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Hippocratic Journal of Unani Medicine

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Review Article

Gulqand (Flower Conserve) in Unani Medicine: Composition, Therapeutic Insights, and Contemporary Relevance

Abstract

Edible flowers have been conventionally utilized to enhance the taste, appearance, and nutritional value of food and beverages since ancient times. Novel evidence regarding the composition and health benefits of these flowers provides a compelling rationale for their consumption. They are a good source of carbohydrates, vitamins, minerals, antioxidants, and several bioactive compounds with strong medicinal properties, including anti-inflammatory, antioxidant, antimicrobial, anti-anxiety, cardioprotective, hepatoprotective, and neuroprotective. In the Unani system of medicine, *Gulqand* is a sweet preserve made from petals of edible flowers, especially rose petals. It is believed to offer various health benefits, including providing nutrition, refreshing the mood, purifying the blood, improving memory, boosting energy, and helping to balance body heat. It acts as a powerful antioxidant and laxative, and resolves gastric and cardiac ailments. In addition to its medicinal uses, *Gulqand* is utilized in Unani medicine to mitigate various side effects of certain herbal drugs and as a binding agent for pills and tablets. Commercially, it is used to enhance the flavor, esthetic appeal, and nutritional value of various processed food products, such as milkshakes, cakes, and pastries. Further exploration of its nutritional and medicinal properties could promote its broader adoption in both traditional and modern healthcare practices while also making it a strong contender for use in the food and beverage industry. This review article aims to emphasize various formulations, compositions, and preparation methods of *Gulqand*, along with its therapeutic benefits, particularly in the context of Unani medicine.

Keywords: *Edible flowers, Gulqand, Rosa damascena, sugar, Unani system of medicine*

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Introduction

Edible flowers such as roses, viola, calendula, lavender, and chamomile have traditionally been utilized for centuries to augment food taste, appearance, and nutritional value worldwide.[1] Roses, for instance, were used in ancient Rome to add sweetness and taste to salads, beverages, and sweets. The blossoms of the calendula plant were included in a variety of salads in medieval France. The unique ability of violets (*Viola odorata* L.) to bring sweetness and color to syrups was the basis for their historical application in the 17th century.[2] Contrary to common opinion, flowers provide a special blend of flavors and nutrients to culinary preparations, making them more than just decorations for savory dishes and sweets. In addition to being used fresh with salads, they may also be used in soups, beverages, desserts, and other foods.[3] Emerging information concerning edible flowers' composition and health-promoting properties is crucial and provides an excellent rationale for their ingestion. These contain carbohydrates, proteins, vitamins, and minerals, and are rich in antioxidants.[4,5] Various bioactive constituents present in edible blossoms are responsible for potential health benefits, such as anti-diabetic, anti-cancer, anti-inflammatory, antimicrobial, antioxidant, neuroprotective, hepatoprotective, and cardioprotective properties.[6] They are primarily represented by phenolic compounds, flavonoids, alkaloids, carotenoids, terpenoids, nitrogen-containing compounds, and organosulfur compounds.[7] Among these, phenolic compounds (gallic acid, chlorogenic acid, *p*-coumaric acid, and *p*-hydroxybenzoic acid) and flavonoids (flavonols, flavones, flavanones, and anthocyanins) are of prime biomedical interest. Their key bioactive property is associated with antioxidant activity, which helps prevent cell damage by neutralizing free radicals. Antioxidants are believed to help reduce the risk of chronic diseases,

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such as heart disease, cancer, and diabetes, by protecting cells from oxidative stress.[8]

In the Unani system of medicine, edible flowers such as rose, hibiscus, chamomile, borage, and viola are highly regarded for their health benefits and are commonly used in the formulations prescribed by Unani physicians. *Gulqand* (flower conserve) is a formulation prepared by preserving edible petals with sugar or hone. It is typically made by using rose petals; however, petals of some other flowers may also be used for the preparation of different types of *Gulqand*.[9] It is believed to offer various health benefits, such as relieving fatigue and lethargy, purifying the blood, improving memory, boosting energy, and helping to balance body heat.[10]

The Persian word *Gulqand* is formed from the words *gul* (rose) and *qand* (sugar). To make *Gulqand,* fresh petals are separated from the rest of the flower, thoroughly rubbed with sugar or honey, and kept for a specific period to get the final product.[9,11] If fresh flowers are not available, then dry flowers should be soaked in water or any suitable *'araq* (distillate) before preparation of *Gulqand*.[12] The *Gulqand,* which is prepared with honey instead of sugar, is called *Gulqand 'Asali* or *Julanjabīn* (rose-petal conserve).[9] When it is prepared by exposing a mixture of flower petals and sugar or honey to sunlight, it is known as *Gulqand Āftābī*. When *Gulqand* is prepared under moonlight, it is termed as *Gulqand Māhtābī*.[13] There is another kind of *Gulqand* known as *Gulqand Ābī,* which is prepared by exposing the closed vessel containing flower petals mixed with sugar or honey to water. The proportion of petals and sugar or honey is recommended to be taken as 1:1 or 1:2.5, but the sweetness may be added up to 4 times the weight of flowers.[9] The addition of sweetness more than the suggested proportion may decrease the efficacy of *Gulqand*.[12]

Methods of Preparation and Other Descriptions of Various *Gulqand*

Gulqand-i-Gulāb

Ingredients: *Gul-i-Surkh* (*Rosa damascena* Mill. flower) and sugar.

Method of preparation: To prepare *Gulqand-i-Gulāb*, one part of rose petals is thoroughly rubbed and mixed with one part of sugar. It is then filled into a jar and kept in sunlight for a week to get the final product.[14]

The second method of preparing *Gulqand-i-Gulāb* involves mixing one part of rose petals with three parts of sugar. A small quantity of *'araq-i-gulāb* (rose distillate) is added, and the mixture is kept in sunlight for 3–4 days.[15]

Therapeutic actions and uses: *Gulqand-i-Gulāb* is beneficial for constipation. It strengthens the brain, liver, and stomach, acts as an exhilarant, and prevents depressive effects. Its use is also indicated in the case of tuberculosis.[13-17] When it is consumed continuously after adding *ustukhuddus* (*Lavandula stoechas* L.) and *banafsha* (*V. odorata* L.), it cures fever, headache, and migraine.[11]

Dose: 10–30 g.[16]

Gulqand-i-Sewtī

Ingredients: *Gul-i-Sewtī* (*Rosa alba* L. flower) and sugar.

Method of preparation: To prepare *Gulqand-i-Sewtī*, one part of *Rosa alba* petals is thoroughly rubbed and mixed with one part of sugar. It is then filled into a jar and kept in sunlight for a week to get the final product.[14]

Gulqand-i-Sewtī can also be prepared by taking 100 pieces of *Rosa alba* and 348 g of sugar. *'Araq-i-bedmushk* (distillate of *Salix caprea* L.) is sprinkled over the flower. The flowers are then mashed, mixed with sugar, and kept in the shade for 4 days.[13,15]

Therapeutic actions and uses: It acts as a cardiotonic, exhilarant, and soothing agent, and is beneficial for palpitations and cardiac insufficiency.[11-16]

Dose: 10–20 g.[16]

Gulqand-i-Gāwzabānī

Ingredients: *Gul-i-Gāwzabān* (*Borago officinalis* L. flower) and sugar.

Method of preparation: One part of fresh flowers is taken, rubbed, and mixed with two parts of sugar to prepare *Gulqand-i-Gāwzabānī*.[11,12] If fresh flowers are unavailable, dry flowers can be used after soaking them in rose distillate.

Therapeutic actions and uses: It tones up the brain and heart, acts as an exhilarant, and expels *sawdāwī maddah* (melancholic matter) from the body.[11,12,18,19]

Gulqand-i-Gurhal

Ingredients: *Gul-i-Gurhal* (*Hibiscus rosa-sinensis* L. flower), *Āb-i-līmū kāghadhī* (*Citrus aurantiifolia* (Christm.) Swingle juice) or *Āb-i-anār tursh* (*Punica granatum* L. juice), and sugar.

Method of preparation: Flowers are rubbed and mixed with sugar, with the addition of *Āb-i-līmū kāghadhī* or *Āb-i-anār tursh,* half of the weight of the flowers.[20]

Therapeutic actions and uses: It acts as an exhilarant and a soothing agent. It is beneficial for palpitations and general debility. It improves blood production and expels yellow bile from the body.[16]

Dose: 10–30 g.[16]

Gulqand-i-Khashkhāsh

Ingredients: *Gul-i-Khashkhāsh* (*Papaver somniferum* L. flower) and sugar.

Method of preparation: Fresh flowers of *Khashkhāsh* are taken, rubbed, and mixed well with three parts of sugar.

It is then kept in sunlight for 3 days to get *Gulqand-i-Khashkhāsh*.[20]

Therapeutic actions and uses: It is astringent in action, prevents diarrhea, and cures cough and catarrh.[20]

Dose: 10–15 g.[16]

Gulqand-i-Amaltās

Ingredients: *Gul-i-Amaltās* (*Cassia fistula* L. flower) and *shakar surkh* (brown sugar).

Method of preparation: Petals of fresh *C. fistula* flowers are taken and mixed with an equal proportion of brown sugar. The mixture is then kept in sunlight to get the final product.

Therapeutic actions and uses: It is laxative in action, cures constipation, and evacuates yellow bile through purgation.[20]

Dose: 20–30 g.[16]

Gulqand-i-Hinā

Ingredients: *Gul-i-Hinā (Lawsonia inermis* L. flower) and purified honey.

Method of preparation: Fresh flowers are taken and mixed well with purified honey. The mixture is then kept in sunlight for 3 days to get the final product.[20]

Therapeutic actions and uses: It purifies blood.[16]

Dose: 10–20 g.[16]

Gulqand-i-Nīm

Ingredients: *Gul-i-Nīm (Azadirachta indica* A. Juss. flower) and purified honey.

Method of preparation: Fresh flowers are taken and mixed well with an equal proportion of purified honey. The mixture is then kept in sunlight to get the final product.

Therapeutic actions and uses: It purifies blood, acts as a deobstruent, and is beneficial in *amrāḍ sawdāwiyya* (melancholic diseases).[20]

Dose: 10–15 g.[16]

Gulqand-i-Nīlūfarī

Ingredients: *Gul-i-Nīlūfar* (*Nymphaea alba* L. flower) and sugar.

Method of preparation:

One part of the fresh flowers of *Nīlūfar* is taken, rubbed, and mixed with two parts of sugar to prepare *Gulqand-i-Nīlūfarī*.[12]

Second method: Take the petals of *Gul-i-Nīlūfar* and unrefined sugar *(khānd)* in a ratio of 1:1. Pour a little rose distillate over the petals, mash them, and mix with sugar. Keep the mixture in sunlight for 10 days or in the moonlight for 20 days.[18]

Therapeutic actions and uses: It is beneficial for headache and cough.[12,18]

Dose: 24 g with *'araq bādiyān* (distillate of *Foeniculum vulgare* Mill.).[12]

Gulqand-i-Māhtābī

Ingredients: *Gul-i-Gurhal* (*H. rosa-sinensis* L. flower) and sugar.

Method of preparation: Mix 100 pieces of flowers in 348 g of sugar, pour a little rose distillate, and mash with hands. Keep it in the moonlight for 3–4 days, then use it.

Therapeutic actions and uses: It helps alleviate palpitations and strengthen the heart.[13,15,18]

Dose: 12–24 g.[15]

Gulqand-i-Āftābī

Ingredients: *Gul-i-Surkh* (*R. damascena* Mill. flower) and sugar.

Method of preparation: To prepare *Gulqand-i-Āftābī,* one part of rose petals is thoroughly rubbed and mixed with two parts of sugar. It is then filled into a jar and kept in sunlight to get the final product.

Therapeutic actions and uses: It acts as a brain tonic, strengthens the stomach, and cures constipation.[12]

Gulqand-i-'Asali

Ingredients: *Gul-i-Surkh* (*R. damascena* Mill. flower) and honey.

Method of preparation: To prepare *Gulqand-i-'Asali*, one part of rose petals is thoroughly rubbed and mixed with three parts of honey. It is then kept in sunlight for 40 days for final preparation.[18]

Therapeutic actions and uses: It is beneficial for *amrāḍ balghamiyya* (phlegmatic diseases), paralysis, arthritis, gout, and constipation. It is lithotriptic in action.[11,12,18] When cumin seeds are added to *Gulqand-i-'Asali*, the whole formulation acts as a carminative and digestive.[11]

Ingredients Used for the Preparation of Different Types of *Gulqand*

Table 1 includes major ingredients used to prepare *Gulqand* as mentioned in Unani classical literature, along with their pharmacological activities and bioactive constituents.

Other Uses of *Gulqand*

Use of *Gulqand* as a Corrective *(Muṣliḥ)*

In Unani medicine*, Gulqand* is also advised to be used as a corrective to minimize the adverse effects of some medicinal herbs, fruits, and cereals such as *Jalāpā* (*Ipomoea purga* Hayne), *'Ūd-i-Ṣalīb* (*Paeonia officinalis* L.), *Kāfūr* (*Cinnamomum camphora* [L.] J. Presl), *Seb* (*Malus*

Table 1: Different ingredients of *Gulqand* mentioned in Unani textbooks

Name of the ingredient with image (flowers)	Botanical name and family	Pharmacological activities	Bioactive constituents responsible for the activity
Gulāb[21] 	*Rosa damascena* Mill., Rosaceae	Antidepressant[22]	Flavonoids and kaempferol[22]
		Memory enhancer[23]	Quercetin[23]
		Laxative[24]	Citronellol and geraniol[24]
		Antioxidant and antibacterial[25]	Galloyl glycosides, ellagitannins, kaempferol-3-glucoside, and quercetin[25]
		Anti-inflammatory[26]	Polyphenols and flavonoids (quercetin)[26]
		Hepatoprotective[27]	Phenolic compounds (gallic acid, quercetin)[27]
		Cardioprotective[28]	Not referred[28]
		Analgesic[29]	Flavonoids (kaempferol, quercetin)[29]
Gurhal[30] 	*Hibiscus rosa-sinensis* L., Malvaceae	Antihypertensive[31]	Anthocyanins[31]
		Antioxidant and antibacterial[32]	Polyphenols, flavonoids, tannins, and anthocyanins[32]
		Anti-inflammatory[33]	Flavonoids, saponins, and steroids[33]
		Cardioprotective[34]	Quercetin, cyanidin, and kaempferol[34]
		Hepatoprotective[35]	Anthocyanins[35]
		Gastroprotective[36]	Flavonoids, tannins, and mucilage[36]
		Anticancer, antidiabetic, and wound healing[37]	Phenolic compounds, alkaloids, glycosides, amino acids, tannins, and flavonoids[37]
Gāwzabān[38] 	*Borago officinalis* L., Boraginaceae	Neuroprotective[39]	Polyunsaturated fatty acids[39]
		Anxiolytic[40]	Polyphenols, flavonoids, and linolenic acid[40]
		Hepatoprotective[41]	Phenolics and flavonoids[41]
		Antioxidant, antibacterial, and anti-inflammatory[42]	Phenolics (gallic acid, pyrogallol, salicylic acid, caffeic acid), flavonoids (myricetin, rutin), and fatty acids (α-linolenic, palmitic, linoleic, and γ-linolenic acids)[42]
		Anticancer[43]	Flavonoids, tannins, carbohydrates, and glycosides[43]
Nīlūfar[44] 	*Nymphaea alba* L., Nymphaeaceae	Antioxidant[45]	Phenolic compounds (gallic and ellagic acids)[45]
		Antibacterial[46,47]	Phenolic compounds, tannins, alkaloids, and flavonoids[46,47]
		Anxiolytic[48]	Phenolic acids, flavonoids, alkaloids, gallic acid, tannic acid, and sterols[48]
		Anti-inflammatory[49]	Not referred[49]
		Hepatoprotective[50]	Not referred[50]
		Antidiabetic and hypolipidemic[51]	Flavonoids, alkaloids, glycosides, phenols, steroids, and tannins[51]
Amaltās[52] 	*Cassia fistula* L., Fabaceae	Antioxidant[53]	Flavonoids, alkaloids, and glycosides[53]
		Anti-inflammatory[54]	Rhein[54]
		Antibacterial[55]	Flavonoids, glycosides, triterpenoids, carbohydrates, anthraquinone, steroids, and saponins[55]
		Anticancer[56]	Rhein[56]
		Laxative[57]	Anthraquinone glycosides like rhein and sennosides[57]
Nīm[58] 	*Azadirachta indica* A. Juss., Meliaceae	Antimicrobial[59]	Quercetin, nimbidin, and nimbin[59]
		Anti-inflammatory[59]	Quercetin, Kaempferol, nimbidin, nimbin, rutin, astragalin, and gallic acid[59]
		Antioxidant[60]	Sesquiterpenes (caryophyllene)[60]
		Anticancer[61]	Nimbolide[61]
		Anxiolytic and antidepressant[62]	Quercetin[62]
		Neuroprotective[63]	Quercetin[63]
		Immunomodulatory[64]	Not referred[64]

Contd...

Table 1: Contd...

Name of the ingredient with image (flowers)	Botanical name and family	Pharmacological activities	Bioactive constituents responsible for the activity
Hinā[65] 	*Lawsonia inermis* L., Lythraceae	Antioxidant, anti-inflammatory, and cytotoxic[66]	Phenolics and flavonoids[66]
'Asal[67] 	Honey	Antioxidant[68] Anti-inflammatory[69] Anti-microbial[70] Cardioprotective[71] Gastroprotective[72] Neuroprotective[73] Anticancer[68]	Phenolics (benzoic acids, cinnamic acids), flavonoids, organic acids (gluconic, malic, and citric acids), and proteins[68] Phenolic acids (ferulic acid), and flavonoids (hesperetin, luteolin)[69] Glucose oxidase, hydrogen peroxide, and some phenolic compounds such as pinocembrin and syringic acids[70] Polyphenols, flavonoids, Vitamin C, and reduced glutathione[71] Flavonoids[72] Apigenin, caffeic acid, catechin, p-coumaric acid, ellagic acid, gallic acid, kaempferol, naringenin, myricetin, and luteolin[73] Phenolic acids (p-coumaric, vanillic, caffeic, p-hydroxybenzoic acid), and flavonoids (chrysin, apigenin, quercetin, acacetin, pinocembrin)[68]

sylvestris [L.] Mill.), *Falsā* (*Grewia asiatica* L.), *Tarbūz* (*Citrullus lanatus* [Thunb.] Matsum. and Nakai), *Ber* (*Ziziphus mauritiana* Lam.), and *Sha'īr* (*Hordeum vulgare* L.).[74]

Use of *Gulqand* as a Binding Agent *(Rābiṭa)* for Pills and Tablets

In Unani medicine, *Gulqand* is occasionally used as a binding agent in the preparation of pills and tablets. When the herbal ingredients in the formula of pills or tablets are dry and coarsely powdered, it becomes challenging to form pills. To overcome this issue, some viscous or mucilaginous substances are added, which help create a viscous paste, making it easier to shape the pills and ensuring they stay intact for longer. Along with other substances such as gum acacia, tragacanth gum, starch, and honey, *Gulqand* is also employed for this purpose.[9]

Use of *Gulqand* in Food Products

For commercial purposes, it is utilized to enhance the taste and texture of various processed food products such as milkshakes, cakes, and pastries.[75] Kanse *et al*. prepared a gelato ice cream with enhanced nutritional value and antioxidant effects by adding *Gulqand*.[76] In the betel vine, *Gulqand* serves the purpose of a sweetener, and it augments its taste.[77] It is a product believed to be high in nutrients and used to improve the nutritional value and storage stability of cow's milk.[78] *Gulqand* imparted an augmented taste, flavor, and aesthetic value to a desiccated coconut chocolate.[79] *Gulqand*-flavored *shrikhand* is a valuable product that helps with gastric and heart-related problems.[75]

Shelf-life

Gulqand prepared with honey has a shelf-life of 4 years; however, it is reduced to 2 years when prepared with sugar.[11]

Storage

Gulqand should always be stored in clean and dry porcelain containers or glass jars and kept in a clean and dry place.[16]

Discussion

In the Unani system of medicine, *Gulqand* is a semi-solid dosage form recommended by Unani physicians for preserving health and curing ailments owing to its incredible health benefits. The most practiced *Gulqand* in Unani medicine is prepared with rose petals. This formulation is prescribed as a laxative, brain tonic, liver tonic, and stomachic. It elevates mood and prevents depressive effects. Nowadays, it has been shown that *R. damascena* possesses neuroprotective and memory-enhancing effects, thereby benefiting memory-related disorders such as dementia. It demonstrates an antidepressant effect by reducing lipid peroxidation and enhancing antioxidants in the cerebral cortex.[80] In an experimental study, it was found that the

rose fragrance improved absolute beta activity, which is linked with alertness, conscious behavior, and increased concentration.[81] *R. damascena* has revealed its laxative action in some studies, which may be brought on by osmotic inflow of fluids into the intestinal lumen, and a stimulatory impact on the smooth muscle of the ileum.[82,83] It has demonstrated significant therapeutic effects in a clinical trial, where its use over 4 weeks resulted in marked improvement in constipation and overall quality of life among pregnant women.[84] It has also been reported to possess antimicrobial, anti-inflammatory, cardioprotective, diuretic, and relaxant properties, which can be attributed to the presence of bioactive compounds such as flavonoids, phenolic acids, terpenes, and anthocyanins in the flower. The beneficial effects of *R. damascena* in scavenging free radicals make it a good antioxidant for helping health conditions.[85]

The indications of various types of *Gulqand* are more or less related to the effects of the petals. For instance, *Gulqand-i-Gāwzabānī* is recommended for brain and cardiac weakness, and melancholic diseases, including anxiety, insanity, melancholia, and palpitations due to black bile. Similarly, flowers of *Borago officinalis* have been reported to have anxiolytic and memory-improving activities in animal studies. The presence of γ-linolenic acid, polyphenols, and flavonoids in borage flower extract supports its anxiolytic effects.[86] *Gulqand-i-Amaltās* has a laxative effect and cures constipation. This property is attributed to the presence of active laxative content, the anthraquinone glycosides, which are concentrated in flowers, leaves, and pods of *C. fistula*.[57] *Gulqand-i-Gurhal* has positive effects on the heart. In an animal study, it was revealed that *H. rosa-sinensis* flower extract enhanced postischemic recovery. The treatment decreased the number of ventricular premature beats and duration of ventricular tachycardia in a dose-dependent manner, suggesting the cardioprotective property of hibiscus.[87,88] *Gulqand-i-Nīm* and *Gulqand-i-Hinā* act as blood purifiers. According to Unani theory, the consumption of blood purifiers helps in the modulation of immunity and the removal of toxins from the body.[89] They can also be ingested in a healthy state as a preventive measure to avoid the buildup of toxins in the body. *Nīm (A. indica* A. Juss.*)* and *Hinā* (*L. inermis* L.) are the key detoxifying agents in Unani medicine that are extensively used for their antiseptic properties. *Nīm* has been revalidated for a broad-spectrum antimicrobial activity against several pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Aspergillus flavus,* and *Candida albicans*.[90] It plays a crucial role in boosting immunity, particularly cell-mediated and humoral immunity. It aids in the synthesis of immunoglobulin IgG and IgM antibodies and stimulates lymphocytes and macrophages through cell-mediated processes.[91] *Hinā* cleanses blood and has shown antibacterial, antifungal, antiparasitic, anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, antipyretic, wound healing, and many other pharmacological effects.[92,93]

Gulqand is also used after the addition of other ingredients to augment its therapeutic potential. When cumin seeds are added to *Gulqand*, it helps to resolve flatulence and improves digestion. Cumin seeds have revealed digestive stimulant action in various studies. It enhances the activities of pancreatic trypsin, chymotrypsin, and amylase, augments small intestinal maltase activity, and has a significant stimulatory effect on bile flow rate and bile acid secretion.[94] If *turbud* (*Operculina turpethum* [L.] Silva Manso) and *karafs* (*Apium graveolens* L.) are added and the whole preparation is consumed frequently, it strengthens the stomach and is beneficial for facial palsy, weakness of muscles, and the initial stage of gout. When *Gulqand* is consumed regularly after adding *ustukhuddus* (*L. stoechas* L.) and *banafsha* (*V. odorata* L.), it helps cure fever, headache, and migraine.[11] *V. odorata* has demonstrated efficacy in the management of migraine attacks in a clinical trial.[95]

Unani scholars suggested the use of *Gulqand* as a corrective for several adverse effects caused due to certain herbal drugs, certain herbal drugs, such as flatulence due to Indian jujube and apple, bladder stones due to camphor, and loss of libido due to watermelon, among others.[74] It can also be used as a binder for pills and tablets due to its consistency, offering a more cost-effective alternative to expensive binding agents.

Gulqand's contemporary relevance lies in its ability to bridge ancient wisdom with modern health and lifestyle trends. With the growing shift toward natural, plant-based foods, *Gulqand* emerges as a chemical-free, wholesome option that supports overall well-being. Beyond its health advantages, *Gulqand* is gaining popularity in fusion cuisine, where it adds a rich floral aroma, depth of flavor, and improved nutritional value to various processed food products. Its ability to blend tradition with innovation makes *Gulqand* a culturally significant and highly adaptable formulation that aligns with today's demand for both functionality and flavor.

Conclusion and Future Aspect

The medicinal and nutritional significance of edible flowers has been admirable since antiquity. Although many edible flowers are used traditionally to treat illnesses, recent studies have confirmed their health benefits, identified the bioactive components, and uncovered the underlying mechanisms. Edible flowers possess strong medicinal properties, namely, anti-diabetic, anticancer, anti-anxiety, anti-inflammatory, antioxidant, antimicrobial, cardioprotective, hepatoprotective, neuroprotective. The Unani system of medicine has long preserved a diverse

range of formulations made with edible flowers. *Gulqand* is an example of such a formulation, regarded as one of the most significant value-added products derived from edible flowers, especially roses. Its consumption provides nutrition, strengthens the body, elevates mood, reduces stress, helps in digestion, removes morbid matter from the body, and has cooling properties. It is a potent antioxidant as well as a strong rejuvenator. The above review provides a fair idea about various types of *Gulqand* described in Unani classical texts, along with their medicinal uses; thus, it will serve as a source of valuable information for researchers of Unani medicine and other traditional medicine systems.

As consumers increasingly seek natural and holistic remedies, the demand for traditional products like *Gulqand* is likely to rise in the future. With its potential health benefits, *Gulqand* may find broader applications in modern health trends. In addition, further research into its medicinal properties could lead to more widespread use in both traditional and contemporary healthcare practices. Its natural flavoring, sweetening, and preservative qualities also make it a potential candidate for use in the food and beverage industry.

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Conflicts of interest

There are no conflicts of interest.

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Review Article

Historical Perspective of Measles in Unani System of Medicine with Special Reference to the Treatment Guidelines

Abstract

In the Unani system of medicine, special importance is placed on the health of children, emphasizing disease prevention to avoid illnesses in later life. One such illness is measles, or "*Ḥaṣba"* considered a bilious disease and most common during hot, dry seasons. Measles is highly contagious, entering through the respiratory tract or conjunctiva, and is most infectious before and shortly after the rash appears, marked by Koplik spots. While extensive vaccination efforts led to an 84% decline in measles cases between 2000 and 2016, there was a 556% surge from 2016 to 2019, highlighting the ongoing public health threat despite significant vaccination success. A comprehensive literature search in PubMed, Google Scholar, Scopus, and Unani books using relevant keywords. The studies were reviewed, and information was gathered using standardized forms. Vaccination programs significantly reduced measles incidence globally, with notable resurgence due to vaccination gaps. Unani medicine provides complementary insights into disease prevention. Combined vaccination and Unani preventive strategies can enhance measles control and reduce disease incidence.

Keywords: *Ḥaṣba, infectious diseases, measles, pediatric health, Unani medicine, vaccination*

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Introduction

In the *Unani system of medicine*, special importance is placed on the health of children. Unani physicians emphasize that by providing appropriate care during childhood and making efforts to prevent diseases, many illnesses can be avoided until old age. Childhood illnesses are given more attention due to the delicate nature and constitution of children, making them quickly susceptible to infectious diseases.[1] *Ḥaṣba*, commonly known as "*Khasra,*" is an extremely infectious disease. According to Unani medicine, it is a *Ṣafrāwī* (bilious) disease and mostly occurs during the hot and dry seasons, especially when there is a prevalence of heat and dryness in the atmosphere. The earliest clear information about measles was provided by *Abu Bakr Muhammad ibn Zakariya al-Razi*. In his work "*Al-Judari wa al-Ḥaṣba*," *Razi* differentiated between smallpox and measles and explained that both are infectious diseases. According to *Zakariya Razi*, in dry air, humors are reduced, but they become very severe because dry air dissolves and evaporates humors, leading to their reduction. The remaining humors tend to be inclined toward biliousness. During this season, it is crucial to be aware that bilious humors are more susceptible to being affected.[2]

Measles is a highly contagious viral disease that affects humans. The virus usually enters the respiratory tract or conjunctiva, spreads to the lymph nodes, and causes viremia. The virus is most contagious 5 days before the skin rash appears and up to 4 days after it disappears. The prodromal phase of measles is characterized by the appearance of Koplik spots, which are small erythematous, white or gray lesions seen on the buccal mucosa. These spots are typically seen before the skin rash in 50%–70% of patients with measles.[3]

The number of reported cases of measles worldwide saw a significant decline of 84% between 2000 and 2016. This reduction in cases was primarily due to the extensive efforts of the World Health Organization (WHO) and its partners in implementing vaccination programs. However, between 2016 and 2019, there was a sharp increase in the number of measles cases reported globally, which

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surged by 556% to almost 870,000, the highest number reported since 1996. The estimated number of deaths caused by measles per year decreased to under 100,000 in 2016, which was a significant achievement for the global health community.[4] This decrease was due to the improved mathematical model used to calculate the number of deaths caused by the disease. However, the number of deaths caused by measles increased again to 207,500 in 2019, indicating that the disease remains a significant threat to public health. Despite the resurgence of measles cases, the WHO estimates that around 25.5 million deaths worldwide were averted by measles vaccination between 2000 and 2019, highlighting the importance of vaccination programs in controlling the spread of the disease. The COVID-19 pandemic has had serious effects on vaccination programs and surveillance systems. Due to the pandemic, many countries have had to redirect their resources toward managing the pandemic, which has led to a decline in vaccination rates and surveillance efforts.[5] It is unclear whether the low number of measles cases reported in 2020 is due to a genuine reduction in cases as a byproduct of COVID-19 sanitation, hygiene, and distancing measures, or a result of underdiagnosis and under-reporting due to fewer medical consultations or a combination of both.[6]

Methodology

Literature search

Database selection

Conducted comprehensive searches in electronic databases including PubMed, Google Scholar, Scopus, and Unani books. These databases were selected for their extensive coverage of medical and health-related research.

Search terms

The search terms used included combinations of keywords such as "Measles," "*Ḥaṣba*," "Khasra," "Unani medicine," "Bilious disease," "Pediatrics health," "Vaccination," "Measles resurgence," and "COVID-19 impact on measles."

Inclusion and exclusion criteria

Inclusion criteria

Peer-reviewed articles, reviews, historical texts, and reports published in English or translated into English, relevant to measles, Unani medicine, pediatric infectious diseases, vaccination programs, and recent epidemiological data.

Exclusion criteria

Articles not related to the topic, nonpeer-reviewed sources, and publications in languages other than English without available translations.

Data extraction

Selection process

Titles and abstracts of the search results were initially screened for relevance. The full-text articles were obtained for the ones that met the inclusion criteria.

Data extraction tool

A standardized data extraction form was used to collect information from each selected study. The form included fields for the study title, authors, publication year, study design, population, interventions, outcomes, and key findings.

Epidemiology

Measles virus is a highly contagious virus that is primarily transmitted through respiratory droplets and secretions. Humans are the only natural hosts of this virus, although nonhuman primates can be experimentally infected to gain knowledge of viral pathogenesis. The incubation period of measles virus ranges from 10 to 14 days, and possibly up to 23 days, with symptoms lasting up to 3 weeks. The infectious period starts 4 days before the onset of the rash and continues for about 4 days after the rash appears.[7] Due to its high contagiousness, a single infected case of measles can lead to 12–18 secondary cases in a completely susceptible population.[8]

Etiology

According to the research of *Unani physicians*, measles occurs due to a bilious (*ṣafrāwī*) condition arising in the blood, which causes the blood to become impure.[9] In this disease, the blood tends to become dry and the bilious matter is scanty.[10] The cause of measles is the blood that contains a large amount of bile, which is why measles becomes ingrained.[10] Mostly, individuals with a dry and bilious temperament are affected by this disease.[1] In addition, weak individuals also tend to contract it quickly.[10] Generally, children are more susceptible to this disease. Furthermore, measles mostly occurs in the autumn and spring seasons.[9] According to *Syed Ismail Jurjani*, if there is excessive heat and dryness in the summer season and this continues into the autumn, the incidence of measles increases significantly at the end of autumn.[10] According to *Al-Razi*, the condition of measles occurs mostly in the autumn, when neither the northern winds blow nor does it rain, but rather the dusty southern winds that cause darkness prevail.[1] These observations also confirm the statement made by *Buqrāṭ* (Hippocrates) in the second section of his book "*Kitab al Fuṣūl*" where he wrote that the departure of the seasons from their usual temperament leads to an increase in diseases.[1] According to modern research, the cause of measles is a type of virus that belongs to the genus paramyxovirus.[11]

Signs and Symptoms

The symptomatic phase of measles starts a few days after the appearance of Koplik spots and is characterized by fever, cough, coryza, conjunctivitis, and a maculopapular rash that spreads from the head to the trunk and limbs. Children with defects in immune function have higher infection rates and mortality because both humoral and cellular measles virus-specific immunity are required for viral clearance and lasting immunity. Acquired immunity after measles virus infection is usually lifelong, but a modified form of measles is an attenuated infection that occurs in individuals with immunity from infection or vaccination and is not highly contagious.[5]

Secondary infections and coinfections are common in patients with measles, and they are mainly due to parainfluenza virus, adenovirus, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Haemophilus influenza*, *Streptococcus pyogenes*, and reactivation of tuberculosis. Measles-associated immune dysfunction may persist for up to 3 years following measles virus infection.[12]

Transmission

Measles stands as one of the most highly contagious infectious diseases, with as many as 9 out of 10 susceptible individuals who come into close contact with a measles patient likely to contract the illness. Transmission occurs through direct exposure to infectious droplets or through airborne dissemination when an infected person breathes, coughs, or sneezes.[13] The measles virus can persist in the air for up to 2 h after an infected individual has vacated the area. This highly contagious nature, facilitated by contact with infected respiratory secretions or airborne particles, underscores the virus's potency, allowing it to infect a significant portion of unvaccinated individuals – up to 90% – in close proximity to the carrier. The infectious period spans from 4 days before the onset of the characteristic rash to 4 days after its appearance.[14] Measles outbreaks pose substantial risks of severe complications and fatalities, particularly among young, undernourished children. Even in regions nearing measles elimination, imported cases from other countries pose a significant risk of reintroducing the infection.[15]

Clinical Features

According to *Zakariya Razi*, if a child has symptoms like back pain, itching in the nose, fright during sleep, heaviness in the head, redness in the eyes, and chills and pain throughout the body, these are the precursors of measles. In *Kitāb al-Ḥāwī, Zakariya Razi* mentioned that in the early stages of measles, the voice becomes heavy, the eyes and cheeks turn red, there is pain in the throat and chest, the tongue becomes dry, the temples swell, the body turns red, tears flow from the eyes, and there is severe nausea.[16]

After that, on the 4th or 5th day, small red spots, about the size of poppy seeds, appear on the skin. According to *Syed Ismail Jurjani*, these spots appear more like patches rather than raised bumps on the skin. These spots first appear on the forehead and face, then spread over the entire body, and continue to emerge for a day or two.[17] During the emergence of these spots, there is intense nasal congestion. Once the spots have fully appeared, the symptoms begin to alleviate. By the 6th or 7th day, the spots start to wither. On the 8th day, the withered spots shed fine scales or crusts, resembling wheat bran. At this stage, there is significant itching on the body.[1] The fever usually subsides by the eighth day. According to physicians, if from the beginning the spots appear black or blue and irregular, these are considered bad signs.[18]

In addition, if there are complaints of distress, delirium, and difficulty breathing, along with black or clay-colored diarrhea, these are fatal signs. If the measles spots are hard and of an indigo color, this is a bad sign. Moreover, if the spots appear greenish and then disappear, it indicates that paralysis may ensue. According to *Ibn-e-Sina*, rapid breathing in a measles patient is a sign of loss of strength or swelling in the diaphragm.[19] If there is intense thirst, increasing distress, and coldness in the body, it signifies that the patient is near death. The presence of palpitations is also a sign of impending death. It is also noted that if bloating occurs late in measles and paralysis occurs frequently, this is a bad sign. If measles spots appear quickly and then resolve, this type is also considered severe. In light of this, many physicians have described techniques to bring out the measles spots.[20]

Preventive Strategies

Live, attenuated measles vaccines are accessible in either monovalent form or as part of measles-containing vaccines, which also include rubella or mumps vaccines.[21] The combined measles-rubella or measles-mumps-rubella vaccines maintain consistent protective immune responses to each antigen and exhibit similar vaccine-associated adverse event profiles. These available measles vaccines are deemed safe and efficacious and can be used interchangeably within vaccination programs. Postvaccination, studies have demonstrated the prolonged presence of neutralizing measles antibodies, lasting up to 33 years, and enduring defense against measles.[22] Nevertheless, it remains uncertain whether a single dose of measles vaccine, without natural boosting from recurrent exposure to measles, confers lifelong protection. Investigations utilizing IgG avidity measurements to distinguish primary vaccination failures from secondary ones suggest that secondary failures may arise infrequently. However, waning immunity has not emerged as a significant risk factor.[14]

Global Goal for Measles Control

The global objective for measles control, outlined in the Global Immunization Vision and Strategy 2006–2015 by the WHO and United Nations Children's Fund, is to

decrease measles-related deaths by 90% by 2010 compared to the estimated figures from 2000.[3] This goal was further endorsed by the World Health Assembly following a review by the WHO's Strategic Advisory Group of Experts on immunization.[23] The primary strategies employed globally to reduce measles mortality include: ensuring high coverage of the first dose of the measles vaccine, aiming for ≥90% coverage at the national level and ≥80% in each district through routine immunization; implementing sensitive laboratory-supported surveillance to detect outbreaks and cases, with confirmation achieved through the detection of measles immunoglobulin M (IgM) in serum from at least two suspected cases; adopting appropriate measles case management, including the provision of Vitamin A to mitigate mortality and complications; and delivering the second dose of the measles vaccine through routine immunization or supplementary immunization activities.[24] In the case of routine immunization, states with ≥80% evaluated coverage for the first dose of measles vaccine are integrating the second dose into routine immunization alongside the DPT booster. For states with <80% coverage, catch-up immunization campaigns have been conducted since 2010 in a phased manner to address measles vaccination gaps.[5]

Complications

Measles virus infection leads to a decline in CD4 lymphocytes, which begins before the onset of the rash and can persist for up to 1 month.[25] This decline results in the suppression of delayed-type hypersensitivity, as evidenced by anergy to skin test antigens, including tuberculosis antigen.[26] The system-wise complications of measles are presented in Table 1.

Preventive Strategies in Unani

Ibn-e-Sina emphasized the significance of environmental factors in the development and prevention of diseases. He proposed the theory of the "multiple factor" cause of diseases and recommended the avoidance of direct contact with infected individuals as a means of preventing infections, thereby suggesting the concept of quarantine.

Table 1: Complications of measles[7]

Organ system	Complication
Respiratory	Pneumonia, pneumothorax, otitis media, mastoiditis, tracheitis, and mediastinal emphysema
Neurological	Febrile convulsion, encephalitis, Reye's syndrome, and Guillain–Barre syndrome
Gastrointestinal	Diarrhea, mesenteric adenitis, hepatitis, pancreatitis, and stomatitis
Ophthalmic	Keratitis, corneal ulceration, corneal perforation, central venous occlusion
Cardiovascular	Myocarditis and pericarditis
Dermatologic	Cellulitis
Other	Nephritis, myositis, renal failure, and malnutrition

Ibn-e-Sina also focused on specific high-risk groups for disease prevention and proposed numerous evacuation methods (*istifrāgh*) to maintain a healthy body, such as addressing diarrhea, vomiting, bloodletting, and cupping. He discussed different kinds, properties, and uses of water. He addressed air contamination and its impacts. He documented the layout and positioning of residences.[27]

Environment (*Muḥīṭ*)

Ibn-e-Sina made several significant contributions to Unani preventive and social medicine. He highlighted the crucial role of environmental factors in both the causation and prevention of diseases. Ibn-e-Sina introduced the idea that diseases arise from "multiple factors," emphasizing that illnesses cannot be attributed to a single cause. He was ahead of his time in recognizing the importance of infection control, advocating for the avoidance of direct contact with infected individuals, a principle that eventually evolved into the practice of quarantine. In addition, Ibn-e-Sina paid special attention to identifying and protecting certain groups who were at a higher risk of contracting illnesses, underscoring his holistic approach to health and preventive care.[28]

Treatment

According to *Zakariya Razi*, it is necessary to protect children who are prone to measles from acute fevers in the spring season, use of antipyretics as much as possible, and use of such light foods that produce thin blood is necessary. Also, do not create density in the skin surface through *Dalk*, *Riyāḍat* and *Ḥammām*.[1] *Zakariya Razi* had quoted from the book *Kitāb al-'Alāmāt* that the elements of *Ṣafrā* are in bad condition at the end of summer and the beginning of autumn, so good management should be taken in these two seasons.[1] If fever occurs with no rashes, then do *Ḥijāma* and *faṣd*.[18] If there is a delay in the rashes coming out, give boiled yellow *Anjīr* (figs) in water, dissolve 128 milligrams of *za'frān* (saffron) and drink it, and give steam of lukewarm water.

According to the opinion given by Unani scholars in this disease, the patient's *Quwā* (the bodily powers or faculties) is monitored and the matter is left largely to *ṭabī'yat*. In the beginning of fever, give the child 4–5 small seeds of true pearls.[20]

Remedies used by Unani physicians for *Ḥaṣba* (measles):

1. Five *Unnab* (jujube) seeds, three yellow *Anjīr* (figs), 13 g of *khāksī*, and 10 g of white sugar candy. Boil all these ingredients in water, strain it, and give it to the patient. If the weakness is severe, then along with this give 2 g of *Khamīra Marwarīd* and sprinkle *khāksī* over the bedding. Ismail Jurjani a famous medieval physician instructs that if the measles rash does not appear, then induce sweating so that the pores open up. This will make the rash appear quickly and the patient will recover

from the disease.[17] If there is also constipation, then add 2 g of violet flowers to the remedies and use 10 ml of *sharbat banafsha* instead of white sugar candy[10]

2. If there is difficulty in the eruption of measles, and there is excessive nausea, vomiting, or shortness of breath, then give the following recipe: 24 g each of *Anjeer* (figs), peeled *Dal* (lentils), *Saunf* (fennel) seeds, and *Laung* (clove), boil them in water, strain it, and give it several times a day.[18] When the rash starts to appear, administer 2 g of *Khamīra Marwārīd*, followed by a decoction of two *Unnāb* (jujube) seeds, three raisins, and 50 ml of *Kewra* (screw pine) water, and add 6 g of *Misri* (rock sugar) and 1 g of *khāksī* to it and administer it
3. Boil three *Mawīz munaqqa* (raisins), two jujube seeds, and half a yellow fig in water, dissolve 12 ml of *sharbat unnab* in it, and sprinkle 2 g of *Khāksi Musallam* over it and administer it.[20]

Zakariya Razi advises against using purgatives before the measles rash appears. If there is diarrhea, use *Ṭabāshīr* (siliceous concretion in bamboo), *Babul gond* (gum acacia), *Gil-e-Armani* (Armenian bole), and rose petals. In case of diarrhea, administer 10 ml of Habbul Aas, if that does not provide relief, then mix 1–2 g of Safoof-e-Teen with rub-e-beh (a thick syrup made from various fruits) and give it. *Ibn-e-Sina* instructs that no poultice of any kind should be applied to the stomach during measles.[2]

Conclusion

The management and prevention of measles from the perspective of Unani medicine offers a unique approach that complements modern medical practices. Unani medicine emphasizes the importance of maintaining a balanced temperament and good health to prevent diseases like measles. The historical insights provided by Unani scholars such as *Zakariya Razi* and *Ibn-e-Sina* highlight the significance of environmental factors and the body's natural ability to heal itself.

Measles, or *Ḥaṣba* is recognized in Unani medicine as a bilious disease predominantly occurring in hot and dry seasons. The disease's etiology is attributed to the bilious condition in the blood, with symptoms and transmission routes well documented by ancient Unani physicians. The preventive strategies outlined in Unani medicine stress the importance of maintaining good health through environmental adjustments, dietary regulations, and the use of specific herbal remedies.

Despite the advances in vaccination and the significant reduction in measles cases globally, the recent resurgence of measles underscores the necessity of continued vigilance in both vaccination efforts and complementary preventive measures. The Unani perspective, with its holistic approach, can provide valuable insights and strategies to support modern public health initiatives in controlling and managing measles.

This review highlights the need for an integrated approach that combines the strengths of modern medicine and traditional practices to effectively combat measles and ensure the health and well-being of children worldwide. Further research and collaboration between conventional and Unani medicine practitioners could enhance our understanding and management of infectious diseases like measles, contributing to better health outcomes on a global scale.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

An Observational Study on Changes in the Color and Viscosity of Urine in Patients with *Fālij* (Paralysis) after Administration of *Munḍij* (Concoctive) Therapy

Abstract

The Unani System of Medicine, over 2500 years old, is based on logical, philosophical, and observational principles. In ancient times, due to limited scientific tools, most observations were subjective. Urinalysis, although urine is a waste product, remains a vital diagnostic tool and is now the third major clinical screening test, improved by modern technology and automation. Conventionally, Unani physicians diagnosed diseases using the three key parameters: *Nabḍ* (pulse), *Bawl* (Urine), and *Barāz* (stool). They believed therapies like *munḍij* (concoctives) altered the *akhlāṭ* (humours) and excretory materials in terms of color, viscosity, odor, and taste-parameters that were hard to quantify. In this study, urine from paralysis patients was analyzed for color and viscosity before and after *munḍij* (concoctive) therapy to objectify these subjective measures. Sixty-five participants were enrolled; forty completed the 12-day study. They consumed a *nuskha munḍij-i-balgham* (phlegmatic concoctive drugs) daily before breakfast. Urine samples were collected on Day 0, Day 4, and Day 12. The mean dynamic viscosity rose from 1.84 to 1.85, and optical density increased from 0.238 to 0.33 by Day 12. Both changes were statistically significant ($P < 0.0001$), showing subjective observations can be quantified through modern methods.

Keywords: *Mundij therapy, nudj, unani medicine, urine color, urine viscosity*

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Introduction

In the Unani system of Medicine, uroscopy is one of the diagnostic tools. Unani physicians believed that a sample of urine shows the inner functioning of the body.[1] The parameters that are observed in urine to assess the status of the body were referred by physicians as *Dalail-e-Bawl* (Indicators of Urine). These are *Alwān al-Bawl* (color of the Urine), *Qiwām* (Consistency/Viscosity), *Safāyi Wa Kadūrat* (Clarity and Turbidity), *Rasūb* (Sediment), *Miqdār* (Quantity), *Kaf/Jhāg* (Foam), and *Bū* (Odor).[2,3] Urine is not only a diagnostic tool but a prognostic tool too. In many diseases, color, viscosity, and sediments, etc., are observed to assess the prognosis of diseases, for example, in *balghami amrād* (phlegmatic diseases,) like *Fālij* (Paralysis), a change in urine color and viscosity during the disease has been mentioned in the classical Unani literature.[3-6]

Fālij is caused mainly by a stroke, which accounts for approximately 5.5 million deaths annually, with 44 million disability-adjusted life-years lost.[7] As a disease of aging, the prevalence of stroke is expected to increase significantly around the world in the years ahead as the global population older than 65 years of age continues to increase by approximately 9 million people per year. In India, paralysis affects 6.98 out of 1000 people ($n = 60$). Males accounted for 34 of the 60 cases, whereas females accounted for 26.[8]

The word "Paralysis" and "Palsy" are used interchangeably. The word comes from the Greek word, meaning "disabling of the nerves."[9] This term is often used vaguely to describe the loss of sensation and movement in any parts of the body.

Diagnostic errors are also a major source of concern for both patients and doctors in case of paralysis. Since ancient times, *fālij* has been misdiagnosed in patients, causing inefficient and even toxic treatment. Many ancient Unani physicians have mentioned their treatment plans and the safety and efficacy studies of the drugs mentioned in Unani medicine have been done. One of the treatment plans, known as *nuskha*

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munḍij-i-balgham (phlegmatic concoctive drugs) has been proven to be clinically effective.

The two main techniques that ancient physicians described and advised for the treatment of such diseases are *nudj* (concoction) and tanqiya (evacuation). Concoction is the method of transforming and changing the viscosity of morbid matter so that it can be easily removed from the site of lodgement. *Munḍijat* (concoctives) is a class of drugs that alter the consistency of morbid *akhlāṭ* to allow it to be evacuated from tissues, vessels, and interstitial spaces.[10] They work by either liquefying the consistency of *ghalīẓ khilṭ*, or by thickening the *raqīq akhlāṭ*; both require consistency changes because *ghalīẓ akhlāṭ* cannot come out due to their viscosity and adherence, and *raqīq akhlāṭ* cannot come out due to their deep penetration into the interstitial tissues.[11] *Munḍijat* modifies the consistency of morbid *khilṭ* with their specific property and action known as "*Taskheen Bil Aitidal*."[2,3,12] *Fālij* is a *balghami maraḍ*.[2,5,13] To evacuate its matters, it is mandatory to give *munḍij-i-balgham* drugs to the patients of *fālij*. The signs of *nuḍj* in *fālij* can be seen clearly and significantly in urine.[14] It is better seen in color and viscosity changes after the administration of *munḍij-i-balgham* drugs.[2,10]

The color of urine in patients with paralysis is white and sometimes red due to *zoaf* (weakness) and the viscosity is *raqīq* (thin).[14,15] As far as the effect of giving *munḍij* drug is concerned, the color of urine changes from *Abyaḍ* (white) *to Zard* (yellow) and the viscosity changes from *Raqīq* (thin) to *Ghaliz* (thick).[3,16] *Fālij* is a disease associated with *māddī marad* (morbid matter), and the disease matter needs to be removed from the body. In the body, *tabi'at* (medicatrix naturae) plays an important role to rectify the *māddā* (morbid matter) by *f'ale nuḍj*. After *nuḍj*, *tabi'at* eliminates the abnormal matter with the help of *quwwat dāfiy'a* (expulsive power).[10] Hence, the Unani physicians have explained the changes that occur in urine before and after *nuḍj,* especially in color and viscosity.[14] The primary aim of this study is to observe the changes occurring in color and viscosity of urine in patients with paralysis after taking *munḍij* therapy.

Materials and Methods

The present study was conducted in the research laboratory of the Department of Mahiyatul Amraz (pathology), National Institute of Unani Medicine Hospital, Bangalore. Before embarking upon the study, a comprehensive protocol was chalked out and put forth for ethical clearance. It was approved by the Institutional Ethical Committee NIUM, Bengaluru under NIUM/IEC/20I9-20/029/MA/01 with trial registration number CTRI/2021/11/038108. The trial lasted for a full year (2021–2022). The patients from the hospital's Neurology inpatient department (IPD) were included in the study. Patients of *balghami amrāḍ,* e.g., *fālij* (paralysis) were included in the study, who are taking *munḍij-i-balgham* drugs, patients more than 40 years of age, and patients of either gender. Patients <40 years of age, pregnant and lactating women were excluded and the patients taking such foods or medicines which can change their urine color were also excluded from the study. There were 40 participants in the observational study, which included a 12-day study protocol. The CONSORT flow-diagram of the study in presented in Figure 1. Assuming the expected population standard deviation to be 0.16, and employing t-distribution to estimate sample size, the study would require a sample size of 40 to estimate a mean with 95% confidence and a precision of 0.052. In other words, if selecting a random sample of 40 from a population, and determining the mean to be say y, one would be 95% confident that the mean in the population lies somewhere between y − 0.052 and y + 0.052.[17] Patient's informed consent was taken.

Preparation of the drug and dosage

Due to the involvement of *balgham* in the etiopathogenesis of this disease, the *mizāj* of *fālij* is referred to as *Barid Ratab*.[14] *Ilaj bil zid* is the basic treatment premise of Unani medicine. Therefore, the test formulation used in *Tanqiya* and *Ta'dīl* was selected from the reputed Unani treatise *Bayaze Kabeer* Vol-2. Before preparing the formulation, all the drugs were properly identified to ascertain their originality. The drugs were crushed, weighed, and mixed in their respective proportions as described in *Qarabadeen* (pharmacopeia). These drugs were dispensed to the patient in the IPD in a transparent plastic lock bag, to avoid any confusion regarding dosage. One packet for one dose is 24 g, the patients were advised to soak these drugs in 250 ml of water for the whole night and the next morning-soaked drugs were allowed to boil in the form of *Joshanda* (decoction) till the water reduces to half (125 ml).[18]

Method of sample collection

On the day of admission of the patient to the hospital, he/she was informed about the study, if the patient fits into inclusion criteria, and then informed consent was taken and given a urine sample container of 20 ml. The patient was instructed how to collect the urine and give the sample to the lab within an hour of collection.

For viscosity determination

The urine sample was transferred into the measuring cylinder of the viscometer instrument which measures about 5 ml of the urine and the sample was run into the instrument, reading was noted.[19,20]

For color determination

Some amount of the sample was transferred into the cuvette by using a dropper and the instrument was set to zero by using blank (normal saline). The cuvette was fitted into the instrument and reading was noted down between the 400 and 700 nm range.[21]

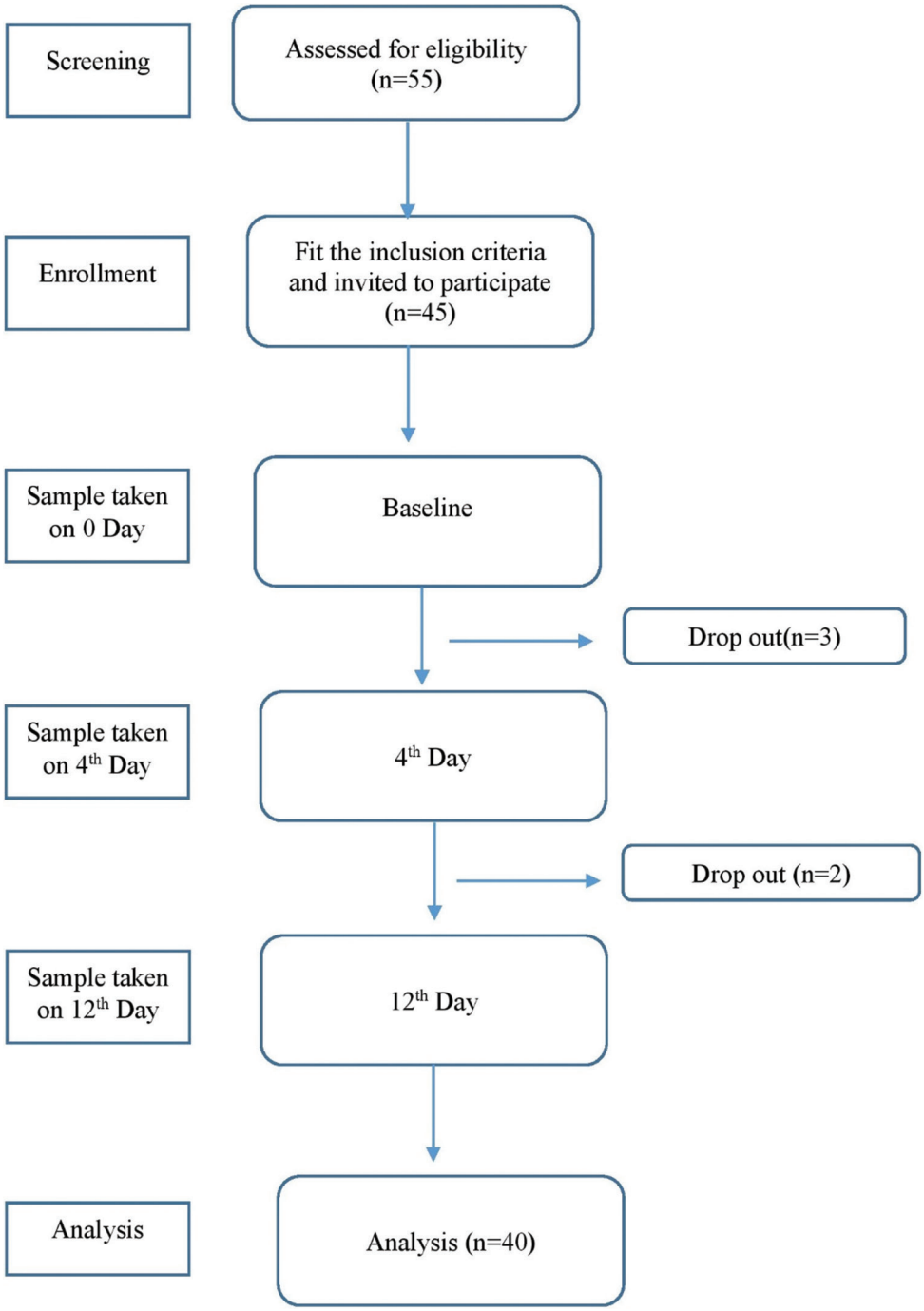

Figure 1: Consort flow-diagram of the study

Procedure

Before starting the *Munḍij-i-balgham* therapy, a morning urine sample were evaluated collected and measured for colorimetric as well as viscometric parameters for color intensity and viscosity, respectively. The second sample was collected on the 4th day. The third sample was taken on the 12th day.[2,3,12] Any color intensity difference was observed at 400–700 nm particularly using blue and yellow filters and then analyzed by the appropriate statistical method.[22] Any change in the viscosity of urine was also recorded and analyzed by the viscometer.[23]

Statistical analysis

The assessment for the color as well as viscosity changes was evaluated through Colorimeter and viscometer, respectively. The change in their values was recorded on a case record form specifically designed for the study. At the end of the study, the data were tabulated and analyzed statistically using IBM, NY, USA SPSS version 20.0. All the

quantitative variables such as dynamic viscosity and optical density were summarized using the descriptive statistics such as mean and standard deviation. Kolmogorov–Smirnov test was used to test for the normality of the data. Repeated measures of the ANOVA test were used to compare the optical density and the Friedman's test was used to compare the dynamic viscosity from baseline to the end of the 12th day. $P < 0.05$ was considered statistically significant. The data were analyzed using IBM, NY, USA SPSS version 20.0.

Results

Dynamic viscosity

The mean dynamic viscosity [Table 1 and Figure 2] decreased from 1.84 to 1.82 on an average between the 0 and 4th day. However, the average change (−0.056) was not found to be statistically significant ($P = 0.09$). Furthermore, the dynamic viscosity increased from 1.84 on day 0 to 1.85 on day 12th and this increase was found to be statistically significant ($P = 0.012$). Wilcoxon's signed–rank test was used to compare the differences between 0 and 4th days and 0th to 12th days, etc.

On an average from the 4th day to the 12th day, there was an increase in the dynamic viscosity from 1.82 to 1.85, and this increase was found to be statistically significant ($P < 0.001$).

Overall using Friedmann's test, we found that the change in the dynamic viscosity from baseline to the 12th day was statistically significant ($P < 0.0001$).

Optical density/absorbance

On average, the mean optical density [Table 2 and Figure 3] increased from 0.23 to 0.29 from 0th to the 4th day (−0.056). However, this increase was not found to be statistically significant ($P = 0.06$).

There was an increase in the optical density from the 0th to the 12th day. On average, the mean optical density was 0.238 at baseline and increased to 0.33 on the 12th day (−0.94) which was statistically significant ($P < 0.001$).

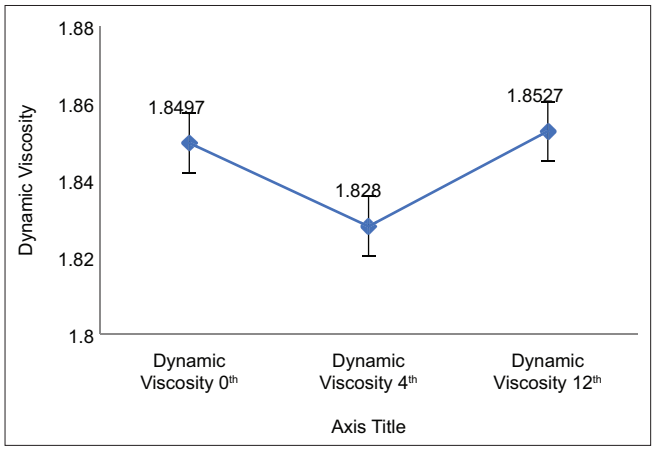

Figure 2: Graph showing changes in dynamic viscosity

On average, there was also an increase in the dynamic viscosity from the 4th day to the 12th day. The average on the 4th day was 0.29, and on the 12th day, it was 0.33. However, this difference was not found to be statistically significant.

Overall, the change in the average optical density from baseline to the 12th day was statistically significant ($P < 0.0001$) using RM ANOVA.

Discussion

This study demonstrated that the classical Unani visual observations of urine, specifically changes in color and viscosity during *munḍij* therapy for *fālij* can be translated into measurable, objective parameters using modern instruments. The study confirmed that patients' urine changed from *Abyad* (white) and *Raqīq* (thin) before therapy to *Zard* (yellow) and *Mu'atdil* (moderate) after therapy, aligning with descriptions found in classical texts.[2,4,16]

These findings validate the traditional Unani understanding that therapies such as *munḍij* induce quantifiable

Table 1: The dynamic viscosity change

	Dynamic viscosity 0th day	Dynamic viscosity 4th day	Dynamic viscosity 12th day
Mean	1.8497	1.828	1.8527
SD	0.16	0.13	0.16
Minimum	1.669	1.7	1.696
Maximum	2.421	2.301	2.31
	Average change in dynamic viscosity from 0th to 4th day	**change in dynamic viscosity from 0th–12th day**	**change in dynamic viscosity from 4th–12th day**
Mean	0.02176	−0.002915	−0.024675
SD	0.14	0.15	0.04
P (Friedmans test)	0.09	0.012*	<0.001**

SD: Standard deviation. *Stands for $p<0.05$, **Stands for $p<0.01$

Table 2: The optical density change

	Optical density 0th day	Optical density 4th day	Dynamic viscosity 12th day
Mean	0.238	0.294	0.33225
SD	0.11	0.14	0.16
Minimum	0.01	0.08	1.696
Maximum	0.55	0.9	2.31
	Average change in optical density from 0th to 4th day	**Change in optical density from 0th to 12th day**	**Change in dynamic viscosity from 4th to 12th day**
Mean	−0.056	−0.002915	−0.024675
SD	0.15	0.15	0.04
P (Friedmans test)	0.066	<0.001*	0.259

SD: Standard deviation. *Stands for $p<0.05$

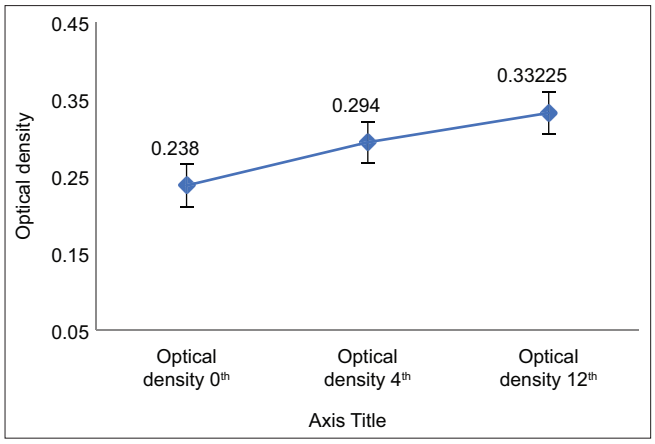

Figure 3: Graph showing changes in optical density/absorbance

physiological changes that can aid the diagnosis and prognosis. Previous literature often cites such visual diagnostic signs but lacks empirical evidence using modern tools.[2,3] By providing measurable data, this study supports the views of early Unani physicians, such as Hakim Ajmal Khan, who emphasized integrating modern scientific methods without losing classical essence.

This study opens the new avenues for standardizing subjective diagnostic methods in Unani medicine, thereby strengthening its scientific basis and facilitating its potential integration into contemporary healthcare. Documenting objective evidence for classical parameters can help revive traditional diagnostic skills among practitioners and encourage confidence among modern physicians and researchers.

The primary limitations include a small sample size, a short study period, and limited urine parameters. These factors restrict the generalizability of the findings and highlight the need for more robust data to confirm and expand upon these results.

Future studies should focus on larger clinical trials with diverse patient populations and longer follow-up durations. Expanding the range of measurable parameters and applying this approach to other *māddī* diseases could help build a stronger scientific bridge between classical Unani diagnostics and modern laboratory medicine. Such work can ultimately contribute to developing validated diagnostic protocols that honor the system's heritage while meeting contemporary scientific standards.

Conclusion

Ancient Unani scholars were well aware of the changes occurring in urine, sputum, feces and other body fluids, and they were able to examine them with naked eye. Urine is a sample obtained that continuously supplies crucial information about the state of the body to physicians. Urine testing was thought to be an expedient way of revealing not only fundamental alterations in the four humor's balance but also the site of disease inside the body. Thus, examination and analysis of urine have been in practice since antiquity. The said practice was utilized for prognostic purposes as well. But nowadays, due to a lack of such physicians and also due to the invention of certain analytical instruments, it is reasonable to observe changes that occur in urine after giving any therapy with the help of instruments. The assimilation of scientific methods in studies in the Unani medicine is not only appealing but also saves time on the one hand; and on the other hand, it reduces the subjectivity into objectivity. Now, quality can be converted into quantity.

This study was conducted to prove the assertion and hypothesis that certain Unani therapies, such as *nudj wa tanqia*, are utilized to eliminate morbid stuff in an altered form, similar to how modern medications are metabolized. In current pharmacology, medication metabolism is mediated by specific enzymes; however in the Unani system of Medicine, changes in a pathological matter are caused by drug activity.

This research is not focused on the drug's efficacy, but rather on the changes in body fluids that occur after taking particular medications.

The colorimeter and viscometer were only utilized as a tool to measure the changes in color and viscosity in this observational study. There is a statistically significant difference between the urine samples taken before and after therapy.

The study not only confirmed the hypothesis but also paved the way for further research.

Based on the result and discussion, it can be concluded that the hypothesis of this study has been proven right i.e., at the end of the study, the color of the urine is found to be changed from *Abyaḍ* (white) *to Zard* (yellow) and became *Mu'tadil* (moderate) in consistency.

The fundamental goal of the treatment for *fālij* survivors is to enhance motor function so that they are less reliant on caregivers and their families are less burdened. The Unani system of Medicine is an alternative therapy that may still be able to treat people with these issues.

This study also validates several claims made by the Unani system of Medicine, which is frequently criticized for being unscientific due to the fact that most of the concepts in Unani medicine are subjective.

As a result of the preceding discussion, it is possible to conclude that the given therapy is effective in the treatment of motor recovery. This treatment plan could aid *fālij* patients in reducing their reliance and improving their quality of life.

The findings create a basis for larger, longer-term studies to standardize such measurable parameters for diagnosing and monitoring *balghami amrād*. Future research should

expand on this approach, validate it across other Unani therapies, and integrate modern lab methods to strengthen the scientific basis of Unani diagnostics and enhance their clinical acceptance.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Standardization and Quality Evaluation of a Traditional Unānī Formulation

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Abstract

Unānī polyherbal formulations are widely used for local application in the management of musculoskeletal disorders, however, standardization data for these externally applied herbal preparations are limited. Establishing standardization parameters is essential to ensure their safety, efficacy, batch-to-batch consistency, and compliance with regulatory requirements, thereby facilitating their integration into evidence-based clinical practice. The present study aims to standardize and evaluate the quality of a traditional Unānī polyherbal formulation used in the management of musculoskeletal disorders, particularly knee osteoarthritis. The formulation comprises *Colchicum luteum* Baker (*Sūranjān talkh*), *Matricaria chamomilla* L.(*Bābūna*), *Trigonella uncata* Boiss. (*Nākhūna*), *Butea monosperma* (Lam.) Taub.(*Gule-tesū*), and *Solanum nigrum* L.(*Mako*), traditionally employed in *Bakhūr* (fumigation) therapy. The formulation underwent comprehensive standardization protocols, including organoleptic assessment, physicochemical evaluation (loss on drying, ash values, and extractive values), phytochemical screening, and thin-layer chromatographic (TLC) profiling. Results revealed significant physicochemical stability with low moisture content (7.87% mean loss on drying), acceptable ash values, and high extractive values, particularly in methanolic extracts. TLC analysis confirmed a diverse phytochemical profile with distinct Rf values across various solvents, aiding in formulation fingerprinting. The findings support the formulation's consistency, quality, and safety, offering a standardized reference framework for its clinical application and regulatory compliance.

Keywords: *Bakhūr (fumigation), physicochemical analysis, phytochemical screening, Polyherbal Unānī formulation, standardization, thin-layer chromatographic, Unānī medicine*

Introduction

Herbal medicines are widely used, and over the past two decades, there has been a notable increase in their use. According to estimates, around 75%–80% of the world's population, primarily in developing countries, relies on herbal medicine to meet their primary healthcare needs.[1,2] Recently, there has been a clear paradigm shift in health trends from synthetic to natural medicine. This is primarily due to the assumption that "natural" always means "safe" and a widespread general belief that herbal remedies are inexpensive, readily available, and compatible with the human body, causing no side effects.[3] Although generally considered safe, the use of herbs can potentially be harmful and toxic. Certain medicinal plants possess inherent toxicity and contain harmful amounts of noxious matter. While some herbs and their products exhibit adverse effects due to purity and quality issues, this may be attributable to several factors; one such factor is the lack of availability of genuine crude drugs, which leads to a break in demand and supply, resulting in various forms of substitution and adulteration with undeclared medicines and substances to meet rising demands and derive more economic benefits.[4] Other reasons may include misidentification, incorrect preparation, and dosage by both consumers and healthcare providers. Furthermore, adverse events may also arise from improper storage and contamination with pathogenic microorganisms and potentially hazardous substances, such as heavy metals and agrochemicals.[5,6] Consequently, this has led to compromises in product safety and quality, which results in substandard products that disappoint both manufacturers and consumers, and in some instances, lead to serious and fatal consequences rather than health benefits.[7] To address these challenges, implementing Good Agricultural and Collection Practices and Good Manufacturing Practices (GMPs) is crucial.[8,9] In addition, developing guidelines, improving regulatory measures,

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and utilizing modern analytical methods can enhance the safety and quality of herbal medicines.[5,10] To address this, it is essential to establish suitable infrastructure, reliable methods, skilled manpower, strict regulatory laws, and guiding principles for evaluating their safety and quality.[3,6] In 1989, the World Health Assembly passed a resolution addressing the need to ensure the quality standard of medicinal plant products by employing more specific modern instrumental methods of analysis to guarantee the purity and quality control of raw materials and finished products.[11] Standardization is a crucial aspect of ensuring the safety and quality of polyherbal formulations, as the combination of more than one ingredient is often necessary to achieve the desired therapeutic effect.[12,13] Using standardization, we can ensure the effectiveness, safety, quality, and tolerability of herbal medications, as well as minimize batch-to-batch variation.[5,12-14]

Unānī polyherbal formulation

Unānī medicines are regarded as a safe and effective therapy for *Waja' al-Mafāṣil* (arthritis) owing to their long history of usage and absence of serious adverse effects. Scientific evidence from human and animal studies shows its effectiveness. Unānī medicines offer a range of single and compound drugs that are scientifically proven to be effective in managing musculoskeletal diseases. Some commonly used drugs are used locally in the management of osteoarthritis (OA), in the form of *Ravghan* (*oil*), *dimād* (poultice), *tilā'* (liniment), *takmīd* (fomentation), *naṭūl* (douche), and *Inkibāb* (vapour bath), etc. These local regimens have been found to be very effective in relieving pain, inflammation, and stiffness in the knee joints. Some of the commonly used Unānī medicines, including *Sūranjān talkh* (*Colchicum luteum* Baker), *Bābūna* (*Matricaria chamomilla* L.), *Nākhūna* (*Trigonella uncata* Boiss.), *Gule-tesū* (*Butea monosperma* (Lam.) Taub.), and *Mako khushk* (*Solanum nigrum* L.), are used for local application traditionally as *Bakhūr* (fumigation or medicated steam) and *Takor* in managing various musculoskeletal disorders.[15,16] The traditional use of these drugs as analgesics and anti-inflammatory agents has therapeutic effects in the management of knee OA.

Bakhūr (fumigation) therapy is among the most renowned regimens of *'Ilāj bi'l Tadbīr* (regimenal therapy).[17] Unānī physicians have utilized *Bakhūr* (fumigation or medicated steam) therapy since antiquity; in this procedure, some herbal medications are boiled in a vessel, then applied to the surface of the afflicted body parts.[18] In *Bakhūr* (fumigation) therapy, some amount of vapour may penetrate the body through the skin and produce the mechanism of diversion of morbid humours from one part of the body to another, called *Imāla*.[19]

Aim and objective

The present study aims to standardize and evaluate the quality of a traditional Unānī polyherbal formulation used in the management of musculoskeletal disorders, particularly knee OA. The formulation comprises *Colchicum luteum* Baker (*Sūranjān talkh*), *Matricaria chamomilla* L.(*Bābūna*), *Trigonella uncata* Boiss. (*Nākhūna*), *Butea monosperma* (Lam.) Taub.(*Gule-tesū*), and *Solanum nigrum* L.(*Mako*), traditionally employed in *Bakhūr* (fumigation) therapy.

Standardization of a polyherbal Unānī formulation

The present research study entails standardizing a polyherbal Unānī formulation comprising *Sūranjān talkh* (*Colchicum luteum* Baker), *Bābūna* (*Matricaria chamomilla* L.), *Nākhūna* (*Trigonella uncata* Boiss.), *Gule-tesū* (*Butea monosperma* (Lam.) Taub.), and *Mako khushk* (*Solanum nigrum* L.) [Table 1.1 and Figure 1]. These drugs are scientifically proven to have analgesic and anti-inflammatory properties, and hence utilized in *Bakhūr* (fumigation) dosage form to treat a variety of musculoskeletal illnesses. To assess the safety, purity, and quality of formulations, they were subjected to morphological studies, powder microscopy, pharmacognostic studies, and phytochemical analysis to set the standard values, which can be used as reference standards to assess the purity and quality control in herbal industries and to overcome batch-to-batch disparity in traditional preparation.

Materials and Methods

Parameters for Standardization: The standardization of the Unānī formulation was carried out using the following parameters.

a) Raw drug procurement, identification, and authentication
b) Organoleptic characteristics of formulation
c) Physicochemical analysis of formulation
   - Loss on drying
   - Ash values
     i. Total ash
     ii. Acid-insoluble ash
     iii. Water-soluble ash.
   - Extractive values in different solvents.
     i. Petroleum ether extract.
     ii. Chloroform extract
     iii. Methanol extract
     iv. Aqueous extract.

Table 1.1: Ingredients of formulations

Ingredients	Botanical name	Parts used	Quantity (g)
Bābūna	*Matricaria chamomilla* L.	Flowers	5
Nākhūna	*Trigonella uncata* Boiss.	Seed-containing pods	5
Sūranjān talkh	*Colchicum luteum* Baker	Dried corms	5
Gule-tesū	*Butea monosperma* (Lam.) Taub.	Flower	10
Mako	*Solanum nigrum* L.	Fruits	10

d) Phytochemical screening
e) Thin-layer chromatographic (TLC) profile of Unānī formulation with different extracts.

Raw drug procurement, identification, and authentication

Procurement, Identification and Authentication of Drugs: All the drugs were procured from the genuine/registered drug seller Universal Biotech, Delhi, and identified and authenticated by Dr. Sunita Garg, Chief Scientist and Head of the Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Policy Research (NIScPR), Delhi. Vouchers of drug specimens were deposited in the RHMD, CSIR-NIScPR, Delhi, given below in Table 1.2.

Organoleptic characteristics of formulation

- Appearance of formulation: Powder form
- Color of formulation: Dark Brown
- Odor of formulation: Characteristic.

Physicochemical analysis of formulation

Loss on drying: The drying process of herbal medicines might cause losses due to their hygroscopic nature, which also creates an excellent environment for the development of bacteria and fungi. The drug's purity, therefore, decreases. As a result, medications devoid of moisture must be assessed to determine the amount of active ingredients.

Table 1.2: Drug specimen with authentication number

Specimens	Scientific name	Authentication number
Bābūna	*Matricaria chamomilla* L.	NIScPR/RHMD/ Consult/2023/4321-22-1
Nākhūna	*Trigonella uncata* Boiss.	NIScPR/RHMD/ Consult/2023/4321-22-2
Gule-tesū	*Butea monosperma* (Lam.) Taub.	NIScPR/RHMD/ Consult/2023/4321-22-3
Mako	*Solanum nigrum* L.	NIScPR/RHMD/ Consult/2023/4321-22-4
Sūranjān talkh	*Colchicum luteum* Baker	NIScPR/RHMD/ Consult/2023/4321-22-5

The powdered drug of the formulation was placed onto a sterile and desiccated Petri dish of known mass (Wp), and the Petri dish, along with the drug sample, was weighed together (Wp + Wo). The Petri dish containing the sample was then dried in an oven at 100°C–105°C for 2 h. After drying, the sample was removed from the oven, allowed to cool, and weighed. The empty Petri dish was subsequently heated in an oven at 105°C for 2 h, removed, and weighed. This heating and weighing process was repeated every 2 h until a constant weight was achieved, or until the difference between two successive weighings was ≤0.25% of the constant weight. Finally, the weight of the medication-packed Petri dish was determined, and the weight loss of the drug after air-drying was calculated using the following formula

Mc (%) = (W2/Wo) × 100 where Wo = Initial weight of the drug sample, Wp = Weight of empty Petri dish, W1 = Final weight obtained (weight of Petri dish + drug sample) after successive drying, Weight of dried drug sample (W2) = (Wp + Wo)–W1 [Table 2.1].

Ash values

The residue that remains after completely burning medicines is known as its ash value. It is critical in determining a pharmaceutical's quality. Drug analysis is beneficial for evaluating the purity, identity, and integrity of a drug, since the presence of dust, earthy particles, and extraneous materials might contribute to its overall weight. The ash value is the total amount of ash, including both water-soluble and acid-insoluble ash. This measurement helps detect the presence of inorganic contaminants and

Figure 1: (a) *Gule-tesū* (*Butea monosperma* (Lam.) Taub.); (b) *Bābūna* (*Matricaria chamomilla* L.); (c) *Nākhūna* (*Trigonella uncata* Boiss.); (d) *Sūranjān talkh* (*Colchicum luteum* Baker); (e) *Mako* (*Solanum nigrum* L.)

adulteration. This is the criterion for judging the identity and purity of a crude drug by performing total ash, acid-insoluble ash, and water-soluble ash tests.[20]

Total ash

A crucible containing 2 g of an air-dried sample of formulation was placed in a muffle furnace and heated at a maximum temperature of 450°C for 8 h. The crucible containing ash was allowed to cool, and then weighed, and the percentage was determined relative to the weight of the ash after it had been dried in the air [Table 2.2].

Acid-insoluble ash

Twenty-five milliliters of hydrochloric acid (1:25) were boiled with the total prepared ash for 5 min. To prevent spattering, the silica dish was covered with a watch glass. The mixture was then filtered through ashless filter paper (Whatman No. 42). The residue was thoroughly rinsed with hot water (above 85°C). The ashless paper containing the residue was dried and then transferred to a muffle furnace, where it was ignited at 550°C for 2 h. The ignition process in the muffle furnace was repeated, followed by cooling and weighing at half-hour intervals until a constant weight was achieved [Table 2.3].

Water soluble ash

To prepare water-soluble ash, the ash was boiled with 25 ml of distilled water in a beaker for 5 min. The mixture was then filtered through ashless filter paper, and the filtrate was collected. The residue was washed with hot water, dried in an oven, and then ignited at 550°C for 2 h. After ignition, the crucible was cooled in a desiccator and weighed. This process was repeated until the difference between two successive weightings was <1 mg [Table 2.4].

Extractive values

The extraction value of a medicine is important for identifying adulteration since it approximates the quantity of particular ingredients. It is essential for setting drug standards, as adulterated or exhausted material may yield different values than the genuine extractive percentage. This includes cold extraction, hot extraction, and successive extraction.

Table 2.1: Loss on drying

Weight of drug Wo (g)	Weight of drug (g) Wo + weight of petri dish (g) Wp (before drying) A (g)	Weight of drug (g)+ weight of petri dish W1 (g) (after drying) (g)	Loss on drying W2 (g)	Loss on drying (%)	Mean±SEM (%)
5	47.7	47.31	0.39	7.8	7.87±0.18
5	50.62	50.21	0.41	8.2	
5	52.03	51.65	0.38	7.6	

SEM: Standard error of mean, Wo: Initial weight of the drug sample, Wp: Weight of empty Petri dish

Table 2.2: Total ash

Weight of drug (g)	Weight of crucible (g)	Weight of crucible (g) + weight of air-dried drug sample (g)	Weight of crucible (g) + weight of total ash (g)	Weight of total ash (g)	Total ash (%)	Mean±SEM (%)
5	25.86	30.86	26.23	0.37	7.4	7.27±0.35
5	31.25	36.25	31.58	0.33	6.6	
5	28.4	33.4	28.79	0.39	7.8	

SEM: Standard error of mean

Table 2.3: Acid insoluble ash

Weight of drug (g)	Weight of crucible (g)	Weight of crucible (g) + weight of air-dried drug sample (g)	Weight of crucible (g) + weight of total ash (g)	Weight of total ash (g)	Total ash (%)	Acid insoluble ash (%)	Mean±SEM (%)
5	24.91	29.9	25.26	25.09	0.18	3.6	3.67±0.29
5	33.12	38.12	33.5	33.33	0.21	4.2	
5	30.65	35.65	30.96	30.65	0.16	3.2	

SEM: Standard error of mean

Table 2.4: Water-soluble ash

Weight of drug (g)	Weight of crucible (g)	Weight of crucible (g) + weight of air-dried drug sample (g)	Total weight of the ashed sample + crucible (g)	Weight of water insoluble ash + crucible (g)	Weight of water-soluble ash (g)	Water-soluble ash (%)	Mean±SEM (%)
5	25.86	30.86	26.23	26.16	0.07	1.4	1.47±0.30
5	31.25	36.25	31.58	31.49	0.09	1.8	
5	28.4	33.4	28.79	28.73	0.06	1.2	

SEM: Standard error of mean

Cold extraction

In a stoppered conical glass flask, 5 g of the formulation powder were soaked with 100 ml of each solvent (petroleum ether, chloroform, methanol, and water). The flask was shaken every six hours and then left to stand for 18 h. The mixture was quickly filtered, and the filtrate was collected in a Petri dish. It was then evaporated in a water bath at 105ºC until fully desiccated. After cooling in a desiccator for 30 min, the weight was determined.

Individual cold extractive values [Tables 3.1-3.4]

Hot extraction

The formulation powder (20 g) was subjected to solvent extraction using a Soxhlet equipment, heated for 6 h, and then evaporated in a water bath. The extraction values were determined using the drug's mass (World Health Organization [WHO], 1998).

Individual hot extractive values [Tables 4.1-4.4].

Successive extraction

The formulation powder (20 g) was extracted in a Soxhlet apparatus using a series of solvents in succession, including petroleum ether, chloroform, methanol, and water. Heated for 6 h and then evaporated in a water bath until it was entirely desiccated. We determined the extraction values by utilizing the drug's mass.

Successive extractive values [Tables 5.1-5.4].

Phytochemical screening

The therapeutic properties of crude drugs are primarily determined by their physiologically active chemical constituents. A lower percentage of these constituents may result in lesser therapeutic value, whereas a high percentage may cause unpredictable biological effects and side effects. Phytochemical findings can help predict drug biological activity and dose response. The phytochemical analysis consisted of extracting a powdered formulation using a Soxhlet apparatus and solvents such as petroleum ether, chloroform, acetone, alcohol, and water. The liquid was then evaporated, and the remaining residue was examined for various phytoconstituents, including alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, resins, and others [Table 6.1].

Table 3.1: Petroleum ether cold extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
5	41.06	41.17	0.11	2.2	2.13±0.18
5	47.45	47.54	0.16	1.8	
5	52.5	52.62	0.08	2.4	

SEM: Standard error of mean

Table 3.2: Chloroform cold extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
5	37.2	37.38	0.18	3.6	3.87±0.17
5	44.32	44.51	0.19	3.8	
5	47.71	47.92	0.21	4.2	

SEM: Standard error of mean

Table 3.3: Methanol cold extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + Weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
5	50.03	50.65	0.62	12.4	12.4±0.37
5	42.12	42.77	0.65	13	
5	53.23	53.82	0.59	11.8	

SEM: Standard error of mean

Table 3.4: Aqueous cold extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
5	49.64	49.87	0.23	4.6	4.73±0.35
5	37.88	38.08	0.21	4.2	
5	41.45	41.72	0.27	5.4	

SEM: Standard error of mean

Table 4.1: Petroleum ether hot extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	48.11	49.58	1.47	7.35	7.63±0.17
20	42.43	44.02	1.59	7.95	
20	50.65	52.17	1.52	7.6	

SEM: Standard error of mean

Table 4.2: Chloroform hot extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	51.2	51.51	0.31	1.55	1.78±0.11
20	47.35	47.62	0.27	1.85	
20	45.27	45.65	0.38	1.9	

SEM: Standard error of mean

Table 4.3: Methanol hot extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	42.8	47.71	4.91	24.5	24.23±0.16
20	46.71	51.50	4.79	23.95	
20	52.65	57.5	4.85	24.25	

SEM: Standard error of mean

Table 4.4: Aqueous hot extract

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	58.72	62.53	3.81	19.05	18.73±0.25
20	37.4	41.05	3.65	18.25	
20	55.24	59.02	3.78	18.9	

SEM: Standard error of mean

Table 5.1: Petroleum ether hot extract (successive)

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	45.97	47.13	1.16	5.80	5.6±0.28
20	50.22	51.23	1.01	5.05	
20	54.68	55.87	1.19	5.95	

SEM: Standard error of mean

Thin-layer chromatographic profile of Unānī formulation with different extracts

One of the most important tools for detecting adulteration and evaluating the caliber of pharmaceuticals is TLC analysis, or TLC. Using several mobile phases, TLC was performed on percolated silica gel 60 F254 TLC plates for the formulation's methanolic, chloroform, and aqueous extracts. Anisaldehyde-sulfuric acid was sprayed on the stained TLC plates, and they were then observed in both daylight and ultraviolet (UV) short and long wavelengths. The standard was established by recording the Rf values of unique spots seen in different solvent systems. It is possible to use the Rf value to determine the number of components in the test drug. The TLC profile may also be the starting point for a quantitative analysis of a drug's active components. The Rf value of spots was determined by the given formula.[21] Rf value = Distance travelled by the Spot/Distance travelled by the solvent.

Results

The study analyzed three extracts from the Unānī formulation: chloroform, methanol, and aqueous extracts, using TLC analysis. The study found that all three extracts had excellent separation spots, with the chloroform extract having the most spots. Short wavelength (UV 254 nm) detected the chloroform extracts, revealing eleven spots with Rf values of 0.04, 0.08, 0.12, 0.15, 0.21, 0.28, 0.40, 0.43, 0.51, 0.56 0.59; long wavelength (UV 366 nm)

Table 5.2: Chloroform hot extract (successive)

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	38.76	39.09	0.33	1.65	2.16±0.27
20	51.12	51.63	0.51	2.55	
20	53.46	53.9	0.46	2.30	

SEM: Standard error of mean

Table 5.3: Methanol hot extract (successive)

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	43.39	46.2	2.87	14.35	14.33±0.23
20	49.37	52.19	2.82	14.1	
20	48.25	51.16	2.91	14.55	

SEM: Standard error of mean

Table 5.4: Aqueous hot extract (successive)

Weight of drug (g)	Weight of petri dish (g)	Weight of petri dish (g) + weight of extract (g)	Weight of extract (g)	Extractive value (%)	Mean±SEM (%)
20	46.7	49.64	2.94	14.7	15.11±0.25
20	52.18	55.2	3.02	15.1	

SEM: Standard error of mean

Table 6.1: Phytochemical screening of powdered formulation

Constituents	Method	Observation
Alkaloids	Dragendorff's test	+
Phenols	Ferric chloride test	+
Protein	Xanthoproteic test	+
Glycosides	Keller-kiliani test	+
Carbohydrate	Molisch's test	+
Flavonoids	Shinoda test	+

Present (+), Absent (−)

revealed four spots with Rf values of 0.13, 0.26, 0.40, and 0.46; and day light revealed five spots with Rf values of 0.17, 0.19, 0.25, 0.37, 0.57; when anisaldehyde sulfuric acid was sprayed, it revealed ten spots with Rf values of 0.06, 0.10, 0.16, 0.24, 0.30, 0.36, 0.42, 0.47, 0.54, 0.60 [Table 6.2 and Figure 2b].

Methanolic extract revealed two spots under daylight, with two spots with Rf values of 0.31 and 0.42 seen in the methanolic extract under daylight. Seven spots with Rf values of 0.02, 0.30, 0.36, 0.54, 0.68, 0.75, and 0.80 were seen under short wave (UV 254 nm), and seven spots with Rf values of 0.07, 0.13, 0.17, 0.33, 0.45, 0.54, and 0.63 were seen under long wave (UV 366 nm). When sprayed with anisaldehyde sulfuric acid, nine spots with Rf values of 0.12, 0.24, 0.32, 0.41, 0.53, 0.60, 0.66, 0.82, 0.90 [Table 6.3 and Figure 2a].

Aqueous extracts showed only one spot in daylight with an Rf value of 0.12. In short wave (UV 254 nm) light, they showed five spots with Rf values of 0.04, 0.09, 0.11, 0.34, and 0.40. In UV 366 nm light, they showed only two spots with Rf values of 0.09 and 0.15. When sprayed with anisaldehyde sulfuric acid, it shows six spots with Rf values of 0.07, 0.15, 0.22, 0.26, 0.31, and 0.36, as shown in Table 6.4 and Figure 2c.

Discussion

Although the standardization of multi-drug formulation is not an easy task, but simple and basic techniques play an important role in quality control of herbal drugs. The Unānī formulation was subjected to physicochemical analysis, which helps find the presence of desired chemical constituents that are therapeutically active. Other standards such as loss on drying ensures the moisture percentage in the drugs which is important factor for the stability as well as safety, higher moisture contents lead to early deterioration of the drug either by bacterial or fungal growth or loss of chemical constituents due to other reasons., ash values help to find out adulterants in the drug sample while extractive values confirm the quantity of desirable constituents in drug. This study is likely to provide the fingerprint data as well as SOPs for future reference in the preparation and dispensing of the drug in a clinical or pharmacy setup.

Ensuring batch-to-batch uniformity in polyherbal preparations is difficult because each plant contributes a variable spectrum of secondary metabolites that can shift with season, geography, and post-harvest handling. Nevertheless, a structured set of "first-line" quality control tests physicochemical limits, preliminary phytochemistry, and chromatographic fingerprinting, remains the backbone of herbal standardization recommended by the WHO and pharmacopoeias.

Table 6.2: Thin layer chromatography of the formulation (chloroform extract)

Medium of extraction	Mobile phase	In day light		In shortwave length 254 (nm)		In long wave length 366 (nm)		Ater spraying anisaldehyde sulphuric acid reagent	
		Rf value	Color of bands	Rf value	Color of bands	Rf value	Color of bands	Rf value	Color of bands
Chloroform	Tauline:Ethyl acetate (3:1)	0.17	Light green	0.04	Light green	0.13	pink	0.06	Violet
		0.19	Light green	0.08	Light green	0.26	Dark brown	0.10	Brown
		0.25	Orange green	0.12	Light green	0.40	Green	0.16	Pink
		0.37	Yellowish green	0.15	Dark green	0.46	Pink	0.24	Green
		0.57	Light yellow	0.21	Light violet			0.30	Light pink
				0.28	Green			0.36	Violet
				0.40	Violet				
				0.43	Dark violet			0.42	Dark violet
				0.51	Light violet			0.47	Light violet
				0.56	Light violet			0.54	Green
				0.59	Light violet			0.60	Light violet

Table 6.3: Thin layer chromatography of the formulation (methanolic extract)

Medium of extraction	Mobile phase	In daylight		In the shortwave length 254 (nm)		In the long wavelength 366 (nm)		After spraying Anisaldehyde sulphuric acid reagent	
		Rf value	Color of bands	Rf value	Color of bands	Rf value	Color of bands	Rf value	Color of bands
Methanol	Tauline: Ethyl acetate: Glacial acetic acid (8:2:0.1)	0.31	Light yellow	0.02	Dark purple	0.07	Light yellow	0.12	Light violet
				0.30	Light purple	0.13	Light yellow	0.24	Orange brown
				0.36	Light purple	0.17	Light yellow	0.32	Light brown
				0.54	Light purple	0.33	Light yellow	0.41	Ash color
		0.42	Light	0.68	Light purple	0.45	Light green	0.53	Light violet
				0.75	Dark purple	0.54	Light yellow	0.60	Light violet
				0.80	Dark purple	0.63	Light yellow	0.66	Light violet
								0.82	Violet
								0.90	Yellow

Table 6.4: Thin layer chromatography of the formulation (aqueous extract)

Medium of extraction	Mobile phase	In daylight		In shortwave length 254 (nm)		In long wavelength 366 (nm)		After spraying anisaldehyde sulphuric acid reagent	
		Rf value	Color of bands	Rf value	Color of bands	Rf value	Color of bands	Rf value	Color of bands
Aqueous	n-Butanol: Acetic Acid: (5:1)	0.12	Light yellow	0.04	Violet	0.09	Ash	0.07	Yellow
				0.09	Light violet	0.15	Ash	0.15	Ash
				0.11	Ash			0.22	Light yellow
				0.34	Ash			0.26	Light violet
				0.40	Ash			0.31	Light violet
								0.36	Ash

Physicochemical parameters such as loss on drying, total/acid-insoluble/water-soluble ash and solvent-specific extractive values are fast, inexpensive proxies for stability, purity and richness of soluble actives. A low moisture content (7.87 %) in the present formulation indicates good shelf life and minimal risk of microbial spoilage, aligning with the WHO guidance that stresses moisture reduction to curb microbial growth and hydrolytic degradation.[22] Ash limits help flag inorganic adulteration; our values fall within ranges proposed for root/flower drugs in the WHO quality control monographs.[11] High methanolic extractives (24 % hot; 12 % cold) confirm efficient recovery of mediumpolarity constituents – flavonoids, phenolics, and alkaloids – correlating with pharmacological activity and echoing the interpretation framework outlined by Kunle.[20]

Preliminary phytochemical screening detected alkaloids, glycosides, flavonoids, and phenolics, providing a qualitative rationale for the formulation's traditional anti-inflammatory use. Although such colour tests lack specificity, they are valuable triage tools before more intensive assays and are explicitly recommended in the WHO checklists for small to medium-scale manufacturers.[22]

TLC fingerprinting delivered reproducible, multi-band profiles in three solvent systems, with the chloroform extract yielding 11 diagnostic spots at 254 nm (Rf 0.04–0.59).

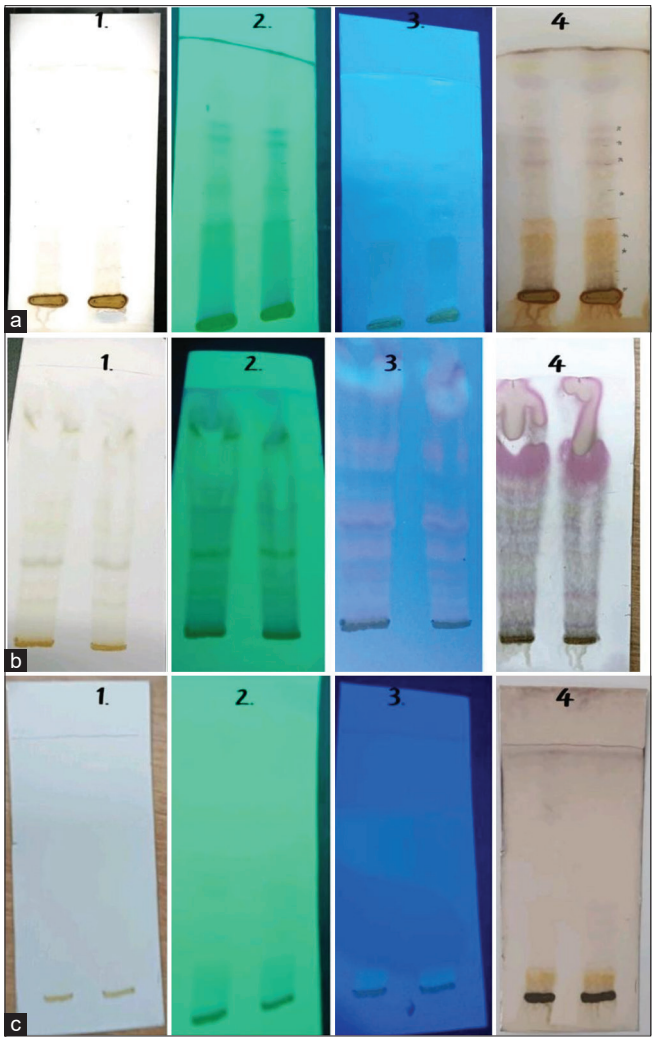

Figure 2: (a) Thin-layer chromatographic (TLC) profile of methanol extract of formulation under (1) Daylight, (2) ultraviolet (UV) short, (3) UV long, and (4) After spraying. (b) TLC Profile of Chloroform Extract of Formulation under (1) Daylight, (2) UV short, (3) UV long, and (4) After spraying. (c) TLC Profile of Aqueous Extract of Formulation under (1) daylight, (2) UV short, (3) UV long, and (4) After spraying

Chromatographic fingerprints are now recognized as the most practical chemometric "identity card" for complex botanicals when single-marker assays are unavailable. Current reviews emphasize TLC/high performance TLC (often coupled with densitometry) as a first-choice platform for regulatory authentication and phytoequivalence assessment, especially in resource-limited settings.[23] The distinct Rf ladder produced here can therefore serve as a comparator for future production lots and enforcement laboratories investigating market samples. Taken together, the data set furnishes a "minimal but sufficient" Standard Operating Procedure for routine quality assurance of this Unānī *Bakhūr* (fumigation) formulation. It translates centuries-old empirical knowledge into measurable criteria compatible with GMP, thereby bridging traditional use and modern regulatory expectations. While advanced techniques (HPLCMS, DNA barcoding, heavy metal and pesticide panels) would further strengthen the dossier, the present tier-one profile already satisfies the baseline recommendations of WHO (1998) for herbal medicines destined for local clinical or pharmacy deployment.[22] Future work should quantify bioactive markers and link chromatographic zones to anti-inflammatory potency through bioassay-guided fractionation, thereby strengthening the fingerprint–efficacy relationship suggested by contemporary chemometric studies.[23]

Conclusion

The present study demonstrates a comprehensive and systematic approach to the standardization and quality evaluation of a traditional Unānī polyherbal formulation used in *Bakhūr* (fumigation) therapy for musculoskeletal disorders. Through organoleptic, physicochemical, phytochemical, and chromatographic (TLC) analyses, specific quality parameters were established, including extractive values, moisture content, ash values, and phytochemical constituents. These findings not only support the therapeutic rationale for its traditional use, particularly its analgesic and anti-inflammatory properties, but also ensure reproducibility, safety, and authenticity in future formulations.

The formulation showed consistency in its physicochemical and phytochemical profiles and produced clear TLC fingerprints that can serve as reference standards for quality control in manufacturing settings. This work aligns with the WHO's guidelines for the quality assurance of herbal medicines and contributes toward bridging the gap between traditional wisdom and evidence-based scientific validation. Establishing such quality benchmarks is vital for ensuring the safety, efficacy, and global acceptance of Unānī Medicine.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Original Article

A comparative Pharmacognostic Study and Evaluation of the Anti-anxiety Effect of Two Species Used as *Barg-i-Gāozabān* (*Borago officinalis* L. and *Anchusa strigosa* Banks and Sol.) in Wistar Albino Rats

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Abstract

Background: *Borago officinalis* L. and *Anchusa strigosa* Banks and Sol. belongs to the family Boraginaceae. For ages, in the Unani System of Medicine, both have been used as *Barg-i-Gāozabān* for the treatment of various ailments, especially for neurological *disorders* such as melancholia, schizophrenia, and anxiety It is also used for the treatment of various other disorders such as palpitation, cough, rhinitis, pneumonitis, and cardiac weakness. **Aim of the Study:** To evaluate the anti-anxiety effect of two species of *Barg-i-Gāozabān* (*B. officinalis* L. and *A. strigosa* Banks and Sol.) in albino Wistar rats. **Materials and Methods:** Twenty-four Wistar albino rats of either sex were divided into 4 groups. Group I (Plain Control) received 0.5% chronic mucocutaneous candidiasis (CMC) orally for 7 days. Group II (standard control) received standard drug Diazepam (DZ) (1.0 mg/kg/bw i. p. 30 min); Group III (Test AAEAS) received an aqueous extract of *A. strigosa* Banks and Sol. Leaves, 817 mg/kg/b. w in 1 ml of distilled water with 0.5% CMC orally for 7 days. Group IV (Test BAEBO) received an aqueous extract of *B. officinalis* L. leaves, 817 mg/kg/b. w in 1 ml distilled water with 0.5% CMC orally for 7 days. **Results:** In the EPM model, the Aq. ext. of AS and BO both showed highly significant (**$P < 0.01$) time spent in the open arm on the 1st, 3rd, and 7th day, as compared with the plain control group, however less significant than standard drug DZ. Moreover, *A. strigosa* was found more effective than *B. officinalis* L. in anti-anxiety-related activities. The same results were noted in the light and dark method as in the EPM model. **Conclusion:** Proved the AS in comparison to the Plain Control group on EPM and light and dark test models. Based on the above findings, it is concluded that Aq. ext. of test drugs BO and AS showed significant antianxiety effects and validated the Unani claim that the drugs are useful in the management of depressive disorders. This activity may be attributed to the phytochemicals flavonoids, alkaloids, triterpenoids, and tannins present in test drugs. The result also proved Unani's concept of Badal (drug substitution) due to the similar effect and phytochemicals present in both plants.

Keywords: *Anchusa strigosa Banks and Sol, Barg-i-Gāozabān, Borago officinalis L.*

Introduction

In the Unani System of Medicine, different parts of plants such as roots stems, leaves, flowers, fruits, twigs, secretions, seeds, barks, and modified plant organs are being used for the treatment of various ailments.[1] According to WHO the current survey suggests that many developed countries have a high proportion of the population, making use of traditional practices of health.[2] The most popular herbs and medicines of Unani Tib are found here like, *Gāozabān* (*Anchusa strigosa* Banks and Sol.), *Jadwār* (*Delphinium denudatum* Wall), etc. which are used for the treatment of various ailments that became a challenge for the modern system of medicine such as melancholia and anxiety, etc. In the present era, the most predominant mental disorders are depression, obsessive–compulsive disorder, anxiety disorders, specific phobias, social anxiety disorder, and separation anxiety disorder.[3] Anxiety is the most common disorder encountered by psychiatric, worldwide. The term anxiety is derived from the Latin word Anxitas meaning "troubled mind."[4] Anxiety is described as a diffuse, unpleasant, vague sense of apprehension, with or without associated autonomic symptoms. It can be defined as a disorder of mental health represented by feelings of worry, anxiety, or fear in response to an apparent threat, that prevents the patient from performing his/her

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daily activities.[5-8] Anxiety is a physiological and mandatory primary emotion without which survival of humans would be impossible.[9] The anxiety is associated with three or more of the below symptoms for at least 6 months: (a) Restlessness, feeling keyed up or on edge, (b) being easily fatigued, (c) inability to focus, or mental fogginess, restlessness, (d) muscle tension, (e) sleep difficulties, and (f) Irritability.[10] According to WHO, 400 million cases of anxiety disorders are present.[10] In the Unani System of Medicine, anxiety disorders fall within the category of Fikr or *Iḍṭirāb-i-Nafsāniyyah*. There are several herbal drugs mentioned in the Unani System of Medicine since extended time for the management of anxiety disorder, single drugs as well as compound drugs. Those are *Brahmī* (*Bacopa moneri* L.), *Sankhāholī* (*Evolvulus alsinoides* Schult.), *Halela Siyāh* (*Terminalia chebuala* Retz), *Halela Zard* (*Terminalia chebula* Retz), *Kāhu* (*Lactuca sativa* L.), *Nīlofar* (*Lotus nymphaea* L.), *Gul-i-Surkh* (*Rosa damascena* Mill), etc. as mentioned in various classical literature.[2,11-14] One of the single drugs *Barg-i-Gaozaban* (*A. strigosa* Bank and Sol.) also known as "*Lisānal-Thūr*" is a leaf that resembles to cow's tongue and is known for its potent anxiolytic activity. It is being used in Unani medicine to treat various neuropsychiatric disorders such as melancholia, mania, and palpitation.[13] Another drug *Borago officinalis* L. also known as *Gāozabān* is found in Europe, and Northern Asia and grown at hill stations in India. This drug is used in various compound formulations as mentioned by the National Formulary of Unani Medicine and other pharmaceutical companies. However, in the market, the drug available as *Barg-i-Gāozabān* is identified as *A. strigosa* Banks and Sol., by Afaq *et al*. and the same is documented in Wealth of India.[15,16] As the plant B. *officinalis* L. is available in the Garden of Regional Research Institute of Unani Medicine (RRIUM), Srinagar, it was selected for the comparative study with a market sample of *Gāozabān* (*A. strigosa* Banks and Sol) for various pharmacognostic characteristics and their anti-anxiety property was done to establish the concept of substitution, as there may be the possibilities of similarity in various features and activities. Unani concept of Badal (drug substitution) or therapeutic interchange is based on the similarity in the action of the drug, temperament, and physical properties. Various pharmacological studies have been done on these two plants. Antiarthritic,[15] antibacterial, and antioxidant activities,[16] but no anti-anxiety study has been done on these two leaves of *Gāozabān*. Consequently, a need has been felt for a preclinical study for the anxiolytic effect and pharmacognostic profile of both species of *Gāozabān*. Hence, the two species of *Gāozabān* (*A. strigosa* Banks and Sol. and *B. officinalis* L.) are taken for an anxiolytic study in albino Wistar rats. In the study, a comparison of the anxiolytic effects of both species of *Gāozabān* (*A. strigosa* Banks and Sol. and *B. officinalis* L.) and also with diazepam (DZ) as standard drug was done. Pharmacognostic evaluations were also done.

Materials and Methods

Ethical approval of the study

Before starting the study, the protocol was submitted for ethical clearance. Accordingly, the Institutional Ethical Committee had approved the protocol (IAEC-Approval) IAEC/RRIUM/*Gāozabān*/19-20/3. On dated September 16, 2020. The study was carried out on albino Wistar rats of either sex. The data were generated from experimental studies at the laboratories of the drug standardization research unit and Animal Experimental Lab, RRIUM Srinagar, Jammu and Kashmir, India.

Collection of plant material

B. officinalis L. was cultivated and collected from Herbal Garden, RRIUM, Srinagar [Figure 1]. and the other species *A. strigosa* Banks and Sol [Figure 2]. was procured in December 2021 from an authentic drug supplier of the local market of Srinagar.

Identification and authentication

The drugs were identified and authenticated by Dr. Akhtar H. Malik, Jr. Scientist-Cum-Curator Centre, for Biodiversity and Taxonomy, Department of Botany, University of Kashmir under specimen voucher No. 4406 KASH and voucher No. 4405KASH for *A. strigosa* Banks and Sol. and *B. officinalis* L., respectively.

Parameter for standardization

Foreign matters

Foreign matters can affect the accuracy and efficacy of a drug. Hence, it should be separated from the drug.[17,18] In this study, foreign matters are present in both samples. The specified quantity of leaves around 100 g was spread on a thin layer of white paper. By visual inspection and by using a magnifying lens (×5), the foreign matters were picked out and the percentage in both was calculated separately [Tables 1 and 2].

Macroscopical characters

The macroscopy of a drug includes its visual appearance to the naked eye, particular systemic examination can be carried out and includes the visual appearance to the naked eye like shape, color, odor, taste, and other external features.[19] The leaves of *A. strigosa* Banks and Sol. and *B. officinalis* L. were evaluated for their size, color, shape odor, and consistency through the naked eyes and other sensory organs [see Tables 3 and 4].

Microscopical evaluation

Powder study microscopic examination of powder drugs aided by stains helps in the distinction of anatomy in adulterants. The cell contents such as starch granules, calcium oxalate crystals, and aleurone grains are scattered in the powder.[19] The size, shape, and relative positions of the different cells and tissues, the chemical nature of the cell walls, and the cell contents were determined. The basic arrangement of tissues in each drug was fairly constant like fibers, sclereids, tracheids, and vessels.[19] The leaves of *B. officinalis* L. and *A. strigosa* Banks and Sol. were powdered and sliced and then boiled in chloral hydrate solutions for a few minutes. A small quantity of powder was taken on a microscopic slide, evenly spread with the help of a brush, stained with phloroglucinol solution and a drop of concentrated hydrochloric acid (HCl), and then a few drops of glycerin (10%) were added to it. The

Figure 1: Dry leaves of *Borago officinalis* L.

Figure 2: *Barg-i-Gaozaban* (*Anchusa strigosa* Banks and Sol. dry leaves)

Table 1: Physicochemical constants of *Borago officinalis* L.

Particulars	**Weight of drug (g)**	**Weight of ash (g)**	**Percentage yield**
Total ash value	5	0.64	9.4
Acid insoluble ash value	5	0.24	4.8
Water soluble ash value	5	0.34	6.8
Extractive values			
Hot extractive value			
Solvent	**Weight of drug (g)**	**Weight of dried extract (g)**	**Percentage yield of extract (w/w)**
Ethanol	5	0.19	3.8
Aqueous	5	0.51	10.2
Cold extractive value			
Solvent	**Weight of drug (g)**	**Weight of dried extract (g)**	**Percentage yield of extract (w/w)**
Ethanol	5	0.1	2
Aqueous	5	0.28	5.6
Foreign organic matter analysis			
Plant part	**Weight of drug (g)**	**Weight of foreign matter (g)**	**Percentage of foreign matter**
Leaf	5.43	0.001	0.016
Loss on drying			
Part used	**Weight of drug (g)**	**Loss on drying (g)**	**Percentage loss on drying**
Leaf	5	0.82	9.4
pH values			
Sample		**pH**	
pH of 1% solution		6.5	
pH of 10% solution		8.2	
Swelling index			
Part used		**Swelling index**	
Leaf		0	
Foaming index			
Part used		**Foaming index**	
Leaf		0	

Table 2: Physicochemical constants of *Anchusa strigosa* Banks and Sol. leaves (market sample)

Particulars	Weight of drug (g)	Weight of ash (g)	Percentage yield
Total ash value	5	0.72	9.8
Acid insoluble ash value	5	0.01	13.6
Water soluble ash value	5	0.76	7.2
Extractive values			
Hot extractive value			
Solvent	**Weight of drug (g)**	**Weight of dried extract (g)**	**Percentage yield of extract (w/w)**
Ethanol	5	0.12	2.4
Aqueous	5	1.04	20.8
Cold extractive value			
Solvent	**Weight of drug (g)**	**Weight of dried extract (g)**	**Percentage yield of extract (w/w)**
Ethanol	5	0.76	15.2
Aqueous	5	0.41	8.2
Foreign organic matter analysis			
Plant part	**Weight of drug (g)**	**Weight of foreign matter (g)**	**Percentage of foreign matter**
Leaf	5.43	0.001	0.016
Loss on drying			
Part used	**Weight of drug (g)**	**Loss on drying (g)**	**Percentage loss on drying**
Leaf	5	0.64	8.4
pH values			
Sample			**pH**
pH of 1% solution			6.8
pH of 10% solution			7.7
Swelling index			
Part used			**Swelling index**
Leaf			0
Foaming index			
Part used			**Foaming index**
Leaf			0

Table 3: Macroscopical and sensory characters of dry leaves of *Borago officinalis* L

Parameters	Findings
Colour	Varies from yellowish-green to greenish-brown
Odour	Characteristic
Taste	Mucilaginous
Surface	Soft, hairy, crumbled
Fracture	Splintery
Margin	Dentate

Table 4: Macroscopical and sensory characters of *Anchusa strigosa* Banks and Sol. leaves (market sample)

Parameters	Findings
Colour	Greenish-brown with white spots like papillae of tongue
Odour	Odourless
Taste	Mucilaginous++
Surface	Rough, glandular, hard spiky projections
Fracture	Splintery

slides were covered with a cover slip and observed under a microscope for various microscopic characters [See in Figures 3 and 4].

Physicochemical evaluation

Ash values

This parameter can be used for the determination of inorganic materials. Heating causes the loss of organic materials in the form of carbon dioxide leaving behind the inorganic constituents. The acid-insoluble ash consists mainly of silica and high acid-insoluble ash is used to estimate the number of inorganic elements.[20,21]

The ash values were determined by the following methods:

Total ash

Ash of both plants' powdered samples 5 g were incinerated separately in a silica crucible in a muffle furnace at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed to get the total ash content [Tables 1 and 2].

Acid insoluble ash

Ash of both plants powdered samples 5 g each were boiled with 25 ml dilute HCl for 5 min separately. The insoluble matter was collected on an ash-less filter paper, and washed with hot water until the filtrate was neutral. Then, it was

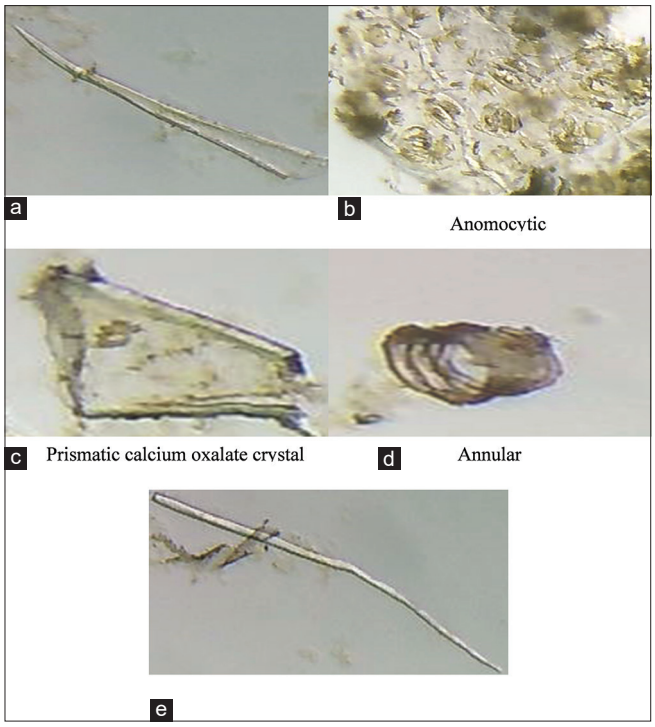

Figure 3: Powder microscopy of Borago officinalis L. showed (a) Trichome, (b) Nomocytic Stomata, (c) Prismatic calcium oxalate crystal, (d) Spiral vessels, (e) Annular vessel and fibre

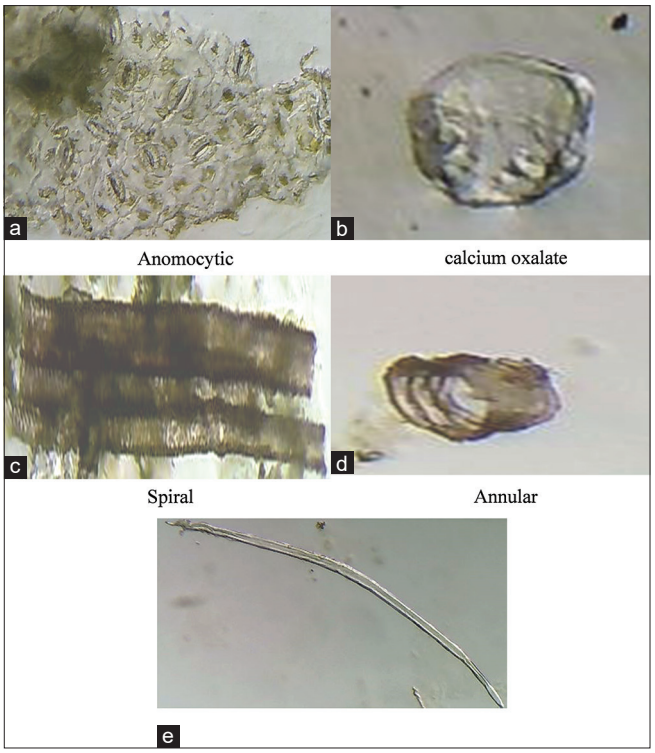

Figure 4: Powder microscopy of Anchusa strigosa Bank & Sol. Showed (a) normocytic stomata, (b) calcium oxalate crystal, (c) spiral vessels, (d) annular vessel, and (e) fiber

ignited in a muffle furnace at a temperature not exceeding 450°C to a constant weight. The residue was allowed to cool in a suitable desiccator for 30 min and weighed without delay. The content of acid-soluble ash concerning air-dried drugs was calculated [Tables 1 and 2].

Water-soluble ash

Ash of both plants powdered samples 5 g were boiled separately with 25 ml of water for 5 min, insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate was neutral, then ignited in a muffle furnace at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of the ash.[21] The difference in the weight represents the water-soluble ash. The percentage of water-soluble ash was calculated regarding the air-dried drug [Tables 1 and 2].

Extractive value

Determination of water-soluble extractive value (cold method)

5 g of coarsely powdered drug of both samples were added with 100 ml distilled water in a closed flask shaken frequently during the first 6 h and allowed to stand for 24 h. After 24 h, it was filtered and 25 ml of filtrate was taken in an accurately weighed Petri dish. The Petri dish was made to dry in a water bath to constant weight and then the water-soluble extractive was calculated by subtracting the original weight of the Petri dish from the weight of the extractive containing the petri dish. The percentage of water-soluble extractives was calculated concerning air-dried drugs taken [see Table 5].[19]

Determination of alcohol soluble extractive value (cold method)

Five gram of air-dried powdered drug of both samples were added with 100 ml alcohol of specified strength and kept in a closed flask for 24 h and shaken frequently during the first 6 h and allowed to stand for 24 h. After 24 h, it was rapidly filtered taking precautions against loss of solvent. Twenty-five milliliters of filtrate was taken in an accurately weighed Petri dish and was made to dry in the water bath to constant weight. The alcohol-soluble extractive value was calculated by subtracting the original weight of the Petri dish from the weight of the Petri dish containing the extract. The percentage of alcohol-soluble extractives was calculated regarding air-dried drugs [see Table 5].[17,19]

Determination of water-soluble extractive value (hot method)

Five gram of air-dried powdered drugs from both samples were taken with 100 ml of distilled water kept in a flask and placed on the hot plate after shaking it well. After boiling, the flask was rapidly filtered taking precautions against loss of solvent. Twenty-five milliliter of filtrate was taken in an accurately weighed Petri dish and was made to dry in the water bath to constant weight. The water-soluble extract value was calculated by subtracting the weight of the Petri dish from the weight of the Petri dish with the extract.

Table 5: Various extractive values of *Borago officinalis* L. and *Anchusa strigose* Banks and Sol.

Name of drug	Solvent	Values (%w/w)
Borago officinalis L.	Petroleum ether	3.87
	Ethanol	20.72
	Hydroalcoholic	17.39
	Aqueous	7
Anchusa strigosa Banks and Sol.	Petroleum ether	2.42
	Ethanol	6.18
	Hydroalcoholic	24.48
	Aqueous	17

According to the air-dried drug, the percentage of the extract that is water soluble was calculated [see Table 5].[17,19]

Determination of alcohol soluble extractive value (hot method)

Five gram of air-dried powdered drugs of each sample was taken with 100 ml of alcohol of specified strength kept in a flask and placed on the hot plate after shaking it well. After boiling, the flask was rapidly filtered taking precautions against loss of solvent. 25 ml of filtrate was taken in an accurately weighed Petri dish and was made to dry on the water bath to constant weight. Alcohol Soluble extract value was calculated by subtracting the weight of the petri dish from the weight of the Petri dish with the extract. The percentage of alcohol-soluble extract was calculated with reference to air-dried drugs.[17,22]

Loss on drying

The powdered samples of both drugs, 5 g each without preliminary drying were placed on a tarred evaporating dish and dried at 105°C for 3 h and weighed. The drying was continued until two successive readings matched each other or the difference between two successive weightings was not >0.25%.[18,21] Constant weight was reached when two consecutive weighing after drying for 30 min in a desiccator, showed not >0.01 g difference.

Determination of pH

pH of 1% solution

One gram of both drug samples were taken in an accurately measured 100 ml of distilled water in a beaker.[23] The extract was filtered and checked pH of the filtrate was with a standardized glass electrode [Tables 1 and 2].

pH of 10% solution: 10 g of both the drug samples were taken in accurately measured 100 ml of distilled water in a beaker. The extract was filtered and checked for pH of the filtrate with a standardized glass electrode [Tables 1 and 2].

Swelling index

1 g of both powdered plant materials previously were taken in a 25 ml of glass stoppered measuring cylinder. 25 ml of water was added and the mixer was shaken thoroughly on every 10 min for 1 h. It was allowed to stand for 24 h at room temperature and the reading was recorded.[18] The mean value of the individual determination was calculated related to 1 g of plant material [Table 1 and 2].

Foaming index

In this study, 1 g of both plant materials was reduced to a coarse powder, weighed accurately and transferred to a conical flask containing 100 ml of boiling water and boiled for 30 min at a moderate temperature, then filtered into a 100 ml volumetric flask after cooling. The decoction was poured into a 10 ml test tube and the volume of liquid in each test tube was up to 10 ml with water. Stoppered the tubes and shaken in a lengthwise motion for 15 s and two shakes per second. Fifteen minute of stand time were given, and the height of the foam was measured by the following method:

If the height of foam in every tube is <10 mm, the foaming index will be <100. If the height of foam in any test tube is 1 cm, the volume of plant material decoction in the test tube was used to determine the index. If this tube will be the first or second tube in a series, prepare an intermediate dilution similarly to obtain a more precise result. If the height of the foam is >1 cm in every tube, the foaming index will be over 1000. In this case, repeat the determination using a new series of decoctions to obtain a result.[19]

The foaming index was calculated using the following formula: $1000/\alpha$ Where a = the volume in ml of decoction used for preparing the dilution in the tube where foaming to a height of 1 cm was observed [Tables 1 and 2].

Fluorescence analysis

The fluorescence character of both sample leaves powders sieved in (40 mesh) was studied both in daylight and ultraviolet (UV) light (255 nm and 366 nm) after treatment with different reagents such as sodium hydroxide, picric acid, acetic acid, HCl, nitric acid, iodine, and ferric chloride.[24] [Table 6a and 6b].

Successive extraction of crude drug material

The dried leaves (powder form) of both the plant samples were collected, cleaned, and dried under shade at room temperature. The dried leaves were pulverized using a stainless-steel mixer grinder. After pulverization, the powder was stored in dried and air-tight glass containers for the phytochemical investigation. The 265 g and 387 g dried, coarsely powdered material of *A. strigosa* Banks and Sol. and *B. officinalis* L. respectively (leaves) were subjected to successive extraction in the Soxhlet apparatus. Soxhlation was performed at 60°C using different solvents such as petroleum ether, ethanol, and hydroalcoholic (40/60) ratio. In each solvent, soxhlation was continued until no color was observed in the siphon tube and evaporated for residue. The absence of residual confirmed the completion of extraction. The extract was evaporated until they completely dry and extractive values were calculated.[25]

Table 6a: Fluorescence analysis of *Borago officinalis* L. leaves

Treatment	Day light	UV (254 nm)	UV (366 nm)
Powder as such	Brown	Dark green	Coffee brown
Powder treated with distilled water	Golden	Olive green	Green
Powder treated with GAA	Golden	Dark green	Brown
Powder treated with concentrated HCl	Brownish green	Green	Black
Powder treated with concentrated HCl + H_2O	Brown	Green	Black
Powder treated with petroleum ether	Green	Lemon green	Reddish yellow
Powder treated with methanol	Light green	Dark green	Orange
Powder treated with ethyl acetate	Light green	Green	Red
Powder treated with concentrated H_2SO_4	Dark green	Dark green	Green
Powder treated with concentrated H_2SO_4 + H_2O	Light brown	Green	Black
Powder treated with picric acid	Golden	Purple green	Black
Powder treated with 5% $FeCl_3$	Green	Dark green	Black
Powder treated with chloroform	Watery	Green	Light red
Powder treated with HNO_3	Ivory	Light green	Black
Powder treated with HNO_3 + H_2O	Brown	Green	Black

GAA: Glacial acetic acid

Table 6b: Fluorescence analysis of *Anchusa strigosa* Banks and Sol. leaves (market sample)

Treatment	Day light	UV (254 nm)	UV (366 nm)
Powder as such	Yellowish green	Milky green	Milky brown
Powder treated with distilled water	Light yellow	Dark green	Milky green
Powder treated with GAA	Light yellow	Green	Dark brown
Powder treated with concentrated HCl	Yellowish green	Green	Dark green
Powder treated with concentrated HCl + H_2O	Light brown	Light green	Black
Powder treated with petroleum ether	Lemon	Lemon green	Light orange
Powder treated with methanol	Light green	Green	Dark brown
Powder treated with ethyl acetate	Light green	Light green	Dark orange
Powder treated with concentrated H_2SO_4	Green	Purple green	Parrot green
Powder treated with concentrated H_2SO_4 + H_2O	Light brown	Green	Black
Powder treated with picric acid	Yellow	Parrot green	Black
Powder treated with 5% $FeCl_3$	Green	Dark green	Black
Powder treated with chloroform	Golden	Green	Ivory
Powder treated with HNO_3	Ivory	Light green	Black
Powder treated with HNO_3 + H_2O	Light brown	light green	Black

GAA: Glacial acetic acid

Preliminary Phytochemical screening of the extract

Petroleum ether, ethanol, hydroalcoholic, and aqueous extracts of *B. officinalis* L. and *A. strigosa* Banks and Sol. were subjected to phytochemical screening. Phytochemical studies were carried out to identify various constituents present in the leaves [Table 7a and b].[26-29]

Tests for alkaloids

A few milligrams of the residual extract of each sample of different solvents were taken separately in 5 ml of 1.5% v/v HCl and filtered.

The following reagents were used to test the alkaloids in these filtrates:

Dragendroff's test

It was prepared by mixing two solutions. Solution A (17 g of bismuth subnitrate + 200 g tartaric acid + 800 ml distilled water) and Solution B (160 g potassium iodide + 4 ml distilled water) were mixed in 1:1 v/v proportion. From this solution, working standard was prepared by taking 50 ml of this solution adding 100 g of tartaric acid and making up to 500 ml with distilled water [see Table 7a and b].

The above Dragendroff's reagent was sprayed on Whatmann No.1 filter paper and the paper was dried. The test filtrates after basification with ammonia were extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendroff's reagent, with the help of a capillary tube. The development of an orange-red color on the paper was observed for the presence of alkaloids.

Mayer's reagent

Each filtrate was treated with a few drops of Mayer's reagent (potassium mercuric iodide) which was added by

Table 7a: Phytochemical screening of pet-ether, ethanol, hydroalcoholic and aqueous extracts of *Borago officinalis* L. Leaves

Tests	Inference extract	Pet ether extract	Ethanol extract	Hydroalcoholic extract	Aqueous extract
Carbohydrates					
Molish's test	Violet ring	−	+	+	+
Fehling's test	Brick red ppt	−	+	+	−
Benedict's test	Brick red ppt	−	−	−	−
Tannins					
5%$FeCl_3$	Yellow colour	−	+	−	+
Lead acetate	White ppt	−	+	+	−
Flavonoids					
Alkaline reagent test	Pink colour	−	+	+	−
Anthraquinone glycosides					
Borntrager's test	Pink colour	−	−	−	+
Cardiac glycosides					
Keller Killiani test	Brown ring at junction	−	−	+	−
Borntragers test	Pink colour	−	+	+	−
Terpenoids					
Salkowski's test	Golden yellow ring at junction	−	−	−	−
Phytosterols					
Liberman's test	Brown ring at junction	−	+	+	−
Alkaloids					
Dragendroff's reagent	Orange ppt	−	−	−	−
Mayer's reagent	Cream ppt	−	−	−	−
Wagers test		−	−	−	−
Hagers test		−	−	−	−
Proteins					
Millons test	Purple colour	+	−	−	−
Biuret test	Blue colour	−	−	−	−

−:Negative, +: Positive

dropping it from the side of the tube and was observed for creamy white precipitation [see Table 7a and b].

Wagner's test (iodine-potassium iodide)

1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml water. A few drops of this reagent were added to the test filtrate, and precipitation of a brown flocculent was observed for the presence of alkaloids [see Table 7a and b].

Hager's test

Filtrates were treated with Hager's reagent (Saturated picric acid solution) and the formation of a yellow precipitate indicates the presence of alkaloids [see Table 7].

Test for glycosides

Each extract of the drug was tested for the presence of glycosides. A few grams of all extracts were dissolved in their respective solvent to make a stock solution.

Anthraquinone glycoside

Borntrager's test

Dilute H2SO4 was added to 3 ml extract and boiled and filtered. To the cold filtrate, equal volume of chloroform was added. Shaken well and the organic solvent was separated, then ammonia was added. The ammoniacal layer turns pink or red, which indicates the presence of anthraquinone glycosides [see Table 7a and b].

Cardiac glycosides

Keller killani Test

Glacial acetic acid, one drop of 5% FeCl3, and conc. H2SO4 were added to 2 ml of extract. The presence of cardiac glycosides was indicated by the formation of reddish-brown color at the junction of two liquid layers and the upper layer appeared bluish-green [see Table 7a and b].

Tests for tannins

The test residue of each extract was taken separately in water, warmed, and filtered. Tests were carried out with the filtrate using the following reagents [see Table 7a and b].

Ferric chloride reagent

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. A few drops of this solution were added to the water of the above filtrate. A dark green or deep blue color was observed for the presence of tannins [see Table 7a and b].

Table 7b: Phytochemical screening of pet-ether, ethanolic, hydroalcoholic and aqueous extracts of *Anchusa strigosa* Banks and Sol. leaves (market sample)

Tests	Inference extract	Petroleum-ether extract	Ethanol extract	Hydroalcoholic extract	Aqueous extract
Carbohydrates					
Molish's test	Violet ring	−	+	+	+
Fehling's test	Brick red ppt	−	+	+	−
Benedict's test	Brick red ppt	−	−	−	−
Tannins					
5% $FeCl_3$	Yellow colour	−	+	−	+
Lead acetate	White ppt	−	−	−	−
Flavonoids					
Alkaline reagent test	Pink colour	−	+	+	−
Phenolics					
Anthraquinone glycosides					
Borntrager's test	Pink Colour	−	−	+	−
Cardiac glycosides					
Keller killiani test	Brown ring at junction	−	+	+	−
Terpenoids					
Salkowski's test	The golden yellow ring at junction	+	+	+	−
Phytosterols					
Libermann's test	Brown ring at junction	−	+	+	−
Alkaloids					
Dragendroff's reagent	Orange ppt	−	−	−	−
Mayer's reagent	Cream ppt	−	−	−	−
Wagers test		−	−	−	−
Hagers test		−	−	−	−
Proteins					
Millons test	Purple colour	−	+	−	−
Biuret test	Blue colour	−	−	−	−

-:Negative, +:Positive

Lead acetate test

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. The precipitate was observed for the presence of Tannins 5. Tests for carbohydrates: 5.1 Molisch's test: The Molisch's reagent was prepared by dissolving 10 g of α-naphthol in 100 ml of 95% alcohol. A few mg of the test residue was placed in a test tube containing 0.5 ml of water, and it was mixed with 2 drops of Molisch's Reagent. 1 ml of conc. sulfuric acid from the side of the inclined test tube was added to this solution so that the acid formed a layer beneath the aqueous solution without mixing with it. The appearance of a red-brown ring at the common surface indicates the presence of sugars [see Table 7a and b].

Benedict's test

The 1 ml of test solution was treated with 2 ml of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on a water bath for 2 min and allowed to stand, the reddish-brown precipitate formed indicating that reducing sugars were present [see Table 7].

Fehling's solution test

1 ml each of Fehling's A and Fehling's B solutions were mixed immediately and boiled for 1 min and an equal volume of test solution was added to the test tube and boiled for 5 min. The formation of yellow precipitate, which turns brick red indicates the presence of reducing sugars [see Table 7a and b].

Tests for flavonoids

Alkaline reagent test

The 2 ml of extract was treated with 10% NaOH solution. The presence of flavonoids is indicated by the appearance of a bright yellow color [see Table 7a and b].

Tests for proteins

Millon's test

The 1 ml of the extract was taken in a test tube, then 1 ml Millon's reagent was added and heated for 3 min, and further 1% sodium nitrate was added. Red or reddish-brown color indicates the presence of tyrosine [see Table 7a and b].

Biuret test

A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it. A drop of 1% solution of copper sulfate followed this. A violet or pink color is formed if proteins are present [see Table 7a and b].

Tests for terpenoid

Salkowaski Test

A few milligrams of the residue of each extract were taken in 2 ml of chloroform and 2 ml of conc. sulfuric acid was added from the side of the test tube. The test tube was shaken for a few minutes. The development of red color in the chloroform layer indicated the presence of sterols [see Table 7a and b].

Test for phytosterols

Libermann's buchard test

The crude extract was mixed with a few drops of acetic anhydride, boiled, and cooled. Concentrated sulfuric acid was then added from the sides of the test tube and the formation of a brown ring at the junction of two layers. The green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids, respectively [see Table 7a and b].

Thin layer chromatography profile

Thin layer chromatography (TLC) is used for qualitative screening of petroleum ether and ethanol extracts of AS and BO for separation and determining the number of compounds present in the solvent extracts. TLC was carried out on precoated aluminium plates, silica gel 60 F 254 (layer thickness 0.25 mm). The extracts of AS and BO were applied to the plates (1 cm above the bottom). It was then kept in the previously saturated developing chamber containing the mobile phase and allowed to run up to 80 mm of the height of the plate. The developed plate was then removed; air dried and observed using the spraying reagent (anisaldehyde) and the Rf value for each spot or compound using the following formula. Rf value = Distance traveled by spot/Distance traveled by the solvent front.[26]

Thin layer chromatography profile

The various extracts of the drug were subjected to TLC to obtain a fingerprint and to identify the number of components resolved in different solvent systems and also to test the purity of the drug [see Table 8]. The following table depicts the Rf values of different components of the drug obtained with the solvent system.

Anti-anxiety study

Experimental animal

A total of 24 healthy albino Wistar rats of either sex (150–200 g and 8–12 weeks old) were taken in this study. The animals were kept under standard laboratory conditions as per the CPCSEA guidelines. They were housed in the polypropylene cages at 220C ± 30C, relative humidity 50% ± 20%, and on 12 h light/dark cycles. They had free access to food and water. Before, the commencement of the experiment the animals were acclimatized for 5 days.

Dose of the test drugs

The dose of *Barg-i-Gāozabān* mentioned in classical Unani literature is up to 7 g for adults.[23,30] As described by Freireich *et al*. the dose for albino rats was calculated by a conversion factor of "7."[1,31,32] The dose of extract was determined concerning the dose of crude drug and found to be 817 mg/kg. The final dose of both test samples was calculated according to the yield obtained. All the drugs were prepared immediately before use and administered orally.

Standard drug

The test drug DZ was obtained from Ranbaxy Laboratories Limited from an authentic drug dealer in Srinagar.

Experimental design

The study was carried out on albino Wistar rats of either sex weighing 150–200 g. The animals were kept at standard environmental conditions with free access to water and food. Albino Wistar rats were taken and divided into four groups, each group consisting of 6 animals. Group I Plain Control group received 0.5% chronic mucocutaneous candidiasis (CMC), Group II standard control was given DZ, and Group III test group was given aqueous extract of *A. strigosa* Banks and Sol.(A-AEAS) and Group IV test received an aqueous extract of *B. officinalis* L. leaves.

- Group I (plain control): This group received 0.5% CMC orally for 7 days
- Group II (standard control): This group received the standard drug DZ (1.0 mg/kg/b. w) intraperitoneally 30 min before the experiment and activities were recorded for 7 days[33]
- Group III (Test A-AEAS): This group received an aqueous extract of *A. strigosa* Banks and Sol. leaves at 817 mg/kg/b. w in 1 ml of distilled water with 0.5% CMC orally for 7 days
- Group IV (Test B-AEBO): This group received an aqueous extract of *B. officinalis* L. leaves, 817 mg/kg/b. w in 1 ml distilled water with 0.5% CMC orally for 7 days. In Group I, III and IV, the effects were estimated after 60 min and in Group II after 30 min of the drug administration in both models i. e. Elevated plus-maze (EPM) and Light and dark arena.

Two models were used for evaluating the anti-anxiety activity:

- Elevated plus-maze[34]
- Light and dark arena.[35]

Elevated plus-maze

EPM is currently one of the most widely used models for evaluating anti-anxiety. The EPM apparatus consisted of two

Table 8: Thin layer chromatography profile of borage *officinalis* L and *Anchusa strigosa* Banks and Sol. leaves extracts

Sample	Solvent system	Spraying reagent	Number of spots	Rf values
Borage officinalis L. (leaves)				
Petroleum ether (cold) extract	Petroleum ether: Ethyl acetate	Anisaldehyde, methanol, glacial acetic acid, sulphuric acid	3	0.16, 0.33, 0.66
Ethanol (cold) extract	Ethyl acetate and methanol	Anisaldehyde, methanol, glacial acetic acid, sulphuric acid	5	0.35, 0.51, 0.66, 0.79, 0.89
Anchusa strigosa Banks and Sol. (leaves)				
Petroleum (cold) extract	Petroleum ether: Ethyl acetate	Anisaldehyde, methanol, glacial acetic acid, sulphuric acid	2	0.40, 0.59
Ethanol (cold) extract	Methanol and toluene	Anisaldehyde, methanol, glacial acetic acid, sulphuric acid	4	0.15, 0.23, 0.27, 0.44

Successive extractives of *Borago officinalis* L and *Anchusa strigosa* Banks and Sol.

open arms (35 cm × 5 cm) and two closed arms (30 cm × 5 cm × 15 cm) that extended from a common central platform (5 cm × 5 cm) to form a plus sign. A slightly raised edge on the open arms (0.25 cm) provided an additional grip for the animals. The maze floor and the closed arms were covered with black adhesive tape that was elevated to a height of 50 cm above floor level by a single central support. Rat was given vehicle, DZ and oral doses of the plant extract. All the drugs were given 60 min before their placement on the EPM except DZ Two models were used for evaluating the anti-anxiety activity: (1) Elevated plus-maze.[34] (2) Light and dark arena.[35] Elevated plus-maze (EPM) EPM is currently one of the most widely used models for evaluating anti-anxiety. The EPM apparatus consisted of two open arms (35 cm × 5 cm) and two closed arms (30 cm × 5 cm × 15 cm) that extended from a common central platform (5 cm × 5 cm) to form a plus sign. A slightly raised edge on the open arms (0.25 cm) provided an additional grip for the animals. The maze floor and the closed arms were covered with black adhesive tape that was elevated to a height of 50 cm above floor level by a single central support. Rat was given vehicle, DZ and oral doses of the plant extract. All the drugs were given 60 min before their placement on the EPM except DZ which was given 30 min before the experiment. The number of entries and the time spent in the open and closed arms were recorded during a 5-min test period. This test was done on the 1st, 3rd, and 7th of the 7-day experiment. The percentage of open arm entries (100xopen/total entries) was calculated for each animal [Figure 5, 6 and Table 9].

The percentage of time spent in the open arm was determined as follows:

$$\% = \frac{\text{Number of seconds spent in the open arm total}}{\text{300 total seconds}} \times 100$$

$$(\text{5 minutes observation periods})$$

Light and dark test

The apparatus consisted of a rectangular box (45 × 27 × 27), partitioned into two compartments connected by a 7.5 × 7.5 cm opening in the wall between compartments. One compartment was painted black and covered with a roof. The other compartment had no roof and was brightly illuminated by a 60 W bulb located above the box. Rats were given oral doses of vehicle, DZ, two test drugs, and after 60 min, except DZ which was observed after 30 min. Each animal was placed in the center of the light compartment and was observed for 5 min experimental apparatus.[33,35]

The time spent in the open (white/light) compartment was recorded. This test was done on the 1st, 3rd, and 7th of the 7-day experiment. The percentage of time spent in the light and dark compartments was recorded and summarized in Table 10.

Results

The results are shown in the following tables [see Table 3-10]:

Discussion

Anxiety is a disorder of emotion rather than a disturbance of thought. When a person has an anxiety disorder, it interferes with daily life, and normal functioning and causes pain for both the person with the disorder and those who care about him or her. It may range from a very mild anxiety condition to severe depression characterized by a persistent depressed mood, feelings of worthlessness, inappropriate guilt, and suicidal thoughts.[35] Various single and compound drugs in Unani medicine have been evaluated for antianxiety effects such as *Aftimūn (Cuscuta reflexa* Roxb, etc.[35] *Barg-i-Gāozabān* (*B. officinalis* L. or *A. strigosa* Banks and Sol.) has been used for a long time for the treatment of various ailments such as melancholia, schizophrenia, anxiety, palpitation, etc. The present study was carried out at the RRIUM, University of Kashmir, Srinagar. In this study, the Elevated Plus Maze Test and Light and dark test methods were followed to calculate the anxiety-related behavior statistically. As the pharmacognostic study is a primary and reliable criterion in the identification and determination of quality and purity of crude drugs in the present study, the plants, namely *B. officinalis* L. and *Anchusa strigose* Banks and Sol. were selected and authenticated by their morphological and organoleptic characters. The techniques

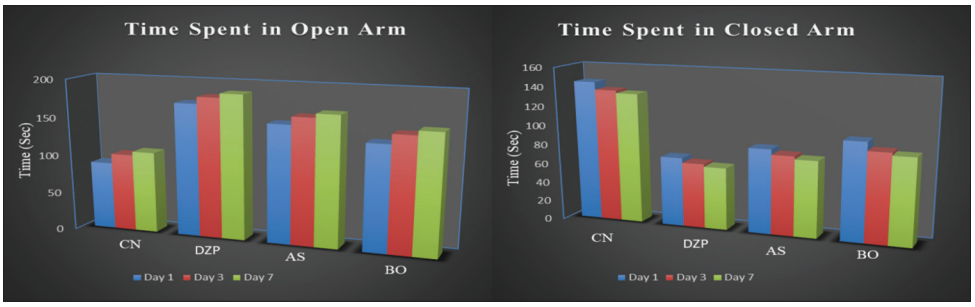

Figure 5: Graphical representation on the effect of aqueous extract of *Anchusa strigosa* Banks and Sol. *Borago officinalis* L. on time spent in open/closed arm in the EPM model

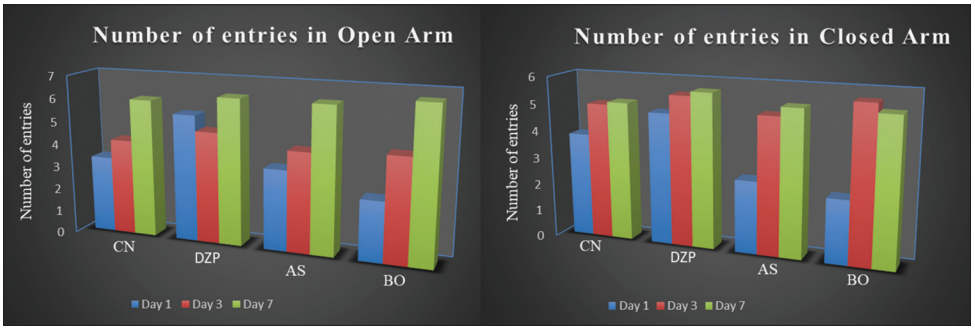

Figure 6: Graphical representation of the effect of aqueous extract of *Anchusa strigosa* Banks and Sol, *Borago officinalis* L. on open/closed arm entries in the EPM model

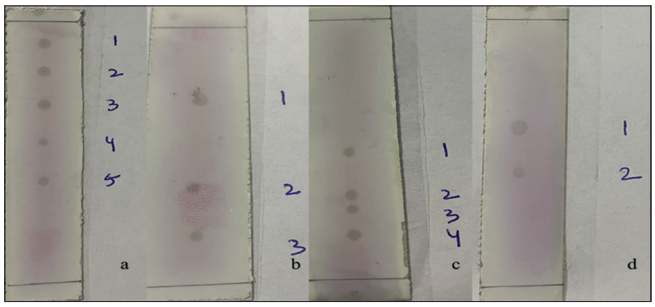

Figure 7: Thin layer chromatography plate showing various spots of *Borago Officinalis* L. (BO) and *Anchusa strigosa* Banks and Sol. (AS), (a) Ethanolic extract of BO, (b) Petroleum ether extract of BO, (c) Ethanolic extract of AS (d) Petroleum ether extract of AS

of physicochemical and phytochemical evaluation and TLC profile were applied for the standardization, which is mentioned in Tables 3-6b, 8 and Figure 7. The macroscopic characteristics of the *B. officinalis* L. were found to be varying from yellowish-green to greenish brown having a mucilaginous taste and soft, hairy, crumbled surface with dentate margin [Table 1]. Similarly, in *A. strigosa* Banks and Sol., the leaves show greenish brown, in color, having mucilaginous taste and rough, hard spiky projections and white spots like papillae on the surface [Table 2]. They are found odourless. But in comparison to *A. strigosa*, *B. officinalis* has no white spots on the surface of the leaves. The microscopic characters of the *B. officinalis* L. and *A. strigosa* Banks and Sol. leaves powder of species both show the presence of stomata, spiral vessels, annular vessels, fiber, and abundant covering trichomes.

For the evaluation of crude drugs, physicochemical constituent determination is an important parameter. The ash values were carried out to know the presence of any earthy material or organic matter. The ash values were carried out and found that *B. officinalis* has a total ash 9.4%, acid-insoluble ash 4.8% and water-soluble ash 6.8% and *A. strigosa* Banks and Sol. has total ash 9.8%, acid-insoluble ash 13.6% and water-soluble ash 7.2% as shown in [Tables 3 and 4]. Different extractive values give an idea about the amount of active ingredients present in a particular given amount of medicinal plant when extracted with different solvents. In cold extraction of *B. officinalis*, the cold extractive value was found to be 2% w/w in ethanol and 5.6% in aqueous extract and for *A. strigosa* Banks and Sol, the maximum extractive value of ethanol extract was observed 15.2% w/w, while in aqueous extract it was found 8.2% w/w. In hot extraction, the maximum extractive value of *B. officinalis* L. was found 3.8% w/w in ethanol and 10.2% w/w in aqueous extract. In *A. strigosa* Banks and Sol. it was observed at 2.4% w/w in ethanolic extract followed by 20.8% w/w in aqueous extract [Table 10]. The foreign matter in *B. officinalis* L. collected from the herbal garden RRIUM, Srinagar was found negligible, while in the market sample of *Barg-i-Gāozabān* (A. *strigosa*), the foreign matter was also negligible, and that was 0.016% [Table 1]. The percentage of active chemical constituents in a crude drug is mentioned on an air-dried basis. Therefore, loss on drying of the plant material should be determined and moisture content should be controlled. The loss on drying of dry powder of *B. officinalis* L. and

Table 9: Anxiety-related behaviour using the elevated plus maze model

Groups	Treatment days	Time spent (s)			Number of entries	
		Latency	Open arm	Closed arm	Open arm entries	Closed arm entries
Plain control	Day 1	66.66±3.5	88.66±6.05	144.66±8.98	3.33±0.23	3.83±0.13
	Day 3	61.05±3.05	101.50±3.06	137±3.05	4.16±0.70	5±0.80
	Day 7	59.16±3.05	106.33±3.06	134.5±3.05	6±0.40	5.1±0.41
Diazepam	Day 1	55.16±3.6 (NS)	173.16±9.18**	71.66±11.37**	5.5±0.42**	4.83±0.30 (NS)
	Day 3	50.83±3.80 (NS)	182.16±7.92**	67±10.28**	4.83±0.47 (NS)	5.5±0.22 (NS)
	Day 7	47.83±3.82 (NS)	187.66±7.89**	64.5±10.21**	6.33±0.49 (NS)	5.66±0.33 (NS)
Aqueous extract of *Anchusa strigosa*	Day 1	59.33±3.10 (NS)	153.5 ±4.89**	87.166±4.22**	3.5±0.34 (NS)	2.66±0.49 (NS)
	Day 3	54.5±3.23 (NS)	163.66±5.09**	81.83±4.06**	4.33±0.61 (NS)	5±0.44 (NS)
	Day 7	52.33±3.10 (NS)	168.83±4.96**	78.83±3.95**	6.33±0.61 (NS)	5.33±0.01 (NS)
Aqueous extract of *Borago officinalis*	Day 1	63.0±3.94 (NS)	137±1.80**	100.83±4.63**	2.6±0.33 (NS)	2.33±0.55*
	Day 3	58.5±3.76 (NS)	149.5±2.11**	92.0±3.42**	4.5±0.34 (NS)	5.66±0.66 (NS)
	Day 7	55.66±3.78 (NS)	155.16±1.81**	89.16±3.50**	6.66±0.71 (NS)	5.33±0.66 (NS)

*P<0.05, **P<0.01, P>0.05 will be considered as significant, highly significant, extremely significant, and insignificant respectively compared with plain control. Data was significantly analysed by ANOVA followed by Dunnet's test. Results are expressed as mean±SEM (n=6). SEM: Standard error of mean, NS: Not significant

Table 10: Anxiety-related behaviour using the light and dark method

Groups	Treatment days	Time spent (s)		Number of entries	
		Light	Dark	Light	Dark
Control	Day 1	93.16±10.10	206.83±12.05	4.5±0.61	9.66±1.3
	Day 3	95.5±10.05	204.5±12.05	4.33±0.5	8.33±1.5
	Day 7	96.5±12.05	203.5±12.05	4.8±0.51	5.66±0.49
Diazepam	Day 1	187.5±14.14**	112.5±14.04**	6.66±0.51*	5.55±0.77*
	Day 3	189±13.25*	111±13.25**	7.5±0.81*	6.33±1.10*
	Day 7	190±13.25**	110±13.25**	7.88±1.40*	7.0±0.5 (NS)
Aqueous extract of *Anchusa strigosa*	Day 1	176.33±2.37**	123.66±2.37**	6.0±0.56*	3.66±0.75
	Day 3	178±1.24*	122±1.23**	7.5±0.66 (NS)	4.35±0.57
	Day 7	179±1.23**	120±1.23**	7.9±0.57*	6.16±0.75
Aqueous extract of *Borago officinalis*	Day 1	155.66±5.01*	144.33±5.18**	3.17±0.49*	5.18±0.7*
	Day 3	157.5±4.32**	142.5±4.32**	4.54±0.76*	7.05±0.57*
	Day 7	158.16±4.17**	141.83±4.17**	4.33±0.57 (NS)	7.33±1.05 (NS)

*P<0.05, **P<0.01, will be considered as significant, highly significant, extremely significant and insignificant respectively compared with plain control. Data was significantly analysed by ANOVA followed by Dunnet's test. Results are expressed as mean±SEM (n=6). SEM: Standard error of mean, NS: Not significant

A. strigosa Banks and Sol was found to be 9.4% and 8.8%, respectively, which is within permissible limits. The idea about the presence of acidic or basic type of constituents present in a plant material, i.e., acidity or basicity of the constituent is evaluated by pH value determination at different concentrations. The pH value of 1% solution and 10% solution in distilled water of *B. officinalis* L. and *A. strigosa* Banks and Sol. was found to be 6.5 and 8.2 and 6.8 and 7.7, respectively, thus the result indicates the presence of basic constituents in both drugs. The swelling index was found to be zero. In fluorescence analysis, the powdered drug material was treated with different acids, bases, and other reagents. All these treatments carried out, indicate the presence of some particular type of constituents in the plants by giving color change of plant material with different reagents and then viewed under visible and UV light [Figures 8 and 9]. The preliminary phytochemical tests on various extracts (petroleum ether, ethanol, hydroalcoholic, aqueous) of *B. officinalis* L. and *A. strigosa* Banks and Sol. were carried out to identify the constituents to support the literature review and the results have been tabulated in [Tables 4 and 9]. No protein was found in any extract of both samples. Further, an attempt has been made to separate the individual chemical constituents of petroleum ether and ethanolic extracts by TLC. First, they were subjected to TLC. Several solvent systems of low to high polarity were tried. The petroleum ether cold extracts of *B. officinalis* and A. *strigosa* showed 3 spots and 2 spots, respectively, while ethanol cold extract showed 05 spots and 04 spots respectively [Table 8].

Furthermore, to evaluate the anti-anxiety effect of two samples of *Barg-i-Gāozabān* (*B. officinalis* L. and *A. strigosa* Banks and Sol.) in albino Wistar rats. The recommended therapeutic dose of *Barg-i-Gāozabān* was mentioned as 7 g, which was found too bulky to be

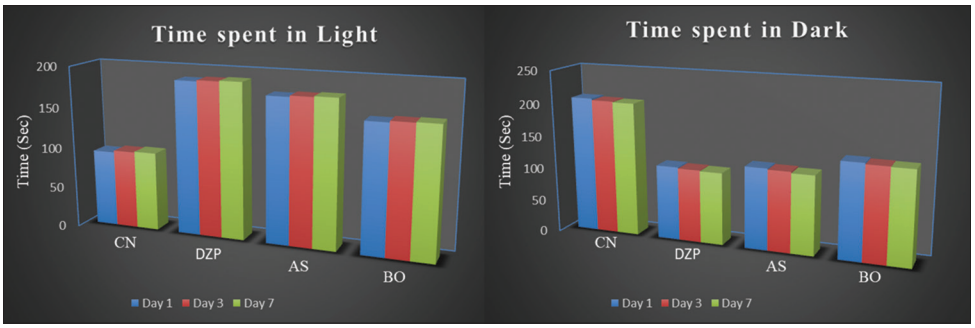

Figure 8: Graphical representation on the effect of aqueous extract of *Anchusa strigose* Banks and Sol. and *Borago Officinalis* L. on time spent in light and dark arena model

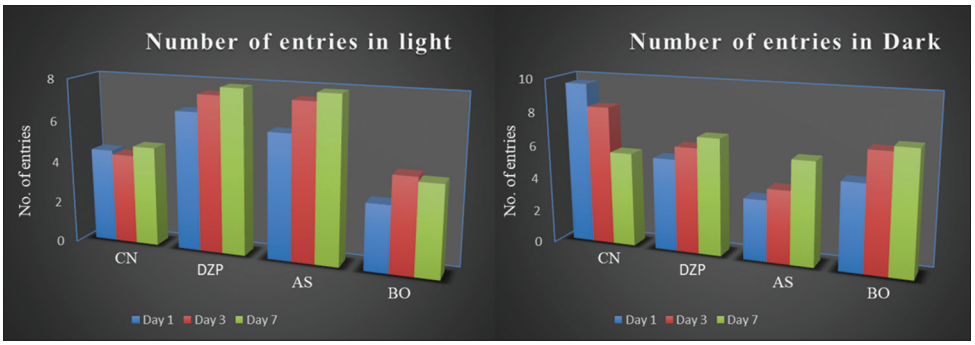

Figure 9: Graphical representation on the effect of aqueous extract of *Anchusa strigosa* Banks and Sol. and *Borago Officinalis* L. time spent in light and dark arena model on the number of entries in light and dark box. CN: Plain Control, DZP: Diazepam, AS: *Anchusa strigosa* Banks and Sol., BO: *Borago Officinalis* L.

administered to the experimental animals; therefore, both samples were extracted in distilled water. Aqueous extract was selected for the study because most drugs in Unani Medicine are used with water. The yield aqueous extract of *B. officinalis* and *A. strigosa* Banks and Sol. were found 7% and 17%, respectively. The dosage for rats was calculated by multiplying the therapeutic dose of the test drug as described in Unani literature, by conversion factor 7, and the dose was found 817 mg/kg/bw for both samples.

In the EPM model, the aqueous extract of *A. strigosa* Banks and Sol. showed the most significant time spent in the open arm of 153.5 ± 4.89, 163.66 ± 5.09, and 168.83 ± 4.96 s, as compared with plain control (distilled water + 0.5% CMC) which showed 88.66 ± 6.05, 101.50 ± 3.06, and 106.33 ± 3.06 s on the 1st, 3rd, and 7th day, respectively. The aqueous extract of *B. officinalis* showed 137 ± 1.80, 149.5 ± 2.11, and 155.16 ± 1.81 s on the 1st, 3rd, and 7th day in open arm, which is again much better than the time spent by Plain Control. The same study was done by using hydroalcoholic extract of *B. officinalis* flowers. It was found that the extract increased both the percentage of time spent in the open arms of the maze and the percentage of entries into the open arms of the maze. In other words, the extract was able to produce an anxiolytic effect in rats.[36] Unfortunately, there was no study related to anxiety or depression was found on *A. strigosa* Banks and Sol., but based on Unani literature, the drug is mentioned for use in anxiety disorder, the study was conducted first time. The results indicate that the aqueous extract of both drugs, *B. officinalis* L. and *A. strigosa* Banks and Sol, showed significant anti-anxiety activity as compared to the Plain Control group but lesser than the standard drug DZ, which showed 173.16 ± 9.18, 182.16 ± 7.92, and 187.66 ± 7.89 s on the 1st, 3rd and 7th day, respectively. All the values were found to be highly significant with $P < 0.01$ Moreover, *A. strigosa* Banks and Sol. is found to be a better performer than *B. officinalis* for anti-anxiety-related activities [Table 9].

In the light and dark method, the results showed that the aqueous extract of *B. officinalis* showed 155.66 ± 5.01, 157.5 ± 4.32, and 158.16 ± 4.17 s on the 1st, 3rd and 7th day in open arm, which is again better than the time spent by the plain control group. The aqueous extract of *A. strigosa* Banks and Sol. is most significant time spent in the lighted area for 176.33 ± 2.37, 178 ± 1.24, and 179 ± 1.23sec on 1st, 3rd, and 7th day as compared with Plain Control (distilled water + 0.5% CMC) which showed 93.16 ± 10.10, 95.5 ± 10.05, and 96.5 ± 12.05 s on 1st, 3rd and 7th day in the lighted area. The results indicate that the aqueous extract of both drugs, *B. officinalis* L. and *A. strigosa* Banks and Sol. again showed statistically significant anti-anxiety activity as compared to the Plain Control group but lesser than the standard drug DZ, which showed 187.5 ± 14.14, 189 ± 13.25, and 190 ± 13.25 s on the 1st, 3rd, and 7th day, respectively. All the values were found to be

highly significant with $P < 0.01$. Moreover, *A. strigosa* Banks and Sol. is again found to be a better performer than *B. officinalis* for anti-anxiety-related activities [Table 10].

Conclusion

In the present study, two species *B. officinalis* and *A. strigosa* known by the name of *Gāozabān* have been evaluated for antianxiety activity against EPM and light and dark test. In the EPM model, Aq. ext. of AS and BO both showed highly significant time spent in the open arm, as compared with the Plain Control group, but less than the standard drug DZ. Moreover, *A. strigosa* is found to be a better performer than *B. officinalis* for anti-anxiety-related activities. The same effect was found in the light and dark methods. These results may be due to the presence of phytochemicals like flavonoids, alkaloids, tri-terpenoids, and tannins in both drugs. Based on the above findings, it is concluded that Aq. ext. of test drugs BO and AS showed significant antianxiety effects and validated the Unani claim that the drugs are useful in the management of depressive disorders.

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Conflicts of interest

There are no conflicts of interest.

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Original Article

Drug Standardization through Pharmacognostic Approaches and *In vitro* Evaluation of Antimicrobial Potential of *Ṣa'tar* (*Zataria multiflora* Boiss.) aerial parts

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Abstract

Objective: The aim of the present work was to determine the chemical composition of various extracts and the evaluation of the antimicrobial activities of the various extracts of *Zataria multiflora* Boiss. aerial parts. **Materials and Methods:** Physicochemical analysis (total ash, acid-insoluble ash, water-soluble ash, and extractive values); phytochemical analysis (alkaloids, glycosides, tannins, and phenols); and thin layer chromatography profile were done according to standard procedures, and antimicrobial activities have been evaluated with reference to the agar well diffusion (AWD), and minimum inhibitory concentration (MIC) assays. **Results:** *Zataria multiflora* Boiss., extracts demonstrated remarkable antimicrobial activities against AWD method and MIC assay. **Conclusion:** It is worthwhile to mention that aerial parts of *Zataria multiflora* Boiss. have a varied chemical composition and significant antimicrobial potential.

Keywords: *Antimicrobial, Ṣa'tar, Unani medicine, Zataria multiflora Boiss.*

Introduction

Medicines derived from plants are the ancient form of the healthcare system and have played a tremendous role in the traditional systems of medicine throughout history.[1] Even many developed nations are making use of medicines derived from plants as per the survey conducted by the WHO. However, the use of traditional medicines has some obstacles in these countries because of the unavailability of experimental evidence and also due to the absence of quality control measures. Therefore, it is the need of the time to have some documentation of all the research works that are being carried out on traditional medicines. Hence, it is important to undergo standardization of plants and plant materials before using them as a medicine. Despite these hindrances, many people in the world have started using alternative or complementary therapies including plant-based medicines.[2,3] According to a survey, about 80% of the world's population is dependent on plant-based products for their primary health needs.[4] Several factors support the use of medicinal drugs by a large fraction of the population in the world such as their easy availability, accessibility by all practitioners all the time, less or negligible side effects, and people's faith from ancient times, especially in rural areas.[5] In recent days, the traditional system of medicines is gaining more popularity due to its cost-effectiveness, better acceptability, better compatibility with the human body, high efficiency, and also because it does not cause any adverse effects as caused by powerful, synthetic allopathic drugs.[6] Synthetic drugs are being used on a large scale throughout the world and have a higher probability of adverse effects on the human body. This has motivated people to go back to the traditional system of medicines for safer remedies. Keeping this in mind, the researchers are working hard on the standardization of crude drugs before using them as medicine through different standardization techniques and methodologies to achieve the goal. The quality control standards are being achieved in a stepwise manner through pharmacognostic and phytochemical studies. These steps and processes are helpful in the identification and quality control of the plant material. The quality control standards of medicinal plants in the traditional system of medicine are becoming

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more important nowadays due to the commercialization of herbal drugs unlike in the past when the traditional doctors would themselves dispense the medicines. Characterization and quality control measurement of starting material is an important step to ensure quality of herbal drug which further helps us to ensure its safety and efficiency.[7-9]

USM is an ancient form of medicinal system, based on the theories of Hippocrates and Galen. It is being practiced in different parts of the world, especially in the Arab and Indian subcontinents which correlate healing and disease and try to cure health-related issues using natural substances.[10] This system is based on natural resources directly available from three sources, namely, plants, minerals, and animals. Out of these three resources, plant-based medication is the main ingredient which is further blended with other ingredients in a compound formulation.[11] All the physicians from the Greek to Arab and Indian subcontinent have followed the concept of disease and their management on the basis of Hippocrates and Galen's theory. *Ibn Sina* (980-1037 AD), *Zakaria Rāzī* (865-925 AD), *Ibn Zohr* (1092-1162AD), *Ajmal Khān* (1864-1927AD), etc. have built a pave and provided materials for research through their world fame treatises such as *Al-Qanūn fi'l Tib*, *Al-Hāwī fi'l Tib*, *Kitāb Al-Taisīr*, and *Ḥāziq*. They covered the management of maximum systemic and local diseases either communicable or noncommunicable, based on their experiences.[12-14] In recent times, the rapid growth of microorganisms has been a matter of concern among scientists and researchers throughout the world. Therefore, it the need for time to develop safer, more potent, efficacious antimicrobial compounds that have novel modes of action for the future that are not toxic to the host. In USM, several herbs are being used for treating various infectious diseases such as fever, pneumonia, boils, and ulcers. The drugs used for this purpose may be classified as *Dafi'-i-'Ufūnat* (anti-infective), *Dafi'-i-Ḥumma* (antipyretic), *Muḥallil* (anti-inflammatory), and *Muṣaffi-i-Dam* (blood purifier).[15] These classes of drugs may be the possible leads for developing herbal antibiotics. Many of them have been proven to have antimicrobial properties in experimental studies, i.e., *Hāshā* (*Thymus vulgaris*),[16] *Lehsun* (*Allium sativum* L),[17] *Lisanul Ibil* (*Salvia officinalis* L),[18] *Podina* (*Mentha arvensis*),[19] *Afsantin* (*Artemisia absinthium*)[20] etc.

Herbal drugs are known for their medicinal, aromatic, and various qualities. It can be viewed as biosynthetic chemical laboratories which produce several chemical compounds. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied topically to the skin. Herbal products often contain a variety of naturally occurring biochemicals from plants, many of which contribute to the plant's medicinal benefits. *Ṣa'tar* (*Zataria multiflora* Boiss.) is one of the most commonly used drugs of plant origin obtained from the family Labiatae. It is an aromatic herb found mainly in Iran, Pakistan, and Afghanistan. It is prescribed by all the Unani Physicians in the management of various diseases such as odontalgia, loosening of gums, food poisoning, ringworm, scabies, helminths, amenorrhea, anuria chest and lung diseases, liver disorders, and diseases related to the stomach. These benefits are attributed to its anti-inflammatory, analgesic, demulcent, expectorant, detergent, deobstruent, diuretic, and carminative properties.[21-24] *Ṣa'tar* (*Zataria multiflora* Boiss.) contains various compounds, particularly glycosides, flavonoids, steroids, phenolic compounds, and terpenes, such as thymol and carvacrol.[25-27] The plant also contains apigenin, luteolin, and 6-hydroxyluteolin glycosides, as well as di-, tri-, and tetra-methoxylated.[28,29] which are likely to be responsible for the therapeutic effects mentioned above.

Hence, in this study, we have adopted various pharmacognostic methods, namely, macroscopical, organoleptic, microscopical, and physicochemical characters and instrumental methods described in standard texts and papers on pharmacognosy. The data obtained from the study were analyzed using appropriate statistical tests wherever necessary. The results obtained from pharmacognostic experiments may be utilized as standard parameters for various species of the plant. The extracts of *Ṣa'tar* leaves were screened out for their antimicrobial activity. In this context, the following strains of bacteria, namely, Gram-positive bacteria, i.e., *Staphylococcus aureus* and *Streptococcus pyogenes* and Gram-negative bacteria, i.e., *Escherichia coli*, *Proteus vulgaris,* and *Pseudomonas aeruginosa* and fungi i.e., *Cryptococcus neoformans and Candida albicans*, for the evaluation of antimicrobial and antifungal were used.

Materials and Methods

Plant sample collection, identification, and authentication

The aerial parts of *Zataria multiflora* Boiss. were obtained from an authorized drug dealer in Srinagar, Jammu and Kashmir. The plant sample was authenticated by the Taxonomist, from the Center for Biodiversity and Taxonomy, University of Kashmir, and submitted to the herbarium under specimen voucher no. 3731-KASH.

Chemicals

The levofloxacin (Himedia)-SD216 and amphotericin B (Himedia)-SD233 were purchased from the supreme syndicate, Nursing Garh, Srinagar, and nystatin (Himedia)-SD271-1CT was obtained from the microbiology laboratory of pharmaceutical science, Kashmir University.

Parameters for drug standardization

Macroscopic evaluation

The crude drug was observed macroscopically and a detailed study of the visual appearance (shape size and color) and sensory profile (odor and taste) and other external features was done by the naked eye and sense organs.[30]

Microscopic evaluation

Microscopic examination of powder drugs helps in the distinction of anatomy in adulterants. The powdered aerial parts of *Z. multiflora* were taken and boiled in chloral hydrate solution for 5 min. A small quantity of boiled powder is taken on a slide and spread evenly with the help of a brush, then covered with a coverslip and observed under a microscope for various characteristics[30] such as size, shape, and relative positions of the different cells and tissues, chemical nature of the cell walls and of the cell contents.

Physicochemical evaluation

Various physicochemical parameters such as total ash, acid-insoluble ash, water-soluble ash, extractive values, loss on drying, fluorescence analysis, and pH determination were carried out for the test sample.[30,31]

Ash values

Ash values are used to determine the proportion of inorganic materials in the sample, such as carbonates, silicates, oxalates, and phosphates. The method was used to ascertain total ash, acid-insoluble ash, and water-soluble ash.[32]

Extractive values

The drug was extracted with different solvents in order of their increasing polarity to get the correct and dependable values. In general, alcohol and aqueous extractives were taken into consideration for fixing the standard of a drug.[31]

Determination of alcohol (ethanol) soluble extractive

Five grams of air-dried powder of *Z multiflora* was added to 100 ml of alcohol (ethanol) of specified strength in a closed flask for 24 h. The drug was shaken and macerated frequently for 6 h and allowed to strength for 18 h, then, the content was filtered and 25 ml of filtrate was evaporated to dryness in a tarred evaporating dish at 105°C to constant weight. The percentage of alcohol (ethanol) soluble extractive was calculated with reference to air air-dried drug.

Loss on drying

The powdered drug samples (5g) without preliminary drying were placed on a tarred evaporating dish and dried at 105°C for 6 h and weighed. The drying was continued until two successive readings matched each other or the difference between two successive weightings was not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 30 min in a desiccator, showed not more than 0.01g difference.[33]

Fluorescence analysis

Many herbs show fluorescence, when cut surface or powder, is exposed to UV light; this can help in their identification. The fluorescence character of the aerial parts of the plant (*Z. multiflora*) powders (40 mesh) was studied both in daylight and UV light (255and 366 nm) after treatment with different reagents such as sodium hydroxide, picric acid, acetic acid, HCl, nitric acid, iodine, and ferric chloride.[34]

Determination of pH

- 1% pH solution: 1g of the drug (*Z. multiflora*) was taken in an accurately measured 100 ml of distilled water. The extract was filtered and checked pH of the filtrate was with a standardized glass electrode
- pH 10% solution: 10 g of the drug (*Z. multiflora*) was taken in accurately measured 100 ml of distilled water. The extract was filtered and checked pH of the filtrate was with a standardized glass electrode.[35]

Extraction of crude drug material

Hot extraction

The dried and coarsely powdered material of the aerial part of *Zataria multiflora* Boiss (400 g) was subjected to successive extraction in a Soxhlet apparatus with different solvents such as ethanol, hydroalcoholic, and aqueous. The extracts were concentrated under reduced pressure using a rotary vacuum evaporator. The extracts were dried and stored in a sealed glass vial in a refrigerator at 4°C before further analysis. The weight of dried hot extracts was obtained as: ethanolic extract 14 g, hydroalcoholic extract 17 g, and aqueous extract 20 g.[36]

Preliminary phytochemical screening of the extracts[37]

The ethanolic, hydroalcoholic, and aqueous extracts of *Zataria multiflora* Boiss were subjected to phytochemical screening. Phytochemical studies were accomplished for identifying different constituents contained in the aerial part of *Zataria multiflora* Boiss.

Test for alkaloids

Each extract of the *Zataria multiflora* Boiss was assessed for the occurrence of alkaloids using Mayer's test, Wager's test, Dragendroff's test, and Hager's Test. A few grams of each extract were mixed in their respective solvents to prepare a stock solution.

Tests for glycosides

Each extract of the drug was tested for the availability of glycosides using Borntrager's test, Keller-Killiani test, and Legal's test. A few grams of each extract were properly mixed in their respective solvents to prepare a stock solution.

Test for tannins

Each extract of the drug was tested for the presence of tannin using the ferric chloride test and lead acetate test. A few grams of each extract were properly mixed in their respective solvents to prepare a stock solution.

Test for carbohydrates

Each extract of the drug was tested for the availability of carbohydrates using Molisch's Test (for the presence of general sugars), Benedict's test (for reducing sugars), and Fehling's test (for reducing sugars).

Test for flavonoids

Each drug extract was tested for the availability of flavonoids using the Shinoda test.

Test for terpenoids

Each drug extract was tested for the availability of terpenoids using Salkowski's test.

Test for phytosterols

Each drug extract was tested for the availability of phytosterols using Libermann's test.

Thin layer chromatography

Thin layer chromatography (TLC) was carried out on precoated aluminum plates and silica gel 60 F 254 (layer thickness 0.25 mm). The ethanol extract of ZM was applied to the plates (1cm above the bottom). It was then kept in the previously saturated developing chamber containing the mobile phase and allowed to run up to 80 mm of the height of the plate. The developed plate was then removed, air-dried, and observed using the spraying reagent anisaldehyde and calculating the R_f value for each spot or compound using the following formula.

R_f value = Distance traveled by spot/Distance traveled by solvent front.[38]

Antimicrobial study

Evaluation of antimicrobial activity

For the evaluation of the antimicrobial activity of aqueous extracts of *Zataria multiflora* Boiss., the first and foremost step was to sterilize all the glassware in an autoclave. After sterilization, the subsequent steps were the preparation of media, selection of test microorganisms, and performing the sensitivity tests for antimicrobial activity of various extracts of *Zataria multiflora* Boiss.

Microbial strains and culture media

Gram-positive and Gram-negative bacterial strains and the fungal strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH) Chandigarh, India. The bacterial strains used were *Pseudomonas aeruginosa* MTCC 1688, *E. coli* MTCC 443, *S. aureus* MTCC 96, *Proteus vulgaris* ATCC 6380, and *S. pyogenes* 0385. The fungal strains used were *Candida albicans* ATCC 1403 and *Cryptococcus neoformans* ATCC 66031. The composition of Muller–Hinton Agar (MHA) was as follows:

Formula per liter:	
Casein acid hydrolysate	17.5g
Beef heart infusion	2.0g
Starch, soluble	1.5g
Agar	17.0g
Final pH (at 25°C)	7.4±0.2.

Procedure

Thirty-eight grams of the medium was suspended in one liter of distilled water. Then, mixed well and heated with frequent agitation, boiled for 1 min, and sterilized at 121°C (15 lbs. of pressure) for 15 min. Cooled to 40°C–45°C and poured into Petri plates.

The composition of Sabouraud Dextrose Agar (SDA) is as follows:

Formula per liter:	
Mycological, peptone	10.0g
Agar	15.0g
Dextrose	40.0g
pH (at 25°C)	5.6±0.2.

Procedure

Sixty-five grams of the medium was suspended in one liter of distilled water. Then, mixed well and heated with frequent agitation, boiled for 1 min, and sterilized at 121°C (15 lbs. of pressure) for 15 min. Cooled to 40°C–45°C and poured into Petri plates. The bacterial strains were grown on MHA plates at 37°C and maintained on MHA slants at 37°C. The fungal strains were grown on SDA plates at 28°C and maintained on SDA slants at 28°C.

Agar well diffusion assay

The antimicrobial susceptibility tests were carried out using the agar well diffusion (AWD) assay. The bacterial cultures were developed for 24 h and fungal cultures were developed for 48 h and later transferred into boiling tubes containing 20 ml of liquid MHA and 20 ml of SDA, respectively. The contents of the tubes were transferred to Petri plates. After 5 min of solidification of the agar, Petri plates were punched in the form of wells with the help of a cork borer of 6 mm diameter. Later, these wells were filled with different volumes (25 µl, 50 µl) having a concentration of 50 mg/ml of *Zataria multiflora* Boiss. aqueous extract for bacterial

assay and fungal assay. Extracts were dissolved in 1 % (v/v) dimethylsulphoxide (DMSO) which was taken as negative control. The incubation period was carried out for 24 h at 37°C for bacteria and 48 h at 28°C for fungi. Triplets of the experiment were maintained for each bacterial and fungal strain to ensure reliability. After the incubation period, the diameter of circular inhibitory zones (zone of inhibition) formed around each well was measured in mm and recorded. The antimicrobial agent (levofloxacin 5 µg/disc) was used as a positive control in case of antibacterial activity while nystatin (50 µg /disc) discs were used as positive control for antifungal activity.[39] The zone of inhibition of Z. mutiflora against different bacterial and fungal strains are presented in Tables 1 and 2. The graphical representation of Zone of inhibition of Zataria multiflora ethanolic and aqueous hot extract against fungal strain Cryptococcus neoform are shown in Figure 1 and the Graphical representation of the zone of inhibition of ethanolic hot extract of *Z. multiflora* against Candida albicans fungal strain are shown in Figure 2.

Minimum inhibitory concentration assay

Minimum inhibitory concentration (MIC) of aqueous extract was determined by the Agar Dilution Method, recommended by the National Committee for Clinical Laboratory Standards (CLSI). A series of two-fold dilutions of the extracts ranging from 2.5 to 40.0 mg/ml was prepared in MHA at 48°C and in SDA at 40°C for antibacterial and antifungal activity, respectively. Plates were dried at room temperature for 30 min before spot inoculation with 3 µl and 2 µl aliquots of culture containing approximately 105 cfu/ml and 103 cfu/ml of each organism for antibacterial and antifungal activity, respectively. The bacterial plates were incubated at 37°C for 18 h while the fungal plates were incubated at 28°C for 48 h and were read visually and MIC was determined. Experiments were performed in triplicate. Inhibition of bacterial growth and fungal growth in the plates containing test extract was judged by comparison with growth in blank control plate. The MICs were determined as the lowest concentrations of extracts inhibiting the visible growth of each organism on the agar plate. The MIC for different strains of bacteria and fungi are presented in Tables 3 and 4. The plates showing MIC

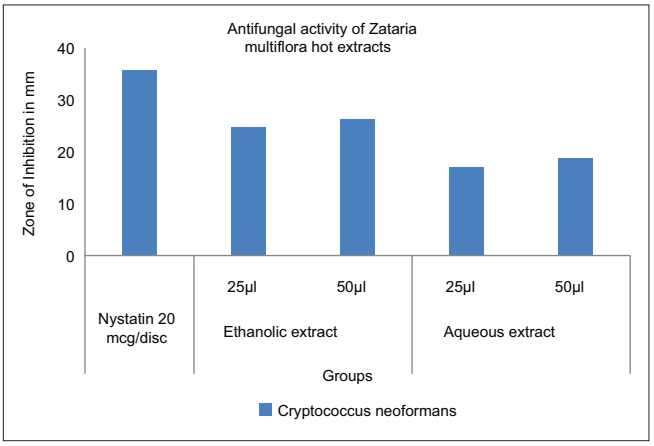

Figure 1: Graphical representation of zone of inhibition of the *Zataria multiflora* Boiss. hot extracts against fungal strain (*Cryptococcus neoformans*)

Table 1: *In vitro* antibacterial activity of *Zataria multiflora* Boiss. hot extracts and reference antibiotics determined with the agar well diffusion method

Test organism	Antibacterial activity of *Zataria multiflora* Boiss. hot extracts having a concentration of 50 mg/mL against the reference strains						Antimicrobial agent used (levofloxacin); levofloxacin (5 µg/disc) ZOI (mm)
	Ethanolic extract ZOI (mm)		Hydroalcoholic extract ZOI (mm)		Aqueous extract ZOI (mm)		
	25 µL	50 µL	25 µL	50 µL	25 µL	50 µL	
Staphylococcus aureus MTCC 96	14.33±0.01	17.30±0.06	13.63±0.04	15.61±0.05	14.62±0.09	16.17±0.05	34.14±0.69
Escherichia coli MTCC 443	19.04±0.14	20.07±0.07	14.09±0.22	15.21±0.20	15.11±0.22	16.14±0.16	33.51±0.57
Proteus vulgaris ATCC 6380	12.24±0.14	13.11±0.01	11.33±0.01	12.31±0.005	14.07±0.06	15.07±0.06	33.51±0.57
Pseudomonas aeruginosa MTCC 1688	14.20±0.01	18.07±0.02	15.01±0.07	16.02±0.02	13.206±0.01	14.25±0.01	31.43±1.16
Streptococcus pyogenes 0385	18.34±0.01	19.12±0.04	11.52±0.01	12.56±0.01	14.25±0.01	15.34±0.01	34.36±0.58

Results are expressed as mean±SD. Data was statistically analyzed by ANOVA followed by Dunnet's test. SD: Standard deviation

Table 2: *In vitro* antifungal activity of *Zataria multiflora* Boiss. hot extracts and reference antibiotic determined with agar well diffusion method

Test organism	Antifungal activity of *Zataria multiflora* Boiss. hot extracts having a concentration of 50 mg/mL against the reference strains					Antifungal agent used (Nystatin and amphotericin B) Amphotericin B (20 µg/disc) ZOI (mm)
	Ethanol extract ZOI (mm)		Aqueous extract ZOI (mm)		Negative control (mm)	
	25 µL	50 µL	25 µL	50 µL	50 µL	
Cryptococcus neoformans ATCC 66031	24.66±0.57	26.33±0.57	17±0.57	18.66±0.57	0	35.66±0.57
Candida albicans ATCC 14053	15.33±0.57	16.33±0.57	0	0	0	18.66±0.57

Results are expressed as Mean±SD. Data was statistically analyzed by ANOVA followed by Dunnet's test. SD: Standard deviation, ZOI: Zone of inhibition

Table 3: Minimum inhibitory concentration of *Zataria multiflora* Boiss. hot extracts (mg/mL) against different bacterial strains

Extract name	Test microorganisms				
	Staphylococcus aureus MTCC 96	*Escherichia coli* MTCC 443	*Proteus vulgaris* ATCC 6380	*Pseudomonas aeruginosa* MTCC 1688	*Streptococcus pyogenes* 0385
Ethanolic extract (mg/mL)	20	10	20	20	20
Hydroalcoholic extract (mg/mL)	40	10	20	20	10
Aqueous extract (mg/mL)	20	10	40	40	10

Table 4: Minimum inhibitory concentration of *Zataria multiflora* Boiss. hot extracts (mg/mL) against different fungal strains

Extract name	Fungal organism	
	Cryptococcus neoformans ATCC 66031	*Candida albicans* ATCC 14053
Ethanolic extract (mg/mL)	40	20
Aqueous extract (mg/mL)	20	0

Table 5: Macroscopic characters of dried aerial part of *Zataria multiflora* Boiss.

Parameters	Findings
Color	Green
Odor	Aromatic and agreeable
Taste	Bitter
Leaves	Elliptical
Size of leaves	1.1–1.5 cm L and 0.7–0.9 cm B (average)
Branches	Pieces of branches in the sample were 2.5 cm L and 0.1 cm B

of *Z. multiflora* ethanolic and aqueous hot extract against to different bacterial strains are shown in Figures 3 and 4.

Statistical analysis

Graph Pad 7.0 Prism (225 Frannklin street. F1.26 Boston, MA02110) and MS (Microsoft) Excel 2007 were used for statistical analysis and diagrammatic construction. Data were represented as mean ± standard deviation and statistical significance was determined using ANOVA followed by Dunnet's test as appropriate, three replications were used in the experiment. $P < 0.05$ was referred to as significant.

Results

Organoleptic evaluation

The organoleptic characters of dried aerial part of *Zataria multiflora* Boiss. are evaluated and are tabulated in Table 5.

Physicochemical parameters

Determination of ash values

The overall ash value of plant material is reported as: the total ash value was 10.4 %, acid insoluble 0.8 %, and water-soluble 10.0 % [Table 6].

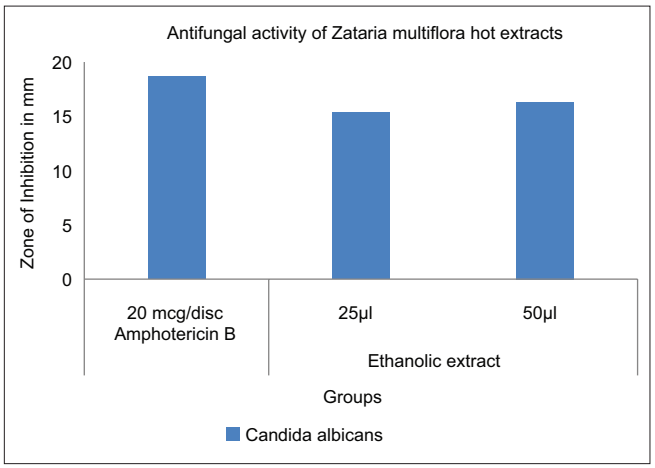

Figure 2: Graphical representation of zone of inhibition of the *Zataria multiflora* Boiss. hot extracts against fungal strain (*v*)

Determination of solvent extraction values

Polar elements such as phenols and alkaloids in the plant sample are indicated by the ethanol-soluble extractive values. As shown in Table 6, the ethanol-soluble extractive value(s) of *Zataria multiflora* Boiss were 19.6% (hot extractive value) and 16.0% (cold extractive value). The water-soluble extractive values were found to be 15.6% and 8.0% as hot extractive value and cold extractive value, respectively.

Losses due to drying

The data pertaining to losses due to drying are tabulated in Table 6. From data, it was found that there was a 7.4% loss on drying of the aerial part of *Zataria multiflora* Boiss

Determination of pH values

The pH values of the aerial part of *Zataria multiflora* Boiss were found to be 6.4 and 5.5 for 1% and 10% solution, respectively, as shown in Table 6.

Analysis for checking powdered drug fluorescence

Fluorescence characteristics of the powdered aerial part of *Zataria multiflora* Boiss are present in Table 7 with various chemical reagents under visible and UV light.

Phytochemical screening

The analysis of phytochemicals of various extracts of the aerial part of *Zataria multiflora* Boiss is tabulated in Table 8. The various phytochemicals present in various

Table 6: Physicochemical parameters of aerial part of *Zataria multiflora* Boiss.

Parameter	Weight of drug (g)	Weight of ash (g)	Percentage yield
Total Ash value	5	0.52	10.4
Acid-insoluble ash value	5	0.04	0.8
Water-soluble ash value	5	0.5	10.0
Extractive values			
Hot extractive value			
Solvent	**Weight of drug (g)**	**Weight of dried extract (g)**	**Percentage yield (w/w%)**
Ethanol	5	0.98	19.6
Aqueous	5	0.4	15.6
Cold extractive value			
Solvent	**Weight of drug (g)**	**Weight of dried extract (g)**	**Percentage yield (w/w%)**
Ethanol	5	0.8	16.0
Aqueous	5	0.4	8.0
Foreign organic matter analysis			
Plant part	**Weight of drug (g)**	**Weight of foreign matter (g)**	**Percentage of foreign matter**
Aerial part	5	0.37	7.4
pH values			
Sample		**pH**	
1% solution		6.4	
10% solution		5.5	

Table 7: Fluorescence analysis of powdered drug with various chemical reagents under visible light, short, and long wavelengths

Treatment	Daylight	UV (254 nm)	UV (366 nm)
Powder as such	Greyish green	Green	Green
Powder treated with distilled water	Brown	Greenish grey	Light brown
Powder treated with GAA	Greenish brown	Green	Chocolate brown
Powder treated with concentrated HCl	Brown	Light green	Dark brown
Powder treated with concentrated HCl + H_20	Light brown	Greenish grey	Yellowish brown
Powder treated with petroleum ether	Greenish brown	Green	Yellowish brown
Powder treated with 10%NaOH.	Coffee brown	Greenish grey	Coffee brown
Powder treated with methanol	Green	Mint green	Greyish brown
Powder treated with ethyl acetate	Green	Green	Grey
Powder treated with concentrated H_2SO_4	Black	Black	Black red
Powder treated with concentrated H_2SO_4 + H_2O	Yellowish brown	Light green	Light brown
Powder treated with picric acid	Yellowish	Greenish	Dark brown
Powder treated with 5%$FeCl_3$	Black	Dark green	Black
Powder treated with chloroform	Dark grey	Light green	Coffee brown
Powder treated with HNO_3	Brown red	Dark red	dark red
Powder treated with HNO_3 + H_2O	Brown orange	Dark orange	Dark orange

GAA: Glacial acetic acid

extracts of the aerial part of *Zataria multiflora* Boiss are alkaloids, glycosides, carbohydrates, tannins, flavonoids, phytosterols, and terpenoids.

Thin layer chromatography profile

The ethanolic extract of the drug was subjected to TLC with the aim to obtain a fingerprint and to identify the number of components resolved in different solvent systems and also for testing the purity of the drug. The following table depicts the Rf values of different components of the drug obtained with the solvent system.

Microscopic evaluation

The histological character of aerial part of *Zataria multiflora* Boiss. is shown in Figure 5.

Discussion

Nowadays, the increasing resistance of microorganisms against available antimicrobial agents is of major concern among scientists, researchers, and clinicians, worldwide. In general, it is observed that pathogenic viruses, bacteria, fungi, and protozoa are more and more difficult to treat

Table 8: Phytochemical screening of ethanol, hydroalcoholic, and aqueous extracts of aerial part of *Zataria multiflora* Boiss.

Tests	Inference	Ethanol extract (H)	Hydroalcoholic extract (H)	Aqueous extract (H)
Carbohydrates				
Molich's test	Violet ring	+	−	−
Benedict's test	Brick red ppt	+	−	−
Fehling's test	Brick red ppt	−	−	−
Tannins				
5%$FeCl_3$	Yellow color	+	+	+
Lead acetate	White ppt	−	+	+
Flavonoids				
Shinoda test	Pink color	+	+	+
Phenolics				
1% $FeCl_3$	Bluish color	+	+	+
Anthraquinone glycosides				
Borntrager's test	Pink color	−	−	+
Cardiac glycosides				
Keller Killiani test	Brown ring at junction	+	+	+
Legal's test	Pink color			
Terpenoids				
Salkowski's test	Golden yellow ring at junction	+	+	+
Phytosterols				
Libermann's test	Brown ring at junction	+	+	+
Alkaloids				
Dragendroff's reagent	Orange ppt	+	−	+
Mayer's reagent	Cream ppt	−	−	−
Wager's test		+	+	−
Hager's test		−	−	−

+=+ve, -=-ve

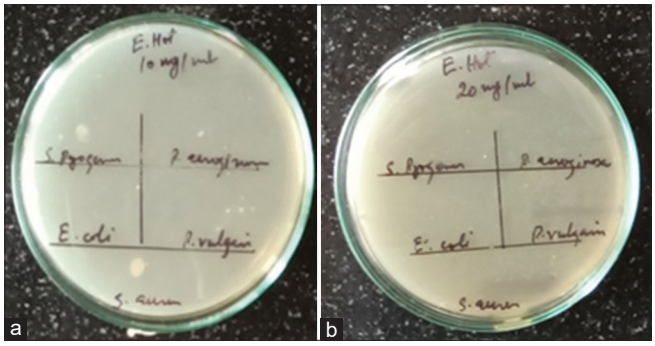

Figure 3: The plates showing MIC of *Zataria multiflora* Boiss. ethanolic hot extract against different bacterial strains where (a) against *Escherichia coli*, (b) *Streptococcus pyogenes*, *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*

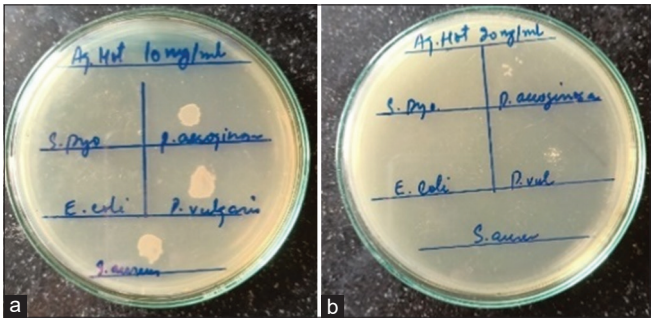

Figure 4: The plates showing MIC of *Zataria multiflora* Boiss. Aqueous hot extract against different bacterial strains where (a) against *Streptococcus pyogenes* and *Escherichia coli*, (b) against *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*

with the existing drugs. Hence, today research directed toward the development of new antimicrobial agents is necessary for several reasons such as the emergence and rapid spread of drug-resistant bacterial pathogens and the spectra of untreatable disease.[13] Several herbs are being used for treating various infectious diseases such as fever, pneumonia, boils, and ulcers in USM. The drugs used for this purpose may be classified as anti-infective, antipyretic, anti-inflammatory, and blood purifier. These classes of drugs may be the possible leads for developing herbal antibiotic drugs. Many of them have been proven to have antimicrobial properties in experimental studies. *Ṣa'tar* (*Zataria multiflora* Boiss.) is one of the most commonly used drugs of plant origin obtained from the family Lamiaceae.[15] It has anti-inflammatory, analgesic, demulcent, expectorant, detergent, deobstruent, and carminative properties and has been used for infectious diseases very successfully for a long time.

The pharmacognostic study is a primary and reliable criterion in the identification and determination of the quality and purity of crude drugs. The techniques of physicochemical and phytochemical evaluation and TLC profile were applied for the standardization, which is

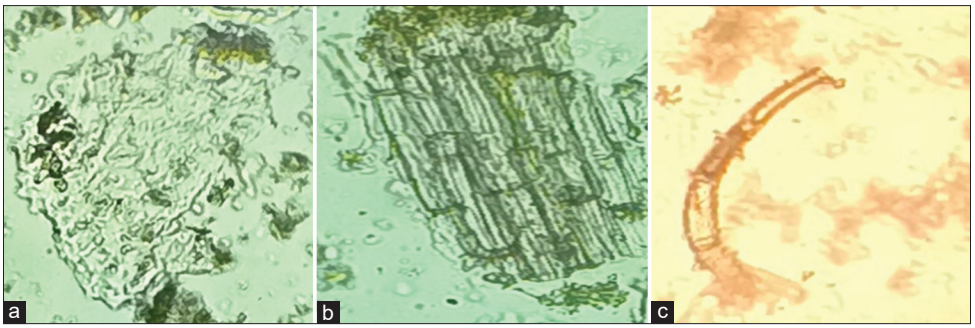

Figure 5: (a) Epidermis showing stomata; (b) epidermal cells; (c) covering trichome

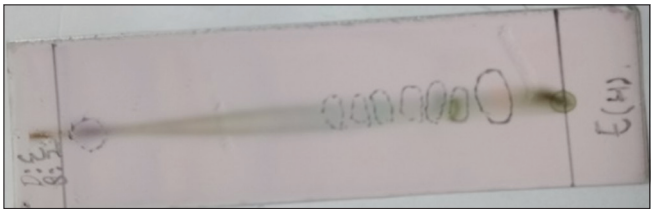

Figure 6: Thin layer chromatography profiling images of ethanolic hot extract

Table 9: Thin layer chromatography of ethanolic hot extract of *Zataria multiflora* Boiss.

Sample	Solvent system	Spraying reagent	Number of spots	Rf values
Ethanol (hot) extract	Petroleum ether: Ethyl acetate	Anisaldehyde	7	0.245, 0.298, 0.333, 0.385, 0.438, 0.473, 0.543

mentioned in Tables 6-9 and Figure 6. The macroscopic characters of the *Ṣa'tar* (*Zataria multiflora* Boiss) reveal that the aerial part of *Zataria multiflora* Boiss is green in color, has an aromatic and agreeable odor, tastes slightly bitter, and has elliptical leaves with size 1.1–1.5 cm in length and 0.7–0.9 cm in breadth. The microscopic characters of the *Zataria multiflora* Boiss. leaves powder revealed the presence of stomata, epidermal cells, and abundant covering trichomes.

For the evaluation of crude drugs, the determination of physicochemical parameters is of paramount importance. The ash values, which represent inorganic residue in the form of carbonates, silicates, and phosphates of sodium, potassium, calcium, and magnesium, were analyzed. Higher ash values indicate adulteration and subsequently the care taken while preparing the crude dug. Determination of these values was carried out to obtain total ash (10.4 %), acid-insoluble ash (0.8 %), and water-soluble ash (10.0 %) and the values are within permissible limits [Table 6]. Different extractive values give an idea about the number of active ingredients present in a particularly given amount of medicinal plant when extracted with different solvents. In hot extraction, the maximum extractive value of 19.6 % w/w was observed in ethanolic extract followed by aqueous extract at 15.6 % w/w. Thus, the results revealed that the plant mainly contains polar substances.[40] The medicinal plant material should be entirely free from foreign matter. The percentage of foreign matter was found to be negligible indicating good quality. The percentage of active chemical constituents in a crude drug is mentioned on a dried basis. Therefore, loss on drying of the plant material should be determined and moisture content should be controlled. The loss on drying of dry powder of *Zataria multiflora* Boiss. was found to be 7.4 % which is within permissible limits. The idea about the presence of an acidic or basic type of constituent present in plant material, i.e., acidity or basicity of the constituent is evaluated by pH value determination at different concentrations. The pH value of 1% solution and 10% solution in distilled water of *Zataria multiflora* Boiss. was found to be 6.4 and 5.5, respectively, thus indicating the presence of acidic constituents. In fluorescence analysis, the powdered drug material was treated with different acids, bases, and other reagents. All these treatments carried out indicate the presence of some particular type of constituents in the plants by giving color change of plant material with different reagents and then viewed under Visible and UV light.

The preliminary phytochemical tests on various extracts (ethanol, hydroalcoholic, and aqueous) of *Zataria multiflora* Boiss were carried out to identify the constituents and the results are tabulated in Table 8. Alkaloids, tannins, flavonoids, terpenoids, phytosterols, and phenolics are present in all the extracts; glycosides are present only in aqueous, ethanol, and hydroalcoholic extracts, while carbohydrates showed presence only in ethanol extract.

Ethanol, hydroalcoholic, and aqueous hot extracts of ZM aerial part were subjected to antimicrobial screening against selected pathogens which included Gram-positive bacterias, i.e., *S. aureus* and *S. pyogenes* and Gram-negative bacterias Figure 7, i.e., *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa,* and fungi, i.e., *Cryptococcus neoformans and Candida albicans*. The hot extracts of *Zataria multiflora* showed strong antimicrobial activity against different bacterial and fungal strains used for screening. In the case of ethanolic hot extract of *Z. multiflora,* the maximum zone of inhibition of bacterial strains was recorded in the order as: *E. coli* > *S. pyogenes* > *P. aeruginosa* >*S. aureus* > *P. vulgaris*. The MIC of ethanolic hot extract ranged from 10-20 mg/ml. The zone

of inhibition was lesser for *P. vulgaris* compared to other bacterial strains [Table 1 and Figures 8-10].

In the case of hydroalcoholic hot extract, the maximum zone of inhibition of bacterial strains was measured as: *P. aeruginosa* > *S. aureus* > *E. coli* > *S. pyogenes* > *P. vulgaris*. The MIC of hydroalcoholic hot extract ranged from 10 to 40 mg/ml. The zone of inhibition was lesser for *P. vulgaris* (12.31mm) compared to other bacterial strains.

In the case of aqueous hot extract, the maximum zone of inhibition of bacterial strains was measured as: *S. aureus* > *E. coli* > *P. vulgaris* > *S. pyogenes* > *P. aeruginosa*. The MIC of aqueous hot extract ranged from 10-40mg/ml. The zone of inhibition was lesser for *P. aeruginosa* (14.25 mm) compared to other bacterial strains.

For the screening of the antifungal activity of ethanolic and aqueous hot extracts, only two fungal strains, namely, *C. neoformans* and *C. albicans* were used. In the case of ethanolic hot extract, the maximum zone of inhibition (26.33 mm) was recorded for *C. neoformans,* and in the case of aqueous hot extract, the maximum zone of inhibition (18.66 mm) was recorded for *C. neoformans*. Levofloxacin (5 mg/disc) was taken as a positive control for antibacterial activity while nystatin (20 µg/disc) was

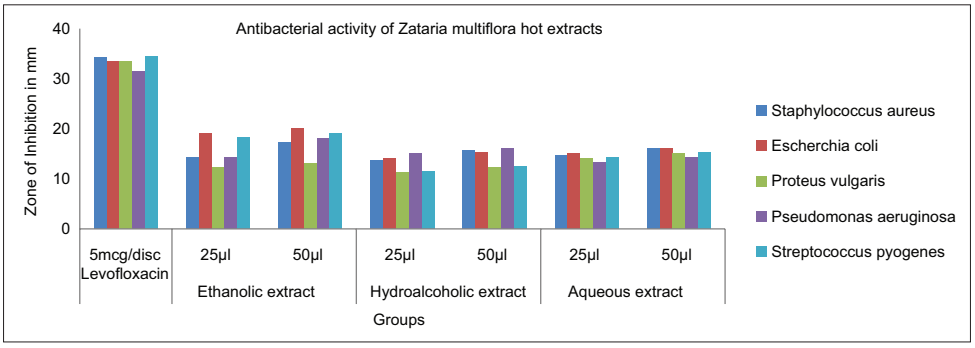

Figure 7: Graphical representation of zone of inhibition of the *Zataria multiflora* Boiss. hot extracts against different bacterial strains

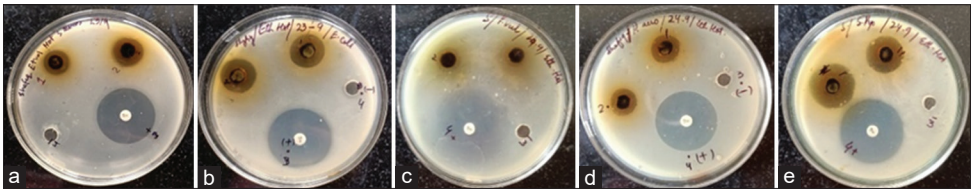

Figure 8: The plates show the effect of *Zataria multiflora* Boiss. ethanolic hot extract (by agar well diffusion) against different bacterial strains. (a) against *Staphylococcus aureus* compared with Levofloxacin; (b) against *Escherichia coli* compared with Levofloxacin; (c) against *Proteus vulgaris* compared with Levofloxacin; (d) against *Pseudomonas aeruginosa* compared with Levofloxacin; (e) against *Streptococcus pyogenes* compared with Levofloxacin

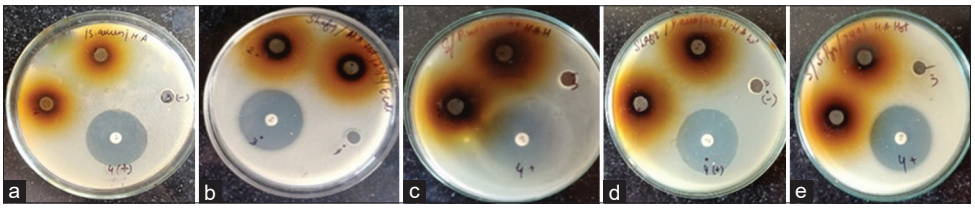

Figure 9: The plates show the effect of *Zataria multiflora* Boiss. hydroalcoholic hot extract (by agar well diffusion) against different bacterial strains. (a) against *Staphylococcus aureus* compared with Levofloxacin; (b) against *Escherichia coli* compared with Levofloxacin; (c) *Proteus vulgaris* compared with Levofloxacin; (d) against *Pseudomonas aeruginosa* compared with Levofloxacin; (e) against *Streptococcus pyogenes* compared with Levofloxacin

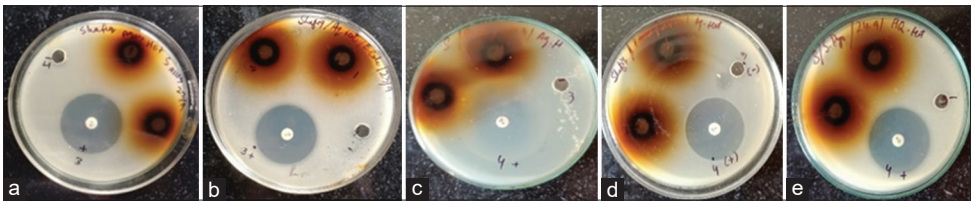

Figure 10: The plates show the effect of *Zataria multiflora* Boiss. aqueous hot extract (by agar well diffusion) against different bacterial strains. (a) against *Staphylococcus aureus* compared with Levofloxacin; (b) against *Escherichia coli* compared with Levofloxacin; (c) against *Proteus vulgaris* compared with Levofloxacin; (d) against *Pseudomonas aeruginosa* compared with Levofloxacin and (e) against *Streptococcus pyogenes* compared with Levofloxacin

taken as a positive control for antifungal activity of *Zataria multiflora*. However, no activity was observed with DMSO which acted as negative control during the whole process.

Conclusion

Based on the findings from the current study, the conclusion can be drawn that *Zataria multiflora* aerial part extracts possess antimicrobial effects by two methods, i.e., AWD assay and MIC assay, which might be useful in preventing bacterial and fungal infections. The current study validates the claims of Unani Medicine that *Ṣa'tar* (*Zataria multiflora*) can be used in various infectious diseases. The study shows that the aerial part of *Zataria multiflora* appears to be a rich source of a drug candidate that can restrict the growth of various infectious diseases. However, it needs further investigation and clinical studies for validation of antimicrobial effects in humans.

Acknowledgments

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Nil.

Conflicts of interest

There are no conflicts of interest.

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