6. DISCUSSION

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

Unani system is a comprehensive medical system, which meticulously deals with the various aspects of health and disease. The Unani system of medicine offers treatment of diseases related to all the systems and organs of the body. Ethno-medicinal plant species were found to be in use for curing several kinds of human or livestock ailments which represent a diverse array of traditional knowledge. This is also an unambiguous illustration of survival of resources- poor communities on meagre earnings and still living a healthy and productive life without putting more than required pressure on natural resources. Therefore, it is need of the hour that such worthy traditional knowledge available at local level to be properly documented and disseminated among all. The present study is an attempt to collect/explore, preserve and express the diversity and richness of the Unani plants and traditional knowledge database available among the rural folks. The sighting of 84 diverse Unani plant species and their usage among the locals amply demonstrate the worthiness of such studies.

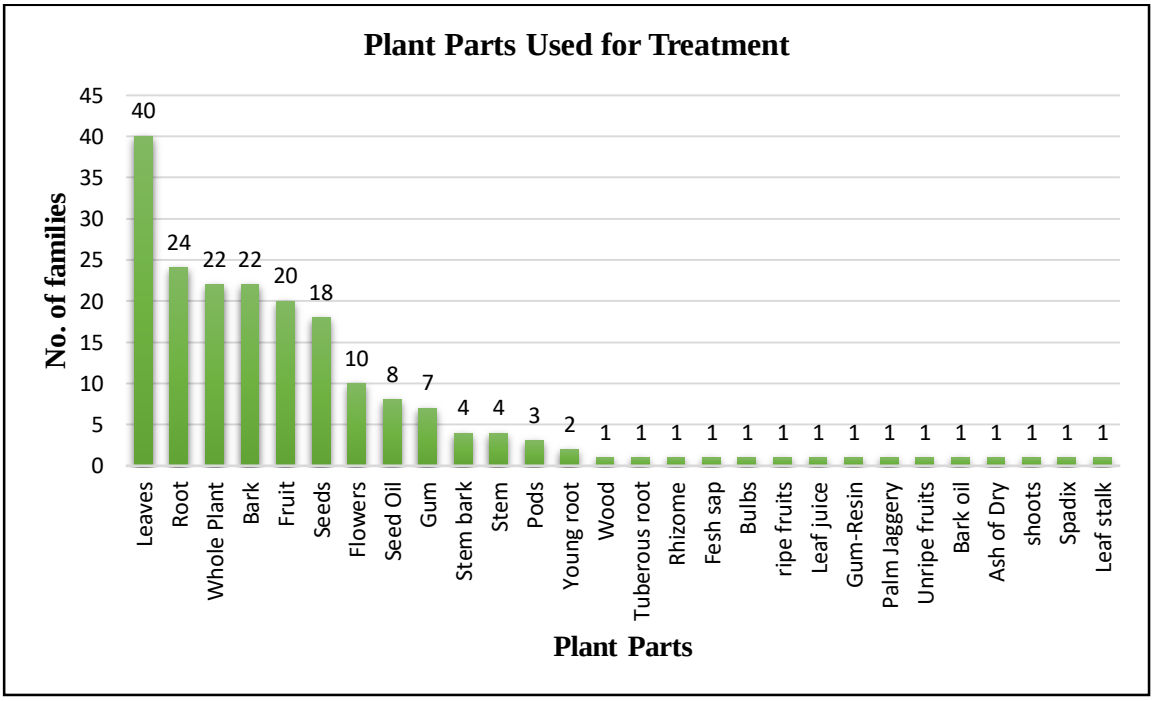

Fig. 80. Ratio of Plant Parts Used per Family

From the above chart we can witness that most common part of the plants is the plant leaves with highest count of 40. By and large, the root count is 24, while whole plant and bark have the similar count i. e. 22 respectively. Fruit counts have been recorded less than whole plant and bark (20) and for seeds and flowers count are 18 and 10 respectively. Rest other are recorded less than 10 and some even only one-one count each. Moreover, we would like to emphasize one thing here is parts which are exposed and easily accessible are used more frequently than other plant parts. Even, we have recorded, calculated and presented the values for the part used have highest count while parts in which processing or extraction is required have less count (Fig. 80).

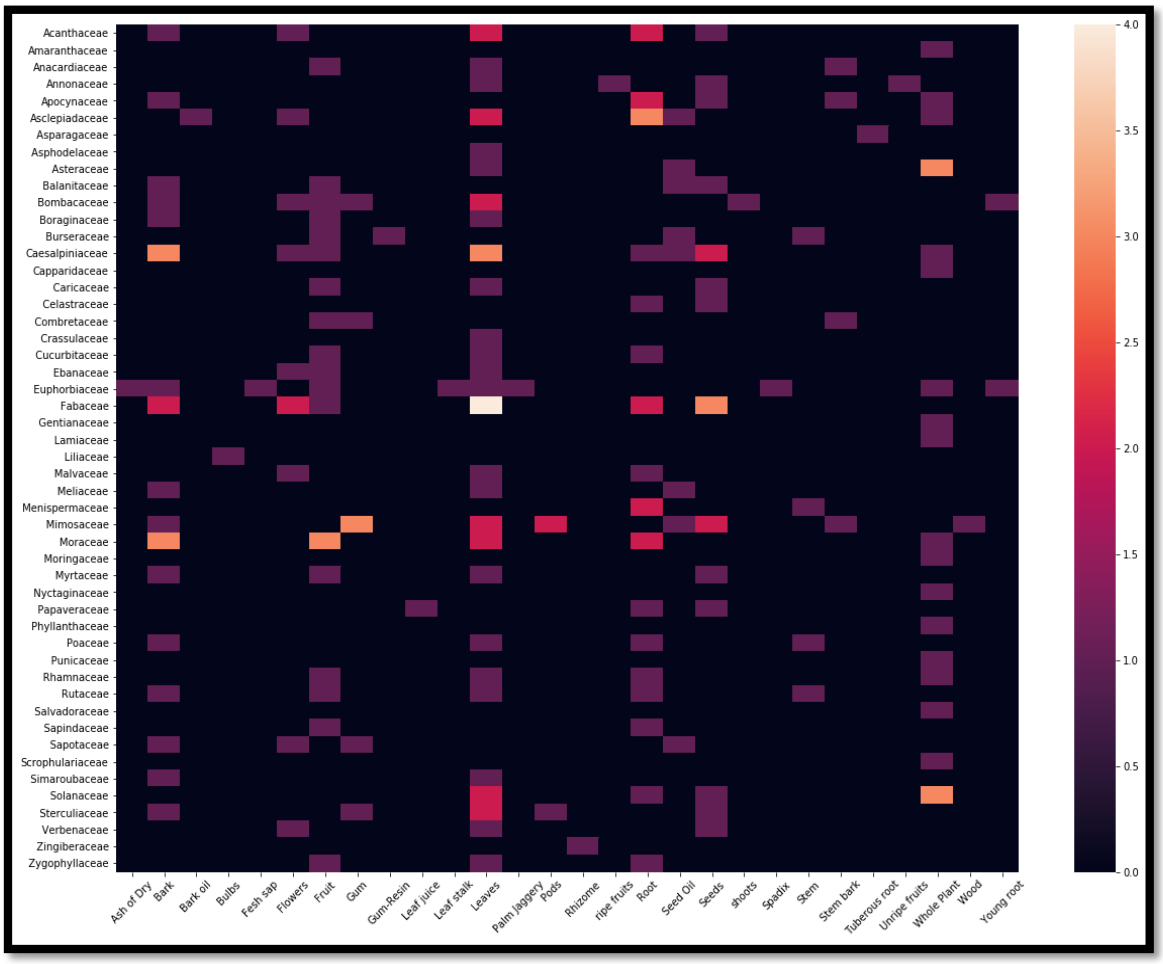

Fig. 81. Heat Map of Families and Plant Parts Used Based on Calculated Frequency

While processing the data we have prepared the above chart is Heat map style where in light color noticed if the frequency is high and dark color is depicting if frequency is

low (Fig. 81). Looking at the above chart we can observe the highest frequency is 4 and lowest is 0. Above plot is the calculated frequency between families recorded and plant parts used in treatment to cure medicinal problems or can be considered as therapeutic in any disorder.

6.2 Batrisu Vasanu: A Polyherbal Formulation from Central Gujarat.

Katlu or Batrisu is the Gujarati approach to feeding the nursing moms as well as the newborn child, which is involving this specific plan in powder structure. Different plant morphological sources and various flavors are additionally utilized generously in this body warming preparation. We have started this particular survey on the ingredients and composition mentioned on the packet from various commercial production houses, which are quite different. The comparative account of folk herbal ingredients depicts the presence of overall 58 ingredients in total from all the samples procured from the market. However, present-day science talks about the nutraceutical nature of any item depending on its phytoconstituents. To focus more on normalization to figure out the adequacy of crude herbal raw materials from plant sources.

This is a primary systematic investigation of Batrisu vasanu items sold over the counter on the lookout in all the raw material dealers. We have also tried to correlate Unani traditional raw material and its efficacy in this formulation. These discoveries are essentially vital to comprehend the various ingredients of Batrisu and the accompaniments in it. Even though customarily Batrisu implies 32 herbs, the current review shows as it were 24-27 ingredients per sample. Meanwhile, it shows 32 herbs only in one sample (PH01) and the rest of the other samples show an inconsistent number of ingredients (PH02, PH03, PH04, PH05). This outcome demonstrates the conflicting numbers of plant ingredients. These observations are significantly important to understand the variation of ingredients. It also indicates the uneven numbers of herbs in Unani marketed samples. The result of the calculated RFC value depicts some of these plants such as Shatavar, Filfil Daraz, Filfil Siyah, Asgandh and Sunth act as a Galactogogues (Luecha & Umehara, 2013; Roqaiya et al., 2015; Goyal, 2017; Kwan & Abdul-Rahmna, 2021) and rest others Heel Khurd, Koch, Jayphal, Javitry, Kababkhanda are known for their diuretic, aphrodisiac, neuroprotective, brain tonic, anti-inflammatory, general tonic, muscle building, digestive, nourishing the body activity etc. (Gogte, 2000). The plants are Shatavar, Asgandh and Filfil Siyah find their

reference as a Galactogogues in Unani as well as Ayurveda (Luecha & Umehara, 2013; Roqaiya et al., 2015; Goyal, 2017). Scientific names of the investigated plants are listed in Table-1. Out of 53 plants Unani system describes 17 plants are galactagogue (Roqaiya et al., 2015; Farzana et al., 2016). Ayurveda describes 32 plants as a galactagogue and referred as Stanyajanan and Kseerjanana (Srikanth et al., 2015). In earlier studies by Khare (2007) and Charola et al. (2022) they have reported that the Gokshura is utilized as a diuretic, calming, anabolic, cardiotonic and for hackasthma. It is also utilized as a component of natural decoction given to new mothers (Jain et al., 2011; Chaturvedi et al., 2017). While in our survey, only one (PH01) sample depicted the presence of Gokshura.

All the commercial samples depict the presence of Galactogogues. From the present study, we have also recorded that all samples have an inconsistent herbal composition in the terms of Galactagogue plants. Out of five samples, having variation in terms of galactagogue plants i.e., PH01 and PH04 has 9; PH02 has 12; PH03 has 10 and PH05 has 8 respectively. Rest others include some Ayurvedic drug plants or they might be using substitution drug plants. As in Ayurvedic literature, substitutive drug plant names already have been mentioned (Farzana et al., 2016). Among various reports of postpartum care, root, leaf, and seed are highly referred plant parts (Kankara et al., 2015). A comparable outcome was acquired as root; fruit & seed are predominately-involved parts in Batrisu vasanu. Further, the result of just 24 plant taxa (48.97%) having RFC more than 0.5 demonstrated a profoundly conflicting natural structure.
In spite of the fact that it appears to be encouraging, clinical exploration in this region needs more consideration as it concerns the soundness of the youngster and mother both. In Asia, herbal and polyherbal combinations for self-treatment and as food enhancements can be handily bought from condimental shops and conventional healers (Al Braik et al., 2008). It frequently does not adhere to any guidelines because of the absence of neighborhood administrative bodies, which represents a greater test intending to somewhere safe, secure and quality worries. Further, we observed that commercial suppliers do not follow any standards during collection and processing for the safety and quality of this 32 vasanu formulation. However, the reported irregularity in samples signals a serious health concern for new mothers as well as newborn. In this way, it is perceived that post-pregnancy wellbeing concerns are serious and require

more consideration due to these customary practices, informal eating regimen, and ethno medicine utilization.

6.3 Determination of Percentage Yield of Extracts

The extraction yields of the five formulations—Majoon-E-Najah (MN), Sufoof-E-Najah (SN), Sufoof-E-Chobchini (SC), Formulation 1 (F1), and Formulation 2 (F2)—revealed significant variability across different solvent systems and extraction methods, highlighting the critical need to optimize extraction conditions for maximizing bioactive compound recovery. Aqueous and hydroalcoholic solvents consistently yielded higher extraction efficiencies, particularly in MN and SN formulations. For instance, aqueous sonication of MN achieved the highest yield of 55%, and hydroalcoholic sonication of SN resulted in a yield of 36.06%. Present study findings suggest that polar solvents, particularly when used with sonication, improve the solubility and recovery of bioactive constituents. Such observations align with previous studies (Pin et al., 2010; Foo et al. 2015; Jha et al., 2022; Mukherjee et al., 2019), which underscore the role of solvent polarity in enhancing extractive yields from herbal formulations.

In contrast, ethanolic extracts generally demonstrated lower yields across most formulations, as seen with SC (ET) and F1 (ET), indicating that ethanol's lower polarity might limit its ability to extract certain hydrophilic phytochemicals. This trend is consistent with the findings of Rajani and Kanaki (2008), who noted the variable efficiency of different solvent systems in herbal extractions. The lower yields from ethanolic extractions in F1 and F2 formulations may reflect the chemical nature of these formulations, where more polar solvents like water or hydroalcoholic mixtures are better suited to solubilize active compounds. The present study's findings reinforce the importance of selecting appropriate solvent systems and extraction methods, as these factors play a pivotal role in optimizing the recovery of pharmacologically active constituents.

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

Despite the availability of numerous synthetic drugs, polyherbal formulations continue to be widely used due to their cost-effectiveness and lower incidence of side effects

(Pandey and Rastogi, 2013). However, systematic studies evaluating their phytochemical and pharmacological profiles are essential to validate their safety and efficacy (Fabricant and Farnsworth, 2001; Aslam et al., 2016). Present study represents the first comprehensive pharmacological evaluation of five Unani polyherbal formulations, employing three solvent extracts—Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET)— to investigate their antibacterial, antifungal and antioxidant activities. The results provide valuable insights into the bioactive potential of these formulations, laying a foundation for future therapeutic applications and optimization.

6.4.1 Antibacterial Activity

The global rise of antibiotic resistance has underscored the need for alternative therapeutic strategies, including traditional herbal remedies. The present study provides the first evaluation of the antibacterial properties of five Unani polyherbal formulations against *Escherichia coli* and *Bacillus megaterium*. Significant variations in antibacterial efficacy were observed depending on the solvent used—AQ, HA, and ET—as well as the phytochemical composition of each formulation. Ciprofloxacin and gentamicin were used as positive controls, establishing a benchmark for comparison. Although no prior studies have directly examined the antibacterial activity of the five Unani polyherbal formulations evaluated in this research, findings from similar polyherbal formulations support the observed antimicrobial potential.

The antibacterial evaluation of **Majoon-E-Najah (MN)** demonstrated the superior efficacy of ethanolic extracts (SX (ET) and SN (ET)) against both *Escherichia coli* and *Bacillus megaterium*. The ethanolic extracts consistently showed larger zones of inhibition (ZOI) across all concentrations, with SN (ET) reaching 10 mm against *E. coli* and 13 mm against *B. megaterium*. In contrast, the aqueous (RF (AQ)) and hydroalcoholic (RF (HA)) extracts exhibited limited to moderate activity, with the hydroalcoholic extract performing slightly better than the aqueous extract but still less effectively than the ethanolic extracts. These results highlight the enhanced antibacterial potential of ethanolic solvents, which are more efficient in extracting bioactive compounds, optimizing the therapeutic efficacy of polyherbal formulations like MN. Ethanol's ability to extract both polar and non-polar compounds was key to its superior antibacterial efficacy, supporting findings by Deattu et al. (2012) and

Chandel et al. (2019), while Al-Taei et, al. (2024) emphasized the limitations of aqueous extracts in isolating lipophilic bioactives.

The antibacterial activity results for **Sufoof-E-Najah (SN)** show that the hydroalcoholic (HA) extract had the strongest effect against *Escherichia coli*, with inhibition zones of 7.4 mm to 13 mm, followed by the ethanolic (ET) extract (7 mm to 8.7 mm). The aqueous (AQ) extract was least effective, with zones of 7.5 mm to 8 mm. Against *Bacillus megaterium*, the ethanolic extract was the most potent (6 mm to 10 mm), while the hydroalcoholic and aqueous extracts were less effective, with inhibition zones up to 8 mm. The Present study findings highlight the superior antibacterial efficacy of ethanolic and hydroalcoholic extracts, which effectively extract bioactive compounds, while the lower activity of the aqueous extract reflects water's limited extraction capacity. This aligns with reports by Dwivedi (2023), Gao et al. (2021), and Saha et al. (2022).

The antibacterial activity results for **Sufoof-E-Chobchini (SC)** demonstrate significant variations among the extracts tested against *Escherichia coli* and *Bacillus megaterium*. The hydroalcoholic (HA) extract exhibited the highest efficacy against *E. coli*, with zones of inhibition (ZOI) ranging from 6.2 mm to 8 mm, while the aqueous (AQ) extract showed moderate activity (ZOI from 5.5 mm to 7 mm). In contrast, the ethanolic (ET) extract had limited activity, with ZOI reaching up to 7.2 mm. Against *B. megaterium*, both HA and ET extracts displayed substantial antibacterial effects, with ZOI ranging from 7.1 mm to 14 mm for HA and 6.3 mm to 8.5 mm for ET. The present results indicate that hydroalcoholic extraction is particularly effective in isolating bioactive compounds with antimicrobial properties, such as tannins and flavonoids (Kuncha, 2019). Additionally, the significant activity of both HA and ET against *B. megaterium* supports findings by Vidhi et al. (2024) on the broad-spectrum extraction capabilities of hydroalcoholic solvents.

Formulation 1 (F1) extracts exhibited significant antibacterial activity against *Escherichia coli*, with hydroalcoholic extracts showing zones of inhibition (ZOI) of 5.5 mm to 8.3 mm and ethanolic extracts ranging from 5 mm to 8.5 mm. Against *Bacillus megaterium*, hydroalcoholic extracts outperformed aqueous extracts (ZOI: 6.5 mm to 9.8 mm), while ethanolic extracts showed ZOI from 6.3 mm to 7.7 mm. Present findings align with previous studies emphasizing the efficacy of hydroalcoholic and ethanolic

solvents in extracting antimicrobial compounds like flavonoids and phenolic acids, as well as earlier research indicating that hydroalcoholic extracts offer superior antimicrobial activity due to their broad-spectrum extraction capabilities (Dwivedi, 2023; Vidhi et al., 2024; Chandel et al., 2019).

Formulation 2 (F2) extracts exhibited notable antibacterial activity, particularly against *Escherichia coli* and *Bacillus megaterium*. The hydroalcoholic (HA) extract demonstrated zones of inhibition (ZOI) ranging from 6 mm to a maximum of 7.2 mm, while the ethanolic (ET) extract showed ZOI between 5 mm and 6.3 mm against *E. coli*. Against *B. megaterium*, the ethanolic extract proved most effective, with ZOI ranging from 5 mm to 8 mm, followed by the hydroalcoholic extract with a ZOI of 7 mm. The aqueous (AQ) extract exhibited comparatively limited activity, with ZOI between 5.1 mm and 6.1 mm. These findings emphasize the superior ability of hydroalcoholic and ethanolic solvents to extract bioactive antibacterial compounds, supporting their role in enhancing the efficacy of polyherbal formulations. This finding aligns with previous studies, such as those by Dwivedi (2023), Vidhi et al. (2024), and Al-Taei et al. (2024), which highlight the synergistic potential of polyherbal formulations in combating common pathogens effectively.

6.4.2 Antifungal Activity

The increasing prevalence of fungal infections, coupled with the rising resistance to conventional antifungal treatments, underscores the urgent need for alternative therapies. Polyherbal formulations from Unani medicine, recognized for their lower side effect profiles and potential therapeutic efficacy, offer a promising solution (Parekh and Chanda, 2008; Naseem et al., 2023). Present study represents the first comprehensive evaluation of the antifungal properties of five Unani formulations—Majoon-E-Najah (MN), Suffof-E-Najah (SN), Suffof-E-Chobchini (SC), Formulation 1 (F1), and Formulation 2 (F2)—using three solvent extracts: Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET). The formulations were tested against *Candida albicans* and *Aspergillus niger*, with Amphotericin as the positive control. The results demonstrated varying antifungal activities, influenced by the solvent used and concentration, reinforcing the role of bioactive compounds in determining efficacy against fungal strains.

Majoon-E-Najah (MN) exhibited notable antifungal activity, particularly in the hydroalcoholic (HA) and ethanolic (ET) extracts. The HA extract was effective against *Candida albicans*, with inhibition zones ranging from 8 to 11.33 mm, while the ET extract showed greater efficacy against *Aspergillus niger*, with inhibition zones up to 17.33 mm. The difference in antifungal efficacy could be attributed to the distinct cell wall structures of the fungi, which respond differently to the bioactive compounds extracted by each solvent. Present findings are consistent with prior studies highlighting the influence of solvents in extracting antifungal phytochemicals (Saba et al., 2023; Veeraswamy et al., 2022).

The antifungal evaluation of **Suffof-E-Najah (SN)** extracts demonstrated distinct activity against *Candida albicans* and *Aspergillus niger*. The aqueous (AQ) and hydroalcoholic (HA) extracts exhibited strong antifungal effects across all tested concentrations, with inhibition zones ranging from 8 to 13 mm for AQ and 9 to 13 mm for HA against *C. albicans*, and 10.66 to 15.66 mm for AQ and 9 to 15.33 mm for HA against *A. niger*. In contrast, the ethanolic (ET) extract showed no significant activity. These results emphasize the role of solvent polarity in extracting bioactive compounds, with aqueous and hydroalcoholic extracts, rich in flavonoids, saponins, and phenolics, being more effective in disrupting fungal cell membranes. This aligns with previous studies (Saba et al., 2023), highlighting the importance of solvent choice for optimizing antifungal activity in polyherbal formulations.

The antifungal evaluation of **Suffof-E-Chobchini (SC)** extracts showed varying effectiveness against *Candida albicans* and *Aspergillus niger*. The ethanolic (ET) extract demonstrated significant activity against *C. albicans*, with inhibition zones ranging from 9 to 11 mm, while the hydroalcoholic (HA) extract was less effective (ZOI 5–6 mm), and the aqueous (AQ) extract showed no significant effects. For *A. niger*, all extracts exhibited antifungal activity, with the ET extract being the most potent (ZOI 9–22.33 mm), followed by AQ (ZOI 8.66–14 mm) and HA (ZOI 14–18 mm). These results underscore the importance of solvent polarity in optimizing the extraction of bioactive compounds. The ethanolic extract's superior activity, particularly against *A. niger*, highlights ethanol's efficiency in extracting potent antifungal agents, supporting findings by Hidayat & Fitriani (2023), Kumar et al. (2011), and Naveenkumar et al. (2018) on the role of solvent choice in enhancing the therapeutic potential of polyherbal formulations.

Formulation 1 (F1) exhibited concentration-dependent antifungal activity against *Candida albicans* and *Aspergillus niger*, with the hydroalcoholic (HA) extract showing the highest efficacy. Inhibition zones ranged from 10 to 12.6 mm for *Candida albicans* and 13 to 17.33 mm for *Aspergillus niger*, indicating the presence of bioactive compounds with broad-spectrum antifungal properties. The ethanolic extract showed moderate activity, likely due to partial extraction of key compounds, while the weaker performance of the aqueous extract suggests limited solubility of essential antifungal phytochemicals in water, as supported by Bahekar et al. (2024), Taleghani et al. (2024), and Marcel et al. (2023).

Formulation 2 (F2) displayed notable antifungal activity, with the aqueous (AQ) extract showing the highest efficacy against Candida albicans (inhibition zones: 8.66 to 11 mm), a result that contrasts with other formulations where hydroalcoholic and ethanolic extracts were more potent. Against Aspergillus niger, the hydroalcoholic (HA) extract exhibited the strongest activity, with inhibition zones of 14 to 20 mm, suggesting its ability to extract bioactive compounds more effective against filamentous fungi. The ethanolic extract showed moderate activity, likely due to its partial solubilization of antifungal constituents, as reported by Thombre et al., 2024; Hidayat & Fitriani, 2023.

6.4.3 Antioxidant activity by DPPH assay

Antioxidant activity is essential for managing oxidative stress, which is linked to chronic diseases such as cardiovascular diseases, cancer, and neurological disorders (Aslam et al., 2016). Unani polyherbal formulations, rich in phenolics and flavonoids, have demonstrated significant antioxidant potential (Ali et al., 2014; Gupta et al., 2023). In the present study, which is the first of its kind, the DPPH assay was utilized to evaluate the free radical scavenging ability of five Unani polyherbal formulations, highlighting their potential to neutralize oxidative damage (Amir et al., 2011). The antioxidant capacity was measured through IC50 values, percentage inhibition, and non-linear regression analysis, providing a comprehensive comparison of the efficacy of different extracts across the formulations. Ascorbic acid served as the reference standard, and the results offer valuable insights into the formulations' ability to address oxidative stress-related conditions, reinforcing their therapeutic importance (Kedare & Singh, 2011; Lobo et al., 2010).

The present study evaluates the antioxidant activity of **Majoon-E-Najah (MN)**, with the ethanolic extract showing the highest efficacy, indicated by a low IC50 value. This suggests ethanol's effectiveness in extracting bioactive compounds with potent antioxidant properties, which is consistent with findings reported by Prior et al. (2005) and Ilomuanya et al. (2018). Conversely, the hydroalcoholic extract exhibited the lowest activity, emphasizing the importance of solvent selection in antioxidant extraction, as previously highlighted by Choi et al. (2002). The dose-dependent response observed in the DPPH assay further underscores the formulation's antioxidant potential. The superior performance of the ethanolic extract may be attributed to the enhanced solubility of phenolic and flavonoid compounds, which are crucial for neutralizing reactive oxygen species (ROS), as noted in recent studies by Inavally et al. (2024) and Ahemad et al. (2022). Notably, MN's antioxidant activity, comparable to ascorbic acid, indicates its potential as a natural antioxidant for reducing oxidative stress, a potential that warrants further in vivo investigation, as suggested by previous research (Dai & Mumper, 2010; Kuna et al., 2022).

The present study reveals that the ethanolic extract of **Suffof-E-Najah (SN)** exhibited superior antioxidant activity, as evidenced by its remarkably low IC50 value, which surpasses that of ascorbic acid. This suggests that SN contains potent antioxidant compounds that are most effectively extracted using ethanol. The results align with previous studies that demonstrate ethanol extracts' capability to inhibit reactive oxygen species (ROS) and nitric oxide production, thereby underscoring their strong antioxidant properties (Kuna et al., 2022; Rao & Chandira, 2020).

The ethanolic extract of **Suffof-E-Chobchini (SC)** consistently demonstrated the highest antioxidant activity across all concentrations, significantly surpassing the hydroalcoholic and aqueous extracts. Present finding reinforces the effectiveness of ethanol in extracting potent antioxidant compounds, as reported by Inavally et al. (2024), Ilomuanya et al. (2018), and Kuna et al. (2022), who highlighted that ethanolic extracts are rich in bioactive phytochemicals, such as flavonoids and phenolics, crucial for neutralizing free radicals.

In contrast to the other formulations, **Formulation 1 (F1)** demonstrated that its aqueous extract exhibited the highest antioxidant activity, highlighting the significance of water-soluble compounds in this formulation. Present finding emphasizes the potential of

aqueous extraction to isolate bioactive compounds contributing to antioxidant efficacy, such as tannins and polysaccharides, as reported by Nimmi and George (2012), Mandal and Reddy (2015), Gupta et al. (2017), and Patwekar et al. (2022).

For **Formulation 2 (F2),** the hydroalcoholic extract demonstrated the highest antioxidant activity, underscoring the effectiveness of hydroalcoholic solvents in preserving antioxidant properties. The present study aligns with previous research indicating that hydroalcoholic solvents can effectively extract both polar and non-polar compounds, maximizing the presence of bioactive phytochemicals known for their radical-scavenging abilities (Venkateswaran et al., 2021; Saini et al., 2019). In particular, phenolic compounds and flavonoids play a crucial role in this process, as their antioxidant activity is believed to involve free radical scavenging through hydrogen or electron donation, thereby reinforcing the function of these extracts as primary antioxidants (Srivastava and Lal, 2020; Behera, 2012).

6.5 Phytochemical characterization

The phytochemical characterization of five selected Unani polyherbal formulations involved quantifying Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). Specifically, two formulations—Formulation 1 (F1) and Formulation 2 (F2)—were analyzed using extracts obtained with three different solvents: aqueous, hydroalcoholic, and ethanolic. Employing Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) provided crucial insights into the secondary metabolites present in these formulations, highlighting their chemical complexity and therapeutic potential. Notably, the ethanolic extracts demonstrated the most robust phytochemical profiles, characterized by a higher concentration of bioactive compounds. Consequently, these extracts were subjected to further detailed analysis using High-Resolution Liquid Chromatography-Mass Spectrometry Quadrupole Time-of-Flight (HRLC-MS-QTOF), enabling the identification of key chemical markers.

6.5.1 The determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The quantification of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) is vital for assessing the bioactive constituents in Unani polyherbal formulations, as

these compounds significantly contribute to the antioxidant, antibacterial, and anti-inflammatory properties of traditional medicines (Cao et al., 1997; Gupta et al., 2023). In the present study, five Unani polyherbal formulations—Majoon-E-Najah (MN), Suffof-E-Najah (SN), Suffof-E-Chobchini (SC), Formulation 1 (F1), and Formulation 2 (F2)—were systematically analyzed for their phenolic and flavonoid content, marking the first comprehensive investigation of their phytochemical profiles. These findings provide a critical foundation for the therapeutic standardization and further research into the efficacy of these formulations.

The results of the present study highlight the significant variation in the extraction efficiency of phenolic and flavonoid compounds depending on the solvent used, underscoring the critical role of solvent selection in optimizing extraction yields. The ethanolic extract of **Majoon-E-Najah (MN)** exhibited the highest phenolic content (15.852 mg GAE/g) and flavonoid content (3.313 mg QE/g), reinforcing the effectiveness of ethanol in extracting both polar and non-polar bioactive compounds, as supported by findings in earlier studies (Demla and Verma, 2012; Wakkumbura et al., 2020). Such findings suggest that ethanol's solvent properties facilitate the efficient recovery of a wide range of antioxidant compounds, particularly flavonoids.

Consistently, across all formulations, ethanolic extracts yielded higher flavonoid content, whereas aqueous extracts demonstrated superior phenolic extraction, particularly in **Suffof-E-Najah (SN)**, **Formulation 1 (F1)**, **and Formulation 2 (F2).** This aligns with existing literature that recognizes the selectivity of solvents in targeting different classes of bioactive compounds, with water-based solvents often being more efficient in extracting phenolics (Kamtekar et al., 2014; Saini et al., 2019).

A notable exception was observed in **Suffof-E-Chobchini (SC)**, where the hydroalcoholic extract exhibited the highest TPC (26.376 mg GAE/g), while the ethanolic extract was more effective in flavonoid extraction (23.313 mg QE/g). This suggests that hydroalcoholic mixtures can effectively extract a broader spectrum of phenolic compounds, while ethanol remains particularly adept at flavonoid extraction, a pattern supported by previous studies (Mandal and Reddy, 2015; Yap et al., 2023). Such results emphasize the importance of selecting appropriate solvents to maximize the extraction of specific bioactive compounds depending on the desired pharmacological activity.

6.5.2 Pearson Correlation and Implications

The Pearson correlation analysis in this study was employed to assess the relationships between pharmacological activities—antibacterial, antifungal, and antioxidant—and phytochemical contents, particularly Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), in five Unani polyherbal formulations: Majoon-E-Najah (MN), Suffof-E-Najah (SN), Suffof-E-Chobchini (SC), Formulation 1 (F1), and Formulation 2 (F2). The findings of the present study provide critical insights into how bioactive compounds extracted through various solvents—aqueous (AQ), hydroalcoholic (HA), and ethanolic (ET)—contribute to the therapeutic efficacy of these formulations.

In the present study, a strong positive correlation between antibacterial, antifungal, and DPPH antioxidant activities in **Majoon-E-Najah (MN)** suggests that similar bioactive compounds contribute to its broad-spectrum therapeutic potential. This finding aligns with prior research indicating that phenolic and flavonoid compounds exhibit multi-functional bioactivity (Ali et al., 2021). However, the observed negative correlation between antifungal activity—particularly against *Aspergillus niger*—and both antioxidant and antibacterial properties is noteworthy. Such inverse relationships have not been extensively highlighted in previous studies on Unani formulations, suggesting that elevated TPC levels may selectively enhance or suppress specific therapeutic effects. This points to the potential need for customized extraction protocols tailored to desired pharmacological outcomes. The emphasis on the dual role of TPC offers novel insights for optimization in polyherbal formulations.

The results for **Suffof-E-Najah (SN)** reveal pathogen-specific bioactivity. TPC and TFC levels showed no significant correlation with antibacterial efficacy against *Escherichia coli*, yet they enhanced activity against *Bacillus megaterium* and demonstrated potent antifungal effects. This observation diverges from earlier studies that typically report a more generalized enhancement of antibacterial activity by phenolics and flavonoids (Llorent-Martinez et al., 2017). The strong positive correlation between antioxidant content and antifungal activity in SN reinforces the importance of phenolic compounds in promoting bioactivity, a theme consistent with other polyherbal formulations. However, the pathogen-specific action here provides a

more nuanced understanding of how SN's phytochemical composition drives its pharmacological efficacy.

Suffof-E-Chobchini (SC) exhibited a complex correlation pattern. In contrast to other formulations, TFC was more closely associated with antifungal and antibacterial activity against *E. coli*, while TPC showed greater effectiveness against *Bacillus megaterium*. Previous studies often emphasize the dominant role of phenolics in antimicrobial activity; however, our findings suggest that flavonoids may be more critical in SC's efficacy against certain pathogens (Kashif et al., 2024). This observation adds depth to the understanding of SC's phytochemical interactions, suggesting that specific compounds, such as flavonoids, may exhibit targeted bioactivity. The lack of correlation between antioxidant activity and antifungal efficacy against *Candida albicans* may indicate the involvement of other, more specialized bioactive compounds, contrasting with generalized antioxidant-linked efficacy in other herbal formulations.

In **Formulation 1 (F1)**, positive correlations between TFC and antibacterial activity against *E. coli* suggest that flavonoids significantly contribute to its antimicrobial effectiveness. However, the inverse correlation between higher phenolic content and reduced efficacy against other strains presents a complex dynamic. While previous research generally associates higher phenolic content with increased antimicrobial activity (Yap et al., 2023), the current findings indicate a potential inhibitory effect in certain contexts. This observation is novel and suggests that competitive interactions between different bioactive compounds within F1 necessitate further investigation to optimize phytochemical balance.

Formulation 2 (F2) demonstrated intricate correlations. Antibacterial efficacy against *E. coli* was enhanced by antioxidant and flavonoid content, while a negative correlation between TPC and efficacy against other strains was noted. Although studies have recognized the broad antimicrobial potential of phenolics, this negative correlation suggests that elevated TPC levels may inhibit specific antibacterial activities (Kashif et al., 2024). These findings underscore the complexity of phytochemical interactions within F2, indicating that fine-tuning extraction protocols may be essential for optimizing its therapeutic potential. This nuanced observation adds a layer of complexity to existing research, emphasizing the need for a more targeted approach in phytochemical extraction for Unani formulations.

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

Phytochemical analysis utilizing TLC and HPTLC was conducted to fingerprint the ethanolic, hydroalcoholic, and aqueous extracts of F1 and F2. These techniques are well-established for profiling phytoconstituents and authenticating herbal formulations (Mukherjee, 2002; Farooqui et al., 2014). TLC provided an initial qualitative assessment, while HPTLC, with its enhanced resolution and quantitative capabilities, generated detailed fingerprints of the formulations (Koll et al., 2003). The utility of TLC and HPTLC in herbal quality control and the exploration of bioactive compounds is widely recognized (Bladt, 2009; Sandhya et al., 2015). The generated fingerprints serve as essential tools for ensuring consistency, potency, and therapeutic efficacy of these polyherbal formulations.

This study represents the first comprehensive analysis employing TLC and HPTLC specifically on these Unani formulations. The generated fingerprints provide novel insights into the chemical composition of both the formulations and their individual plant ingredients. To ensure accurate separation of phytoconstituents, various solvent systems were initially tested. The optimal systems selected for HPTLC were Toluene: Ethyl acetate: Formic acid: Methanol (9:9:2.4:0.6 v/v/v/v) for F1, and Toluene: Ethyl acetate: Formic acid (10:8:2 v/v/v) for F2.

In HPTLC analysis, extracts from each formulation were systematically applied to silica gel plates, allowing for direct comparison between extracts and individual plant parts. Tracks 1, 2, and 3 were assigned to the aqueous (AQ), hydroalcoholic (HA), and ethanolic (ET) extracts of both Formulation 1 and Formulation 2. Individual plant part extracts were applied to subsequent tracks—tracks 4, 5, 6, and 7 for Formulation 1, and tracks 4, 5, 6, 7, and 8 for Formulation 2. This systematic approach allowed for a direct comparison between the extracts and individual plant parts, elucidating the phytoconstituents profiles of the formulations on a single TLC plate. HPTLC's capacity to analyze multiple samples simultaneously enhances its efficiency for fingerprint generation (Ram et al., 2011; Sandhya et al., 2015; Lalhriatpuii, 2020).

After development, the plates were visualized at multiple wavelengths (254 nm, 366 nm, and 540 nm), revealing distinct banding patterns for each extract (Figures 48, 49,

52, 53). Derivatization with 10% methanolic sulfuric acid enhanced visibility, producing more pronounced bands representing specific phytoconstituents (Bladt, 2009; Sandhya et.al., 2015). The HPTLC plates, visualized at different wavelengths, revealed distinct banding patterns, with the ethanolic extracts displaying the highest number of bands, followed by the hydroalcoholic and aqueous extracts in both Formulation 1 and Formulation 2.

The development of these HPTLC fingerprints is crucial for ensuring the authenticity, purity, quality, and safety of the Unani drugs used in these formulations (Beg et.al., 2021; Khan et.al., 2023). By comparing the phytochemical profiles of the extracts with those of the individual plant parts, this analysis confirms the presence of key bioactive compounds and validates the integrity of the polyherbal formulations (Sudberg et.al., 2010; Nicoletti et.al., 2013). Additionally, these detailed fingerprints will serve as reference tools for future quality control and standardization of Unani medicines (Ahmad et.al., 2020; Naaz et.al., 2021).

The identification of unique banding patterns, especially in ethanolic extracts, highlights the richness and complexity of the phytochemical composition, further emphasizing the therapeutic potential of the formulations. These fingerprints provide a foundation for future research into the pharmacological efficacy of the bioactive compounds present in the formulations, contributing to the growing scientific support for traditional Unani medicine (Alam et.al., 2020).

Densitograms from HPTLC provide comprehensive data on peak height, area, and Rf values, facilitating a detailed comparison across individual tracks and formulations (Bladt, 2009; Lalhriatpuii, 2020; Beg et.al., 2021). The Rf values obtained reveal both shared and unique compounds between the two formulations. Shared peaks indicate common phytoconstituents, while unique peaks reflect the diversity in their phytochemical makeup (Mukherjee, 2002). Such fingerprinting is crucial for standardization, ensuring that polyherbal formulations meet quality control criteria necessary for reproducibility in therapeutic applications (Devi et al., 2024). Variations in band intensity and the number of phytoconstituents may also be attributed to external factors such as geographical origin, climate, and extraction methods (Singh et al., 2020; Rani and Manju, 2020).

6.5.4 HR-LCMS-QTOF Analysis

The HR-LCMS-QTOF analysis conducted in this study marks a significant step in unraveling the complex metabolite compositions of Formulation 1 and Formulation 2. As the first study to apply this advanced analytical technique to these traditional Unani polyherbal formulations, it provides novel insights into their metabolite profiles. Previous reports have not included HR-LCMS-QTOF analysis for these formulations, highlighting the originality of this work.

The selection of ethanolic extracts for HR-LCMS-QTOF analysis was confirmed by prior HPTLC fingerprinting results, which demonstrated superior separation and a higher number of distinct bands compared to the aqueous and hydroalcoholic extracts. This suggests a richer diversity of bioactive compounds in ethanolic extracts, making them particularly suitable for detailed metabolite profiling. HR-LCMS-QTOF analysis was conducted using both ESI positive and negative ionization modes to ensure comprehensive profiling of polar and non-polar compounds. The resulting chromatograms visually depicted the separation and detection of compounds, with key peaks annotated with their respective retention times, m/z values, and areas under the curve, emphasizing the metabolite complexity of both formulations (Kurella and Addepally, 2023).

The results revealed a total of 49 metabolites tentatively identified in the positive ESI mode and 64 metabolites in the negative ESI mode for Formulation 1 (F1). For Formulation 2 (F2), 64 metabolites were identified in the positive ESI mode and 43 in the negative ESI mode. The identification process was based on key parameters, such as molecular formula, retention time (RT), observed mass, mass-to-charge ratio (m/z), and area under the curve. A significant outcome of the HR-LCMS-QTOF analysis was the identification and classification of numerous bioactive compounds into major metabolite groups, including alkaloids, phenols, fatty acyls, flavonoids, and others. Cross-referencing these compounds with multiple databases (KEGG, Drug Bank, HMDB, Chemspider, and PubChem) confirmed their identities and provided valuable insights into their potential pharmacological activities (Choudhary et al., 2024). This classification and cross-referencing process has further enhanced the understanding of the therapeutic potential of these formulations, supporting their traditional use in Unani medicine.

The classification of major metabolite groups in the ethanolic extracts of Formulations 1 and 2, illustrated through charts (Fig. 56 to 59) and supported by data in Tables 61 to 64, provides significant insights into the chemical complexity of these formulations. The use of HR-LCMS-QTOF enabled the identification and characterization of various metabolites, revealing the richness of their phytochemical profiles (Islam et al., 2024).

In **Formulation 1 (F1)**, the positive ESI mode highlighted the relative abundance of key metabolite groups, including alkaloids, fatty acyls, and amino acids along with their derivatives. Alkaloids are known for their diverse biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties (Badri et al., 2019). The presence of fatty acyls suggests potential health benefits, as they are associated with antimalarial, antimycobacterial, antifungal agents, and lipid-regulating effects (Carballeira, 2008). Amino acids, essential for protein synthesis, play critical roles in metabolic pathways, further underscoring the therapeutic potential of Formulation 1 (Odia and Esezobor, 2017).

Conversely, the negative ESI mode of Formulation 1 revealed different metabolites, notably benzene and its derivatives, tannins, and terpenes. Tannins, recognized for their antioxidant, anticancer, and antimicrobial properties, may contribute to the overall efficacy of the formulation (Pizzi, 2021). Terpenes, known for their aromatic qualities and potential health benefits, suggest multifaceted therapeutic action (Cox-Georgian et.al., 2019). The presence of benzene derivatives indicates the formulation’s chemical diversity and complexity, warranting further exploration of their specific bioactive roles.

For **Formulation 2 (F2)**, the positive ESI mode similarly identified a relative abundance of alkaloids, fatty acyls, and lipids along with their derivatives. The presence of lipids indicates the formulation's potential in promoting skin health and metabolic balance, as lipids are crucial for cellular structure and function (Park et al., 2021). The consistency of alkaloids and fatty acyls across both formulations points to a shared pharmacological foundation, potentially contributing to their traditional uses (Shi et al., 2014; Carballeira, 2008).

In the negative ESI mode for Formulation 2, notable metabolites included steroids and their substituted derivatives, terpenoid and their derivatives, and fatty acyls. The

identification of steroids suggests anti-inflammatory, antimicrobial, antioxidant and hormonal regulatory properties, while terpenoid are often associated with various therapeutic effects, including antimicrobial and anti-inflammatory actions (Ericson-Neilsen and Kaye, 2014; Marahatha et.al., 2021; Cox-Georgian et.al., 2019; Mabou and Yossa, 2021). The fatty acyls noted in both formulations emphasize their significance as essential components, likely contributing to the formulations' overall efficacy.

The comparative analysis of metabolite profiles from both ESI modes highlights the rich diversity of phytochemical constituents in these formulations, with varying prevalence of specific metabolite groups suggesting unique therapeutic potentials. This differentiation may guide future pharmacological investigations and the exploration of synergistic effects in traditional medicine. While the HR-LCMS-QTOF analysis has provided tentative metabolite identifications, further studies using techniques like NMR or targeted MS are recommended to confirm these findings and elucidate their bioactive roles. Overall, this study enhances the understanding of the metabolite compositions of these Unani formulations and sets the stage for future pharmacological research.