ASSESSMENT OF FOLK AND TRADITIONAL MEDICINAL KNOWLEDGE IN MODERN ERA WITH REFERENCE TO UNANI SYSTEMS OF MEDICINES

Ph. D. SYNOPSIS SUBMITTED

TO

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA

BY

Ms. KAMARTAHA I. SAIYED

UNDER THE GUIDANCE OF

Prof. Vinay M. Raole

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BOTANY

DEPARTMENT OF BOTANY, FACULTY OF SCIENCE,

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA,

VADODARA – 390002

JANUARY 2024

1. INTRODUCTION

Traditional systems of medicine have historically played a crucial role in global healthcare, and their significance is expected to persist in the future. In India, the indigenous systems of medicine, whether of Indian origin or assimilated from other cultures, are collectively known as Indian Systems of Medicine, encompassing Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homoeopathy, administered under the AYUSH ministry (Prasad, 2002; Ravishankar & Shukla, 2007). Recent initiatives, prompted by the pandemic and other factors, have led to increased interest in collecting and processing plant resources for potential pathogenicity solutions. This focus extends to the rich biodiversity of Northwest Gujarat, a region characterized by diverse forest vegetation and unique topography, making it a significant area for natural wealth documentation (Mevada et.al., 2020). Despite being a biodiverse region, there is limited documentation on the ethnomedicinal aspects of Northwest Gujarat, prompting a fresh perspective on its exploration and study.

The World Health Organization (WHO) has recognized the Unani system of Medicine as an alternative system to cater to the health care needs of human population. Unani system of health care has its origin in Greece. It is believed to have been established by the great physician and philosopher- Hippocrates (460-377 BC), Galen (130-201 AD). The principal source of drugs in the Unani system of medicine is plant origin drugs, Animal origin drugs and Mineral origin drugs (Mishra et.al., 2016). According to the basic principles of Unani, the body is made up of four basic elements i.e. Earth, Air, Water and Fire which have different Temperaments i. e. Cold, Hot, Wet, and Dry. They give rise, through mixing and interaction, to new entities (Ravishankar & Shukla, 2007). The Unani system of medicine, with its roots deeply embedded in historical traditions, emphasizes the holistic approach to healing by Natural remedies. Unani medicine encompasses a rich tapestry of polyherbal formulations, representing a harmonious blend of multiple herbs to achieve therapeutic synergy. As the demand for traditional medicinal practices grows globally, ensuring the quality, safety, and efficacy of Unani polyherbal formulations becomes paramount. Standardization, in the context of Unani medicine, involves establishing rigorous parameters and protocols to consistently produce formulations of known quality. This is crucial for both traditional practitioners and the broader healthcare community, ensuring reproducibility and reliability in therapeutic outcomes. This research delves into the intricate process of standardization on Unani

medicine and its polyherbal formulations, aiming to address the challenges associated with variability in herbal sources, extraction methods, and formulation techniques. By establishing standardized practices, this study seeks to contribute to the integration of Unani medicine into mainstream healthcare, fostering a bridge between ancient wisdom and contemporary scientific standards.

1.1 OBJECTIVES

1. To document the diversity and traditional knowledge of Unani medicinal plants from Northwest Gujarat.
2. To select, collect, and authenticate the plant sources of Unani drugs for a selected polyherbal formulation.
3. To prepare the selected Unani polyherbal formulations and their extraction using various techniques.
4. To conduct *In vitro* pharmacological screening of selected Unani polyherbal formulations.
5. To perform Phytochemical characterization of selected Unani polyherbal formulations.

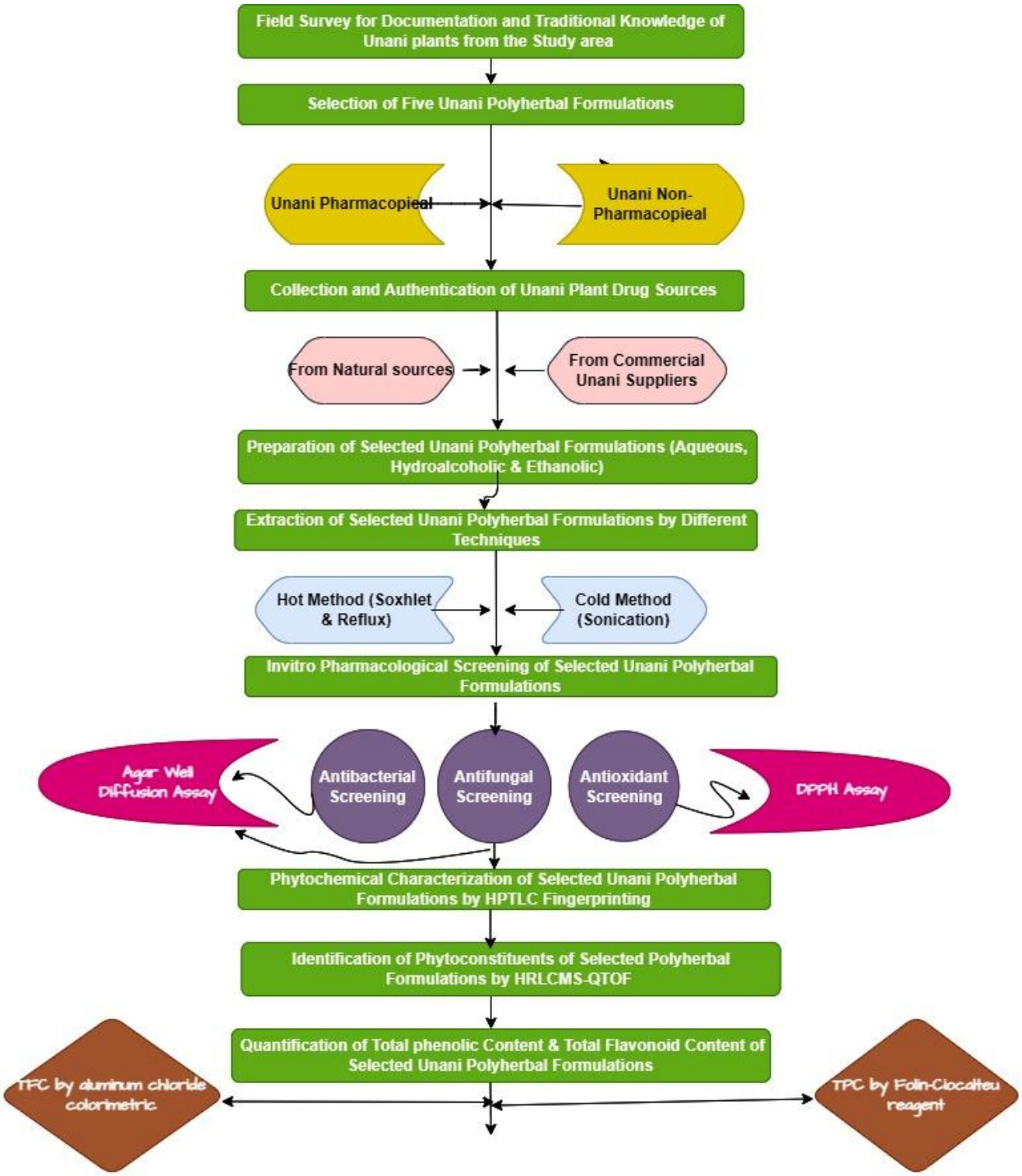
2. REVIEW OF LITERATURE

During recent years, there has been a wide concern to collect more and more ethno botanical information, especially from folk and traditional systems. Ethno botanical study in Gujarat is a result published made by Mac and Prabia (1889), Thaker (1926), Cooke (1958), Menon and Gopal (1981), Shah and Gopal (1982), Vashi (1985), Contractor (1986), Bhatt (1987), Pandya (1989), a Jain (1991). Bhatt et. al. (1999). Passing references to the plants of coastal areas were made by Reddy (1987) Uma Devi (1988) and Punjani, B. (2002,2003, 2006, 2010,2014), Hitesh Patel and R.S. Patel (2013), NB Patel and KB Patel (2015) and Chaudhari et.al, (2019) has studied the Ethno botanical Aspects of some Plants in general and some selected area of North Gujarat. Patel and Desai (2014) have focused their studies on Fruit and seed Drugs. Gadhvi and Modi and Gupta Urvi (2019, 2014), Maru and Patel (2012, 2014) have studied the Traditional Ethno medicinal plants used by Tribal communities. Most of these works provides information and clues for materials to be tested for pharmacological and clinical research, provided new distribution areas for raw drugs and a broad base for interaction with other systems of medicines.

2.1 Review of previous works on Unani medicinal system

The Unani system of medicine is a traditional healing art and science that has been practiced widely in countries such as Persia, Pakistan, India, South Africa, and England (Che et.al., 2017). It is based on the theory of four humors - blood, phlegm, yellow bile, and black bile - and aims to treat the body, mind, and soul. The World Health Organization (WHO) has recognized the Unani System of Medicine as an important traditional medicine system (Hongal et.al., 2014). Several studies have been conducted on Unani polyherbal formulations (UPFs) to evaluate their safety and efficacy in treating various ailments. UPFs are being prescribed for the treatment of various diseases, and the preparations of the UPFs also require animal products such as honey and umber (Siddique, 2023). Some of the studies have evaluated the impact of herbal Unani formulations on melanogenesis mechanism in the treatment of hypopigmentation disorder (Ghali et.al., 2022). Another study evaluated the safety and efficacy of a polyherbal Unani formulation in dyslipidemia and concluded that the formulation was equally effective in controlling dyslipidemia (Ain et.al., 2022). A study conducted on Dawa ul Kurkum, a widely used Unani formulation, showed the presence of p-coumaric acid, cinnamaldehyde, citral, crocin, isovaleric acid, guggulsterone, and dehydrocostus lactone (Gupta et.al., 2023). The study supported the use of Dawa ul Kurkum as a conventional medicine and could be used as a reference for the standardization of the formulation. However, more research is required for UPFs on experimental models along with case-controlled studies to validate their efficacy and safety (Siddique, 2023).

3. METHODOLOGY



4.RESULTS & DISCUSSION

4.1 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 belong to 73 genera, representing 49 families, and are extensively discussed. The findings are based on a literature survey from various sources, providing a comprehensive understanding of the utilization of Unani plants and their applications in treating various diseases among tribal communities in the state. The results are enumerated with details including Botanical name, Family, Folk/Local name, Unani name, Ayurveda name, and Siddha name, offering a holistic perspective on the traditional knowledge associated with these medicinal plants.

4.2 Antibacterial activity

Five Unani polyherbal formulations were tested against gram-negative (Escherichia coli) and gram-positive (Bacillus megaterium) bacterial strains using the Agar Well diffusion assay. Concentration-dependent effects were evident, with higher concentrations yielding larger inhibition zones. Ciprofloxacin and Gentamicin served as positive controls, and solvents were used for the negative control. Clear inhibition zones around the impregnated wells suggested antimicrobial efficacy. Mean ± SD of the inhibition zones' diameters across three replicates was measured and reported.

4.2.1 Majoon-E-Najah (MN)

The antibacterial activity of Majoon Najah formulation was systematically assessed against gram-negative (Escherichia coli) and gram-positive (Bacillus megaterium) strains using the agar well diffusion assay. Concentrations ranging from 0.2 to 10.0 mg/ml and various extraction methods (RF AQ, RF HA, SN AQ, SN HA, SX ET, SN ET) were employed. Results revealed concentration-dependent variations in the antibacterial efficacy against E. coli, with SX (ET) and SN (ET) extracts consistently exhibiting robust activity across all concentrations. In contrast, RF (AQ) extract showed weaker effects. Similar trends were observed against B. megaterium, where SX (ET) and SN (ET) extracts displayed significant antibacterial activity, outperforming other extracts.

4.2.2 Safoof-E-Najah (SN)

The assay involved the use of three different solvents (Aqueous (AQ), Hydro alcoholic (HA), and Ethanolic (ET)) for extraction, and varying concentrations (1.5, 2, 2.5, 3, 4, 5, 10 mg/ml) of the formulations were tested. The results indicate that the SN (HA) and SN (ET) extracts demonstrated significant antibacterial activity across all tested concentrations, whereas the SN (AQ) extract exhibited a comparatively weaker antibacterial effect against *E. coli* strains. In the case of *B. megaterium*, the SN (ET) extract displayed a strong antibacterial effect compared to the other two extracts (SN (HA) and SN (AQ)).

4.2.3 Safoof –E-Chobchini (SC)

The assay involved the use of three different solvents (Aqueous (AQ), Hydro alcoholic (HA), and Ethanolic (ET)) for extraction, and varying concentrations (1.25, 2.5, 5, 10 mg/ml) of the formulations were examined. The results indicate that SC (HA) extract displayed a robust antibacterial activity compared to the other two extracts (SC (AQ) & SC (ET)) against the *E. coli* strain. In the case of *B. megaterium*, both SC (HA) and SC (ET) extracts exhibited a strong antibacterial effect compared to SC (AQ) extract.

4.2.4 Formulation 1 (F1)

The assay utilized three different solvents (Aqueous (AQ), Hydro alcoholic (HA), and Ethanolic (ET)) for extraction, with varying concentrations (1.25, 2.5, 5, 10 mg/ml) of the formulations tested. The results suggest that F1 (HA) and F1 (ET) extracts demonstrated a more potent antibacterial effect compared to F1 (AQ) extract against the *E. coli* strain. Concerning *B. megaterium*, both F1 (HA) and F1 (ET) extracts exhibited a stronger antibacterial effect than F1 (AQ) extract.

4.2.5 Formulation 2 (F2)

The assay involved the utilization of three distinct solvents, namely Aqueous (AQ), Hydro alcoholic (HA), and Ethanolic (ET), for the extraction process. Various concentrations (1.5, 2, 2.5, 3, 4, 5, 10 mg/ml) of the formulations were employed for testing. The results suggest that F2 (HA) and F2 (ET) demonstrated a more robust antibacterial activity compared to F2 (AQ) extract against *E. coli*. Specifically, in the case of *B. megaterium*, F2 (ET) displayed a more potent antibacterial effect than both F2 (HA) and F2 (AQ) extracts.

4.3 Antifungal activity

In the agar well diffusion assay assessing antifungal activity of five Unani polyherbal formulations against Candida albicans and Aspergillus niger, substantial efficacy was observed. Using three solvents (Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET)) for extraction and testing various concentrations, all formulations consistently produced clear inhibition zones around the wells. The antifungal activity exhibited a concentration-dependent pattern, with increasing concentrations resulting in larger inhibition zones. Amphotericin served as the positive control, and solvents were used for the negative control. Mean ± SD of the inhibition zone diameters across three replicates is presented.

4.3.1 Majoon-E-Najah (MN)

Various concentrations (62.5, 125, 250, 500, 1000 ug/ml) were tested against *Candida albicans*, and (312.5, 625, 1250, 2500, 5000 ug/ml) were tested against *Aspergillus niger* for the formulations. The results indicate that the MN (HA) extract exhibited a significant antifungal effect, whereas the other two extracts, MN (AQ) and MN (ET), did not show significant antifungal activity against *Candida albicans*. In the case of *Aspergillus niger*, the MN (ET) extract exhibited a significant antifungal effect, whereas the other two extracts, MN (HA) and MN (AQ), did not show significant antifungal activity.

4.3.2 Safoof-E-Najah (SN)

Various concentrations (62.5, 125, 250, 500, 1000 ug/ml) were tested against *Candida albicans*, and (312.5, 625, 1250, 2500, 5000 ug/ml) were tested against *Aspergillus niger* for the formulations. The results suggest that the antifungal efficacy of the SN formulation against C. albicans varies with concentration. Notably, these three extracts consistently displayed robust and statistically significant antifungal activity across all tested concentrations. Moreover, the SN (AQ & HA) extract exhibited comparatively strong and pronounced antifungal effects, while the SN (ET) extract didn't show efficacy against *Aspergillus niger*.

4.3.3 Safoof –E-Chobchini (SC)

Various concentrations (62.5, 125, 250, 500, 1000 ug/ml) were tested against *Candida albicans*, and (312.5, 625, 1250, 2500, 5000 ug/ml) were tested against *Aspergillus niger* for the formulations. The results suggest that SC (ET) extract exhibited significant activity across all tested

concentrations, whereas SC (HA) exhibited a weak antifungal effect, and SC (AQ) didn't show significant antifungal activity against *Candida albicans*. In the case of *Aspergillus niger*, all three extracts showed significant activity across all tested concentrations, out of which SC (ET) exhibited a strong antifungal effect compared to SC (AQ & HA) extracts.

4.3.4 Formulation 1 (F1)

Various concentrations (62.5, 125, 250, 500, 1000 ug/ml) were tested against *Candida albicans*, and (312.5, 625, 1250, 2500, 5000 ug/ml) were tested against *Aspergillus niger* for the formulations. The results suggest that F1 (HA) exhibited a strong antifungal effect, while F1 (ET) exhibited a moderate effect, and F1 (AQ) exhibited less antifungal activity against *Candida albicans*. In the case of *Aspergillus niger*, all three extracts exhibited significant activity across all tested concentrations, out of which F1 (HA) exhibited a strong effect, and F1 (ET & AQ) exhibited antifungal activity.

4.3.5 Formulation 2 (F2)

Various concentrations (62.5, 125, 250, 500, 1000 ug/ml) were tested against *Candida albicans*, and (312.5, 625, 1250, 2500, 5000 ug/ml) were tested against *Aspergillus niger* for the formulations. The results suggest that F2 (AQ) extract exhibited significant activity across all tested concentrations, while F2 (HA & ET) extracts didn't show significant antifungal effect against *Candida albicans*. In the case of *Aspergillus niger*, F2 (HA) exhibited strong antifungal activity, and F2 (ET & AQ) exhibited moderate antifungal activity.

4.4 Antioxidant activity by DPPH assay

In the assessment of antioxidant activity through the DPPH assay, three distinct extracts (Aqueous (AQ), Hydro alcoholic (HA), and Ethanolic (ET)) from Unani polyherbal formulations were scrutinized. These extracts displayed noteworthy antioxidant potential, evident in their capacity to scavenge DPPH radicals. The observed antioxidant activity exhibited a concentration-dependent pattern, with higher concentrations of the extracts correlating with increased radical scavenging. The results affirm that the chosen Unani polyherbal formulations harbor significant antioxidant capabilities, suggesting their potential effectiveness in mitigating oxidative stress.

4.4.1 Majoon-E-Najah (MN)

In the comparison of antioxidant potential among the extracts, it was consistently observed that MN (ET) extract displayed the highest scavenging activity across all tested concentrations when compared to extracts MN (AQ & HA).

4.4.2 Safoof-E-Najah (SN)

When assessing the antioxidant potential among the extracts, consistent observations indicated that SN (AQ) extract consistently displayed the highest scavenging activity across all tested concentrations, outperforming extract MN (ET&HA).

4.4.3 Safoof –E-Chobchini (SC)

When comparing the antioxidant activities of these extracts, a consistent observation emerged, revealing that SC (HA) extract consistently exhibited the highest scavenging activity across all tested concentrations, when compared to extract SC (ET&AQ).

4.4.4 Formulation 1 (F1)

In the comparison of antioxidant potential among the extracts, F1 (ET) extract consistently exhibits the highest activity compared to F1 (HA & AQ) extracts.

4.4.5 Formulation 2 (F2)

In the comparison of antioxidant potential among the extracts, F2 (HA) extract consistently exhibits the highest activity compared to F2 (ET & AQ) extracts.

4.5 PHYTOCHEMICAL CHARACTERIZATION

The phytochemical characterization of the selected Unani polyherbal formulations was conducted through a comprehensive approach. The selected Unani Polyherbal formulations underwent assessment through techniques such as TLC, HPTLC, and HRLCMS-QTOF. Furthermore, the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of five Unani Polyherbal formulations were determined.

4.5.1 HPTLC ANALYSIS

HPTLC analysis of Ethanolic extracts from Unani polyherbal formulations (Formulation 1 & 2) employed silica gel 60 F254-coated aluminum plates. Fifteen microliter (15 µL) extracts were

applied, and separation was achieved. Formulation 1 used a toluene: ethyl acetate: formic acid: methanol solvent system in a ratio of 9:9:2.4:0.6 v/v/v/v/v/v/v. Formulation 2 employed a toluene: ethyl acetate: formic acid solvent system in a ratio of 10:8:2 v/v/v. Plates were sprayed with 10% methanolic sulfuric acid reagent, developed up to 8 cm, and air-dried. Scanning at 254 nm, 366 nm, and 540 nm revealed separated phytoconstituents. Derivatization at 105°C using an Automated CAMAG Derivatizer produced color, with subsequent scanning. Chromatograms, along with calculated Rf values, depicted diverse compounds for both formulations, offering a comprehensive HPTLC fingerprint at various wavelengths.

4.5.2 HRLCMS-QTOF PROFILING

Identification and profiling of compounds in the ethanolic extracts of two Unani polyherbal formulations (Formulation 1 & Formulation 2) were successfully accomplished using High-Resolution Liquid Chromatography-Mass Spectrometry Quadrupole Time-of-Flight (HRLCMS-QTOF) analysis in both positive and negative Electrospray Ionization (ESI) modes. The study generated a comprehensive list of identified compounds, including names, retention times, compound formulas, molecular masses, m/z values, and hits. This thorough analysis facilitated precise identification and characterization of multiple constituents in the extract. The diverse range of compounds identified underscores the formulation's complexity, with associated peak areas providing a quantitative measure of their abundance. These findings offer valuable insights into chemical composition, forming a foundation for further exploration of potential bioactive compounds and their therapeutic implications.

4.5.3 TOTAL FLAVANOID CONTENT (TFC)

The assessment of total flavonoid content was conducted on three different extracts of the five selected Unani polyherbal formulations. The results revealed varying levels of flavonoid compounds across the extracts. The total flavonoid contents of the three crude extracts of five formulations were assessed, and the calibration curve of the quercetin standard yielded the equation $y = 0.0292\times-0.1205$ with an R2 value of 0.9941.

4.5.3.1. Majoon-E-Najah (MN)

In the analysis of the three crude extracts, MN (ET) extract demonstrated the highest total flavonoid content, with a value of 3.313356 mg QE/g, followed by MN (HA) at 3.090754 mgQE/g, and MN (AQ) at 2.962329 mgQE/g.

4.5.3.2 Safoof-E-Najah (SN)

In the analysis of the three crude extracts, SN (HA) extract exhibited the highest amount of total flavonoid content compounds at 10.63356mgQE/g, followed by SN (AQ) at 9.306505 mgQE/g, and SN (ET) at 8.58733 mgQE/g.

4.5.3.3 Safoof –E-Chobchini (SC)

In the analysis of the three crude extracts, SC (ET) extract exhibited the highest amount of total flavonoid content compounds at 23.31336 mgQE/g, followed by SC (HA) at 17.85103 mgQE/g, and SC (AQ) at 6.429795 mgQE/g.

4.5.3.4 Formulation 1 (F1)

In the analysis of the three crude extracts, F1 (ET) extract exhibited the highest amount of total flavonoid content compounds at 13.03082 mgQE/g, followed by F1 (HA) at 8.784245 mgQE/g, and F1 (AQ) at 7.157535 mgQE/g.

4.5.3.5 Formulation 2 (F2)

In the analysis of the three crude extracts, F2 (ET) extract exhibited the highest amount of total flavonoid content compounds at 11.27569 mgQE/g, followed by F2 (HA) at 7.82534 mgQE/g, and F2 (AQ) at 5.77911 mgQE/g.

4.5.4 TOTAL PHENOLIC CONTENT (TPC)

The assessment of total phenolic content was conducted on three different extracts of the five selected Unani polyherbal formulations. The following results revealed varying levels of phenolic compounds across the extracts. The total phenol contents of three crude extracts of the five formulations, reported as gallic acid equivalents using the Folin-Ciocalteu method, were determined. The calibration curve generated from gallic acid exhibited maximum absorbance at a wavelength of 765 nm (equation $y = 0.0299x + 0.2257$, $R2 = 0.9738$).

4.5.4.1 Majoon-E-Najah (MN)

The total Phenolic content analysis revealed that among the three crude extracts, MN (ET) extract exhibited the highest total phenol content at 15.85229 mgGAE/g, followed by MN (HA) at 6.604795 mgGAE/g, and MN (AQ) at 4.748607 mgGAE/g.

4.5.4.2 Safoof-E-Najah (SN)

The total Phenolic content analysis revealed that among the three crude extracts, SN (AQ) extract exhibited the highest total phenol content at 37.32943 mgGAE/g, followed by SN (HA) at 37.19565 mgGAE/g, and SN (ET) at 32.26254 mgGAE/g.

4.5.4.3 Safoof –E-Chobchini (SC)

The total Phenolic content analysis revealed that among the three crude extracts, SC (HA) extract exhibited the highest total phenol content at 26.37626 mgGAE/g, followed by SC (AQ) at 22.95931 mgGAE/g, and SC (ET) at 17.10647 mgGAE/g.

4.5.4.4 Formulation 1 (F1)

The total Phenolic content analysis revealed that among the three crude extracts, F1 (AQ) extract exhibited the highest total phenol content at 36.72742 mgGAE/g, followed by F1 (HA) at 35.04403 mgGAE/g, and F1 (ET) at 30.46767 mgGAE/g.

4.5.4.5 Formulation 2 (F2)

The total Phenolic content analysis revealed that among the three crude extracts, F2 (AQ) extract exhibited the highest total phenol content at 21.77 mgGAE/g, followed by F2 (HA) at 17.02285 mgGAE/g, and F2 (ET) at 7.100885 mgGAE/g.

5. CONCLUSION

1. The systematic documentation of Unani medicinal plants in Northwest Gujarat has produced a comprehensive checklist, serving as a vital resource for researchers, practitioners, and conservationists.
2. Meticulous collection and authentication of Unani drugs in polyherbal formulations ensure botanical identity and quality control.
3. The extraction and chemical composition analysis of these formulations reveal promising antioxidant, anti-inflammatory, and antimicrobial activities, suggesting their potential for

further research and development in therapeutic applications for both traditional and modern medicine.

4. Phytochemical characterization provides valuable insights into their diverse composition, supporting potential therapeutic efficacy and targeted applications.

6. FUTURE PROSPECTS

1. Detail phytochemical characterization of these five polyherbal formulations using modern tools is needed.
2. Pre-clinical and in-depth clinical studies of these five polyherbal formulations are warranted.

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