103061848	0.13	10
5	Gulonic acid	C ₆ H ₁₂ O ₇	Medium-chain hydroxy acids and derivatives	86.32	1.214	196.0581	195.0509	118912	2214147	0.96	10
6	2-Galloylglucose	C ₁₃ H ₁₆ O ₁₀	Galloyl esters (Benzoic acids and derivatives)	96.73	1.299	332.0742	331.0671	1550237	45467960	0.36	10
7	1-O-Caffeoyl-(β-D-glucose 6-O-sulfate)	C ₁₅ H ₁₈ O ₁₂ S	Hydroxycinnamic acid glycosides	56.51	1.345	422.0493	481.0632	623263	20981234	6.07	1

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
8	N-Acetyl-L-glutamic acid	C ₇ H ₁₁ N O ₅	Glutamic acid and derivatives	83.02	1.366	189.0637	188.0565	126718	2519655	0.3	10
9	Gallic acid 3-O-(6-galloylglucoside)	C ₂₀ H ₂₀ O ₁₄	Hydrolyzable tannins	95.24	1.607	484.0864	483.0793	565441	17828758	-2.21	8
10	Methyl 2-furoate	C ₆ H ₆ O ₃	Furoic acid esters	84.78	1.644	126.0324	125.0251	525183	18290274	-5.41	9
11	Gallic acid	C ₇ H ₆ O ₅	Gallic acid (Benzoic acids and derivatives) Flavonoids	98.44	1.649	170.0219	169.0147	2640054	87315072	-2.45	4
12	Punicacortein B	C ₂₇ H ₂₂ O ₁₈	Hydrolyzable tannins	93.51	1.764	634.0803	633.0732	139183	9166539	0.53	4
13	Granatin A	C ₃₄ H ₂₄ O ₂₂	Hydrolyzable tannins	87.87	2.291	784.0746	783.0677	182538	15727672	1.63	3
14	2,6-dihydroxybenzoic acid	C ₇ H ₆ O ₄	Salicylic acids (Benzoic acids and derivatives)	86.29	2.694	154.027	153.0198	138157	6882696	-2.81	9

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
15	4-Hydroxyphthalate	C ₈ H ₆ O ₅	Member of phenols and a hydroxybenzoic acid	97.33	2.82	182.0222	181.0149	1055114	64652712	-3.48	4
16	Patuletin	C ₁₆ H ₁₂ O ₈	Flavonols	93.73	3.627	332.0544	377.0527	655327	24481898	-3.53	10
17	Sanguin H11	C ₄₁ H ₂₈ O ₂₇	Hydrolyzable tannins	94.8	3.665	952.0797	951.0727	224157	12888558	2.19	3
18	N-Pyruvoyl-5-methoxy-3-hydroxyanthranilate	C ₁₁ H ₁₁ N O ₆	Methoxybenzoic acid.	85.82	3.726	253.0587	252.0515	231041	6274689	-0.41	10
19	Heterophyllin A	C ₃₄ H ₂₆ O ₂₂	Hydrolyzable tannins	97.88	3.954	786.0908	785.0837	605284	32065122	1.02	4
20	Pedunculagin	C ₃₄ H ₂₄ O ₂₂	Ellagitannins	69.09	4.254	784.0761	783.069	406748	11656669	-0.28	5
21	Punicacortein B	C ₂₇ H ₂₂ O ₁₈	Hydrolyzable tannins	26.13	4.331	634.0872	633.0747	580349	33339886	-10.46	4

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
22	6-Hydroxyangelicin	C ₁₁ H ₆ O ₄	Furanocoumarin	96.11	4.483	202.0273	247.0255	281938	7759740	-3.28	7
23	Mallotinic acid	C ₃₄ H ₂₆ O ₂₃	Tannin	97.46	4.705	802.0867	801.0796	192864	5018758	-0.26	7
24	Heterophyllin A	C ₃₄ H ₂₆ O ₂₂	Hydrolyzable tannins	98.62	4.739	786.0921	785.0848	1430403	64875600	-0.71	4
25	Pancratistatin	C ₁₄ H ₁₅ N O ₈	Isoquinoline alkaloid	79.12	4.881	325.0812	324.0738 26	237854	5803719	-4.26	4
26	Methyl 6-O-galloyl-beta-D-glucopyranoside	C ₁₄ H ₁₈ O ₁₀	Galloyl esters	76.91	4.947	346.0916	345.0844	182686	3760026	-4.6	9
27	1,2',5-Tri-O-galloylhamamelofuranose	C ₂₇ H ₂₄ O ₁₈	Hydrolyzable tannin	95	5.072	636.0978	635.0907	174257	6007774	-2.49	8
28	Sanguisorbic acid dilactone	C ₂₁ H ₁₀ O ₁₃	Hydrolyzable tannins	84.9	5.18	636.0978	469.0076	154087	5156827	-5.75	2
29	PG(18:0/20:3(8Z,11Z,14Z))	C ₄₄ H ₈₁ O ₁₀ P	Phosphatidylglycerols (Glycerophospholipids)	15.49	5.279	800.5636	859.5775	125153	2685334	-8.58	2

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
30	TS-TM-calix(4)arene	C ₃₂ H ₃₂ O ₁₆ S ₄	Arene sulfonic acid and a substituted calixarene	42.2	5.288	800.0617	859.0755	145457	4339171	-5.45	1
31	Fenitropan	C ₁₃ H ₁₅ N O ₆	Amphetamines (Fungicides)	78.9	5.685	281.0913	280.0841	207187	5804946	-5.01	6
32	3,4-O-Dimethylgallic acid	C ₉ H ₁₀ O ₅	Gallic acid and derivatives	72.98	5.734	198.0546	197.0474	564229	7872752	-9.07	10
33	3'-(2'',3''-Digalloyl-4'',6''-hexahydroxydiphenoyl)-phloroacetophenone	C ₄₂ H ₃₂ O ₂₅	Tetracarboxylic acids and derivatives	92.66	6.03	936.125	981.1235	189023	17094596	-1.84	2
34	Quercetin 3-(2-galloylglucoside)	C ₂₈ H ₂₄ O ₁₆	Flavonoid-3-O-glycosides	86.89	6.678	616.1092	615.1021	158419	3926817	-4.46	2
35	Kaempferol 7-O-glucoside	C ₂₁ H ₂₀ O ₁₁	Flavonoid-7-O-glycosides	73.18	7.113	448.1046	447.0973	1756389	44743608	-8.97	10

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
36	Phenethyl 6-galloylglucoside	C ₂₁ H ₂₄ O ₁₀	Galloyl esters	71.39	7.298	436.141	435.1337	3815284	83332432	-9.34	3
37	Cis-3-(3-Carboxyethenyl)-3,5-cyclohexadiene-1,2-diol	C ₉ H ₁₀ O ₄	Cyclic alcohols and derivatives	74.27	7.485	182.0596	181.0524	185785	2382898	-9.33	10
38	Pyrazolate	C ₁₉ H ₁₆ Cl ₂ N ₂ O ₄ S	Pyrazole herbicides	29.28	7.682	438.0265	497.04	191070	5658084	-12.95	1
39	(3x,5x,10x)-9,10-Didehydroisohumbertiol O-[rhamnosyl-(1->4)-rhamnosyl-(1->2)-[rhamnosyl-(1->6)]-glucoside]	C ₃₉ H ₆₂ O ₁₈	Oligosaccharides	24.21	9.49	818.3843	817.3793	267769	10836266	11.4	1
40	Erinacine G	C ₂₅ H ₃₆ O ₈	Furopyran	18.73	9.551	464.2336	523.2495	290733	10364499	15.93	3

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
41	6-O-Oleuropeoylsucrose	C ₂₂ H ₃₆ O ₁₃	Saccharolipids	84.3	9.746	508.2156	567.2294	173969	7399021	-0.03	4
42	Auxin a	C ₁₈ H ₃₂ O ₅	Monocyclic monoterpenoids	78.14	10.02	328.2265	327.2193	240018	7543897	-4.5	4
43	Momordicoside E	C ₃₇ H ₆₀ O ₁₂	Steroidal glycosides (steroid saponin)	97.76	10.053	696.4092	695.4021	130830	3621026	-1.03	6
44	Ponasteroside A	C ₃₃ H ₅₄ O ₁₁	Steroidal glycosides (steroid saponin)	20.53	10.058	626.358	685.374	269863	9631726	13.8	2
45	9S,12S,13S-trihydroxy-10E-octadecenoic acid	C ₁₈ H ₃₄ O ₅	Long-chain fatty acids (Fatty acyls)	85.95	10.647	330.2428	329.2356	661220	19268338	-6.44	3
46	Madasiatic acid	C ₃₀ H ₄₈ O ₅	Triterpenoids	69.19	13.177	488.3524	487.3453	315233	6252934	-4.58	10
47	Gnidilatin	C ₃₇ H ₄₈ O ₁₀	Diterpenoid	45.12	14.724	652.3208	711.3369	164252	6269428	6.07	4

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
48	8-Acetoxy-4-acoren-3-one	C ₁₇ H ₂₆ O ₃	Cyclohexenones	85.65	14.865	278.1885	277.1813	134203	2442210	-1.07	10
49	alpha-(p-Methoxyphenyl)-6-methyl-2-pyridineacrylic acid	C ₁₆ H ₁₅ N O ₃	Monocarboxylic acid	84.5	15.36	269.1059	268.0986	212236	3166591	-2.53	6
50	Pyropheophorbide a	C ₃₃ H ₃₄ N ₄ O ₃	Chlorins	80.51	16.041	534.2613	593.2753	232252	5835132	3.37	6
51	Azukisapogenol	C ₃₀ H ₄₈ O ₄	Triterpenoid saponins	87.98	16.297	472.3575	471.3504	215556	10879609	-4.7	10
52	Azafrin	C ₂₇ H ₃₈ O ₄	Triterpenoid	66.54	16.867	426.2797	425.2725	253088	4661904	-6.28	1
53	2-[4,6-Bis(2,4-dimethylphenyl)-1,3,5-triazin-2-yl]-5-(octyloxy)phenol	C ₃₃ H ₃₉ N ₃ O ₂	Phenol ethers	62.02	17.305	509.304	554.3047	347773	12265038	0.49	8

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
54	LysoPE(20:2(1 1Z,14Z)/0:0)	C ₂₅ H ₄₈ N O ₇ P	1-acyl-sn-glycero-3-phosphoethanol amines (Glycerophospholipids)	66.4	17.308	505.3173	504.312	126504	3640790	-0.82	2
55	CPA(18:1(9Z)/0:0)	C ₂₁ H ₃₉ O ₆ P	1-acyl-sn-glycerol-2,3-cyclic-phosphates (Glycerophospholipids)	57.83	17.415	418.25	477.2656	334044	19350528	-3.76	5
56	Salannin	C ₃₄ H ₄₄ O ₉	Triterpenoids	88.33	17.607	596.2989	595.2919	661483	18809842	-0.7	2
57	Usambarensine	C ₂₉ H ₂₈ N ₄	Harmala alkaloid	81.62	18.035	432.2307	431.2234	260693	9143780	1.56	6
58	Euglobal VII	C ₂₈ H ₃₈ O ₅	Germacrane sesquiterpenoids	65.44	18.281	454.2717	453.2658	201654	14074253	0.59	10
59	APGPR Enterostatin	C ₂₁ H ₃₆ N ₈ O ₆	Benzoyl derivatives	71.42	19.096	496.2765	555.2868	129616	4370307	-1.5	3

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
60	Ganoderic acid H	C ₃₂ H ₄₄ O ₉	Triterpenoid.	85.48	19.255	572.2991	571.292	402938	17219006	-0.95	6
61	LPA(0:0/18:2(9Z,12Z))	C ₂₁ H ₃₉ O ₇ P	2-acylglycerol-3-phosphates (Glycerophospholipids).	79.88	20.332	434.2465	433.2393	868204	52559256	-7.38	4
62	Alloxanthin	C ₄₀ H ₅₂ O ₂	Diterpenoid.	50.11	22.285	564.3938	609.3944	293517	12553808	5.15	5
63	3'-N-Acetyl-4'-O-(9-octadecenoyl) fusarochroman one	C ₃₅ H ₅₄ N ₂ O ₆	2,2-dimethyl-1-benzopyrans (Benzopyrans)	59.52	22.979	598.3997	597.3946	177519	6222036	-2.49	4
64	3-Hydroxy-b,e-caroten-3'-one	C ₄₀ H ₅₄ O ₂	Xanthophylls(Tetraterpenoids).	51.12	24.214	566.4095	611.41	748446	29518652	5.04	10

Table 70. HRLCMS QTOF Profiling of Ethanol Extract (Formulation 2) for Metabolite Identification in Positive ESI Mode

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
1	Dihydrocaffeic acid 3-O-glucuronide	C ₁₅ H ₁₈ O ₁₀	Phenolic glycosides	65.22	1.071	358.087	381.0764	232677	5084852	8.29	2
2	Pregabalin	C ₈ H ₁₇ N O ₂	Gamma amino acids and derivatives (GABA)	79.52	1.282	159.1247	160.132	284506	9269958	7.71	3
3	3-Carboxy-4-methoxy-N-methyl-2-pyridone	C ₈ H ₉ N O ₄	Member of methylpyridines and a pyridone	31.78	1.311	183.0551	184.0624	157741	5247150	-10.51	6
4	Retronecine	C ₈ H ₁₃ N O ₂	Pyrrolizidine alkaloids	81.81	1.327	155.0938	156.1007	1059519	24328662	5.5	3
5	(R)-Pelletierine	C ₈ H ₁₅ N O	Piperidine alkaloids	81.07	1.337	141.1143	142.1216	667916	26662982	7.55	10
6	6-Acetamido-3-oxohexanoate	C ₈ H ₁₃ N O ₄	Medium-chain keto acids and derivatives	96.03	1.346	187.0853	210.0745	804784	17657430	-4.38	8
7	Hypoglycin	C ₇ H ₁₁ N O ₂	Alpha amino acids (Phytotoxins)	80.36	1.35	141.0801	164.0693	229912	5653550	-7.9	7

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
8	Methyl o-methoxyhippuric acid	C ₁₁ H ₁₃ N O ₄	Amino acid derivative (N-acyl-amino acid)	69.41	1.421	223.0826	224.0899	394408	11214710	8.15	4
9	(-)-Hygroline	C ₈ H ₁₇ N O	Alkaloides & derivatives	79.73	1.473	143.1299	144.1371	2021189	47765324	8.02	3
110	Isovaleryl sarcosine	C ₈ H ₁₅ N O ₃	N-acyl-alpha amino acids	80.03	3.41	173.1055	196.0949	154833	6020024	-1.91	10
11	2-amino-8-oxo-9,10-epoxy-decanoic acid	C ₁₀ H ₁₇ N O ₄	Fatty Acyls [Oxo fatty acids]	82.67	3.485	215.1163	238.1055	174472	6730392	-2.34	3
12	2,4,6-Trihydroxytoluene	C ₇ H ₈ O ₃	Methoxyphenols	79.88	3.505	140.0462	141.0535	177142	6208104	8.35	10
13	Ethyl 2-hydroxy-3-(3-indolyl)propanoate glucoside	C ₁₉ H ₂₅ N O ₈	Fatty acyl glycosides of mono- and disaccharides	96.7	3.978	395.157	418.1462	310240	11100345	2.66	1
14	Tecostanine	C ₁₁ H ₂₁ N O	Member of piperidines (ALKALOIDES)	72.53	4.717	183.1624	206.1522	181473	5553396	-0.24	3
15	LysoPE(0:0/16:1(9Z))	C ₂₁ H ₄₂ N O ₇ P	2-acyl-sn-glycerol-3-phosphoethanol	82.6	4.751	451.2669	474.2563	307822	6753936	6.56	5

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
			mines (Glycerophospholipids)								
16	Petasinine	C ₁₃ H ₂₁ N O ₃	Pyrrolizidines	66.25	5.135	239.1498	240.1572	162358	4985124	10.01	6
17	Oxopurpureine	C ₂₁ H ₁₉ N O ₆	Aporphines (isoquinoline alkaloid)	77.49	5.307	381.1181	382.1253	173055	4667502	8.33	2
18	R-2-Hydroxy-3-methylbutanoic acid 3-Methylbutanoyl	C ₁₀ H ₁₈ O ₄	Fatty acid esters (Fatty Acyl)	97.66	5.317	202.1212	225.1104	431226	11267470	-3.19	10
19	11-amino-undecanoic acid	C ₁₁ H ₂₃ N O ₂	Amino fatty acids (fatty acyl)	83.9	5.874	201.1736	224.1628	5973610	15023806 4	-3.54	4
20	1,4-Ipomeadiol	C ₉ H ₁₄ O ₃	Heteroarene (Heteroaromatic compounds)	80.93	5.913	170.0949	193.0841	149697	5846224	-3.3	10
21	para-Trifluoromethyl phenol	C ₇ H ₅ F ₃ O	Trifluoromethylbenzenes	82.13	6.502	162.0303	163.0375	439453	8951406	-6.27	10
22	Mahaleboside	C ₁₅ H ₁₆ O ₈	Coumarin glycosides	79.18	6.517	324.0816	325.0889	187169	5009312	8.87	1

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
23	Pirimicarb	C ₁₁ H ₁₈ N ₄ O ₂	Carbamate insecticides (Dialkylarylamines)	34.93	6.718	238.1448	239.1521	212584	4149302	-7.79	2
24	Thalicpureine	C ₂₂ H ₂₇ N O ₅	6,6a-secoaporphines (Alkaloids and derivatives)	62.7	7.809	385.1858	386.1931	371125	10756671	8.18	3
25	11-Hydroxy-9-tridecenoic acid	C ₁₃ H ₂₄ O ₃	Long-chain fatty acids (fatty acyls)	83.77	8.501	228.1727	251.162	239010	8121540	-0.56	10
26	Dihydrocapsaicin	C ₁₈ H ₂₉ N O ₃	Methoxyphenols, capsaicinoid, fatty amides (fatty acyl)	75.63	9.147	307.212	308.2194	258744	6348612	8.99	3
27	2,3-Dihydrowithanolide E	C ₂₈ H ₄₀ O ₇	Withanolide (Steroid lactones)	73.77	9.63	488.2733	489.2807	210504	6664764	8.37	5
28	2,6-Dimethoxyphenol	C ₈ H ₁₀ O ₃	Methoxy phenols	83.73	10.318	154.0637	177.0529	173811	5217466	-4.38	10
29	10'-apo-beta-carotenal	C ₂₇ H ₃₆ O	Sester terpenoids (Apo carotenoid)	48.23	11.397	376.2716	377.279	441222	18845004	13.4	2

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
30	Octadecanoic acid-1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethyl ester	C ₂₁ H ₃₅ F ₇ O ₂	Fluorinated esters	93.23	11.55	452.2523	453.2595	272445	9662247	0.61	5
31	Pholcodine	C ₂₃ H ₃₀ N ₂ O ₄	Morphinans (Alkaloids and derivatives)	81.95	11.77 1	398.2209	399.2283	335000	5230860	-0.82	3
32	Sodium glycocholate	C ₂₆ H ₄₃ N O ₆	Bile salt.	93.07	11.77 3	465.3072	488.2964	1577566	27728110	3.86	3
33	Ramipril	C ₂₃ H ₃₂ N ₂ O ₅	Dipeptides	80.78	11.77 5	416.2315	417.2389	721770	11755226	-1.02	4
34	Oxacyclotetradecan-2-one	C ₁₃ H ₂₄ O ₂	Macrolides and analogues	87.3	12.00 8	212.1777	235.1669	270843	12740926	-0.29	10
35	Estradiol cypionate	C ₂₆ H ₃₆ O ₃	Steroid ester (hormonal 36agent)	72.94	12.76	396.2647	419.2541	184739	3356041	4.34	4
36	Santene	C ₉ H ₁₄	Branched unsaturated hydrocarbons	78.05	13.12 2	122.1106	145.0998	157813	4639622	-8.3	1

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
37	Deoxycholic acid glycine conjugate	C ₂₆ H ₄₃ N O ₅	"Sterol Lipids (bila acid derivatives) Glycine conjugates"	94.49	14.17 4	449.3127	472.3021	554650	9219691	3.09	6
38	1-Monopalmitin	C ₁₉ H ₃₈ O ₄	Glycerolipids (1-monoacylglycerols)	97.55	14.88 1	330.2764	353.2657	633117	25377846	1.92	7
39	3-Methylcyclopentadecanone	C ₁₆ H ₃₀ O	Cyclic ketones	86.59	14.88 2	238.2295	261.2187	157114	5476038	0.89	4
40	Adlupulone	C ₂₆ H ₃₈ O ₄	M-benzoquinones	76.91	15.39 4	414.2756	437.265	326183	5824369	3.33	4
41	Sphinganine	C ₁₈ H ₃₉ N O ₂	1,2-aminoalcohols (Sphingolipids)	93.25	15.66 7	301.2971	324.2866	320185	12148622	3.39	2
42	(Z)-4-Dodecenal	C ₁₃ H ₂₄ O	Fatty aldehydes (Fatty acyl)	86.6	15.75 5	196.183	219.1723	280640	11990128	-1.66	10
43	1-Oleoyl Lysophosphatidic Acid	C ₂₁ H ₄₁ O ₇ P	1-acylglycerol-3-phosphates (Glycerophospholipids)	79.83	15.77 9	436.258	437.2654	272765	5488370	2.2	4

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
44	(6E,8E)-4,6,8-Megastigmatriene	C ₁₃ H ₂₀	Branched unsaturated hydrocarbons	85.11	16.24 ₃	176.1571	199.1464	420199	24936498	-3.45	3
45	(E)-2-(2-Octenyl) cyclopentanone	C ₁₃ H ₂₂ O	Cyclic ketones	84.83	16.28 ₃	194.1677	217.157	408648	18727520	-3.27	10
46	(-)-7,7-Dimethyl-2-methylenebicyclo[2.2.1]heptane	C ₁₀ H ₁₆	Bicyclic monoterpenoids	77.89	16.30 ₃	136.1265	159.1158	797447	34200496	-9.49	10
47	19-Noretiocholanolone	C ₁₈ H ₂₈ O ²	Estrogens and derivatives	67.98	16.43 ₁	276.2065	277.2138	1126150	31375332	8.88	6
48	MG(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0/0:0)	C ₂₅ H ₃₈ O ₄	1-monoacylglycerols (Glycerolipids)	70.26	16.50 ₆	402.2771	425.2652	1703492	33063722	-0.22	2
49	MG(22:5(4Z,7Z,10Z,13Z,16Z)/0:0/0:0)	C ₂₅ H ₄₀ O ₄	2-monoacylglycerols (Glycerolipids)	74.76	16.97 ₄	404.2914	427.2807	172936	2845016	3.22	6
50	Palmitic Acid	C ₁₆ H ₃₂ O ₂	Long-chain fatty acids (Fatty acyls) Saturated fatty acids	99.33	17.00 ₂	256.2403	279.2295	928165	41786508	-0.12	10

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
51	Methyl 2-(10-heptadecenyl)-6-hydroxybenzoate	C ₂₅ H ₄₀ O ₃	o-Hydroxybenzoic acid esters (phenols and a benzoate ester)	97.03	17.045	388.2968	411.2861	441940	7409326	2.36	5
52	MG(22:4(7Z,10Z,13Z,16Z)/0:0/0:0)	C ₂₅ H ₄₂ O ₄	1-monoacylglycerols (Glycerolipids)	97.35	17.047	406.3073	429.2965	1558783	26809972	2.48	6
53	(13R,14R)-7-Labdene-13,14,15-triol	C ₂₀ H ₃₆ O ₃	Diterpenoids	80.73	17.108	324.2639	325.2712	650943	27664652	7.91	3
54	Stearic acid	C ₁₈ H ₃₆ O ₂	Long-chain fatty acids (Fatty acyl)	84.66	17.174	284.271	307.2603	164336	7367386	1.77	10
55	(Z)-4-Dodecenal	C ₁₃ H ₂₄ O	Fatty aldehydes (Fatty acyl)	82.2	18.014	196.183	219.1722	159173	4863606	-1.41	10
56	Sphinganine	C ₁₈ H ₃₉ N O ₂	1,2-aminoalcohols (Sphingolipids)	85.35	18.269	301.2977	324.2869	2470536	36528124	1.28	2
57	(Z)-1,5-Tridecadiene	C ₁₃ H ₂₄	Alkadiene	84.84	18.479	180.1884	203.1777	201260	4657334	-3.39	7

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hits (DB)
58	3-Ketosphinganine	C ₁₈ H ₃₇ N O ₂	Sphingolipids	81.33	18.82 9	299.2799	300.2873	1128594	20304956	8.32	4
59	Oleoyl Ethanolamide	C ₂₀ H ₃₉ N O ₂	N-acyl ethanolamines (Fatty acyls)	60.89	19.66 7	325.295	326.3024	367868	7508578	9.38	1
60	2R-hydroxy-stearic acid	C ₁₈ H ₃₆ O ₃	Hydroxy fatty acids (fatty acyls)	85.22	19.77 7	300.2661	323.2554	785514	32699188	1.07	7
61	Citronellyl hexanoate	C ₁₆ H ₃₀ O ₂	Carboxylic ester (Fatty alcohol esters)	85.55	19.77 7	254.2246	277.214	521089	22652286	-0.21	10
62	Dioctyl hexanedioate	C ₂₂ H ₄₂ O ₄	Carboxylic ester (Fatty alcohol esters)	58.39	20.42 3	370.3048	393.2941	154896	5184507	9.54	3
63	Myxalamid C	C ₂₄ H ₃₇ N O ₃	Fatty amide (Non-ribosomal peptide/polyketide hybrids)	80.46	20.57 3	387.2745	388.2818	493867	9750070	7.42	1
64	N,N-Dimethylsphingosine	C ₂₀ H ₄₁ N O ₂	1,2-aminoalcohols (Sphingolipids)	61.85	21.39 2	327.3106	328.3179	210098	3579852	9.6	2

Table 71. HRLCMS QTOF Profiling of Ethanol Extract (Formulation 2) for Metabolite Identification in Negative ESI Mode

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hit (DB)
1	Nigerose (Sakebiose)	C ₁₂ H ₂₂ O ₁₁	Glycosylglucose (o-glycosyl)	82.99	1.03	342.1168	341.1096	381088	4517820	-1.8	10
2	7-Hydroxy-6-methyl-8-ribityllumazine	C ₁₂ H ₁₆ N ₄ O ₇	Pteridine & Derivatives	83.97	1.033	328.1016	387.1155	304367	3331691	0.79	10
3	Hamamelose	C ₆ H ₁₂ O ₇	Polyols (Carbohydrate)	97.61	1.174	196.0589	195.0517	523904	10422110	-3.25	10
4	Brompheniramine	C ₁₆ H ₁₉ Br N ₂	Pyridines and derivatives (pheniramine)	70.62	1.183	318.0732	377.0867	1286742	42491628	-0.13	10
5	Isoacteoside	C ₂₉ H ₃₆ O ₁₅	Coumaric acids and derivatives	87.88	6.457	624.2081	623.2011	8548945	243206624	-4.29	5
6	Licorice Saponin G2	C ₄₂ H ₆₂ O ₁₇	Triterpenoid Saponin (Terpene glycosides)	69.18	9.487	838.4041	837.3975	337942	8929511	-6.45	4
7	(3x,5x,10x)-9,10-Didehydroisohumbertiol O-[rhamnosyl-(1->4)-	C ₃₉ H ₆₂ O ₁₈	Oligosaccharide	30.47	9.494	818.386	817.3811	308714	13752539	9.29	1

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (pp m)	Hit (DB)
	rhamnosyl-(1->2)-[rhamnosyl-(1->6)]-glucoside]										
8	Erinacine G	C ₂₅ H ₃₆ O ₈	Furopyran	23.34	9.56 8	464.2349	523.25 09	385048	1582136 3	13.1 9	3
9	Acetyl-maltose	C ₁₄ H ₂₄ O ₁₂	o-glycosyl (glycoside)	83.18	10.0 05	384.1248	383.11 76	846840	3450674 4	5.2	10
10	Flecainide	C ₁₇ H ₂₀ F ₆ N ₂ O ₃	Benzamides (Benzoic acids and derivatives)	90.23	10.1 82	414.1359	413.12 87	619952	2241702 8	4.59	10
11	9S,12S,13S-trihydroxy-10E-octadecenoic acid	C ₁₈ H ₃₄ O ₅	Pinellic acid, Fatty Acyls [FA]	61.18	10.6 65	330.2438	329.23 66	191141 5	5641500 4	-9.74	3
112	Hordatine B glucoside	C ₃₅ H ₅₀ N ₈ O ₁₀	2-arylbenzofuran flavonoids	31.11	11.5 33	742.372	801.38 79	340871	1092711 8	-9.42	1
13	6alpha-Fluoro-17beta-hydroxyandrost-4-en-3-oneacetate	C ₂₁ H ₂₉ F O ₃	Estrane steroids	92.79	11.9 71	348.2105	393.20 87	174552 1	7533885 6	-1.28	10

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hit (DB)
14	Quinestrol	C ₂₅ H ₃₂ O ₂	C18 steroids (estrogens) and derivatives	58.03	13.048	364.2428	423.2568	473225	7223440	-7.18	1
15	Desoximetasone	C ₂₂ H ₂₉ F O ₄	Synthetic corticosteroid with glucocorticoid activity	92.44	13.075	376.2057	375.1985	921525	40767840	-1.95	10
16	11S-HpODE	C ₁₈ H ₃₂ O ₄	lineolic acids and derivatives (Fatty Acyls)	63.1	13.133	312.2331	311.2258	2246668	33549008	-9.61	8
17	Melongoside G	C ₄₅ H ₇₄ O ₁₇	Steroid Saponin	74.97	14.044	886.4984	921.4677	1712802	58665524	-6.49	7
18	9,10-DiHOME	C ₁₈ H ₃₄ O ₄	Long-chain fatty acids (Fatty Acyls)	56.96	14.179	314.2493	313.242	6128840	102947048	-11.45	6
19	b-Chlorogenin 3-[2'',4''-dirhamnosyl]glucoside]	C ₄₅ H ₇₄ O ₁₇	Steroid Saponin	33.1	14.32	886.4996	931.4978	827567	16510644	-7.86	10

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hit (DB)
20	MG(0:0/15:0/0:0)	C ₁₈ H ₃₆ O ₄	2-monoacylglycerols (Glycerolipids)	69.01	14.853	316.2652	315.2579	1628781	27913902	-12.04	4
21	16 beta-Fluoro-17beta-hydroxyandrost-4-en-3-onepropionate	C ₂₂ H ₃₁ F O ₃	Steroid ester (PC)	77.88	14.887	362.2283	421.2421	457242	12154753	-6.99	10
22	2,3-Dihydroxycyclopentane undecanoic acid	C ₁₆ H ₃₀ O ₄	Long-chain fatty acid (Fatty acyls) (PC)	70.64	15.11	286.2179	285.2106	1339318	16333962	-12.05	10
23	Strictosamide	C ₂₆ H ₃₀ N ₂ O ₈	Alkaloids (Alkaloids derived from tryptophan and anthranilic acid Indole alkaloids)	82.27	15.338	498.1973	543.1959	648966	10537742	5.88	5
24	Trigoneoside XIb	C ₄₄ H ₇₄ O ₁₉	Steroidal Saponins	56.69	15.408	906.479	905.4739	305835	6741316	3.81	3
25	(9S,10S)-10-hydroxy-9-(phosphonooxy)octadec	C ₁₈ H ₃₇ O ₇ P	Derivative of stearic acid (long-chain fatty	24.72	15.847	396.2225	441.2247	345630	21828600	13.1	10

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hit (DB)
	anoicacid		acids-Fatty Acyls) (PC)								
26	12-Hydroxy-8,10-octadecadienoic acid	C ₁₈ H ₃₂ O ₃	Linoleic acids and derivatives. (long fatty acid) (	53.59	16.055	296.239	295.2316	5209635	110210312	-12.95	10
27	10-Oxo-11-octadecen-13-olide	C ₁₈ H ₃₀ O ₃	Macrolides and analogues	52.89	16.38	294.2234	293.2162	990110	34641684	-13.38	10
28	Hordatine A	C ₂₈ H ₃₈ N ₈ O ₄	2-arylbenzofuran flavonoids (Amino acid related compounds)	77.27	16.948	550.2983	595.2966	348770	9082738	5.94	3
29	21-Fluoroprogesterone	C ₂₁ H ₂₉ F O ₂	Corticosteroid hormone	61.3	17.042	332.2149	331.2098	818894	32515124	0.79	2
30	Pateamine	C ₃₁ H ₄₅ N ₃ O ₄ S	Member of 1,3-thiazoles (marine macrodiolide)	56.84	17.253	555.3158	554.3093	1039709	40695988	-4.85	4

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hit (DB)
31	LysoPE(20:2(11Z,14Z)/0:0)	C ₂₅ H ₄₈ N O ₇ P	1-acyl-sn-glycero-3-phosphoethanolamines (Glycerophospholipids)	56.31	17.269	505.3241	504.3171	676728	19500036	-14.36	2
32	(+)-Prosopinine	C ₁₈ H ₃₅ N O ₃	Sphingolipid (Piperidine alkaloids)	22.19	18.142	313.2568	358.2573	310174	6432205	15.77	1
33	LysoPE(0:0/18:0)	C ₂₃ H ₄₈ N O ₇ P	2-acyl-sn-glycero-3-phosphoethanolamines (Glycerophospholipids)	28.02	17.278	565.3457	480.313	271285	7338300	-7.11	3
34	16-Hydroxy hexadecanoic acid	C ₁₆ H ₃₂ O ₃	Long-chain fatty acids (Fatty Acyls)	64.68	17.417	442.3007	271.2319	399808	7822082	-14.64	1
35	Ganoderic acid H	C ₃₂ H ₄₄ O ₉	Triterpenoid (Prenol lipids)	57.48	17.717	505.3247	571.2966	1365371	61900480	-9.36	6

Results

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hit (DB)
36	9S,12S,13S-trihydroxy-10E- octadecenoic acid	C ₁₈ H ₃₄ O ₅	Octadecanoids (Fatty acyls)	32.52	17.718	555.3162	389.2528	397650	9025109	11.46	3
37	cis-p-Coumaroylcorosolic acid	C ₃₉ H ₅₄ O ₆	Triterpenoid (Prenol lipids)	30.04	20.12	618.4	617.3928	658250	16866968	-12.88	3
38	Hydroquinidine	C ₂₀ H ₂₆ N ₂ O ₂	Alkaloids	66.65	20.208	326.1966	325.1898	442932	20947824	8.53	9
39	PA(P-16:0e/18:2(9Z,12Z))	C ₃₇ H ₆₉ O ₇ P	1-(1Z-alkenyl),2-acyl-glycerophosphates (Glycerophospholipids)	27.83	20.743	656.4849	701.4854	364445	14954972	-10.4	1
40	Hydrocortisone cypionate	C ₂₉ H ₄₂ O ₆	C21 steroids (gluco/mineralocorticoids, progestogens) and derivatives (Sterol lipids)	79.17	20.774	486.2985	485.2913	1056441	48911076	-0.72	5

Results

SR. NO.	Name	Formula	Phytogroup	Score	RT	Mass	m/z	Height	Area	DB Diff (ppm)	Hit (DB)
41	3-Hydroxy-10'-apo-b,y-carotenal	C ₂₇ H ₃₆ O ₂	Sester terpenoid (Prenol lipids)	47.2	20.901	392.2741	391.2691	437290	8575282	-6.47	3
42	Tsangane L 3-glucoside	C ₁₉ H ₃₄ O ₇	Terpene glycoside (Prenol lipids)	99.19	21.433	374.2304	433.2442	1258068	69500272	0.27	1
43	Hydrocortisone cypionate	C ₂₉ H ₄₂ O ₆	C21 steroids (gluco/mineralocorticoids, progestogens) and derivatives (Sterol lipids)	95.71	21.849	486.2992	485.292	649988	30175732	-2.23	5
44	12,15-cis-Squamostatin A	C ₃₇ H ₆₆ O ₈	Annonaceous acetogenins (Fatty Acyls)	63.3	23.307	638.4746	683.4747	570545	24694766	1.83	10

5.6.4.1 Identification and Classification of Metabolites.

For each formulation, a comprehensive list of metabolites was identified in both ESI positive and negative ionization modes. These compounds were systematically classified into groups such as alkaloids, phenols, fatty acyls, flavonoids, macrolides, benzene derivatives, and others. To enhance the accuracy and reliability of the identification process, each compound was cross-referenced against multiple databases. Database identifiers such as KEGG ID, Drug Bank ID, HMDB ID, ChemSpider ID, Lipid ID and PubChem CID were assigned to the identified compounds, as detailed in Tables 72–113 (Formulation 1) and Tables 114–143 (Formulation 2). This cross-referencing not only validated the compound identities but also provided valuable insights into their potential pharmacological relevance.

Classification and Identification of Metabolites in Ethanol Extract (Formulation 1) Using HRLCMS QTOF in Positive ESI Mode

Table 72. Glycosides with Identification Details

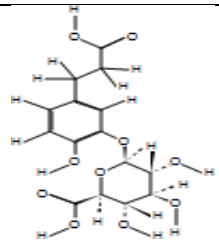
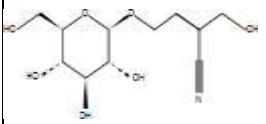
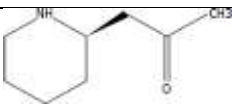
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	Structure
1	Dihydrocaffeic acid 3-O-glucuronide (C ₁₅ H ₁₈ O ₁₀)	Phenolic glycosides	49844972	HMDB0041720	—	
2	Sarmentosin (C ₁₁ H ₁₇ N O ₇)	Cyanogenic glycosides	5281123	—	C08340	

Table 73. Alkaloids with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
3	(R)-Pelletierine (C ₈ H ₁₅ N O)	Piperidines	3034881	HMDB0030324	—	2299257	

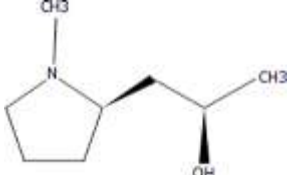
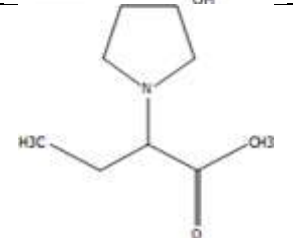
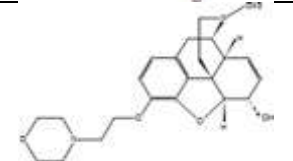
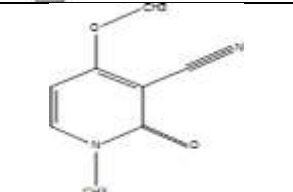
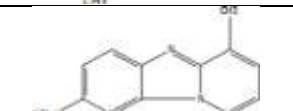
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
4	(-)-Hygroline (C ₈ H ₁₇ N O)	Pyrrolidine alkaloids	442642	—	C10152	—	
5	3-(1-Pyrrolidinyl)-2-pentanone (C ₉ H ₁₇ N O)	N-alkylpyrrolidines	67699479	HMDB0039833	—	35014888	
6	Pholcodine (C ₂₃ H ₃₀ N ₂ O ₄)	Morphinans (Alkaloids and derivatives)	5311356	HMDB0041984	—	4470854	
7	Ricinine (C ₈ H ₈ N ₂ O ₂)	3-pyridinecarbonitriles (pyridine alkaloid)	10666	HMDB0042006	C01526	10216	
8	Glu-P-1 (C ₁₁ H ₁₀ N ₄)	Imidazopyridines	49971	HMDB0029749	C19244	45327	

Table 74. Terpenes with Identification Details

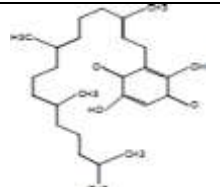
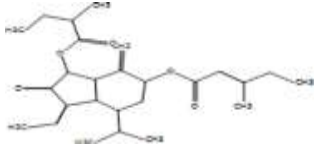
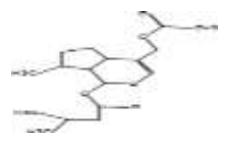
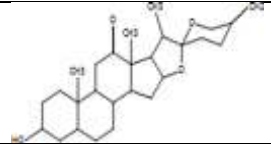
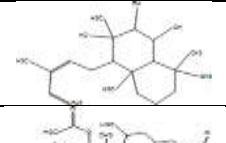
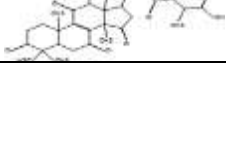
Sr. No.	Compound name	Super class	Pubchem ID	HMDB ID	ChemSpider ID	Structure
1	Boviquinone 4 (C ₂₆ H ₃₆ O ₄)	Diterpenoids	15379535	HMDB0030057	30776806	
2	2-(2-Methylbutanoyl)-9-(3-methyl-2E-pentenoyl)-2b,9a-dihydroxy-4Z,10(14)-oplopadien-3-one (C ₂₆ H ₃₈ O ₅)	Sesquiterpenoids	131753007	HMDB0040994	35015068	
3	Valdiate (C ₁₇ H ₂₆ O ₅)	Iridoid monoterpenoid (Iridoids and derivatives)	129715809	HMDB0040980	35015063	
4	Schidigeragenin B (C ₂₇ H ₄₀ O ₄)	Triterpenoid (Sterol Lipids)	10502675	HMDB0035506	—	
5	Austroinulin (C ₂₀ H ₃₄ O ₃)	Diterpenoids	19073908	HMDB0036802	13936970	
6	Ganoderic acid F (C ₃₂ H ₄₂ O ₉)	Triterpenoid	23247895	HMDB0035988	—	

Table 75. Amino acids and Derivatives with Identification Details

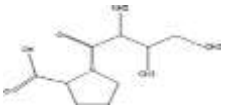
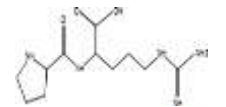
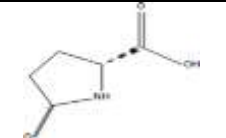
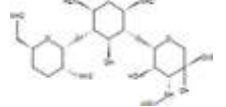
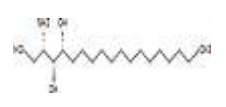
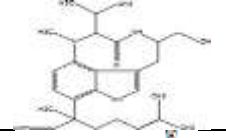
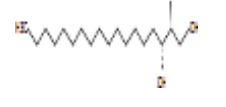
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider Id	DrugBank Id	Structure
1	L-isoleucyl-L-proline (C ₁₁ H ₂₀ N ₂ O ₃)	Dipeptides	444876	HMDB0011174	—	392673	—	
2	Prolyl-Arginine (C ₁₁ H ₂₁ N ₅ O ₃)	Proline Arginine dipeptide	9814075	HMDB0029011	—	7989825	—	
3	(R)-(+)-2-Pyrrolidone-5-carboxylic acid (C ₅ H ₇ N O ₃)	Alpha amino acids and derivatives	439685	HMDB00805	C02237	—	DB03088	
4	Gentamicin C1a (C ₁₉ H ₃₉ N ₅ O ₇)	Aminocyclitol glycosides	582362	HMDB0252688	—	506179	—	
5	Phytosphingosine (C ₁₈ H ₃₉ N O ₃)	1,3-aminoalcohols (Amines)	122121	HMDB0004610	C12144	108921	DB14119	
6	Lyngbyatoxin (C ₂₇ H ₃₉ N ₃ O ₂)	Alpha amino acid amides	91706	—	—	10472344	—	
7	Sphinganine (C ₁₈ H ₃₉ N O ₂)	1,2-aminoalcohols (Amines)	91486	HMDB0000269	C00836	82609	—	

Table 76. Lipids and Derivatives with Identification Details

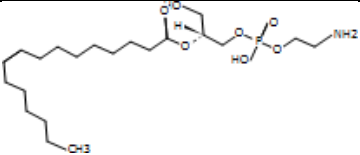
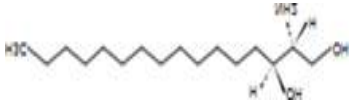
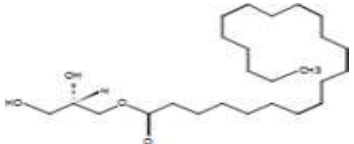
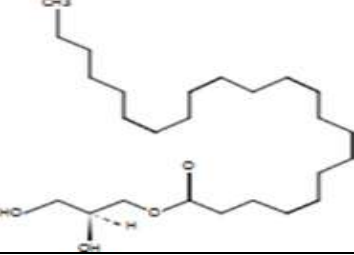
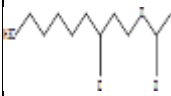
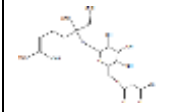
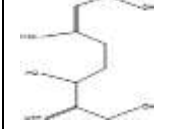
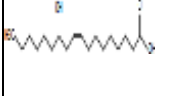
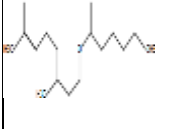
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider Id	Structure
1	LysoPE(0:0/16:1(9Z)) (C ₂₁ H ₄₂ N O ₇ P)	2-acyl-sn-glycero-3-phosphoethanolamines (Glycerophospholipids)	53480923	HMDB0011474	—	24769355	
2	C16 Sphinganine (C ₁₆ H ₃₅ N O ₂)	Hexadecasphinganine (Sphingolipids)	656816	—	C13915	—	
3	MG(22:4(7Z,10Z,13Z,16Z)/0:0/0:0) (C ₂₅ H ₄₂ O ₄)	1-monoacylglycerols (Glycerolipids).	53480992	HMDB0011584	—	—	
4	MG(22:5(4Z,7Z,10Z,13Z,16Z)/0:0/0:0) (C ₂₅ H ₄₀ O ₄)	1-monoacylglycerols (Glycerolipids)	53480993	HMDB0011585	—	24765803	

Table 77. Fatty acyls with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	3-Nonanon-1-yl acetate (C ₁₁ H ₂₀ O ₃)	Fatty alcohol esters	12678469	HMDB0037179	—	23346472	
2	D-Linalool 3-(6"-malonylglucoside) (C ₁₉ H ₃₀ O ₉)	Fatty acyl glycosides of mono- and disaccharides	131752917	HMDB0040729	—	—	
3	6-Methyl-2-methylene-6-octene-1,3,8-triol (C ₁₀ H ₁₈ O ₃)	Fatty alcohols	131752432	HMDB0038692	—	35014639	
4	12-Methyltridecanal (C ₁₄ H ₂₈ O)	Fatty aldehydes (Fatty Acyls)	3018619	HMDB0032432	—	2286111	
5	Ricinoleic acid (C ₁₈ H ₃₄ O ₃)	Long-chain fatty acids (Fatty Acyls)	5282942	HMDB0034297	C08365	4446069	
6	Citronellyl hexanoate (C ₁₆ H ₃₀ O ₂)	Fatty alcohol esters (Fatty Acyls)	114416	HMDB0038958	—	102474	

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
7	Dehydrofalcarinone (C ₁₇ H ₂₀ O)	ynone (Oxygenated hydrocarbons) (Fatty acyls)	5281145	—	—	—	

Table 78. Phenol with Identification Details

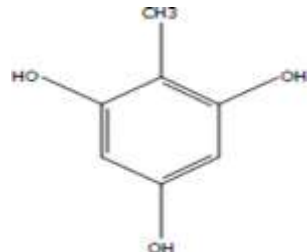
Sr. No.	Compound name	Super class	PubChem ID	KEGG ID	Structure
1	2,4,6-Trihydroxytoluene (C ₇ H ₈ O ₃)	Trihydroxytoluene (Phenols)	66606	C16401	

Table 79. Flavanols with Identification Details

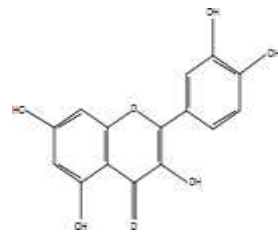
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	Quercetin (C ₁₅ H ₁₀ O ₇)	Flavanols	5280343	HMDB0005794	C00389	4444051	DB04216	

Table 80. Withanolides and Derivatives with Identification Details

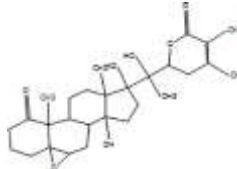
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
1	2,3-Dihydrowithanolide E (C ₂₈ H ₄₀ O ₇)	Withanolides and derivatives	131751517	HMDB0034057	

Table 81. Benzene and Derivatives with Identification Details

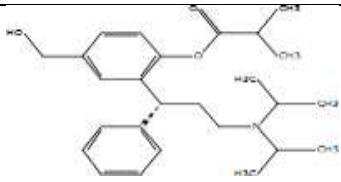
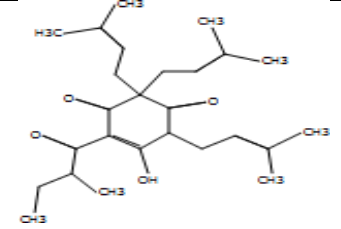
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	DrugBank ID	Structure
1	Fesoterodine (C ₂₆ H ₃₇ N O ₃)	Diphenylmethanes (Benzenoids)	6918558	HMDB0015648	5293755	DB06702	
2	Adlupulone (C ₂₆ H ₃₈ O ₄)	M-benzoquinones	9909740	HMDB0030140	35013143		

Table 82. Esters and Derivatives with Identification Details

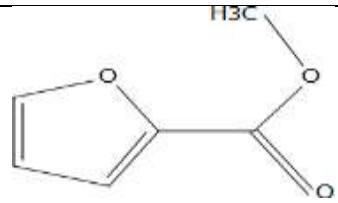
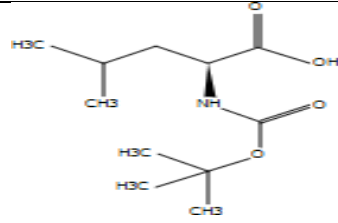
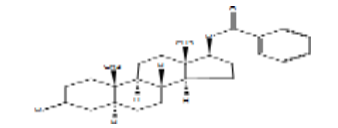
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Methyl 2-furoate (C ₆ H ₆ O ₃)	Furoic acid esters (Furans)	11902	HMDB0029750	—	11408	
2	N(alpha)-t-Butoxycarbonyl-L-leucine (C ₁₁ H ₂₁ N O ₄)	carbamate ester and a L-leucine derivative.	83170	—	C04301	—	
3	Stanolone benzoate (C ₂₆ H ₃₄ O ₃)	Steroid esters	251107	HMDB0258482		219955	

Table 83. Steroids and Derivatives with Identification Details

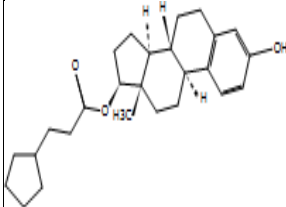
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	Estradiol Cypionate (C ₂₆ H ₃₆ O ₃)	C18 Steroids (Estrogens) and derivatives	9403	HMDB0251962	C14640	9033	DB13954	

Table 84. Carboximic acid with Identification Details

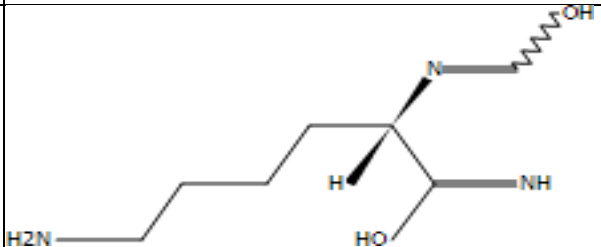
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
1	Apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)] (C ₇ H ₁₅ N ₃ O ₂)	Carboximidic acid	25244460	HMDB0059607	

Table 85. Macrolides and analogues with Identification Details

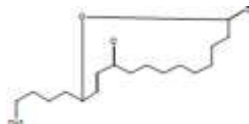
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	10-Oxo-11-octadecen-13-olide (C ₁₈ H ₃₀ O ₃)	Macrolides and analogues	9882599	HMDB0029786	8058274	

Table 86. Herbicide with Identification Details

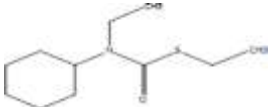
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	Chem Spider ID	Structure
1	Cycloate (C ₁₁ H ₂₁ N O S)	Thiocarbamic acid derivatives	14337	HMDB0250636	C18780	13698	

Table 87. Bile acids with Identification Details

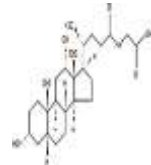
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	Chemspider ID	Structure
1	Deoxycholic acid glycine conjugate (C ₂₆ H ₄₃ N O ₅)	Glycinated bile acids and derivatives	22833539	HMDB0000631	C05464	17215983	

Table 88. Cyclohexanols with Identification Details

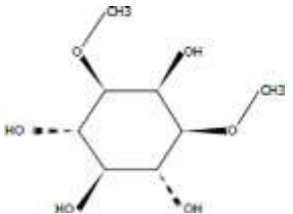
Sr. No.	Compound name	Super class	PubChem ID	Structure
1	Dambonitol (C ₈ H ₁₆ O ₆)	Cyclohexanols	21627888	

Table 89. Insecticide with Identification Details

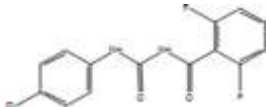
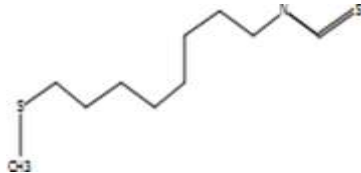
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Diflubenzuron (C ₁₄ H ₉ Cl F ₂ N ₂ O ₂)	N-benzoyl-N'-phenylureas (Benzenoids)	37123	HMDB0031778	C14427	34065	

Table 90. Isothiocyanates with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Chemspider ID	Structure
1	1-Isothiocyanato-8-(methylthio) octane (C ₁₀ H ₁₉ N S ₂)	Isothiocyanates	10171359	HMDB0038447	8346864	

Classification and Identification of Metabolites in Ethanol Extract (Formulation 1) Using HRLCMS QTOF in Negative ESI Mode

Table 91. Glycosides and Derivatives with Identification Details

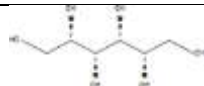
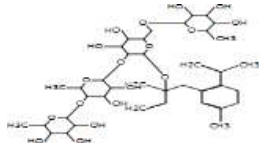
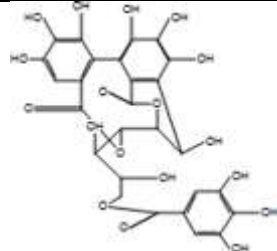
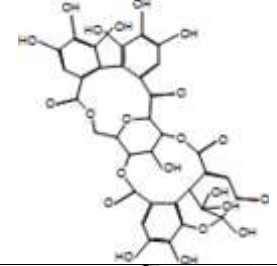
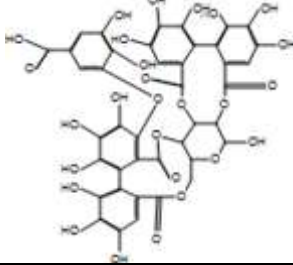
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	Structure
1	L-Iditol (C ₆ H ₁₄ O ₆)	Sugar alcohols	5460044	HMDB0011632	C01507	
2	(3x,5x,10x)-9,10-Didehydroisohumbertiol O-[rhamnosyl-(1->4)-rhamnosyl-(1->2)-[rhamnosyl-(1->6)]-glucoside] (C ₃₉ H ₆₂ O ₁₈)	Oligosaccharides	131752907	HMDB0040687	—	

Table 92. Tannins with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
1	Gallic acid 3-O-(6-galloylglucoside) (C ₂₀ H ₂₀ O ₁₄)	Hydrolyzable tannins	73157749	HMDB003932 5	

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
2	Punicacortein B (C ₂₇ H ₂₂ O ₁₈)	Hydrolyzable tannins	20056239	HMDB003920 1	
3	Granatin A (C ₃₄ H ₂₄ O ₂₂)	Hydrolyzable tannins	131752596	HMDB003927 1	
4	Sanguiin H ₁₁ (C ₄₁ H ₂₈ O ₂₇)	Hydrolyzable tannins	85368941	HMDB003926 0	

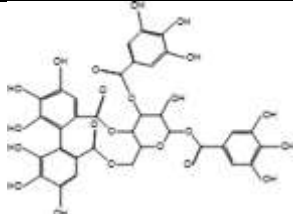
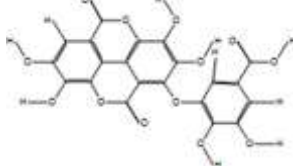
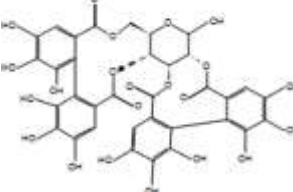
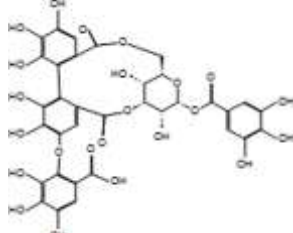
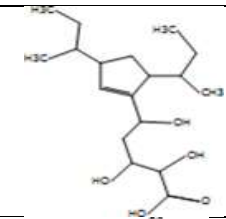
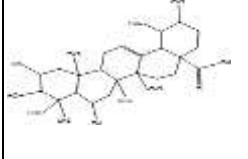
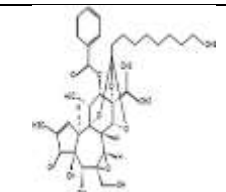
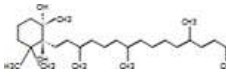
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
5	Heterophyllin A (C ₃₄ H ₂₆ O ₂₂)	Hydrolyzable tannins	471120	HMDB003926 4	
6	Sanguisorbic acid dilactone (C ₂₁ H ₁₀ O ₁₃)	Hydrolyzable tannins	15934028	HMDB002927 4	
7	Pedunculagin (C ₃₄ H ₂₄ O ₂₂)	Ellagitannins	442688	—	
8	Mallotinic acid (C ₃₄ H ₂₆ O ₂₃)	Tannin	10056140	—	

Table 93. Terpenoids with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	Chemspider ID	Lipid ID	Structure
1	Auxin a (C ₁₈ H ₃₂ O ₅)	Monocyclic Monoterpenoids	92772	HMDB0038483	—	83749	—	
2	Madasiatic acid (C ₃₀ H ₄₈ O ₅)	Triterpenoids	23132225	HMDB0035118	—	16161196	—	
3	Gnididilatin (C ₃₇ H ₄₈ O ₁₀)	Diterpenoid	442032	—	C09101	—	—	
4	Azafrin (C ₂₇ H ₃₈ O ₄)	Triterpenoid	73744576	HMDB0035258	—	35013886	—	

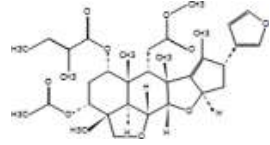
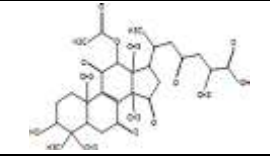
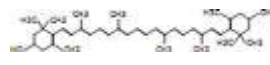
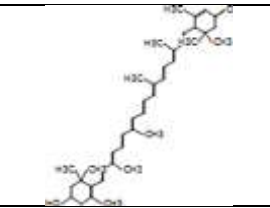
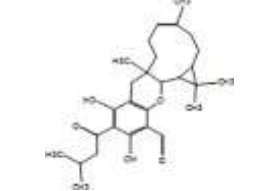
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	Chemspider ID	Lipid ID	Structure
5	Salannin (C ₃₄ H ₄₄ O ₉)	Triterpenoids	6437066	—	—	—	—	
6	Ganoderic acid H (C ₃₂ H ₄₄ O ₉)	Triterpenoid	73657194	HMDB0035987	—	—	—	
7	Alloxanthin (C ₄₀ H ₅₂ O ₂)	Diterpenoid	6443740	—	—	—	LMPR01070053	
8	3-Hydroxy-b, e-caroten-3'-one (C ₄₀ H ₅₄ O ₂)	Xanthophylls (Tetraterpenoids)	5471692	HMDB0003448	—	4581803	—	
9	Euglobal VII (C ₂₈ H ₃₈ O ₅)	Germacrane Sesquiterpenoids	131750947	HMDB0030037	—	—	—	

Table 94. Alkaloids and Derivatives with Identification Details

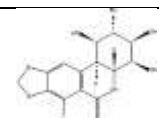
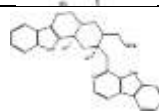
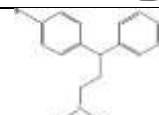
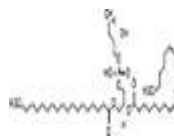
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	Pancratistatin (C ₁₄ H ₁₅ N O ₈)	Isoquinoline alkaloid	441597	—	C08535	—	—	
2	Usambarensine (C ₂₉ H ₂₈ N ₄)	Harmala alkaloid	5281413	—	—	—	—	
3	Brompheniramine (C ₁₆ H ₁₉ Br N ₂)	Pheniramines (Pyridines and derivatives)	6834	HMDB0001941	—	6573	DB00835	

Table 95. Lipids and Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	Structure
1	PG(18:0/20:3(8Z, 11Z,14Z)) (C ₄₄ H ₈₁ O ₁₀ P)	Phosphatidylglycerols (Glycerophospholipids)	52927150	HMDB0010609	—	24768108	—	

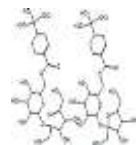
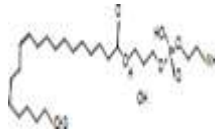
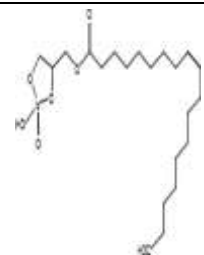
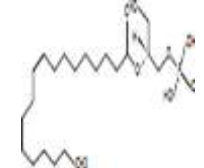
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	Structure
2	6-O-Oleuropeoylsucrose (C ₂₂ H ₃₆ O ₁₃)	Saccharolipids	131752310	HMDB0038117	—	—	—	
3	LysoPE (20:2(11Z,14Z)/0:0) (C ₂₅ H ₄₈ N O ₇ P)	1-acyl-sn-glycerol-3-phosphoethanolamines (Glycerophospholipids)	52925140	HMDB0011513	—	24769391	LMGP02050021	
4	CPA(18:1(9Z)/0:0) (C ₂₁ H ₃₉ O ₆ P)	1-acyl-sn-glycerol-2,3-cyclic-phosphates (Glycerophospholipids)	11962187	HMDB0007006	—	10136430	—	
5	LPA(0:0/18:2(9Z,12Z)) (C ₂₁ H ₃₉ O ₇ P)	2-acylglycerol-3-phosphates (Glycerophospholipids)	53478601	HMDB07852	C00416	—	LMGP10050044	

Table 96. Fatty acyls with Identification Details

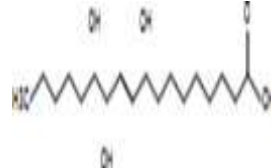
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	9S,12S,13S-trihydroxy-10E-octadecenoic acid (C ₁₈ H ₃₄ O ₅)	Long-chain fatty acids (Fatty acyls)	9858729	HMDB0004708	C14833	8034429	

Table 97. Flavanols with Identification Details

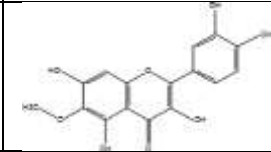
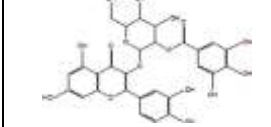
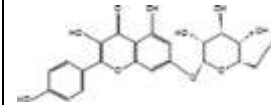
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Patuletin (C ₁₆ H ₁₂ O ₈)	Flavonols	5281678	HMDB0030802	C10118	—	
2	Quercetin 3-(2-galloylglucoside) (C ₂₈ H ₂₄ O ₁₆)	Flavonoid-3-O-glycosides	13889202	HMDB0037367	—	—	
3	Kaempferol 7-O-glucoside (C ₂₁ H ₂₀ O ₁₁)	Flavonoid-7-O-glycosides	10095180	HMDB0303599	—	8270716	

Table 98. Phenols with Identification Details

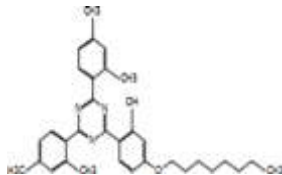
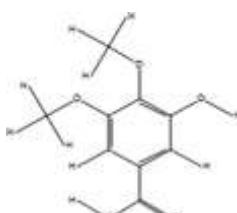
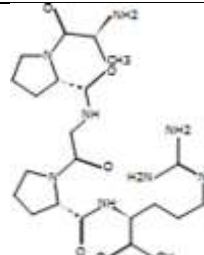
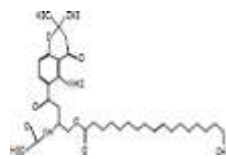
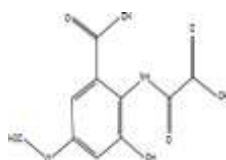
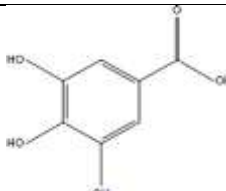
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	2-[4,6-Bis(2,4-dimethylphenyl)-1,3,5-triazin-2-yl]-5-(octyloxy) phenol (C ₃₃ H ₃₉ N ₃ O ₂)	Phenol ethers	135414248	HMDB0037802	—	10632726	

Table 99. Benzene and Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	3,4-O-Dimethylgallic acid (C ₉ H ₁₀ O ₅)	Gallic acid and derivatives	74709	HMDB0041662	—	67282	

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
2	APGPR Enterostatin (C ₂₁ H ₃₆ N ₈ O ₆)	Benzoyl derivatives	3082883	HMDB0006117	—	2340236	
3	3'-N-Acetyl-4'-O-(9-octadecenoyl) fusarochromanone (C ₃₅ H ₅₄ N ₂ O ₆)	2,2-dimethyl-1-benzopyrans (Benzopyrans).	102146306	HMDB0038567	—	35014605	
4	N-Pyruvoyl-5-methoxy-3-hydroxyanthranilate (C ₁₁ H ₁₁ N O ₆)	Methoxybenzoic acid.	443220	—	C11467	—	
5	Gallic acid (C ₇ H ₆ O ₅)	Gallic acid (Benzoic acids and derivatives) Flavonoids	370	HMDB0005807	C01424	361	

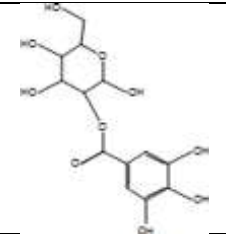
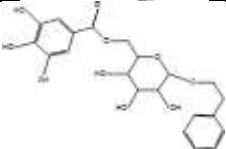
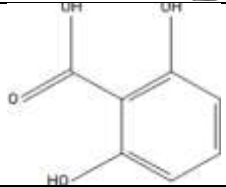
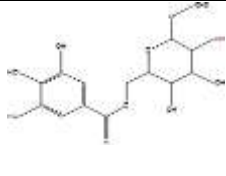
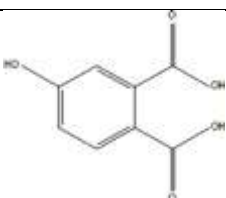
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
6	2-Galloylglucose (C ₁₃ H ₁₆ O ₁₀)	Galloyl esters (Benzoic acids and derivatives)	14034252	HMDB0039625	—	—	
7	Phenethyl 6-galloylglucoside (C ₂₁ H ₂₄ O ₁₀)	Galloyl esters	468285	HMDB0034573	—	—	
8	2,6-dihydroxybenzoic acid (C ₇ H ₆ O ₄)	Salicylic acids (Benzoic acids and derivatives)	9338	HMDB0013676	—	8974	
9	Methyl 6-O-galloyl-beta-D-glucopyranoside (C ₁₄ H ₁₈ O ₁₀)	Galloyl esters	5319658	—	—	35014782	
10	4-Hydroxyphthalate (C ₈ H ₆ O ₅)	Member of phenols and a hydroxybenzoic acid	11881	—	C06680	—	

Table 100. Steroids with Identification Details

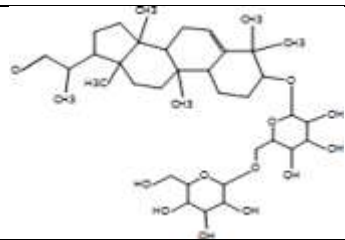
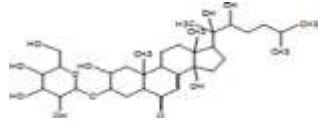
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	Momordicoside E (C ₃₇ H ₆₀ O ₁₂)	Steroidal glycosides (Steroid Saponin)	131751850	HMDB0035697	—	
2	Ponasteroside A (C ₃₃ H ₅₄ O ₁₁)	Steroidal glycosides (Steroid saponin).	12314455	HMDB0034091	19990519	

Table 101. Hydroxy acids and Derivatives with Identification Details

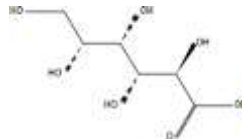
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Gulonic acid (C ₆ H ₁₂ O ₇)	Medium-chain hydroxy acids and derivatives	152304	HMDB0003290	C00800	134243	

Table 102. Alpha amino acids and Derivatives with Identification Details

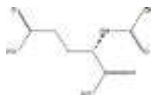
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	N-Acetyl-L-glutamic acid (C ₇ H ₁₁ N O ₅)	Glutamic acid and derivatives	70914	HMDB0001138	C00624	64077	DB04075	

Table 103. Cyclic alcohols and Derivatives with Identification Details

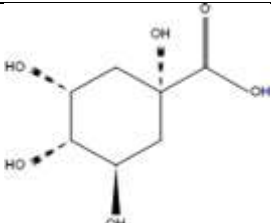
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Quinic acid (C ₇ H ₁₂ O ₆)	Quinic acids and derivatives	6508	HMDB0003072	C00296	10246715	

Table 104. Ketones with Identification Details

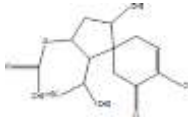
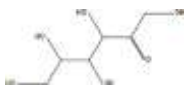
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	8-Acetoxy-4-acoren-3-one (C ₁₇ H ₂₆ O ₃)	Cyclohexenones	85288994	HMDB0030974	—	35013295	
2	Cpd 3: L-Sorbose; C ₆ H ₁₂ O ₆	Ketoses (Monosaccharides)	439192	—	C00247	—	

Table 105. Hydroxy cinnamic acid esters with Identification Details

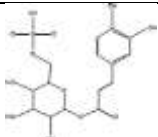
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
1	1-O-Caffeoyl-(b-D-glucose 6-O-sulfate) (C ₁₅ H ₁₈ O ₁₂ S)	Hydroxy cinnamic acid glycosides	131753055	HMDB0041173	

Table 106. Furans with Identification Details

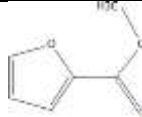
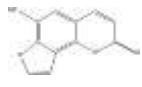
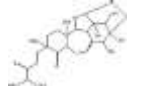
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Methyl 2-furoate ($C_6 H_6 O_3$)	Furoic acid esters	11902	HMDB0029750	—	11408	
2	6-Hydroxyangelicin ($C_{11} H_6 O_4$)	Furanocoumarin	10465441	—	C18085	—	
3	TS-TM-calix (4)arene ($C_{32} H_{32} O_{16} S_4$)	Arene sulfonic acid and a substituted calixarene	656718	—	C13704	—	
4	Erinacine G ($C_{25} H_{36} O_8$)	Furopyran	10552092	—	—	—	

Table 107. Fungicide with Identification Details

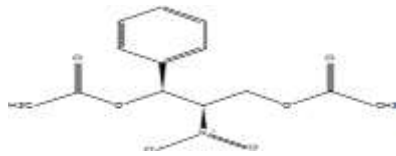
Sr. No.	Compound name	Super class	PubChem ID	KEGG ID	Structure
1	Fenitropan ($C_{13} H_{15} N O_6$)	Amphetamines (Fungicides)	93358	C18938	

Table 108. Herbicide with Identification Details

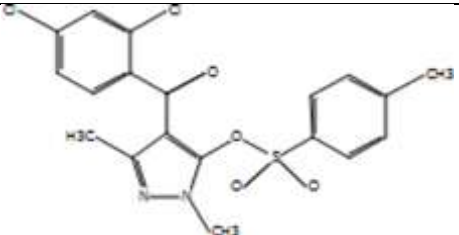
Sr. No.	Compound name	Super class	PubChem ID	KEGG ID	Structure
1	Pyrazolate (C ₁₉ H ₁₆ C ₁₂ N ₂ O ₄ S)	Pyrazole herbicides	42619	C11123	

Table 109. Cyclic alcohols and Derivatives with Identification Details

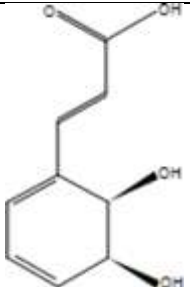
Sr. No.	Compound name	Super class	PubChem ID	KEGG ID	Structure
1	cis-3-(3-Carboxyethenyl)-3,5-cyclohexadiene-1,2-diol (C ₉ H ₁₀ O ₄)	Cyclic alcohols and derivatives	5282145	C12622	

Table 110. Carboxylic acid and Derivatives with Identification Details

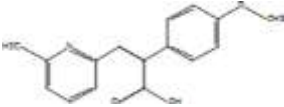
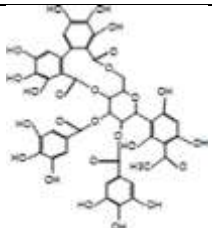
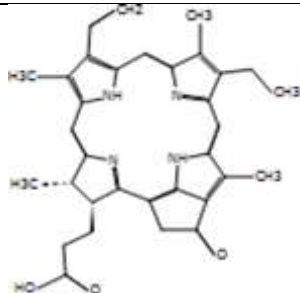
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	Structure
1	alpha-(p-Methoxyphenyl)-6-methyl-2-pyridineacrylic acid (C ₁₆ H ₁₅ N O ₃)	Monocarboxylic acid	6300471	—	C15122	
2	3'-(2'',3''-Digalloyl-4'',6''-hexahydroxydiphenoyl)glucosyl)-phloroacetophenone (C ₄₂ H ₃₂ O ₂₅)	Tetracarboxylic acids and derivatives	131752875	HMDB0040628	—	

Table 111. Tetra pyrroles & derivative with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	KEGG ID	Structure
1	Pyro pheophorbide a (C ₃₃ H ₃₄ N ₄ O ₃)	Chlorins	161456	C18064	

Classification and Identification of Metabolites in Ethanol Extract (Formulation 2) Using HRLCMS QTOF in Positive ESI Mode

Table 112. Glycosides with Identification Details

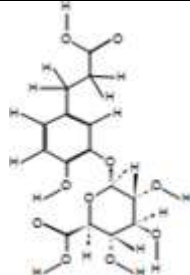
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	Dihydrocaffeic acid 3-O-glucuronide (C ₁₅ H ₁₈ O ₁₀)	Phenolic glycosides	49844972	HMDB0041720	30777618	

Table 113. Coumarin and Derivatives with Identification Details

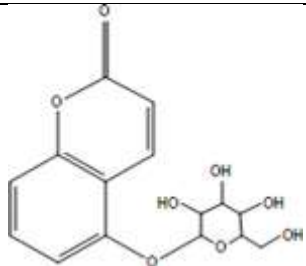
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
1	Mahaleboside (C ₁₅ H ₁₆ O ₈)	Coumarin glycosides	131751353	HMDB0032984	

Table 114. Amino acids and Derivatives with Identification Details

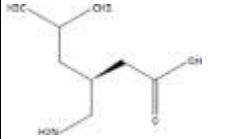
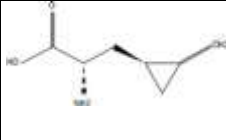
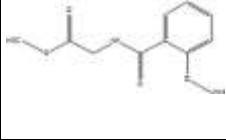
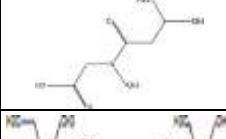
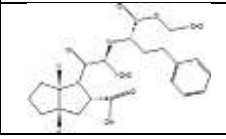
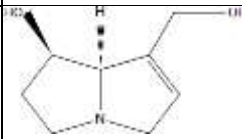
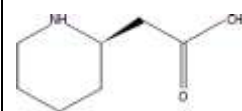
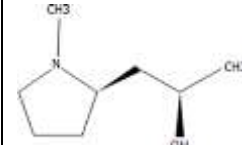
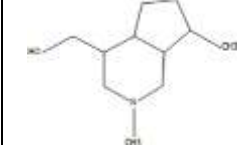
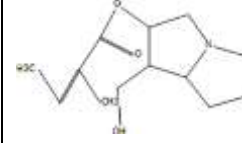
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	Pregabalin (C ₈ H ₁₇ N O ₂)	Gamma amino acids and derivatives (GABA)	5486971	HMDB0014375	—	4589156	DB00230	
2	Hypoglycin (C ₇ H ₁₁ N O ₂)	Alpha amino acids (Phytotoxins)	9081	HMDB0029427	C08287	8728	—	
3	Methyl o-methoxyhippuric acid (C ₁₁ H ₁₃ N O ₄)	Amino acid derivative (N-acyl-amino acid)	—	—	—	11854286	—	
4	Isovalerylsarcosine (C ₈ H ₁₅ N O ₃)	N-acyl-alpha amino acids	—	HMDB0002087	—	121068	—	
5	Myxalamid C (C ₂₄ H ₃₇ N O ₃)	Fatty amide (Non-ribosomal peptide/polyketide hybrids)	11954001	—	C12160	—	—	
6	Ramipril (C ₂₃ H ₃₂ N ₂ O ₅)	Dipeptides	5362129	HMDB0014324	—	4514937	DB00178	

Table 115. Alkaloids with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	Retronecine (C ₈ H ₁₃ N O ₂)	Pyrrolizidine alkaloids	10198	—	C06177	—	—	
2	(R)-Pelletierine (C ₈ H ₁₅ N O)	Piperidine alkaloids	92987	HMDB0030324	C06182	2299257	—	
3	(-)-Hygroline (C ₈ H ₁₇ N O)	Alkaloides & derivatives	442642	—	—	237999	—	
4	Tecostanine (C ₁₁ H ₂₁ N O)	Member of piperidines (ALKALOIDES)	120773	—	C09989	—	—	
5	Petasinine (C ₁₃ H ₂₁ N O ₃)	Pyrrolizidines	131751042	HMDB0030516	—	35013218	—	

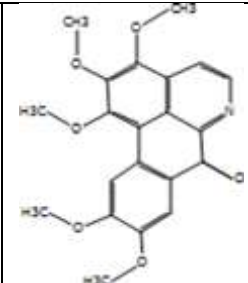
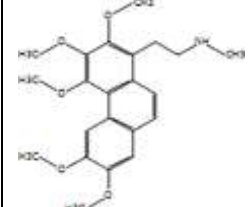
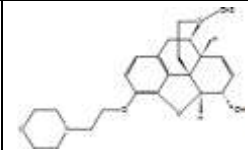
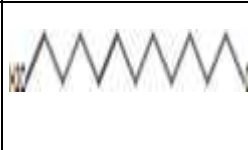
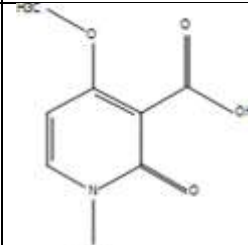
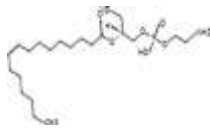
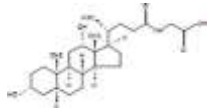
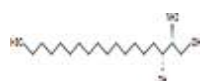
6	Oxopurpureine (C ₂₁ H ₁₉ N O ₆)	Aporphines (Isoquinoline alkaloid)	284998	HMDB0033522	—	251181	—	
7	Thalicpureine (C ₂₂ H ₂₇ N O ₅)	6,6a-secoaporphines (Alkaloids and derivatives)	10430190	HMDB0033032	—	8605618	—	
8	Pholcodine (C ₂₃ H ₃₀ N ₂ O ₄)	Morphinans (Alkaloids and derivatives)	5311356	HMDB0041984	—	4470854	DB09209	
9	(Z)-1,5-Tridecadiene (C ₁₃ H ₂₄)	Alkadiene	12793708	HMDB0041081	—	30777533	—	
10	3-Carboxy-4-methoxy-N-methyl-2-pyridone (C ₈ H ₉ N O ₄)	Member of Methylpyridines and a Pyridone	440344	—	—	—	—	

Table 116. Lipids and Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	Structure
1	LysoPE(0:0/16:1(9 Z)) (C ₂₁ H ₄₂ N O ₇ P)	2-acyl-sn-glycero-3-phosphoethanolamines (Glycerophospholipids)	53480923	HMDB0011474	—	24769355	—	
2	Deoxycholic acid glycine conjugate (C ₂₆ H ₄₃ N O ₅)	Sterol Lipids, (bile acid derivatives) Glycine conjugates	3035026	—	C05464	—	LMST05030006	
3	1-Monopalmitin (C ₁₉ H ₃₈ O ₄)	Glycerolipids (1-monoacylglycerols)	—	—	—	14201	—	
4	Sphinganine (C ₁₈ H ₃₉ N O ₂)	1,2-aminoalcohols (Sphingolipids)	91486	HMDB0000269	C00836	82609	—	
5	1-Oleoyl Lysophosphatidic Acid (C ₂₁ H ₄₁ O ₇ P)	1-acylglycerol-3-phosphates (Glycerophospholipids)	5311263	HMDB07855	—	—	LMGP10050008	

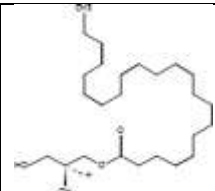
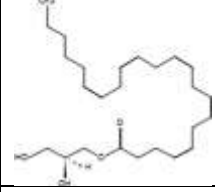
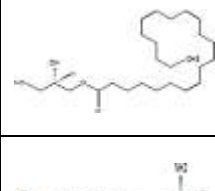
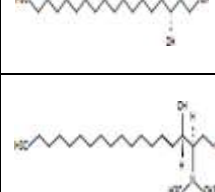
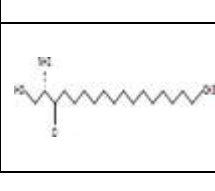
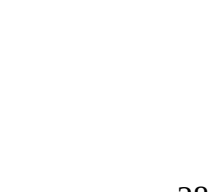
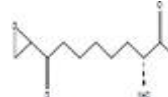
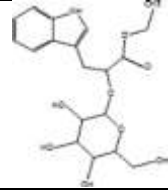
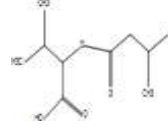
6	MG(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0/0:0) (C ₂₅ H ₃₈ O ₄)	1-monoacylglycerols (Glycerolipids)	53480995	HMDB0011587	—	—	—	
7	MG(22:5(4Z,7Z,10Z,13Z,16Z)/0:0/0:0) (C ₂₅ H ₄₀ O ₄)	2-monoacylglycerols (Glycerolipids)	53480970	HMDB0011555	—	—	—	
8	MG(22:4(7Z,10Z,13Z,16Z)/0:0/0:0) (C ₂₅ H ₄₂ O ₄)	1-monoacylglycerols (Glycerolipids)	53480992	HMDB0011584	—	—	—	
9	Sphinganine (C ₁₈ H ₃₉ N O ₂)	1,2-aminoalcohols (Sphingolipids)	91486	HMDB0000269	C00836	82609	—	
10	N, N-Dimethylsphingosine (C ₂₀ H ₄₁ N O ₂)	1,2-aminoalcohols (Sphingolipids)	5282309	HMDB0013645	C13914	4445480	—	
11	3-Ketosphinganine (C ₁₈ H ₃₇ N O ₂)	Beta-hydroxy ketones (Sphingolipids) Sphinganines	439853	HMDB0001480	C02934	388895	LMSP01020002	

Table 117. Fatty acyls with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	DrugBank ID	Structure
1	(Z)-4-Dodecenal (C ₁₃ H ₂₄ O)	Fatty aldehydes (Fatty acyl)	10976419	HMDB0032247	—	9151620	LMFA06000256	—	
2	2-amino-8-oxo-9,10-epoxy-decanoic acid (C ₁₀ H ₁₇ N O ₄)	Fatty Acyls (Oxo fatty acids)	16061039	—	C12027	—	LMFA01060176	—	
3	Ethyl 2-hydroxy-3-(3-indolyl)propanoate glucoside (C ₁₉ H ₂₅ N O ₈)	Fatty acyl glycosides of mono- and disaccharides	131751633	HMDB0034873	—	—	—	—	
4	R-2-Hydroxy-3-methylbutanoic acid 3-Methylbutanoyl (C ₁₀ H ₁₈ O ₄)	Fatty acid esters (Fatty Acyl)	22922043	HMDB0031510	—	28505508	LMFA07011009	—	
5	11-amino-undecanoic acid (C ₁₁ H ₂₃ N O ₂)	Amino fatty acids (fatty acyl)	17083	—	C19325	—	LMFA01100004	—	

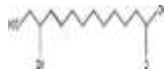
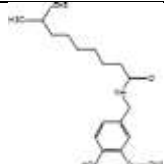
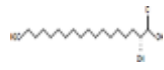
6	11-Hydroxy-9-tridecenoic acid (C ₁₃ H ₂₄ O ₃)	Long-chain fatty acids (fatty acyls)	5318367	HMDB0035881	—	4476964	LMFA01050437	—	
7	Palmitic Acid (C ₁₆ H ₃₂ O ₂)	Long-chain fatty acids (Fatty acyls) Saturated fatty acids	985	—	C00249	960	—	DB03796	
8	Stearic acid (C ₁₈ H ₃₆ O ₂)	Long-chain fatty acids (Fatty acyl)	5281	HMDB0000827	C01530	5091	—	DB03193	
9	Dihydrocapsaicin (C ₁₈ H ₂₉ N O ₃)	methoxyphenols, capsaicinoid, fatty amides (fatty acyl)	107982	HMDB0038457	C16952	97096	LMFA08020318	—	
10	Oleoyl Ethanolamide (C ₂₀ H ₃₉ N O ₂)	N-acylethanolamines (Fatty acyls)	5283454	HMDB0002088	—	4446574	LMFA08040015	—	
11	2R-hydroxy-stearic acid (C ₁₈ H ₃₆ O ₃)	Hydroxy fatty acids (fatty acyls)	439885	—	C03042	—	LMFA02000195	—	
12	Oleoyl Ethanolamide (C ₂₀ H ₃₉ N O ₂)	N-acylethanolamines (Fatty acyls)	5283454	HMDB0002088	—	4446574	LMFA08040015	—	

Table 118. Terpenoids with Identification Details

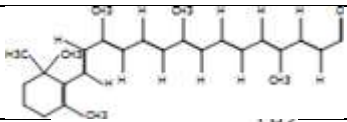
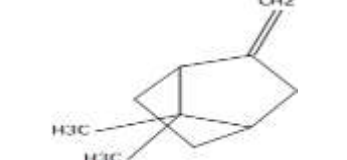
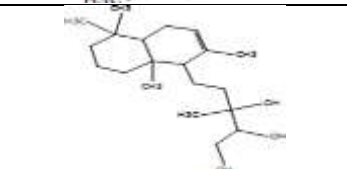
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	10'-apo-beta-carotenal (C ₂₇ H ₃₆ O)	Sesterterpenoids (apo carotenoid)	6450190	HMDB0059605	4952807	
2	(-)-7,7-Dimethyl-2-methylenebicyclo [2.2.1] heptane (C ₁₀ H ₁₆)	Bicyclic monoterpenoids	28930	HMDB0035625	26910	
3	(13R,14R)-7-Labdene-13,14,15-triol (C ₂₀ H ₃₆ O ₃)	Diterpenoids	340130	HMDB0034958	301536	

Table 119. Heteroaromatic compound with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	1,4-Ipomeadiol (C ₉ H ₁₄ O ₃)	Heteroarene (Heteroaromatic compounds)	13186539	HMDB0030471	19991118	

Table 120. Steroids and Derivatives with Identification Details

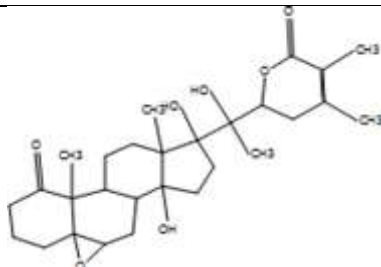
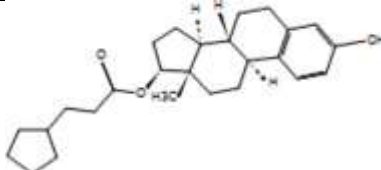
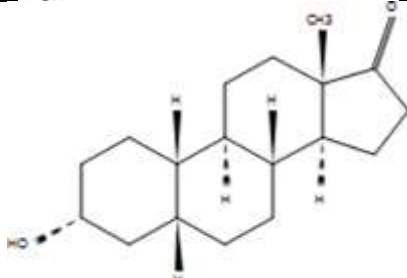
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	2,3-Dihydrowithanolide E (C ₂₈ H ₄₀ O ₇)	Withanolide (Steroid lactones)	131751517	HMDB0034057	—	
2	Estradiol Cypionate (C ₂₆ H ₃₆ O ₃)	Steroid ester (hormonal agent)	9403	—	—	
3	19-Noretiocholanolone (C ₁₈ H ₂₈ O ₂)	Estrogens and derivatives	14009228	HMDB0005886	19347817	

Table 121. Ester and Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	R-2-Hydroxy-3-methylbutanoic acid 3-Methylbutanoyl (C ₁₀ H ₁₈ O ₄)	Fatty acid esters	22922043	HMDB0031510	28505508	
2	Citronellyl hexanoate (C ₁₆ H ₃₀ O ₂)	Carboxylic ester (Fatty alcohol esters)	114416	HMDB0038958	102474	
3	Methyl 2-(10-heptadecenyl)-6-hydroxybenzoate (C ₂₅ H ₄₀ O ₃)	O-Hydroxybenzoic acid esters (phenols and a benzoate ester)	131752389	HMDB0038523	30777263	
4	Dioctyl hexanedioate (C ₂₂ H ₄₂ O ₄)	Carboxylic ester (Fatty alcohol esters)	31271	HMDB0041619	29011	

Table 122. Benzene and Substituted Derivatives with Identification Details

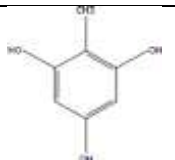
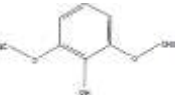
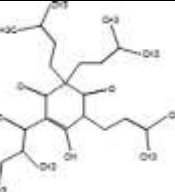
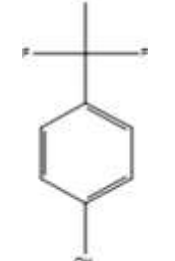
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	2,4,6-Trihydroxytoluene (C ₇ H ₈ O ₃)	Methoxyphenols	71648	HMDB0132905	C16401	64709	—	
2	2,6-Dimethoxyphenol (C ₈ H ₁₀ O ₃)	Methoxyphenols	7041	HMDB0034158	C10787	6774	—	
3	Adlupulone (C ₂₆ H ₃₈ O ₄)	M-benzoquinones	9909740	HMDB0030140	—	35013143	—	
4	Para-Trifluoromethylphenol (C ₇ H ₅ F ₃ O)	Trifluoromethyl benzenes	67874	HMDB0014023	—	61191	DB03610	

Table 123. Unsaturated hydrocarbons with Identification Details

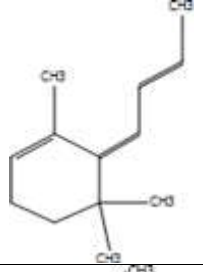
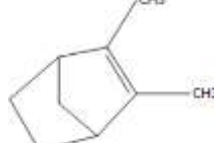
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	(6E,8E)-4,6,8-Megastigmatriene (C ₁₃ H ₂₀)	Branched unsaturated hydrocarbons	5369483	HMDB0035180	4520530	
2	Santene (C ₉ H ₁₄)	Branched unsaturated hydrocarbons	10720	HMDB0038140	10269	

Table 124. Macrolides and Analogues with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	Oxacyclotetradecan-2-one (C ₁₃ H ₂₄ O ₂)	Macrolides and analogues	15596	HMDB0040451	14837	

Table 125. Keto acids and Derivatives with Identification Details

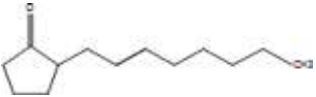
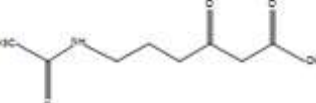
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	3-Methylcyclopentadecanone (C ₁₆ H ₃₀ O)	Cyclic ketones	10947	HMDB0034181	—	10483	
2	(E)-2-(2-Octenyl) Cyclopentanone (C ₁₃ H ₂₂ O)	Cyclic ketones	5367848	HMDB0038897	—	—	
3	6-Acetamido-3-oxohexanoate (C ₈ H ₁₃ N O ₄)	Medium-chain keto acids and derivatives	194080	HMDB0012150	C05548	168403	

Table 126. Insecticide with Identification Details

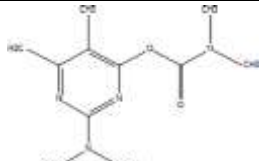
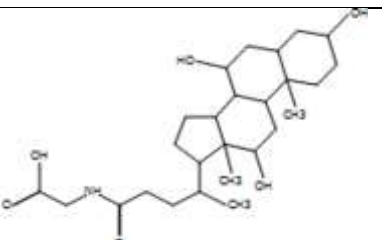
Sr. No.	Compound name	Super class	PubChem ID	KEGG ID	ChemSpider ID	Structure
1	Pirimicarb (C ₁₁ H ₁₈ N ₄ O ₂)	Carbamate insecticides (Dialkylarylamines)	31645	C11079	29348	

Table 127. Bile Salt with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	ChemSpider ID	Structure
1	Sodium Glycocholate (C ₂₆ H ₄₃ N O ₆)	Bile salt	23670522	2016449	

Classification and Identification of Metabolites in Ethanol Extract (Formulation 2) Using HRLCMS QTOF in Negative ESI Mode

Table 128. Glycosides with Identification Details

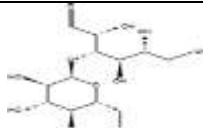
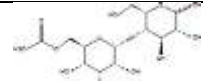
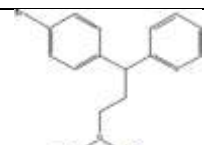
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Nigerose (Sakebiose) (C ₁₂ H ₂₂ O ₁₁)	Glycosylglucose (o-glycosyl)	97050062	HMDB0029882	C01518	30776797	
2	(3x,5x,10x)-9,10-Didehydroisohumbertiol O-[rhamnosyl-(1->4)-rhamnosyl-(1->2)-[rhamnosyl-(1->6)]-glucoside] (C ₃₉ H ₆₂ O ₁₈)	Oligosaccharide	131752907	HMDB0040687	—	—	
3	Acetyl-maltose (C ₁₄ H ₂₄ O ₁₂)	O-glycosyl (glycoside)	10552092	—	—	—	

Table 129. Alkaloids with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	Brompheniramine (C ₁₆ H ₁₉ Br N ₂)	Pyridines and derivatives (pheniramine)	6834	HMDB0001941	C06857	6573	DB00835	

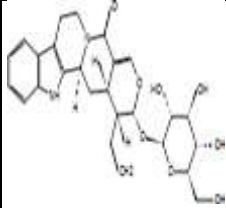
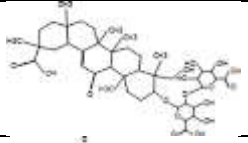
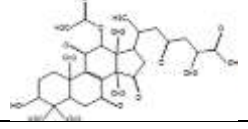
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
2	Strictosamide (C ₂₆ H ₃₀ N ₂ O ₈)	Alkaloids (Alkaloids derived from tryptophan and anthranilic acid Indole alkaloids)	10345799	—	C16724	—	—	
3	(+)-Prosopinine (C ₁₈ H ₃₅ N O ₃)	Sphingolipid (Piperidine alkaloids)	442654	—	C10167	—	—	

Table 130. Terpenoids and Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
1	Licorice Saponin G2 (C ₄₂ H ₆₂ O ₁₇)	Triterpenoid Saponin (Terpene glycosides)	14891565	HMDB0039318	—	
2	Ganoderic acid H (C ₃₂ H ₄₄ O ₉)	Triterpenoid (Prenol lipids)	73657194	HMDB0035987	—	

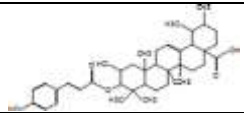
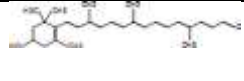
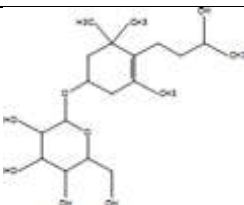
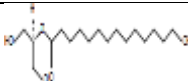
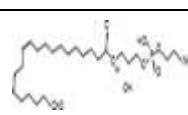
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	ChemSpider ID	Structure
3	cis-p-Coumaroylcorosolic acid (C ₃₉ H ₅₄ O ₆)	Triterpenoid (Prenol lipids)	14335959	HMDB0040495	35014949	
4	3-Hydroxy-10'-apo-b,y-carotenal (C ₂₇ H ₃₆ O ₂)	Sester terpenoid (Prenol lipids)	87443544	HMDB0039019	35014718	
5	Tsangane L 3-glucoside (C ₁₉ H ₃₄ O ₇)	Terpene glycoside (Prenol lipids)	73981648	HMDB0040824	—	

Table 131. Lipids and Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	Structure
1	MG(0:0/15:0/0:0) (C ₁₈ H ₃₆ O ₄)	2-monoacylglycerols (Glycerolipids)	537297	HMDB0011532	—	467962	—	
2	LysoPE (20:2(11Z,14Z)/0:0) (C ₂₅ H ₄₈ N O ₇ P)	1-acyl-sn-glycero-3-phosphoethanolamines (Glycerophospholipids)	52925140	HMDB0011513	—	24769391	LMGP02050021	

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	Structure
3	LysoPE(0:0/18:0) (C ₂₃ H ₄₈ N O ₇ P)	2-acyl-sn-glycero-3-phosphoethanolamines (Glycerophospholipids)	53480667	HMDB0011129	—	—	—	
4	PA(P-16:0e/18:2(9Z,12Z)) (C ₃₇ H ₆₉ O ₇ P)	1-(1Z-alkenyl),2-acylglycerophosphates (Glycerophospholipids)	52929743	HMDB0011155	C15647	24766521	—	
5	MG(0:0/15:0/0:0) (C ₁₈ H ₃₆ O ₄)	2-monoacylglycerols (Glycerolipids)	537297	HMDB0011532	—	467962	—	

Table 132. Fatty acyls with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	Structure
1	9S,12S,13S-trihydroxy-10E-octadecenoic acid (C ₁₈ H ₃₄ O ₅)	Pinellic acid, Fatty Acyls [FA]	9858729	HMDB0004708	C14833	8034429	LMFA02000014	
2	11S-HpODE (C ₁₈ H ₃₂ O ₄)	lineolic acids and derivatives (Fatty Acyls)	5281026	HMDB0062281	C07338	—	LMFA02000115	

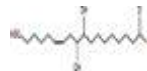
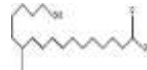
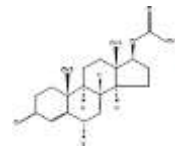
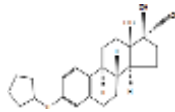
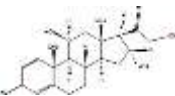
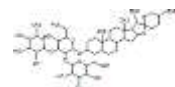
3	9,10-DiHOME (C ₁₈ H ₃₄ O ₄)	Long-chain fatty acids (Fatty Acyls)	9966640	HMDB0004704	C14828	26324121	LMFA02000229	
4	2,3-Dihydroxycyclopentane undecanoic acid (C ₁₆ H ₃₀ O ₄)	long-chain fatty acid (Fatty acyls)	243756	—	C14987	—	—	
5	(9S,10S)-10-hydroxy-9-(phosphonooxy) octadecanoic acid (C ₁₈ H ₃₇ O ₇ P)	derivative of stearic acid (long-chain fatty acids-Fatty Acyls)	23724633	HMDB0059632	C15989	—	LMFA00000008	
6	12-Hydroxy-8,10-octadecadienoic acid (C ₁₈ H ₃₂ O ₃)	lineolic acids and derivatives. (long fatty acid)	131750940	HMDB0029998	—	35013116	—	
7	16-Hydroxy hexadecanoic acid (C ₁₆ H ₃₂ O ₃)	Long-chain fatty acids (Fatty Acyls)	10466	HMDB0006294	C18218	10034	LMFA01050051	
8	9S,12S,13S-trihydroxy-10E-octadecenoic acid (C ₁₈ H ₃₄ O ₅)	Octadecanoids (Fatty acyls)	9858729	HMDB0004708	C14833		LMFA02000014	
9	12,15-cis-Squamostatin A (C ₃₇ H ₆₆ O ₈)	Annonaceous acetogenins (Fatty Acyls)	5321489	HMDB0033423		4479212	—	

Table 133. Steroids and Substituted Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Lipid ID	Drug Bank ID	Structure
1	6alpha-Fluoro-17beta-hydroxyandrost-4-en-3-one acetate (C ₂₁ H ₂₉ F O ₃)	Estrane steroids	—	—	—	—	—	—	
2	Quinestrol (C ₂₅ H ₃₂ O ₂)	C18 steroids (estrogens) and derivatives	9046	HMDB0015579	C07619	8694	LMST02010037	DB04575	
3	Desoximetasone (C ₂₂ H ₂₉ F O ₄)	Synthetic corticosteroid with glucocorticoid activity	5311067	HMDB0014687	—	4470604	—	DB00547	
4	Melongoside G (C ₄₅ H ₇₄ O ₁₇)	Steroid Saponin	131752997	HMDB0040961	—	—	—	—	

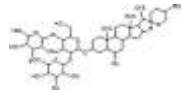
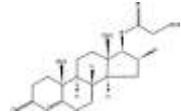
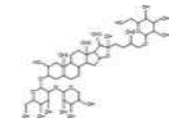
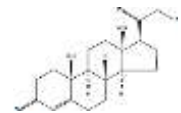
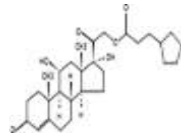
5	b-Chlorogenin 3-[2",4"-dirhamnosyl]glucoside] (C ₄₅ H ₇₄ O ₁₇)	Steroid Saponin	72964728	HMDB0036245	—	—	—	—	
6	16beta-Fluoro-17beta-hydroxyandrost-4-en-3-one propionate (C ₂₂ H ₃₁ F O ₃)	Steroid ester	11954138	—	C15253	—	—	—	
7	Trigoneoside XIb (C ₄₄ H ₇₄ O ₁₉)	Steroidal Saponins	131752001	HMDB0036485	—	—	—	—	
8	21-Fluoroprogestosterone; (C ₂₁ H ₂₉ F O ₂)	Corticosteroid hormone	200138	—	C14488	—	—	—	
9	Hydrocortisone Cypionate (C ₂₉ H ₄₂ O ₆)	C21 steroids (Gluco/mineralo corticoids, progestogins) and derivatives (Sterol lipids)	223253	—	C08176	—	LMST02030121	—	

Table 134. Flavonoids and Derivatives with Identification Details

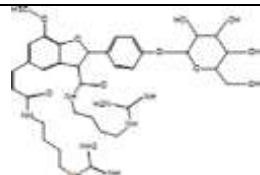
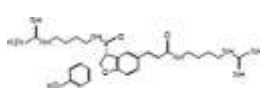
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Hordatine B glucoside (C ₃₅ H ₅₀ N ₈ O ₁₀)	2-arylbenzofuran flavonoids	131751022	HMDB0030460	—	—	
2	Hordatine A (C ₂₈ H ₃₈ N ₈ O ₄)	2-arylbenzofuran flavonoids (Amino acid related compounds)	45485025	HMDB0030461	C08307	35013202	

Table 135. Macrolides and Analogues with Identification Details

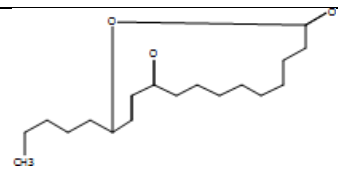
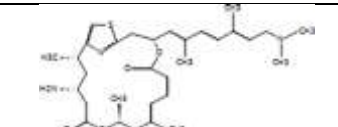
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	10-Oxo-11-octadecen-13-olide (C ₁₈ H ₃₀ O ₃)	Macrolides and analogues	9882599	HMDB0029786	—	8058274	
2	Pateamine (C ₃₁ H ₄₅ N ₃ O ₄ S)	Member of 1,3-thiazoles (marine macrodiolide)	10053416	—	C19917	25053851	

Table 136. Benzene and Substituted Derivatives with Identification Details

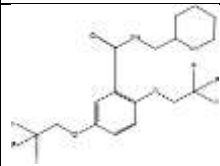
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	DrugBank ID	Structure
1	Flecainide (C ₁₇ H ₂₀ F ₆ N ₂ O ₃)	Benzamides (Benzoic acids and derivatives)	3356	HMDB0015326	C07001	3239	DB01195	

Table 137. Coumaric acids and Derivatives with Identification Details

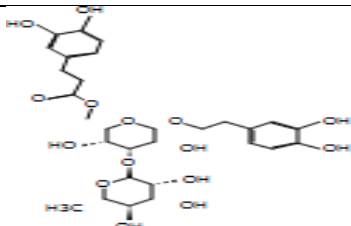
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	Structure
1	Isoacteoside (C ₂₉ H ₃₆ O ₁₅)	Coumaric acids and derivatives	13889686	HMDB0041025	

Table 138. Fuopyran with Identification Details

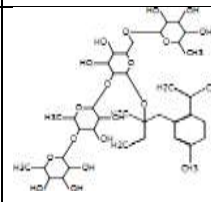
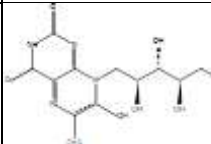
Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	Erinacine G (C ₂₅ H ₃₆ O ₈)	Fuopyran	10552092	HMDB0029655	—	8727483	

Table 139. Pteridines and Derivatives with Identification Details

Sr. No.	Compound name	Super class	PubChem ID	HMDB ID	KEGG ID	ChemSpider ID	Structure
1	7-Hydroxy-6-methyl-8-ribityl lumazine (C ₁₂ H ₁₆ N ₄ O ₇)	Pteridine & Derivatives	1043	HMDB0256912	C07581	1014	

5.6.4.2 Classification of Major Metabolite Groups in Ethanol Extracts of Formulations 1 and 2

The classification of major metabolite groups in Formulations 1 and 2 was depicted through schematic charts (Figures 74–77), derived from the comprehensive data in Tables 68–71. In Formulation 1, analysis in the positive Electrospray Ionization (ESI) mode revealed a predominance of metabolites such as alkaloids, fatty acyls, amino acids, and their derivatives. Conversely, the negative ESI mode identified a higher number of metabolites, including benzene derivatives, tannins, and terpenes.

For Formulation 2, the positive ESI mode showcased a relative abundance of alkaloids, fatty acyls, lipids, and their derivatives. In contrast, the negative ESI mode revealed a significant presence of steroids and their substituted derivatives, terpenoids and their derivatives, as well as fatty acyls. Comparative analysis of these metabolite profiles highlighted the rich and diverse composition of both formulations, with specific metabolite groups being more prevalent in one formulation than the other. This detailed classification underscores the potential pharmacological significance of each formulation's unique metabolite composition.

Fig. 74. Schematic Representation of Classification of Major Metabolites in the Ethanol Extract (Formulation 1) Identified by HR-LCMS QTOF in Positive ESI Mode.

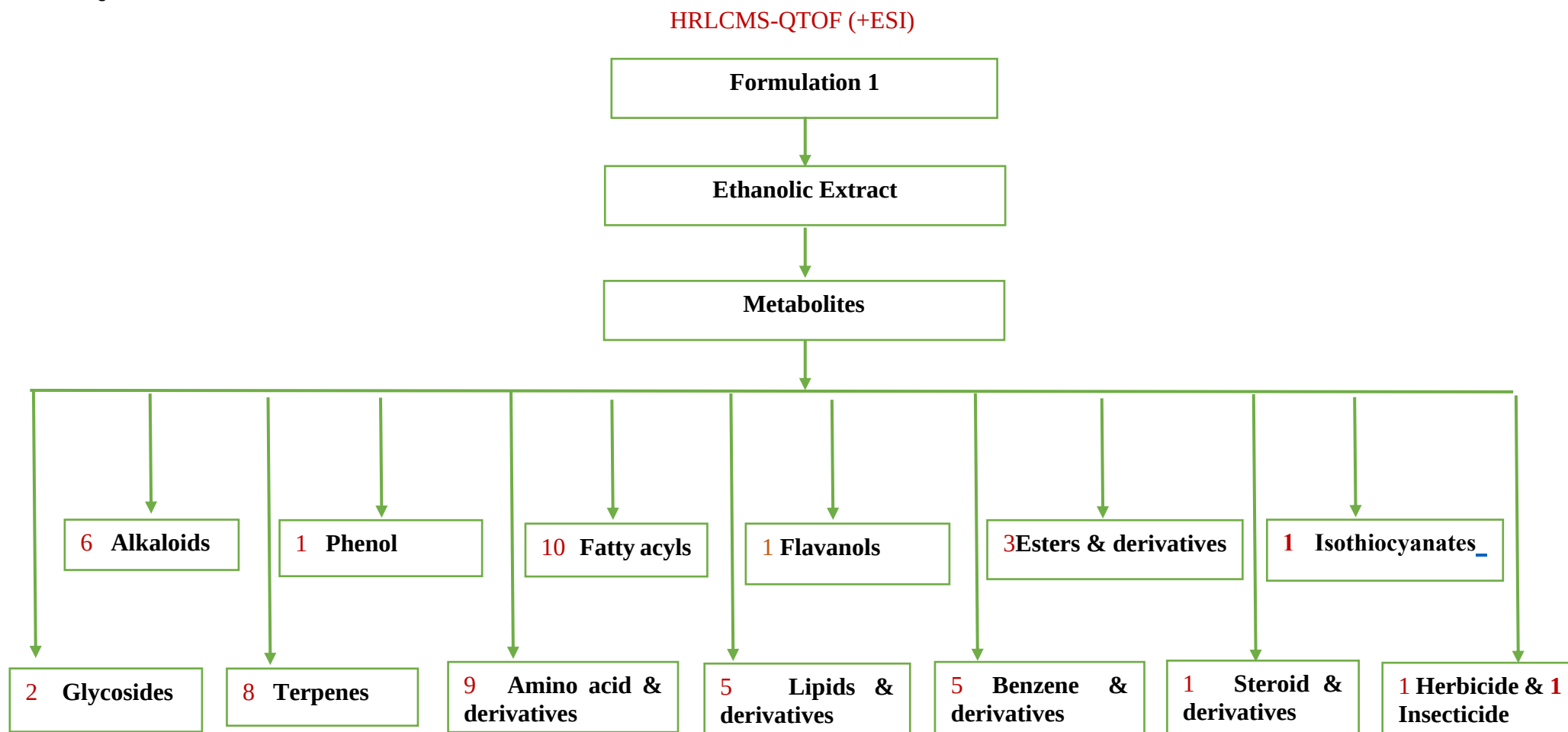


Fig. 75. Schematic Representation of Classification of Major Metabolites in the Ethanol Extract (Formulation 1) Identified by HR-LCMS QTOF in Negative ESI Mode.

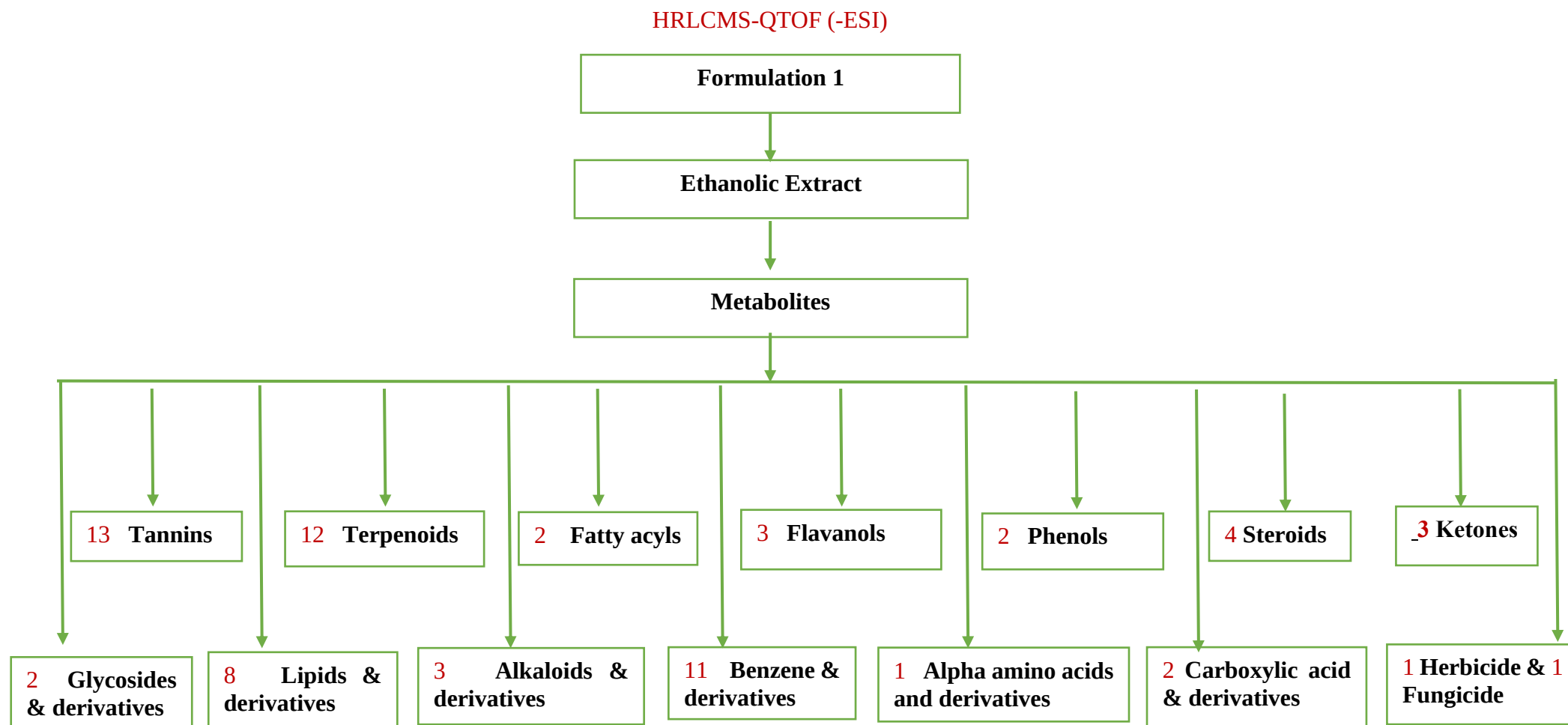


Fig. 76. Schematic Representation of Classification of Major Metabolites in the Ethanol Extract (Formulation 2) Identified by HR-LCMS QTOF in Positive ESI Mode.

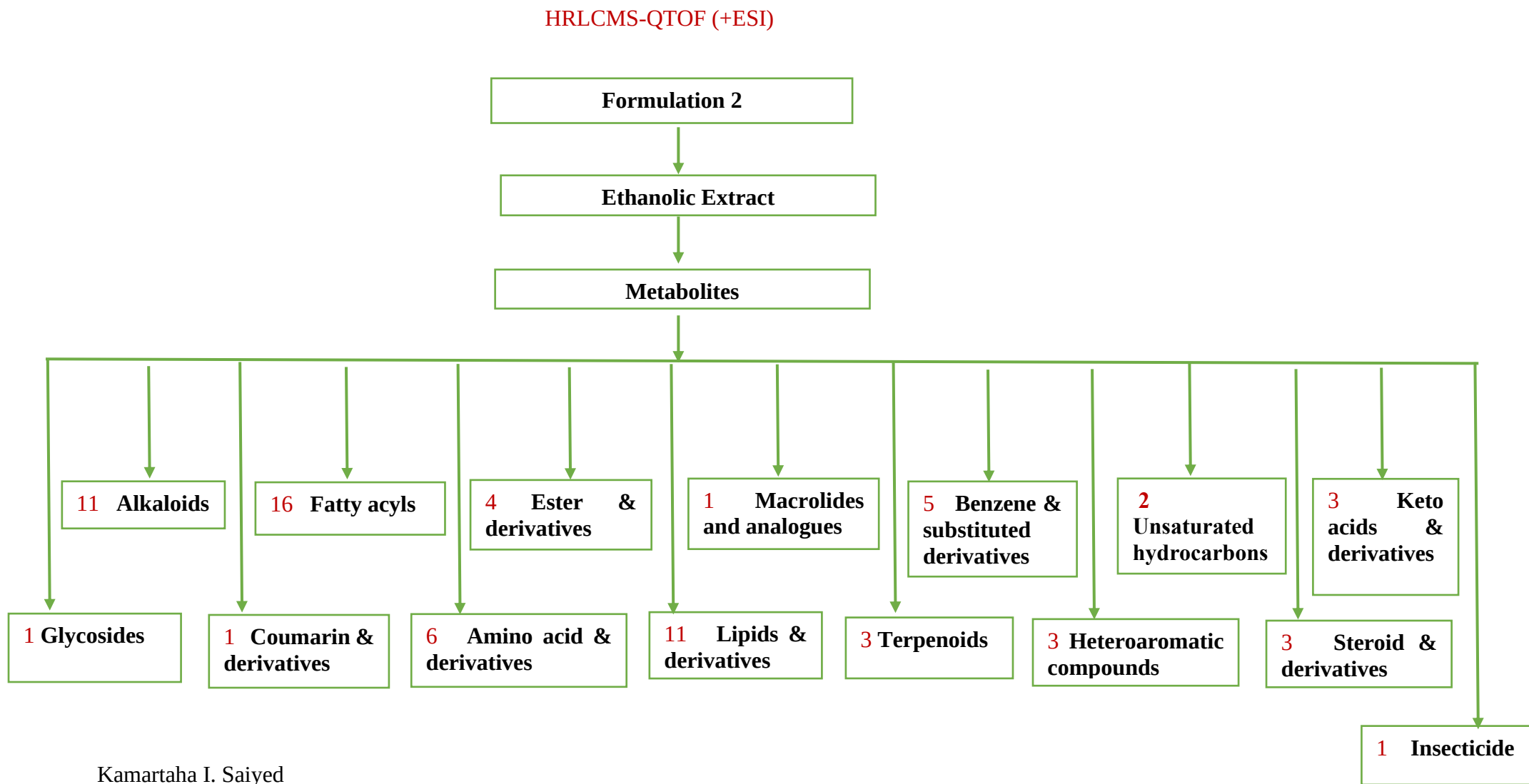
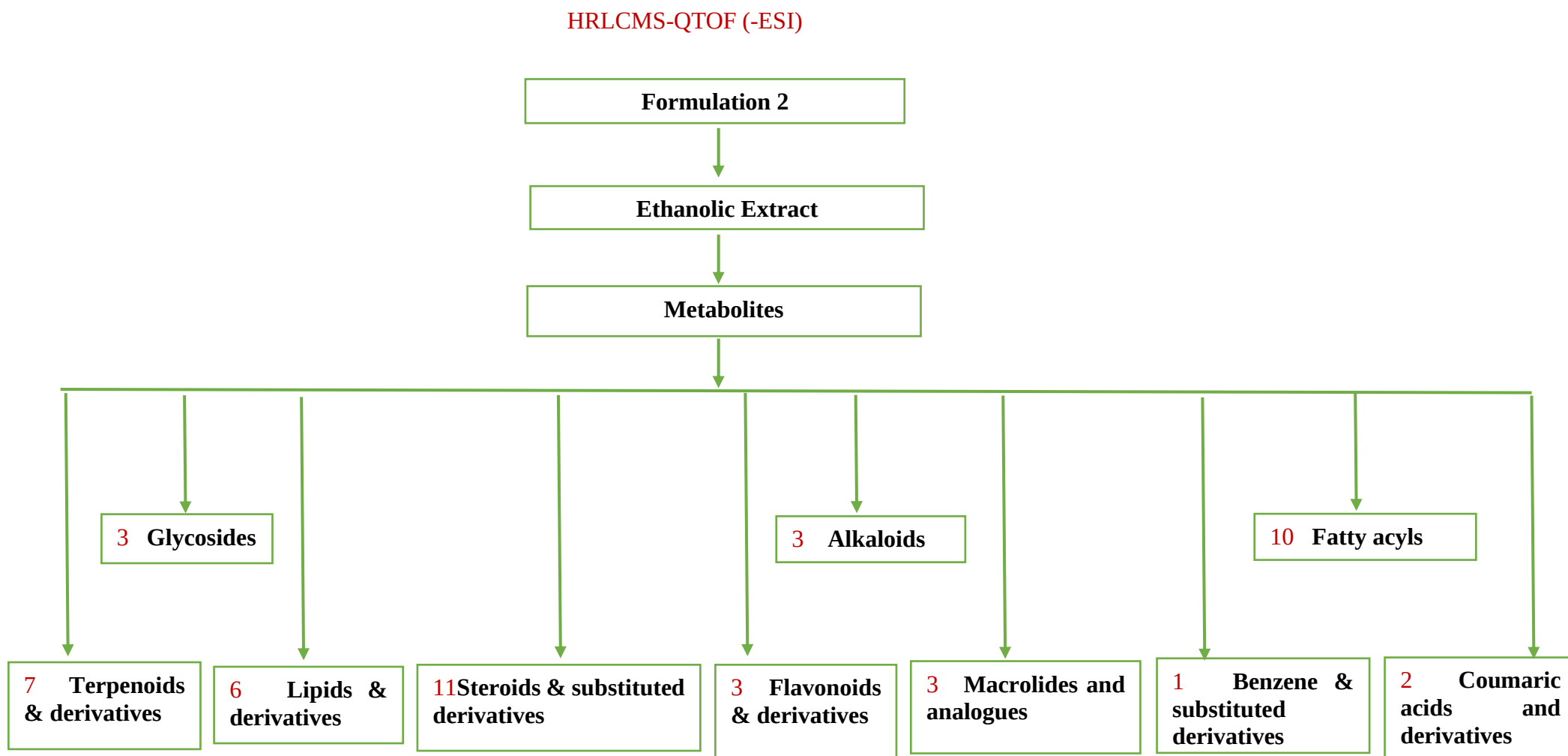


Fig. 77. Schematic Representation of Classification of Major Metabolites in the Ethanol Extract (Formulation 2) Identified by HR-LCMS QTOF in Negative ESI Mode.



5.6.4.3 5.8 HR-LCMS-QTOF Chromatographic Profiling of Formulations 1 and 2.

Chromatograms obtained from HR-LCMS-QTOF analysis of the ethanolic extracts of Formulations 1 and 2 in both ESI positive and negative modes provided a visual representation of the separation and detection of compounds (Fig. 78 and 79). Peaks corresponding to major compounds were identified, with their respective RT, m/z, and area under the curve analyzed and annotated in the chromatograms. These chromatograms highlighted the complexity and diversity of the metabolites in both formulations, emphasizing the presence of significant bioactive compounds.

5.6.4.3.1 Formulation 1

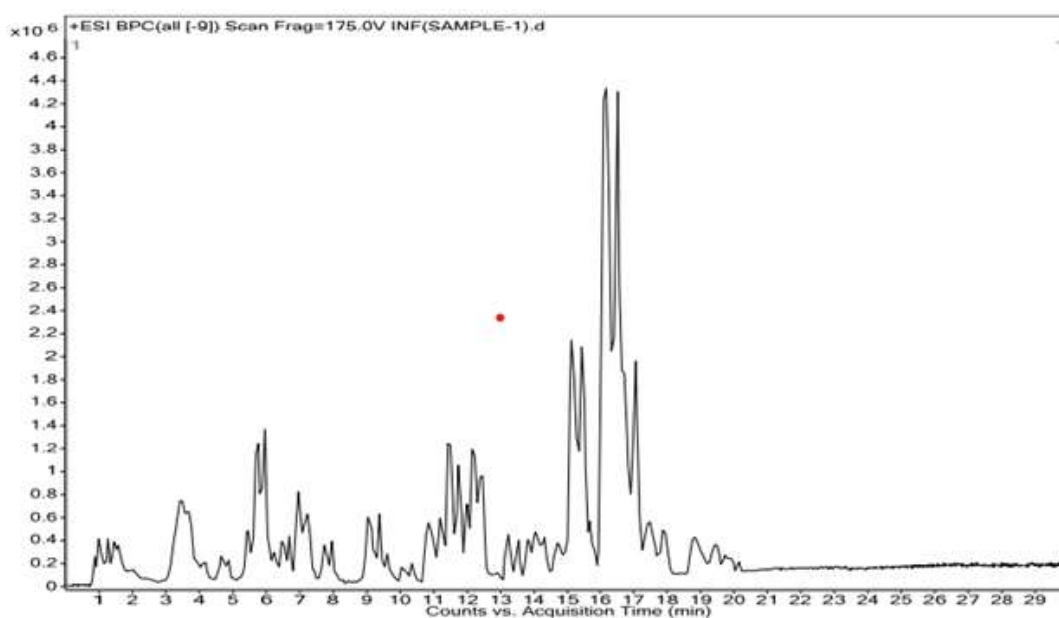
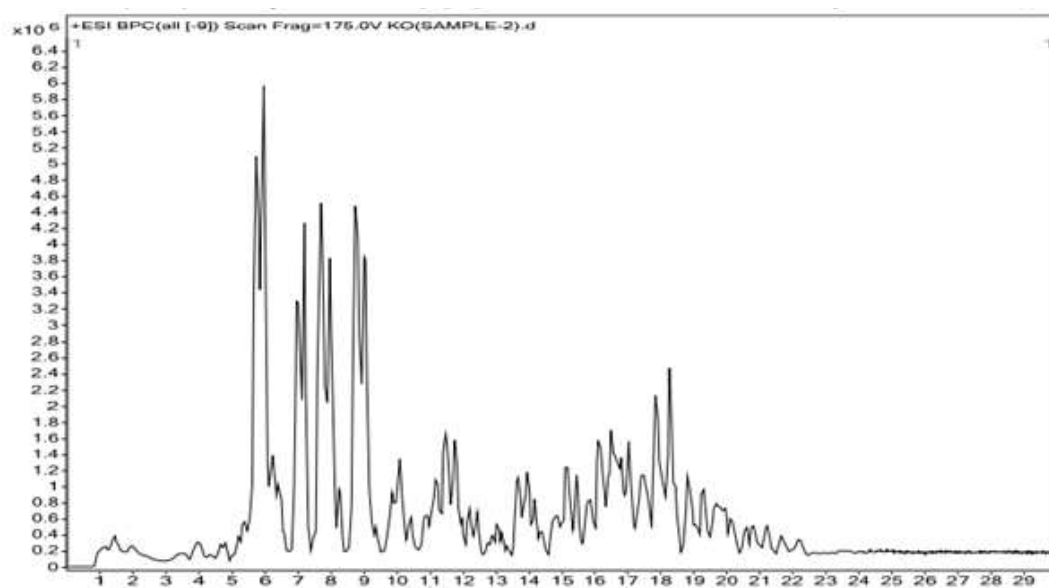


Fig. 78. HR-LCMS Chromatogram of the Ethanolic Extract of Formulation 1 (F1)

5.6.4.3.2 Formulation 2**Fig. 79. HR-LCMS Chromatogram of the Ethanolic Extract of Formulation 2 (F2)**