

3. REVIEW OF LITERATURE

3.1 Ethnobotanical and Traditional Knowledge Studies in India with a Focus on the Unani System

In recent years, there has been a growing interest in gathering ethnobotanical information, especially from folk and traditional systems. Numerous studies have documented the ethnobotanical landscape of Gujarat, a region rich in plant diversity and traditional knowledge. Early efforts include studies by Thaker (1910), Saxton and Sedgwick (1918) and Cooke (1958), which laid the groundwork for subsequent ethnobotanical research in the region. Later, Shah et.al. (1981), Shah and Gopal (1982), Vashi (1985), Bhatt and Sabnis (1987) expanded this body of work. Notably, Jain (1991) and Bhatt et al. (1999) contributed extensive ethnobotanical data, providing insights into plant use across various communities in Gujarat.

Additional references to plants from coastal areas were made by Reddy (1987), Uma Devi (1988), and Punjani (1997, 2002, 2003, 2006, 2010, 2014). Studies by Patel and R.S. Patel (2013), NB Patel, and KB Patel (2015) as well as Chaudhari et al. (2019) examined the ethnobotanical aspects of specific plants in North Gujarat. Meanwhile, Patel et.al., (2014) focused on the medicinal uses of fruit and seed drugs. Research by Gadhvi and Modi (2019), Gupta (2014), and Maru and Patel (2012) explored traditional ethnomedicinal plants used by tribal communities. These studies collectively highlight the diversity and potential pharmacological value of Gujarat's flora, setting a foundation for future pharmacological and clinical research by offering new distribution areas for raw drugs and fostering interactions with other medicinal systems.

Beyond Gujarat, significant ethnobotanical research has emerged from other states in India, such as Rajasthan, Madhya Pradesh, and Chhattisgarh (Singh and Pandey, 1980; Shah and Gopal, 1982; Vartak and Ghate, 1990; Maheswari, 1996; Dam and Hajra, 1997; Varma et.al., 1999; Choudhury et al., 2008; Mohapatra et al., 2008; Kala, 2009; Meena and Yadav, 2010; Dahare and Jain, 2010; Sawant and Rodrigues, 2015). In India's Union Territories, Jammu and Kashmir (Lone et al., 2014; Dhyani and Wani, 2015; Kanta et al., 2018), Dadra, Nagar Haveli, and Daman (Sharma and Singh, 2001), and the Andaman and Nicobar Islands (Bhargava, 1983; Sharief, 2007; Pullaiah et al., 2017; Chander and Vijayachari, 2018) have also been well documented, indicating the country's rich heritage of ethnobotanical knowledge.

Across India, traditional knowledge of medicinal plants has been explored over the years, highlighting the use of plants in monoherbal and polyherbal remedies to treat a range of ailments, such as rheumatism, gynecological disorders, respiratory issues, sexual health, chronic diseases, and as contraceptives or abortifacients (Mali et al., 2006; Sharma et al., 2009; Vijay et al., 2009; Kumar and Mishra, 2011; Manjula et al., 2013; Maruthamuthu and Ramanathan, 2016). Studies by Aruna et al. (2015) and Kaur et al. (2015) specifically explored the traditional knowledge of plants used by women, while Tripathi and Srivastava (2010) documented 11 ethnomedicinal species of Euphorbiaceae employed in treating dermatological problems. Additionally, Simon et al. (2011) described single-drug remedies from Idukki district, Kerala, for managing chronic conditions.

While recent years have seen significant efforts to document and systematize traditional medicinal knowledge, relatively limited work has been undertaken to preserve the foundational knowledge of the Unani system, particularly in Gujarat. The Traditional Knowledge Digital Library (TKDL) project has initiated the documentation of ancient medical wisdom, including the Unani system (Ansari et.al., 2020). However, studies specifically focused on Unani formulations remain sparse, highlighting a need for more in-depth research.

This gap underscores the importance of developing standardization protocols to ensure the safety, efficacy, and consistent quality of Unani formulations. Standardization would not only support the preservation of traditional knowledge but also facilitate its integration into contemporary healthcare practices. Therefore, further research on Unani medicine's pharmacological properties, coupled with efforts toward formulation standardization, is essential for validating and safeguarding its therapeutic potential.

3.2 Polyherbal Formulations: Therapeutic Importance and Applications

Polyherbal formulations have become a focal point in traditional and modern therapeutic research, driven by the non-toxic nature of medicinal plants and their cost-effectiveness as alternative treatments for a range of diseases (Chusri et.al., 2017). According to the World Health Organization, approximately 60–80% of the population in developing nations relies primarily on medicinal plants for their primary healthcare needs, reflecting the deep-rooted reliance on herbal medicine systems. This growing preference has fueled the global herbal medicine market, which is projected to reach

approximately USD 117 billion by 2024, showcasing the increasing integration of plant-based treatments in global healthcare systems (Yadav et.al., 2020).

Historically, both developed and developing countries have utilized natural herbs and extracts to address a wide array of health conditions. The chemical complexity of these natural products results in an abundance of biologically active compounds that possess diverse pharmacological properties. This intrinsic richness has positioned natural products as a valuable resource in drug discovery, often providing new lead compounds and chemical scaffolds for pharmaceutical development. Consequently, natural products are viewed as crucial contributors in the quest for effective drugs to treat human diseases, meeting the urgent demand for innovative and reliable therapeutic agents (Yuan et.al., 2016). The challenges presented by synthetic antimicrobial agents—such as toxicity, low efficacy, the emergence of antimicrobial resistance, and high costs—further underscore the need for potent, plant-derived alternatives. Developing effective plant-based therapies could offer significant advantages in combating microbial diseases (Gupta et.al., 2018; Kanchibhotla et.al., 2022).

Polyherbal preparations, composed of various medicinal plants, offer a synergistic therapeutic approach by combining multiple bioactive constituents that interact to enhance therapeutic effectiveness (Famewo et.al., 2017; Balamithra et.al., 2019). Due to their broad therapeutic range and high efficacy, polyherbal formulations have been traditionally used to treat a wide variety of conditions, including diabetes, arthritis, liver and kidney disorders, respiratory issues, fever, asthma, and tuberculosis (Balamithra et.al., 2019). Notably, these formulations exhibit potent antiviral, antibacterial, and antifungal activities, contributing to their efficacy in managing diverse infectious and inflammatory conditions (Famewo et.al., 2017; Singh et.al., 2015; Pandya et.al., 2011; Muthubalaji et.al., 2013; Zonyane et.al., 2013).

This review underscores that polyherbal formulations play a pivotal role in the treatment and management of various diseases, displaying significant antimicrobial, anti-inflammatory, and antioxidant activities. The wealth of traditional knowledge and clinical experience associated with polyherbal formulations has made them indispensable in the global pursuit of integrative healthcare. The scientific community has responded with heightened research interest, particularly in India, which leads in the number of publications on polyherbal formulations, followed by Nigeria and

Pakistan (Figure 2). This trend demonstrates the increasing recognition of polyherbal formulations as a valuable, evidence-based option for managing various health conditions.

3.3 Antimicrobial Activity of Polyherbal Formulations

Polyherbal formulations, traditionally derived from ethnobotanical research, have increasingly been identified as valuable sources of antimicrobial agents. These natural plant-based formulations, comprising diverse bioactive compounds such as alkaloids, phenols, glycosides, saponins, flavonoids, tannins, salicylic acid, and terpenes, are promising alternatives for developing novel antimicrobial drugs, offering unique compounds to combat infections on a global scale (Awodele et.al., 2013). Studies indicate that the synergistic interaction of these bioactive constituents enhances the therapeutic efficacy of polyherbal formulations against a wide range of microorganisms (Raslin and Rajkumar, 2017; Aziz et.al., 2017; Afolabi et.al., 2012; Kajaria et.al., 2012; Sreekumar and Abraham, 2020).

For instance, tannins—polyphenols abundant in plant foods—have exhibited notable antibacterial activity. Known for their protein-precipitating properties, tannins inhibit the growth of a variety of microorganisms, including bacteria, fungi, yeasts, and viruses, by deactivating microbial enzymes, hindering microbial adherence, and interacting with polysaccharides to form complexes (Mary and Christudhas, 2015; Nazar et.al., 2020). Similarly, flavonoids have demonstrated antimicrobial properties by forming complexes with extracellular proteins and binding to bacterial cell walls, disrupting cell membranes in the process. Compounds such as alkaloids, saponins, and steroids also contribute substantially, each bringing unique bioactivities: alkaloids offer bactericidal, analgesic, and antitumor properties; saponins exhibit anticancer, antioxidant, antimicrobial, and anti-inflammatory effects; while steroids are recognized for their anti-inflammatory properties (Mary and Christudhas, 2015; Dev et.al., 2019; Abbas et.al., 2021).

The effectiveness of polyherbal formulations is also observed to be concentration-dependent, with antimicrobial activity varying based on the microbial strains tested. Select formulations, including THR-SK004, THR-SK010, and THR-SK011, demonstrated substantial antibacterial activity against methicillin-resistant *Staphylococcus aureus* (*S. aureus*) with minimum inhibitory concentrations (MICs) of

4-8 µg/mL, meeting criteria for potent antimicrobial activity as per Cos et al. (2006). Other polyherbal formulations, such as Ambrex, Ya-Sa-Marn-Phlae, and several designated as KWTa, KWTb, KWTc, and HBts, exhibited MIC values below 50 µg/mL, signifying strong antimicrobial potential (Raslin and Rajkumar, 2017; Chusri et.al., 2017; Famewo et.al., 2017).

Additional research has highlighted the broad-spectrum antimicrobial efficacy of certain formulations like Chyawanprash, Plashbijadi churna, and Vranahitkara ghruta, which demonstrated MIC values ranging between 0.625-10 mg/mL and comparable efficacy to standard drugs in microbial assays (Gupta et.al., 2018; Kanchibhotla et.al., 2022). Some of these, including NOQ19 and Triple Bark formulations, even outperformed control drugs in terms of antibacterial activity and showed promising antiviral potential, with NOQ19 eradicating 100% of viral particles at 0.9 mg/mL and an IC₅₀ of 0.2 mg/mL (Kanchibhotla et.al., 2022). Certain polyherbal remedies also showed significant anti-tubercular activity, with KWTa, HBfs, and HBts inhibiting *Mycobacterium tuberculosis* at concentrations as low as 1.562 µg/mL, suggesting potential roles for these formulations in managing tuberculosis (Famewo et.al., 2017, 2018).

While many polyherbal formulations exhibit strong antimicrobial properties, some formulations—such as Leone Bitters, Chandra Kalka, and Sharkaradi Kalka—lacked notable antimicrobial activity. This variability underscores the necessity for comprehensive preclinical and clinical studies on polyherbal remedies to ascertain their effectiveness and safety profiles (Famewo et.al., 2017). Variations in MIC values across polyherbal formulations are attributed to the direct inhibition of microbial growth, disruption of cell membrane integrity, genetic differences among pathogens, and synergistic interactions of bioactive phytochemicals.

Overall, polyherbal formulations represent a viable alternative for managing microbial infections. However, additional research is warranted to expand the range of tested pathogens, assess large-scale effectiveness, and investigate potential side effects. These findings illustrate the promising role of polyherbal formulations in modern healthcare, advocating for rigorous safety and efficacy studies to harness their full therapeutic potential (Aladejana et.al., 2023).

3.4 Antioxidant Activity of Polyherbal Formulations

Polyherbal formulations (PHFs) have emerged as potential sources of antioxidants, which protect the human body against oxidative stress by neutralizing free radicals. These free radicals, especially reactive oxygen species (ROS), are linked to oxidative damage at the cellular level, which can result in a variety of health complications, including DNA mutations that may lead to carcinogenesis (Chamara et.al., 2018; Sreekumar and Abraham, 2020). The antioxidants present in PHFs counteract oxidative stress by multiple mechanisms, including scavenging free radicals, chelating metal ions, and enhancing endogenous antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). This, in turn, protects cells from oxidative damage, preserving cellular integrity and minimizing the risk of ROS-related diseases (Abbas et.al., 2021; Kumar et.al., 2018).

The antioxidant properties of PHFs are typically attributed to bioactive phytochemicals like phenols, flavonoids, tannins, and saponins, which exhibit substantial free radical scavenging capacities (Kumaravel and Alagusundaram, 2014; Agidew, 2022). For instance, flavonoids form complexes with proteins and can bind to bacterial cell walls, while lipophilic flavonoids disrupt microbial cell membranes, further contributing to their therapeutic potential (Abbas et.al., 2021). These natural antioxidant agents have gained attention for their health benefits and are increasingly preferred over synthetic antioxidants due to their biocompatibility and lower risk of adverse effects (Kumar et.al., 2018; Wilson et.al., 2016).

Research has demonstrated the antioxidant efficacy of several polyherbal formulations using various methods, such as DPPH radical scavenging, FRAP assay, and ABTS radical cation scavenging assays, as well as in vivo models. Some formulations have shown promising results comparable to standard antioxidants. For example, the polyherbal remedy used for urinary tract infections exhibited a scavenging activity of 98.63%, surpassing natural antioxidants like rutin (60.32%) and synthetic BHT (64.40%), suggesting superior antioxidative efficacy (Abbas et.al., 2021). Additionally, studies on Thalipathiri Chooranam, traditionally used for treating cough, have shown high nitric oxide radical scavenging activity, which was comparable to that of gallic acid, a positive control, thereby indicating its potential antioxidant capacity (Mary and Christudhas, 2015).

The efficacy of polyherbal formulations is often attributed to their high phenolic content, which correlates strongly with DPPH radical scavenging activities, enabling these extracts to eliminate free radicals efficiently in biological systems (Abbas et.al., 2021). Additionally, formulations like Habb-e-Asgand have been shown to protect against oxidative stress-induced damage, such as that caused by paracetamol toxicity, by significantly enhancing the activity of antioxidant enzymes like glutathione reductase and catalase (Ali et.al., 2014). Similarly, polyherbal formulations such as Livergen, PNK, Karisalai Karpam, and HP-4 have been reported to increase the levels of antioxidant enzymes while reducing lipid peroxidation markers, indicating potent antioxidant action (Arsul et.al., 2011; Shah et.al., 2011; Padmanabhan and Jangle, 2014; Sen et.al., 2015).

Conversely, not all Polyherbal formulations yield positive outcomes; some, such as certain bitters and herbal mixtures, have been linked to decreased hepatic antioxidant enzyme levels due to the overproduction of free radicals, leading to depletion of key enzymes like GSH and CAT in experimental models. This highlights the importance of dosage optimization and careful selection of ingredients in PHFs, as improper formulation can result in adverse effects. It is crucial to continue research on the dosage, duration, and potential side effects of these formulations to ensure both efficacy and safety (Emaleku, 2018).

Overall, the antioxidant potential of polyherbal formulations supports their therapeutic application in preventing oxidative stress-induced diseases by scavenging free radicals, upregulating endogenous antioxidant enzymes, and mitigating oxidative damage. This antioxidant activity positions PHFs as promising alternatives for managing conditions associated with oxidative stress, with further studies required to establish safety profiles and optimal therapeutic use (Aladejana et.al., 2023).

3.5 Review of Recent Pharmacological Research on Unani Polyherbal Formulations

The Unani system of medicine, rooted in ancient Persia and widely practiced in regions such as Pakistan, India, South Africa, and England, operates on the concept of four humors (blood, phlegm, yellow bile, black bile) and treats the body, mind, and soul holistically (Che et al., 2017). Azami (1995, 1998) laid the groundwork by detailing the fundamental concepts and research methods pertaining to the history of Unani

medicine. Recognized by the World Health Organization (WHO) as a vital traditional medicine system (Hongal et al., 2014), Unani medicine increasingly relies on scientific validation of its polyherbal formulations (UPFs) to ensure safety and efficacy in treating various ailments (Mannan et.al., 2020). This validation is crucial for establishing the safety and efficacy of these formulations in treating a variety of health conditions, bridging traditional knowledge with modern healthcare standards (Itrat et.al., 2016; Naaz et.al., 2021; WHO, 2022).

UPFs, often comprising both plant and animal-derived ingredients like honey and amber, are traditionally prescribed for a wide range of conditions (Siddique, 2023). Ghali et al. (2022) explored the effect of herbal Unani formulations on melanogenesis, relevant to hypopigmentation treatments, while Ain et al. (2022) confirmed a UPF's safety and efficacy in managing dyslipidemia. Despite promising findings, further experimental and case-controlled studies remain essential to bolster UPFs' evidence base (Siddique, 2023).

Neuroprotective research reveals Unani medicine as a natural alternative with reduced side effects for neurodegenerative disorders, identifying 43 herbs and 38 formulations for cognitive, neuroprotective, and antioxidant benefits, particularly against Alzheimer's and Parkinson's. While several formulations show efficacy, additional trials are needed for wider clinical adoption (Imran et al., 2024). Ahmad et al. (2021) demonstrated that *Safoof-e-Pathar phori* (SPP), an antiurolithiatic Unani formulation, significantly reduced markers like urinary calcium and serum creatinine in rats, with no long-term toxicity, supporting its safe therapeutic use.

Clinical studies also validate UPFs. In a study on *Pityriasis versicolor* (PV), an Unani polyherbal treatment significantly reduced symptoms compared to standard therapy, with no adverse effects (Lone et al., 2012). Similarly, Husain et al. (2022) highlighted the anti-inflammatory potential of a coded Unani formulation, reinforcing Unani medicine's role in managing inflammation. Safety studies on formulations like *Jawarish Shahi* (JS) revealed no adverse effects at doses up to 2000 mg/kg in rats (Urooj et al., 2018), while *Qurs Tabasheer* demonstrated effective antihyperglycemic and antihyperlipidemic actions in diabetic rats, surpassing Glimpiride (Ahmed et al., 2012).

Further studies reveal Unani's cosmetic potential. Bibi et al. (2024) demonstrated the efficacy of a polyherbal formulation for improving skin quality in photoaged skin without irritation, presenting a safer alternative to tretinoin. Similarly, Siddiqui et al. (2023) reviewed the potential of UPFs for neurodegenerative conditions, emphasizing their affordability and low side effects as alternatives to conventional treatments. Findings on *Majoon-e-Nisyan* showed cognitive enhancement in mice, suggesting potential for dementia management (Shaikh et al., 2024). A UPF tested against Telmisartan for Stage-2 hypertension showed equivalent effectiveness, supporting Unani formulations as viable antihypertensive options (Siddiqui et al., 2024).

Other studies have validated UPFs for their biochemical efficacy, including *Dawa ul Kurkum* (Duk), where key phytochemicals were identified to support its therapeutic use (Gupta et al., 2023). Khanam et al. (2024) demonstrated a UPF's effectiveness in reducing fever and pain in animal models, confirming its analgesic and antipyretic potential. Toxicity assessments of *Habb-e-Azaraqi* (HAZ) showed no significant adverse effects, supporting its use as a nervine treatment, with detoxification processes enhancing safety (Ara et al., 2021). In liver protection, *Habb-e-Asgand* reduced paracetamol-induced liver toxicity in mice, suggesting its role in antioxidant therapy (Ali et al., 2013).

Standardization studies ensure quality control in UPFs, such as *Sufoof-e-Mohazzil*, an anti-obesity formulation analyzed by Patil et al. (2013), which demonstrated anatomical markers, favorable alcohol extractive values, and an HPTLC fingerprint profile, while *Safoof Razyana* (SR) was characterized for quality control using ash values, density, extractive values, and compound identification (Ikram et al., 2019).

This review underscores the ongoing pharmacological research into Unani polyherbal formulations, validating their safety, efficacy, and standardization, while highlighting the need for more rigorous experimental models and clinical trials to solidify their role in modern medicine.

3.6 Review on selected Unani polyherbal formulations:

3.6.1 Majoon-E-Najah (MN) and Safoof-E-Najah (SN)

According to the *Unani Pharmacopoeia of India* (UPI, Pt. II, Vol. 3, Government of India, 2016), Majoon-e-Najah is a traditional Unani polyherbal formulation recognized

for its therapeutic benefits, particularly its blood-purifying (Musaffi-e-Dam) and nervine tonic (Muqawwi-e-Asab) properties. The formulation is used in the treatment of various conditions, including melancholia (Malikhuliya), colic (Qulanj), and hysteria (Ikhtenaq-ur-Raham), and plays a significant role in Unani medicine for both mental and physical health. The following sections provide detailed insights into its identification, physicochemical properties, therapeutic uses, and dosage instructions, as outlined in the UPI (2016).

3.6.1.1 Identification and Microscopic Analysis

Majoon-e-Najah is composed of various plant ingredients with distinctive microscopic characteristics. According to the UPI (2016), the microscopic examination of the formulation reveals the following features:

- Collapsed schizogenous canals and cortical cells (Aftimoon),
- Epidermal cells that elongate to form hair-like protuberances (Post-e-Balela),
- Tabular and polygonal epidermal cells (Aamla),
- Uni- and biseriate medullary rays, and resin cells (Turbud),
- Single branched and stellate hairs, and numerous smooth pollen grains (Ustukhuddus),
- Pigmented parenchyma (Bisfayej), and
- Thin-walled parenchymatous cells containing rosette crystals of calcium oxalate (Halela).

These microscopic features are critical for the authentication and quality control of the formulation.

3.6.1.2 Thin Layer Chromatography (TLC) Analysis

The standardization of Majoon-e-Najah is confirmed through TLC analysis. The UPI (2016) specifies that 5 g of the sample is extracted with 60 ml of alcohol, refluxed for 10 minutes, filtered, and concentrated to 4 ml. The extract is then applied to a silica gel G TLC plate. The mobile phase consists of a chloroform: methanol mixture in a 7.4:2.6 ratio. The developed TLC plate is sprayed with a 2% ethanolic sulfuric acid reagent and

heated at 105°C. The resulting chromatogram reveals five distinct spots with Rf values at:

- **0.10** (greenish black),
- **0.24** (greenish black),
- **0.62** (bluish black),
- **0.76** (bluish black), and
- **0.86** (brownish black).

These spots indicate the presence of multiple active compounds in the formulation.

3.6.1.3 Physicochemical Parameters

The physicochemical properties of Majoon-e-Najah comply with the standards set by the UPI (2016). Key parameters include:

- **Total Ash:** Not more than 1.00% (w/w),
- **Acid Insoluble Ash:** Not more than 0.50% (w/w),
- **Alcohol Soluble Matter:** Not less than 14.00% (w/w),
- **Water Soluble Matter:** Not less than 55.00% (w/w),
- **pH of 1% Aqueous Solution:** Ranges from 3.38 to 3.43,
- **Reducing Sugar:** Not less than 25.00% (w/w),
- **Non-reducing Sugar:** Not more than 20.00% (w/w).

The formulation also meets microbial load limits and is free from aflatoxins, pesticidal residues, and heavy metals, ensuring its safety and efficacy.

3.6.1.4 Actions and Therapeutic Uses

Majoon-e-Najah is primarily known for its blood-purifying (Musaffi-e-Dam) and nervine tonic (Muqawwi-e-Asab) actions. It is commonly used in the treatment of the following conditions:

- Malikhuliya (Melancholia),

- Qulanj (Colic),
- Ikhtenaq-ur-Raham (Hysteria).

These therapeutic uses highlight formulation's value in addressing both neurological and physical health issues.

3.6.1.5 Dosage and Administration

According to the guidelines in the UPI (2016), the recommended dosage of Majoon-e-Najah is 5-10 g per day. It is typically administered with water in the morning to enhance absorption and optimize its therapeutic effects.

3.6.1.6 Storage and Stability

For maintaining the formulation's potency and safety, it is recommended to store Majoon-e-Najah in a cool, dry place, in tightly closed containers, and away from light and moisture, as per the UPI (2016) guidelines.

3.6.1.7 Modern Research on Majoon-E-Najah

Majoon-e-Najah (MN) has long been recognized in Unani medicine for its effectiveness in managing neurological and psychological disorders, particularly epilepsy, hysteria, and melancholia (Afrin et al., 2017). Modern pharmacological studies continue to validate MN's neurotherapeutic potential, particularly for conditions related to the nervous system.

Recent studies have provided evidence of MN's anticonvulsant properties. Afrin et al. (2019) explored MN's efficacy in reducing seizure activity, particularly in models such as Increased Current Electroshock (ICES) and Pentylentetrazole (PTZ)-induced convulsions. These studies suggest that MN may serve as a natural alternative for managing seizures.

Further research by Urooj et al. (2020) tested MN's anticonvulsant and antidepressant effects using animal models. While the formulation did not show significant seizure reduction in electroshock and PTZ-induced convulsion models, it exhibited considerable antidepressant activity, demonstrating a reduction in immobility in the forced swim test—an established measure for depressive behavior in rodents. This

finding indicates that MN may be more effective as an antidepressant than as an anticonvulsant.

In addition to its neurological benefits, MN has demonstrated hepatoprotective, antioxidant, and anti-inflammatory properties. Singh et al. (2023) examined the hepatoprotective effects of MN's hydroalcoholic extract (MNHE), finding it to be effective in protecting liver function and mitigating oxidative stress caused by cadmium chloride (CdCl₂). Despite limited histopathological recovery in liver damage models, the hydroalcoholic extract showed promise in reducing oxidative stress and enhancing antioxidant enzyme activity.

MN is also noted for its potential in hormonal balance, with studies by Naikoo et al. (2007) suggesting its effectiveness in alleviating menopausal symptoms, highlighting its role in promoting hormonal stability and addressing age-related symptoms.

3.6.1.8 Future Research Directions

While much of the research on MN has focused on hydroalcoholic extracts, there remains a lack of *In vitro* studies and comprehensive phytochemical profiling. Future research should aim to investigate the effects of aqueous and ethanolic extracts in greater detail and standardize the formulation's bioactive components.

3.6.1.9 Safoof-e-Najah (SN)

Safoof-e-Najah (SN), a powdered variant of Majoon-e-Najah without honey, remains largely unexplored in scientific literature. This formulation represents an untapped therapeutic opportunity within Unani medicine. The current study aims to conduct *In vitro* analyses of both Majoon-e-Najah and Safoof-e-Najah across three different extracts: aqueous, hydroalcoholic, and ethanolic. By doing so, the research seeks to enhance the understanding of MN and SN's pharmacological properties, support phytochemical standardization, and validate their therapeutic potential within Unani medicine.

3.6.2 Majoon-e-Najah Formulation: Ingredient Overview

The **Majoon-e-Najah (MN)** formulation is a traditional Unani polyherbal remedy comprising eight key ingredients, each contributing distinct pharmacological and

therapeutic benefits. These ingredients have a longstanding history in Unani medicine for addressing various health conditions, ranging from digestive issues to neurological disorders. Below is a concise review of the primary ingredients and their therapeutic actions:

3.6.2.1 *Terminalia chebula* Retz. (Post-e-Halela Kabli)

Post-e-Halela Kabli known for its purgative, stomachic, and diuretic properties, *T. chebula* supports digestive health and balances the humors. It is used as a brain tonic and memory enhancer, addressing conditions like melancholia, epilepsy, and dementia. Recent studies validate its antioxidant, neuroprotective, immunomodulatory, and antidiabetic effects, emphasizing its broad therapeutic potential for mental and metabolic health (Afrin et al., 2017; Raman et al., 2015; Chandra et al., 2012).

3.6.2.2 *Terminalia bellerica* (Gaertn.) Roxb. (Post-e-Balela)

T. bellerica acts as a stomachic, astringent, and expectorant, benefiting gastrointestinal and respiratory health. It also exhibits antimicrobial, antihypertensive, and hepatoprotective effects, reinforcing its utility in managing infections, liver health, and metabolic conditions (Bibhabasu et al., 2010; Vaseem et al., 2012; Mary & Gayathri, 2015; Afrin et al., 2017).

3.6.2.3 *Emblica officinalis* Gaertn. (Amla)

Amla is a potent cardio tonic and brain tonic, enhancing memory and vitality (Kanthimathi & Soranam, 2013). It supports digestive health and has antioxidant, hepatoprotective, and anti-inflammatory properties. It is also valued for its role in managing diabetes, liver conditions, and gastrointestinal disorders (Khare, 2007; Venil et al., 2008; Mani et al., 2010; Afrin et al., 2017).

3.6.2.4 *Terminalia chebula* Retz. (Halela Siyah)

Similar to *T. chebula*, this variety offers diuretic, cardi tonic, and neuroprotective effects. It also aids in digestion and alleviates conditions such as dyspepsia and constipation (Afrin et al., 2017). Modern research confirms its antioxidant, antibacterial, and antiulcerogenic benefits, enhancing its role in general health and wellness (Hua-Yew et al., 2003; Ajay et al., 20017; Rubini et al., 2013).

3.6.2.5 *Operculina turpethum* (L.) Silva Manso (Turbud)

Turbud Known for its powerful purgative properties, aids in expelling phlegm and bile, supporting respiratory and digestive health (Afrin et al., 2017). It has shown anti-secretory, ulcer-protective, and anti-inflammatory actions, validating its traditional uses in treating gastrointestinal and liver disorders (Anbuselvam et al., 2007; Kohli et al., 2010; Anju and Radhamany, 2014).

3.6.2.6 *Polypodium vulgare* Linn. (Bisfayej)

Bisfayej is valued for its purgative, cardiotonic, and anti-inflammatory effects. It has antioxidant, antimicrobial, and anti-arthritic properties, supporting digestive, cardiovascular, and joint health (Afrin et al., 2017). Modern studies confirm its therapeutic relevance in managing oxidative stress and infections (Ghani and Al-Adwiya, 2010; Gaamoune et al., 2015).

3.6.2.7 *Cuscuta reflexa* Roxb. (Aftimoon)

Aftimoon is an aphrodisiac, tonic, and diuretic, traditionally used for conditions like jaundice, paralysis, and neurological issues such as epilepsy (Afrin et al., 2017). It has demonstrated anxiolytic, anti-inflammatory, and anticonvulsant effects, as well as antibacterial and cytotoxic properties, suggesting its potential in managing anxiety, infections, and cancer (Pavan et al., 2012; IbnSina, 2007; Sujith et al., 2015).

3.6.2.8 *Lavandula stoechas* Mill. (Ustukhuddus)

Ustukhuddus acts as a nervine tonic, anticonvulsant, and hepatoprotective agent. It supports mental clarity and respiratory health while offering antimicrobial and anti-inflammatory benefits (Afrin et al., 2017). Recent research highlights its potential for managing neurological disorders, chronic inflammation, infections and potential cancer treatment (Barkat & Laib, 2012; Seema & Jon, 2014; Miraj & Sepideh, 2016; Shakeri et al., 2016).

The **Majoon-e-Najah** formulation is a well-rounded Unani remedy, harnessing the therapeutic properties of its eight ingredients to support a wide range of bodily systems. From promoting digestive health and enhancing cognitive function to offering anti-inflammatory, antimicrobial, and neuroprotective effects, MN's ingredients are

validated by modern scientific studies. The formulation's comprehensive therapeutic profile underscores its potential as a natural remedy for various health conditions, particularly for managing gastrointestinal, neurological, and metabolic disorders.

3.6.3 Sufoof-e-Chobchini Formulation (SC)

According to the *Unani Pharmacopoeia of India* (UPI, Pt. II, Vol. 1, Government of India, 2009), *Sufoof-e-Chobchini* is a traditional formulation known for its various therapeutic uses, including its anti-inflammatory and analgesic properties. The formulation consists of several herbal ingredients, with *Chobchini* (the root of *Smilax china* Linn.) being the main constituent. It is commonly used in Unani medicine to alleviate pain, reduce inflammation, and treat arthritic conditions, including joint pain (*Waj-ul-Mafasil*), gout (*Niqras*), and syphilis (*Aatishak*). Additionally, it is recognized for its expectorant (*Munaffis-e-Balgham*), antibilious (*Daf-e-Safra*), and blood purifying (*Musaffi-e-Dam*) properties (UPI, Pt. II, Vol. 1, Government of India, 2009). The following sections provide details on its identification, composition, and physicochemical characteristics as outlined in the UPI (2009).

3.6.3.1 Microscopic Identification:

Microscopic examination of *Sufoof-e-Chobchini* reveals several distinct features:

- Paracytic stomata (*Sana*)
- Pigmented parenchyma (*Bisfayej*)
- Muller-shaped starch granules (*Suranjan*)
- Spherical tricolpate-shaped pollen grains (*Gul-e-Surkh*)
- Barrel-shaped pitted vessels with tail-like projections (*Sandal Safaid*)
- Collapsed schizogenous canals associated with cortical cells (*Aftimoon*)
- Mucilaginous parenchyma with raphides of calcium oxalate (*Chobchini*)
- Starch grains within the cortical parenchyma (*Ushba Maghribi*)

3.6.3.2 Thin Layer Chromatography (TLC):

The ethanolic extract of *Sufoof-e-Chobchini* was analyzed by TLC using silica gel “G” plates with chloroform: methanol (9:1) as the mobile phase. The chromatogram showed

eight distinct spots at the following Rf values: 0.14, 0.30, 0.37, 0.39, 0.43, 0.68, 0.80, and 0.87. These spots were visualized after exposing the plate to vanillin-sulfuric acid reagent and heating at 105°C for 10 minutes. This TLC analysis provides a chromatographic fingerprint that can be used for the standardization and quality control of the formulation.

3.6.3.3 Physicochemical Parameters:

The physicochemical characteristics of *Sufoof-e-Chobchini* comply with standard specifications:

- **Total Ash:** Not more than 4.00%
- **Acid Insoluble Ash:** Not more than 1.50%
- **Alcohol Soluble Matter:** Not less than 10.00%
- **Water Soluble Matter:** Not less than 17.00%
- **Loss on Drying:** Not more than 12.00% at 105°C
- **pH (1% aqueous solution):** 5.00 to 5.50
- **pH (10% aqueous solution):** 5.10 to 5.50

The formulation complies with microbial load limits and is free from aflatoxins, pesticidal residues, and heavy metals, ensuring its safety for use.

3.6.3.4 Storage:

Sufoof-e-Chobchini should be stored in a cool place, in a tightly closed container, protected from light and moisture to maintain its efficacy.

A clinical trial by Khan et al. (2021) assessed the efficacy of *Sufoof-e-Chobchini*, where patients were administered 3.0 g of the powder along with 20 mL of *Sharbat-e-Buzoori* and *Raughan-e-Baboona* oil for 42 days. The study demonstrated significant improvement in inflammation and pain symptoms, supporting its therapeutic benefits. However, despite this promising trial, there is limited research on this formulation, with only this single clinical study available to date. Further investigation is required to substantiate its clinical applications.

Sufoof-e-Chobchini has long been recognized for its therapeutic potential, particularly in treating joint pain, gout, and related inflammatory conditions. Despite its historical and clinical significance, there remains a significant lack of modern research or experimental studies on this formulation. The available pharmacological details primarily stem from the Unani Pharmacopoeia of India (2009), which outlines its established therapeutic uses and provides guidelines for dosage and administration. Further research is essential to validate these traditional claims and explore the underlying mechanisms of action of this Unani formulation.

3.6.4 Safoof-e-Chobchini Formulation: Ingredient Overview

The Safoof-e-Chobchini (SC) formulation is a blend of eight traditional Unani ingredients, each renowned for its distinct therapeutic properties. Below is a concise review of the key ingredients and their contributions to the formulation's efficacy:

3.6.4.1 *Smilax china* Linn. (Chobchini)

Chobchini is valued in Unani medicine for its demulcent, anti-inflammatory, and blood-purifying properties. It aids in detoxification, addresses syphilis, leprosy, and various inflammatory conditions. Modern research supports its antioxidant, anti-inflammatory, and neuroprotective effects, validating its use in inflammatory, infectious, and neurological disorders (Ibn Rushd, 1980; Adebajo, 2010). Typical dosage: 6 g.

3.6.4.2 *Cassia angustifolia* Vahl (Senna)

Senna is a powerful purgative, also serving as a diuretic, blood purifier, and cardiac tonic. It treats conditions such as constipation, jaundice, and scabies, with traditional uses for arthritis, sciatica, and digestive disorders (Qayoom et al., 2022). Its leaves, used topically with *Lawsonia inermis*, are effective in treating skin rashes. Dosage: 7–9 g for purgative effects (Kirtikar and Basu, 2012; Khan, 2014; Ghani and Al-Adwiya, 2010).

3.6.4.3 *Smilax aristolochiifolia* Mill. (Ushba Maghribi)

Ushba Maghribi Known for its anti-inflammatory and antioxidant properties, supports blood purification, diabetes management, and weight loss. It is also used as a diuretic and for promoting perspiration (Qing-Feng et al., 2009; Argueta and Gallardo-Vazquez,

2013). This herb's versatility extends to both medicinal and culinary applications (Amaro et al., 2014).

3.6.4.4 *Polypodium vulgare* Linn. (Bisfayej)

Bisfayej is effective as a purgative, expelling excess phlegm and black bile, contributing to detoxification and overall well-being, as detailed in the Majoon-E-Najah formulation (Afrin et.al., 2017).

3.6.4.5 *Colchicum luteum* Baker (Suranjan)

Suranjan is widely recognized for its anti-inflammatory and analgesic properties, particularly in the treatment of arthritis, gout, and rheumatism. It also acts as a blood purifier and is beneficial in managing joint pain, sciatica, and digestive issues (Nadkarni, 2007). Dosage: 3–6 g of powdered root (Bhattacharjee, 2004).

3.6.4.6 *Cuscuta reflexa* Roxb. (Aftimoon)

Aftimoon is a blood-purifying herb with a broad therapeutic profile, addressing conditions like epilepsy, anxiety, and skin disorders. It is also known for its anticancer, anti-inflammatory, and antioxidant effects (Suresh et al., 2011; Thomas et al., 2015; Sharma et al., 2012). Dosage: 3–21 g (Anonymous, 2007).

3.6.4.7 *Rosa damascena* Herrm. (Damask Rose)

Damask Rose offers a wide range of medicinal benefits, including heart and liver toning, as well as digestive support (Ibn Baitar, 2003). It is known for its soothing effects on the nervous system and is used for conditions like sore throat, abdominal pain, and uterine hemorrhages (Najmul Ghani, 2011; Boskabady et al., 2011).

3.6.4.8 *Santalum album* L. (Sandalwood)

Sandalwood is valued for its calming and therapeutic effects, treating conditions such as respiratory infections, fever, and inflammation. It also supports heart health and blood purification, with research supporting its antiviral, anti-inflammatory, and hepatoprotective properties (Battaglia, 2007; Sindhu et al., 2010; Misra and Dey, 2013).

The Safoof-e-Chobchini formulation combines the medicinal qualities of these eight Unani ingredients to address a broad spectrum of health conditions, including

inflammation, digestive disorders, skin diseases, and blood purification. Its formulation is rooted in traditional use, and modern research supports the efficacy of these ingredients in treating a variety of ailments, confirming their relevance in contemporary therapeutic practices. This blend exemplifies the holistic approach of Unani medicine, utilizing natural ingredients to promote overall health and well-being.

3.6.5 Formulation 1 (F1) and Formulation 2 (F2)

Formulation 1 (F1) and Formulation 2 (F2) are novel Unani polyherbal formulations developed by a local Unani practitioner. These formulations, composed of distinct blends of herbs, have not been explored in existing literature, making this study crucial to understanding their therapeutic potential within Unani medicine.

Formulation 1 (F1), consisting of five ingredients, is designed to improve female reproductive health and enhance immunity. Its therapeutic uses include acting as an immunoenhancer, aphrodisiac, and antioxidant, as well as supporting female infertility, offering antidiabetic benefits, antimicrobial effects, and neuroprotective properties.

Formulation 2 (F2), made of four ingredients, focuses on female infertility and impotence, with therapeutic uses that include treating female reproductive disorders, acting as an aphrodisiac, uterotonic, and ovulation-inducing agent, as well as providing phytoandrogenic effects, and offering antistress and insulin-sensitizing benefits.

The present study conducted a comprehensive phytochemical analysis, standardization, and *in vitro* pharmacological evaluation of both formulations to assess their therapeutic potential, thereby bridging the research gap and laying the groundwork for future clinical applications in Unani medicine.

3.6.5.1 Formulation 1 (F1): Ingredient Overview

Formulation 1 (F1) is a carefully composed Unani polyherbal formulation comprising four significant ingredients, each contributing unique therapeutic benefits to support various bodily systems. Below is a summary of the pharmacological properties associated with each ingredient.

3.6.5.1.1 *Withania somnifera* (L.) Dunal (Baikh Asgand)

Withania somnifera, known as Baikh Asgand in Unani medicine, is a renowned adaptogenic herb traditionally used as a general tonic (Muqawwie aam) and uterine tonic (Muqawwie reham). It provides notable anti-inflammatory (Muhallile waram), deobstruent (Muffatehe sudad), and blood-purifying (Musaffie khoon) effects, which enhance circulation and help in managing bodily blockages (Kabeeruddin, 2007). It is also recognized for its sedative (Munawwim) and relaxant (Musakkin) qualities, promoting restful sleep and reducing stress. Additionally, it is known to enhance immune health, gastrointestinal function, and memory, while contributing antioxidant and immunomodulatory effects (Hakim et al., 2005; Ghani and Al-Adwiya, 2010; Saiyed et al., 2016).

3.6.5.1.2 *Barleria prionitis* L. (Baikh Piyabansa)

Baikh Piyabansa is highly valued for its bioactive compounds, including iridoid glycosides, which contribute antibacterial activity against pathogens such as *Bacillus cereus* and *Pseudomonas aeruginosa*, and antifungal effects against organisms like *Candida albicans* (Shukla et al., 2011). *Barleria prionitis* has demonstrated antioxidant activity, likely due to its high phenolic content, and is effective in scavenging radicals in various assays (Chetan et al., 2011). It further exhibits antidiabetic, anthelmintic, antiviral, and antifertility effects, showcasing its broad therapeutic applications (Bhattacharya et al., 2020; Dheer & Bhatnagar, 2010).

3.6.5.1.3 *Woodfordia fruticosa* (L.) Kurz (Gule Dhawa)

Gule Dhawa is appreciated for its anti-hyperglycemic, antidepressant, and anti-inflammatory properties, aiding in the management of diabetes, mood disorders, and inflammatory conditions. It also possesses antibacterial, antioxidant, and gastro protective effects, supporting infection control and gastrointestinal health. Additionally, it has shown hepatoprotective, immunostimulatory, anticancer, and wound-healing potential, making it highly beneficial in therapeutic applications (Giri et al., 2023; Sagar et al., 2024, 2024a).

3.6.5.1.4 *Nymphaea alba* L. (Gule Nilofar)

Gule Nilofar, known for its aphrodisiac (Muqawwi Bah), blood-purifying (Musaffi dam), and nervine tonic (Musakkin asab) properties, is widely used in Unani medicine to support cardiovascular, reproductive, and nervous system health. This ingredient also provides anti-inflammatory, antipyretic, anxiolytic, and hepatoprotective benefits, aligned with its traditional use in treating conditions such as fever, insomnia, heart palpitations, and digestive issues (Ghani & Al-Adwiya, 2010; Khan et al., 2019). Its pharmacological properties further include antioxidant, antidiabetic, and antifertility activities, demonstrating broad therapeutic relevance (Anindya et al., 2013; Jacob et al., 2013; Selvakumari et al., 2016; Sumathi & Sethamarai, 2015).

Each ingredient of Formulation 1 (F1) contributes distinct therapeutic properties, making this Unani formulation versatile and supportive for various health conditions. Together, these ingredients provide a synergistic effect, enhancing the formulation's potential as an adaptogenic and revitalizing agent suitable for promoting overall health and well-being in line with traditional Unani practices. This formulation exemplifies a holistic approach, addressing multiple physiological systems with emphasis on inflammation control, immune support, gastrointestinal health, and mental well-being.

3.6.5.2 Formulation 2 (F2)

Formulation 2 (F2) is a thoughtfully composed blend of five Unani ingredients, each contributing unique pharmacological properties:

3.6.5.2.1 *Mucuna pruriens* (L.) DC. (Kocha)

Mucuna pruriens seed known for its broad therapeutic applications, seeds serve as carminative, hypoglycemic, aphrodisiac, diuretic, and anti-parkinsonian agents in Unani medicine. Studies have demonstrated its effectiveness in Parkinson's disease, highlighting its role in enhancing mitochondrial function and neurotransmitter levels without the dyskinesic side effects associated with conventional levodopa treatment (Katzenschlager et al., 2004; Manyam et al., 2004). Additional research indicates that the seed extract provides neuroprotection and improves motor coordination in preclinical models of Parkinson's disease (Kasture et al., 2009a; Lieu et al., 2012).

3.6.5.2.2 *Withania somnifera* (L.) Dunal (Baikh Asgand)

The root of *Withania somnifera*, also known as Baikh Asgand, acts as a revitalizing tonic in Unani medicine. Known for its adaptogenic and neuroprotective properties, it is a significant component in strengthening the nervous system and improving overall vitality, as previously detailed in Formulation 1.

3.6.5.2.3 *Daucus carota* L. (Gajar bij)

The whole plant of *Daucus carota*, especially its subspecies *D. carota* ssp. *carota*, shows notable anticancer, antibacterial, and antioxidant properties. Its essential oils, containing β -caryophyllene and α -humulene, have demonstrated cytotoxicity against colon and breast cancer cells and efficacy in inhibiting both bacterial and fungal pathogens (Ambrož et al., 2017; Zeinab et al., 2011; Shebawy et al., 2013; Asilbekova et al., 2017). The plant also has anti-inflammatory effects, comparable to NSAIDs, and offers gastric protection, making it a valuable component for holistic health in Unani therapeutics (Wehbe et al. 2009).

3.6.5.2.4 *Asparagus racemosus* Willd. (Satavar)

Asparagus racemosus known as Satavar in Unani medicine, the root of *Asparagus racemosus* serves as an aphrodisiac, uterine tonic, and galactagogue, promoting female reproductive health and reducing inflammation in reproductive organs (Ghani and Adviya, 2010). It also has antioxidant, anti-ulcer, and hepatoprotective properties, attributed largely to its saponin content, which provides additional therapeutic effects such as immunomodulation and anti-inflammatory benefits (Sharma and Bhatnagar, 2011; Alok et al., 2013; Shah et al., 2014).

3.6.5.2.5 *Hygrophila auriculata* (Schumach.) Heine (TaalmaKhana)

The whole plant is used traditionally for conditions like jaundice and urinary infections, *Hygrophila auriculata* exhibits hepatoprotective, antidiabetic, and antioxidant activities. Studies have shown its capacity to protect liver health, lower blood glucose, and reduce oxidative stress markers in diabetic models (Singh and Handa, 1995; Vijayakumar et al., 2006). It also demonstrates diuretic, antimicrobial, and antinociceptive effects, offering a broad therapeutic profile useful for managing pain, infections, and inflammation (Shanmugasundaram et al., 2005; Hussain et al., 2009).

Each ingredient in F2 is selected based on its specific pharmacological effects and contributes to the formulation's overall therapeutic potential, aligning with the holistic principles of Unani medicine. This combination enhances neuroprotective, anti-inflammatory, and antioxidant capabilities, supporting its use in treating neurodegenerative conditions and promoting general health.

Based on the objectives outlined for this study, it is clear that traditional knowledge plays a vital role in the continued use of Unani medicine in the modern era. By documenting the diversity of Unani medicinal plants in Northwest Gujarat and selecting authentic plant sources for polyherbal formulations, this study aims to not only preserve traditional knowledge but also validate it scientifically through pharmacological screening and phytochemical characterization. The findings of this research will contribute to bridging the gap between folk medicinal knowledge and modern pharmacology, thus supporting the integration of Unani polyherbal formulations into mainstream healthcare. Furthermore, the standardization and rigorous testing of these formulations will lay the foundation for their broader therapeutic use, ensuring that traditional practices can evolve into safe, effective, and scientifically validated therapeutic options in contemporary medicine.