7. SUMMARY OF KEY FINDINGS

The Present study represents the first comprehensive evaluation of five Unani polyherbal formulations—Majoon-E-Najah (MN), Suffof-E-Najah (SN), Suffof-E-Chobchini (SC), Formulation 1 (F1), and Formulation 2 (F2)—using advanced analytical and pharmacological methods. Among these, Formulations 1 and 2 are novel and were investigated for the first time.

7.1 Diversity assessment and traditional knowledge of Unani plants

A survey conducted during 2020–2021 explored the diversity and traditional knowledge of medicinal plants in Northwest Gujarat, documenting 137 species, with 84 referenced in the Unani system of medicine. These plants belong to 73 genera across 49 families, predominantly Fabaceae and Solanaceae, each represented by 5 species. Detailed data, including botanical names, local names, and traditional uses, were collected through field visits and interactions with local Hakims and Vaidyas. The study also compared these plants with other traditional medicinal systems, highlighting their cross-system medicinal relevance. The findings provide a foundational checklist of Unani medicinal plants in the region, emphasizing their phytodiversity and traditional medicinal significance.

A study in Central Gujarat (2020–2021) explored the polyherbal formulation *Batrisu Vasanu* (Katlu), traditionally used for post-pregnancy care. Surveys across three districts identified 53 plant species from 34 families, with roots, fruits, and seeds being the most utilized parts. Nine species were consistently present in all samples, emphasizing their prominence. Twelve species were validated for gynecological uses in Unani medicine. This work complements the Northwest Gujarat study, showcasing the diversity and application of Unani medicinal plants.

7.2 Selection, Authentication and preparation of selected Unani Polyherbal formulations

The study focused on five Unani polyherbal formulations, including three traditional formulations—Majoon-E-Najah (MN), Sufoof-E-Najah (SN), and Sufoof-E-Chobchini (SC)—prepared as per the Unani Pharmacopoeia (UPI) and National Formulary of Unani Medicine (NFUM), alongside two novel formulations, Formulation 1 (F1) and Formulation 2 (F2), which incorporate contemporary modifications by Hakims.

Ingredients were sourced from natural habitats, authenticated using taxonomic literature, and deposited in the BARO Herbarium for reference. The formulations were prepared following standardized methods, ensuring consistency and reliability for further pharmacological studies.

7.3 Organoleptic properties of the five Unani polyherbal formulations

The organoleptic evaluation of the five Unani polyherbal formulations revealed distinct sensory attributes critical for therapeutic efficacy and patient acceptance. Majoon-E-Najah (MN) was dark brown with a semi-solid consistency, sweet with slight bitterness, and had a pleasant aroma. Sufoof-E-Najah (SN) appeared yellowish-brown with a distinctly bitter taste and characteristic odor, while Sufoof-E-Chobchini (SC) was yellowish-green, slightly bitter, and aromatic. Formulation 1 (F1) was brown, bitter, with a strong odor, and Formulation 2 (F2) was yellowish, slightly bitter, with a characteristic odor. These consistent sensory traits align with traditional standards, ensuring quality and acceptability.

7.4 Extraction Efficiencies

The extraction yields of five Unani polyherbal formulations (Majoon-E-Najah, Sufoof-E-Najah, Sufoof-E-Chobchini, Formulation 1, and Formulation 2) were determined using various solvent systems (Aqueous, Hydroalcoholic, and Ethanol) and extraction methods (Reflux, Sonication, and Soxhlet). Results showed variability in extraction efficiencies, with sonication generally providing the highest yields, particularly for aqueous and hydroalcoholic solvents.

7.5 Antibacterial activity

The antibacterial activity of the extracts from *Majoon-E-Najah* (MN), *Suffof-E-Najah* (SN), *Suffof-E-Chobchini* (SC), **Formulation 1 (F1)**, and **Formulation 2 (F2)** was evaluated against *Escherichia coli* and *Bacillus megaterium*.

7.5.1 Majoon-E-Najah (MN):

The ethanolic extract exhibited the strongest antibacterial effects, showing larger inhibition zones against both bacterial strains, outperforming the aqueous and hydroalcoholic extracts.

7.5.2 Suffof-E-Najah (SN):

The hydroalcoholic extract showed the highest efficacy against *E. coli*, while the ethanolic extract demonstrated the strongest activity against *B. megaterium*. The aqueous extract showed the least antibacterial effects for both bacteria.

7.5.3 Suffof-E-Chobchini (SC):

The hydroalcoholic extract exhibited the strongest activity against *E. coli,* and both the hydroalcoholic and ethanolic extracts demonstrated significant activity against *B. megaterium*. The aqueous extract was the least effective for both strains.

7.5.4 Formulation 1 (F1):

The hydroalcoholic and ethanolic extracts showed significant antibacterial activity, with zones of inhibition ranging from 5 to 9.8 mm. The aqueous extract showed minimal activity for both bacterial strains.

7.5.5 Formulation 2 (F2):

The Ethanolic (ET) and Hydroalcoholic (HA) extracts demonstrated the strongest antibacterial activity against *Escherichia coli* and *Bacillus megaterium*. The ET extract showed notable activity against both bacteria, while the Aqueous (AQ) extract exhibited minimal to no antibacterial effects. The HA extract was also effective, particularly against *E. coli*.

7.6 Antifungal activity

The antifungal activity of *Majoon-E-Najah* (MN), *Suffof-E-Najah* (SN), *Suffof-E-Chobchini* (SC), **Formulation 1 (F1)**, and **Formulation 2 (F2)** was evaluated using aqueous, hydroalcoholic, and ethanolic extracts against *Candida albicans* and *Aspergillus niger*.

7.6.1 Majoon-E-Najah (MN):

The hydroalcoholic (HA) extract showed significant activity against *C. albicans*, while the ethanolic (ET) extract demonstrated strong antifungal effects against both fungi, particularly at higher concentrations. The aqueous extract showed minimal or no activity.

7.6.2 Suffof-E-Najah (SN):

Both the aqueous (AQ) and hydroalcoholic (HA) extracts demonstrated strong antifungal activity against both *C. albicans* and *A. niger*, with inhibition zones ranging from 8 to 15 mm. The ethanolic (ET) extract showed minimal activity.

7.6.3 Suffof-E-Chobchini (SC):

The ethanolic (ET) extract showed significant activity against both fungi, with larger inhibition zones. The aqueous (AQ) extract displayed moderate effects against *A. niger*, while the hydroalcoholic (HA) extract showed weak antifungal activity.

7.6.4 Formulation 1 (F1):

The hydroalcoholic (HA) extract showed the strongest antifungal effects against both fungi, while the ethanolic (ET) extract exhibited moderate activity. The aqueous (AQ) extract displayed the least efficacy.

7.6.5 Formulation 2 (F2):

The aqueous (AQ) extract exhibited significant activity against *C. albicans*, while the hydroalcoholic (HA) and ethanolic (ET) extracts showed no significant effects. For *A. niger*, the HA extract demonstrated strong activity, with moderate effects observed for both AQ and ET extracts.

7.7 Antioxidant activity

The antioxidant activity of five Unani polyherbal formulations—Majoon-E-Najah (MN), Suffof-E-Najah (SN), Suffof-E-Chobchini (SC), Formulation 1 (F1), and Formulation 2 (F2)—was assessed using the DPPH assay. Three extracts were tested for each formulation: Aqueous (AQ), Hydroalcoholic (HA), and Ethanolic (ET).

7.7.1 Majoon-E-Najah (MN):

The ethanolic (ET) extract exhibited the highest antioxidant activity with the lowest IC50 value (32.94±1.21 µg/ml), while the aqueous (AQ) and hydroalcoholic (HA) extracts showed moderate to low antioxidant potential, respectively.

7.7.2 Suffof-E-Najah (SN):

The ethanolic (ET) extract demonstrated superior antioxidant activity with an IC50 value of 3.79 ± 0.28 µg/ml, outperforming both the aqueous (AQ) and hydroalcoholic (HA) extracts. Notably, the ethanolic extract showed higher activity than ascorbic acid, suggesting the presence of potent antioxidant compounds.

7.7.3 Suffof-E-Chobchini (SC):

The ethanolic (ET) extract displayed the highest antioxidant activity with an IC50 value of 22.72 ± 0.53 µg/ml, surpassing the aqueous (AQ) and hydroalcoholic (HA) extracts, indicating the efficacy of ethanolic extraction for isolating active antioxidant compounds.

7.7.4 Formulation 1 (F1):

The aqueous (AQ) extract demonstrated the highest antioxidant activity with an IC50 value of 73.58 ± 0.33 µg/ml, followed by the hydroalcoholic (HA) extract. The ethanolic (ET) extract exhibited the lowest antioxidant activity, suggesting aqueous extraction is the most effective for isolating antioxidant compounds in F1.

7.7.5 Formulation 2 (F2):

The hydroalcoholic (HA) extract exhibited the highest antioxidant activity with an IC50 value of 91.56 ± 0.51 µg/ml, followed by the ethanolic (ET) extract. The aqueous (AQ) extract showed the least activity, highlighting hydroalcoholic extraction as the most effective for isolating antioxidant compounds in F2.

7.8 The total phenolic content (TPC)

The total phenolic content of extracts from five distinct Unani polyherbal formulations was assessed using the Folin-Ciocalteu method, with results expressed as gallic acid equivalents (GAE).

The total phenolic content (TPC) of Majoon-E-Najah (MN) extracts, assessed using the Folin-Ciocalteu method, revealed the highest phenolic content in the ethanolic (ET) extract (15.852 mg GAE/g), followed by the hydroalcoholic (HA) (6.605 mg GAE/g) and aqueous (AQ) extracts (4.749 mg GAE/g). This highlights the superior efficiency of ethanolic extraction for isolating phenolic compounds in MN.

The total phenolic content (TPC) of Suffof-E-Najah (SN) extracts was highest in the aqueous (AQ) extract (37.329 mg GAE/g), followed by the hydroalcoholic (HA) (37.196 mg GAE/g) and ethanolic (ET) extracts (32.263 mg GAE/g). This suggests that the aqueous extraction method is particularly effective for isolating phenolic compounds in SN.

The total phenolic content (TPC) of Suffof-E-Chobchini (SC) was highest in the hydroalcoholic (HA) extract (26.376 mg GAE/g), followed by the aqueous (AQ) (22.959 mg GAE/g) and ethanolic (ET) extracts (17.106 mg GAE/g). This highlights the hydroalcoholic extraction method as the most effective for SC.

The total phenolic content (TPC) of Formulation 1 (F1) was highest in the aqueous (AQ) extract (36.727 mg GAE/g), followed by the hydroalcoholic (HA) (35.044 mg GAE/g) and ethanolic (ET) extracts (30.468 mg GAE/g). This highlights the effectiveness of the aqueous extraction method for F1.

The total phenolic content (TPC) of Formulation 2 (F2) was highest in the aqueous (AQ) extract (21.770 mg GAE/g), followed by the hydroalcoholic (HA) extract (17.023 mg GAE/g), with the ethanolic (ET) extract showing the lowest content (7.101 mg GAE/g). Aqueous extraction proved most effective for F2.

7.9 Total flavonoid content (TFC)

The total flavonoid content of three different extracts (Aqueous, Hydroalcoholic, and Ethanolic) from five distinct Unani polyherbal formulations was evaluated using the colorimetric method with quercetin as the standard.

The ethanolic (ET) extract of Majoon-E-Najah (MN) exhibited the highest total flavonoid content (3.313 mg QE/g), followed by the hydroalcoholic (HA) extract (3.091 mg QE/g) and the aqueous (AQ) extract (2.962 mg QE/g). Ethanolic extraction proved most effective for flavonoid isolation in MN.

The hydroalcoholic (HA) extract of Suffof-E-Najah (SN) showed the highest total flavonoid content (10.634 mg QE/g), surpassing the aqueous (AQ) extract (9.307 mg QE/g) and the ethanolic (ET) extract (8.587 mg QE/g), indicating the efficiency of hydroalcoholic extraction for flavonoid isolation.

The ethanolic (ET) extract of Suffof-E-Chobchini (SC) exhibited the highest total flavonoid content (23.313 mg QE/g), followed by the hydroalcoholic (HA) extract (17.851 mg QE/g) and the aqueous (AQ) extract (6.430 mg QE/g), highlighting the superior efficiency of ethanolic extraction for this formulation.

In Formulation 1 (F1), the ethanolic (ET) extract exhibited the highest flavonoid content (13.031 mg QE/g), followed by the hydroalcoholic (HA) extract (8.784 mg QE/g) and the aqueous (AQ) extract (7.158 mg QE/g), indicating the superior efficiency of ethanolic extraction for flavonoid isolation.

In Formulation 2 (F2), the ethanolic (ET) extract had the highest flavonoid content (11.276 mg QE/g), followed by the hydroalcoholic (HA) extract (7.825 mg QE/g) and the aqueous (AQ) extract (5.779 mg QE/g), emphasizing the efficiency of ethanolic extraction for flavonoid isolation.

7.10 Pearson correlation analysis

The Pearson correlation analysis of the five formulations reveals complex interactions between antimicrobial, antifungal, and antioxidant activities, with total phenolic content (TPC) and total flavonoid content (TFC) playing crucial roles. These findings highlight the varying contributions of bioactive compounds across different extracts and formulations, emphasizing their importance in mediating pharmacological effects.

7.11 HPTLC Fingerprinting

The HPTLC analysis of Formulation 1 (F1) and Formulation 2 (F2) revealed distinct phytochemical profiles with unique fingerprints for each formulation. The ethanolic extracts showed the highest number of bands, with derivatization enhancing the visibility of specific phytoconstituents. These fingerprints provide valuable insights into the formulations' composition, aiding in their standardization, quality control, and the identification of marker compounds for further study.

7.12 HR-LCMS-QTOF analysis

The HR-LCMS-QTOF analysis of Formulations 1 and 2 identified a wide range of metabolites, including alkaloids, phenols, fatty acyls, flavonoids, and more, in both positive and negative ESI modes. A total of 49 metabolites were identified in the positive ESI mode and 64 in the negative mode for F1, while F2 had 64 in the positive

and 43 in the negative mode. Cross-referencing with various databases confirmed their identities and potential pharmacological significance. This analysis aids in the standardization of these formulations by providing a detailed and reproducible metabolite profile, supporting their use in Unani medicine.

This preliminary investigation provides a foundational understanding of the phytochemical and pharmacological properties of these Unani formulations. The findings emphasize the efficacy of ethanolic extraction, the correlation between phenolic and flavonoid content with biological activities, and the therapeutic potential of these formulations. The novel formulations (F1 and F2) and their distinct phytochemical profiles contribute significantly to the field of Unani medicine, laying the groundwork for further clinical studies and standardization protocols.