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Pharmaceutico-analytical standardization, characterization, and clinical evaluation of sedative and hypnotic effects of *Roghan Banāfshā'* (*Viola odorata* oil) in patients with primary insomnia

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Both traditional healers and conventional researchers have reported that either an extract obtained from *Viola odorata* flowers, or its oil has potential to produce marked sedative and hypnotic effects. Unani physicians prescribe *Roghan Banāfshā'* for the treatment of many disorders mainly insomnia and headache. The present study aimed at evaluating its pharmaceutico-analytical standardization, characterization, and sedative and hypnotic effects in patients with primary insomnia. The pharmaceutico-analytical standardization includes physicochemical properties, HPTLC, and GC-MS fingerprint profile of *Roghan Banāfshā'*. Its sedative and hypnotic effects were evaluated on a single group of patients with primary insomnia by observing improvements in sleep patterns and insomnia severity index. The findings of physicochemical and analytical indices of *Roghan Banāfshā'* were found to be within normal limits. HPTLC fingerprinting conducted under Short UV scanning and Long UV scanning has identified seven and nine distinct bands, respectively. GC-MS fingerprint profile of *Roghan Banāfshā'* exhibited the presence of 10 bioactive constituents, including 3-carene and oleic acid, linked to sedative effects. The developed physicochemical constants, HPTLC, and GC-MS chromatograms may be referred to in the future. The findings drawn from the preliminary clinical study have suggested that massage with *Roghan Banāfshā'* over scalp and forehead along with lifestyle and dietary modifications, is useful in relieving primary insomnia.

Keywords: Hypnotic, *Roghan Banāfshā'*, Sedative, Standardization, Unani medicine

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In the present era, medicinal plants are gaining wide global acceptance for managing various diseases, including lifestyle disorders and insomnia. The complex nature of crude medicinal plants and finished products, supplied to a wide range of markets throughout the world, aroused paramount quality concerns and sought appropriate quality control methods. The traditional quality control measures used to set the pharmacopeial standards for herbs confronted a wide-ranging challenge varying from quality to safety and even regulatory issues. In this scientific era, various outstanding advanced techniques, like microscopy, chromatography, spectrophotometry, DNA barcoding, etc., are available for setting-up pharmacopeial standards for processing, authenticating, preparing, and quality assurance of herbal products[1]. *Roghan Banāfshā'*, a Unani pharmacopeial preparation, is used in treating headaches, insomnia, and ankylosing arthritis. It is prepared with *Viola odorata* flowers and sesame oil[2]. The Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) described insomnia as the subjective experience of difficulty with sleep initiation, maintaining sleep, and/ or early awakening for at least three nights a week for three consecutive months, not caused by physical or cerebral diseases[3]. Based on onset, duration, severity, presentation, and etiological factors, insomnia is classified into acute, subacute, chronic, mild, moderate, severe, sleep-onset, sleep-maintenance, end-of-characteristics, primary, secondary, etc[4]. Primary insomnia usually occurs due to excessive consumption of caffeine and alcohol, irregular sleep patterns, poor sleep hygiene, mental stress,

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consumption of certain medications, etc[5]. Whereas the secondary insomnia is caused by psychiatric disorders, medical conditions, other sleep-related disorders, drug abuse, etc[6]. The prevalence of insomnia has been estimated at 6-10% among the general population, especially in women and old age[7]. A cohort study conducted on 880 aged subjects in a city of southern India, Bengaluru, has estimated the prevalence of insomnia in 64.9% of subjects, commonly in females (72.3%)[8]. The sleeping pills used in conventional medicine can effectively relieve sleep deprivation but may produce abuse, dependence, withdrawal symptoms, rebound insomnia, cognitive impairment, dementia, etc[9]. Owing to these reasons, patients with insomnia usually prefer self-help activities, massage therapy, and herbal medicines[10]. In the present era, evidence-based therapeutic approaches to manage lifestyle disorders and other pathological conditions using standardized herbal products are sought. The standardization and clinical validation of age-old claims of Unani pharmacopeial interventions using modern analytical and clinical methods are the need of the hour[11]. Based on the empirical evidence, either *Roghan Banāfshā'* or its chief ingredient, *Gul-i-Banāfshā'* (*Viola odorata* flowers), has been used as sedative, hypnotic, anti-depressant, stress reliever, and anxiolytic in Unani medicine. This study aims to ensure that *Roghan Banāfshā'* is consistent in quality by using modern testing methods, including physicochemical analysis, microbial load, heavy metal analysis, and fingerprinting profile. In addition, the study also aimed to evaluate the efficacy and safety of *Roghan Banāfshā'* in patients with primary insomnia by applying clinical and laboratory parameters.

Materials and Methods

Procurement of chemicals and reagents

All the chemicals and reagents used in the physicochemical standardization were procured from authorized and certified suppliers.

Study drug preparation

A total of 2.8 kg of fresh flowers of *Viola odorata* was macerated into 11.2 litres of sesame oil in a glass jar closed with the iron lid for 14 days at room temperature. Thereafter, it was filtered through a clean muslin cloth and packed into a 70 mL airtight glass container for dispensing purposes[2]. The batch number given to the prepared oil was 01/2021-2022, and the expiry was September 2024.

Physicochemical analysis of *Roghan Banāfshā'*

Different parameters, such as organoleptic properties, saponification value, acid value, peroxide value, pH, and refractory index were carried out to evaluate the physicochemical standards of *Roghan Banāfshā'*[12,13].

Phytochemical analysis of *Roghan Banāfshā'*

Secondary metabolites present in the drug were evaluated by following the standard procedures[14].

Heavy metal analysis of *Roghan Banāfshā'* by Atomic Absorption Spectrophotometry (AAS)

Sample preparation for AAS

Around 0.5 mL of *Roghan Banāfshā'* was added to 6 mL of Conc. HNO_3 in a 50 mL TFM-PTFE vessel. The digestion was carried out using Anton Paar Microwave digestion devices (Multiwave GO Plus). The temperature was set to 0 to 200°C for 30 min for complete digestion. The vessels were then slowly opened to release nitric acid fumes, and the distilled water was added. The solution was then transferred into the 100 mL standard volumetric flask using a funnel filtered with Whatman (No. 1) filter paper and made up to the mark[15].

Microbial load

Sample preparation

One mL of oil sample was emulsified with 1% Polysorbate 20 and diluted to 10 mL. The emulsion was prepared by warming the sample to below 45°C for not more than 15 min. This stock solution was used for serial dilution in further microbial analysis.

Microbial load determination

The microbial quality of the samples, including the isolation and identification of pathogenic bacteria, was assessed following the Unani Pharmacopeia (2016), WHO standards (2007), and US FDA BAM method (2009)[12,13,16]. One mL of sample was transferred to 9 mL of peptone broth to facilitate microbial growth. Subsequently, 1:10 dilutions were prepared by transferring 1 mL from the previous dilution into 9 mL of peptone water until the desired dilution factor was achieved (up to 105, depending on microbial load). From last three dilutions each 1 mL of sample was pour-plated onto Nutrient Agar and Sabouraud Dextrose Agar, incubated at 37°C for 24-48 h for bacteria and 25°C for 48-72 h for fungi. *E. coli* (ATCC 25922) was used as a Positive Control

to validate microbial growth conditions. Sterile peptone water without the sample was incubated under the same conditions to confirm the absence of contamination. After incubation, colony-forming units per gram (CFU/g) were calculated using the average colony count × dilution factor.

Aflatoxin test using afla-test fluorometer

The process involves mixing oil sample with methanol, filtering, and adding purified water. The diluted extract was then passed through an AflaTest WB column, eluted with HPLC-grade methanol, and collected in a VICAM cuvette. The eluate was then placed in a fluorometer for concentration measurement.

High-Performance Thin Layer Chromatography (HPTLC)

Sample preparation

One g of *Roghan Banāfshā'* was dissolved in 20 mL of n-hexane and placed into a sample vial for HPTLC application.

Methodology of HPTLC

The HPTLC fingerprint profile of *Roghan Banāfshā*' was analyzed utilizing a CAMAG HPTLC system, along with a pre-coated silica gel 60 F_{254} HPTLC plate measuring 5 x 10 cm. A volume of 5 µL of the sample was applied to the plate using the CAMAG ATS 4 sample applicator. The mobile phase consisted of a mixture of hexane, ethyl acetate, and formic acid in a ratio of 8:1.5:0.1 (v/v). The plate was developed up to a distance of 8 cm from the bottom of the plate at ambient temperature within a CAMAG twin-through chamber (10 x 10 cm), with a saturation period of 30 min. Following the development, the plate was allowed to dry at room temperature. The resulting bands on the plates were analysed using the CAMAG UV Visualizer 2 at wavelengths of 254 nm (D2 lamp, absorption mode), 366 nm (Hg lamp, fluorescence mode) and under white light (W lamp, absorption mode). Chromatograms were scanned with a CAMAG TLC scanner 4, and the fingerprint data along with R_f values were documented using the WINCATS planar chromatography manager software[17].

Gas Chromatography-Mass Spectrometry (GC-MS)

Sample preparation

The oil sample was filtered through the nylon syringe filter of pore size 0.45 µm and taken for GC-MS analysis.

Methodology of GC-MS

The secondary metabolites present in *Roghan Banāfshā'* was analysed using QP2010 Ultra, Shimadzu brand of Gas Chromatography-Mass Spectrometry equipped with Rtx-5 SilMS (30 m × 0.25 mm × 0.25 µm). An electron ionization energy system with ionization energy of 70e was employed for detection purpose in the GC-MS. Helium gas at a concentration of 99.99% was applied as a carrier gas at a constant flow rate of 1 ml/ min and an injection volume 1 µL were injected into the instrument with a split ratio of 1:10. The injector temperature and Ion-source temperature were set at 200°C during the chromatographic run. The temperature of the oven was set at 80°C for 2 min with an increase of 5°C per minute up to 300°C for 2 min. The mass spectra were detected at 70 eV, at the scan interval of 0.5 s, with a scan range of 45-1000 m/z at the transfer line and ion-source temperature of 240°C. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The software used to record the mass spectra and chromatograms was a GC-MS solution ver.2.53 with NIST 11 and WILEY 8 library[18].

Clinical evaluation of *Roghan Banāfshā'* in primary insomnia

Subject selection

An open-label and single-arm clinical trial was conducted on 112 patients with primary insomnia during 04.12.2021 - 02.08.2023. The protocol was approved by the IEC of RRIUM, Chennai, vide reference 2-21/IEC/Tech/RRIUM/2020-21/740. The research project was registered with the Clinical Trails Registry of India; vide REF/2021/04/042585(DE). Patients of either gender in the age group of 18 - 65 years with the symptoms of primary insomnia were selected for the study. The known cases of psychiatric disorders, cardiac diseases, migraine, cluster headache, COPD, Parkinson's disease, epilepsy, dementia, chorea, eczema, scabies, psoriasis, pregnant and lactating women, subjects on antihistaminics, glucocorticoids, and any other medications, and subjects who were not willing to participate in the study, were excluded.

Study drug intervention and dietary advice

A training session on the self-massage technique in the manner of kneading, squeezing, and deep circular rubbing was given to every patient before enrolling them in the study. The patients were then advised to do self-massage using 5 mL of *Roghan Banāfshā'* over the forehead and scalp for 10 min at bedtime daily for 14 days. The patients were also advised to take plenty of fluids, green leafy vegetables, fruits,

milk, and fiber-containing food items and not to take tea or coffee at bedtime.

Efficacy and safety evaluation

The efficacy of the test drug was clinically assessed at 0, 7th and 14th days. The clinical signs and symptoms, including sleepiness during the day, general tiredness, irritability, problems in concentration, and total sleeping time, were assessed using Visual Analogue Scale (VAS). The Insomnia Severity Index (ISI) was assessed to evaluate the efficacy of the test drug. The socioeconomic status of the patients was also assessed at baseline using Kuppuswamy's Socioeconomic Status Scale to know the incidence of primary insomnia. The safety of the test drug was assessed through reporting adverse effects and relevant clinical pathological and biochemical parameters.

Statistical analysis

A per patient's protocol analysis was employed to evaluate the efficacy and safety of the test drug. The discrete variables were expressed as frequency (percentage) and continuous variables as mean with standard deviation (Mean ± SD), with a 95% confidence interval. The effect size was calculated separately for the two primary outcome measures, namely Visual Analogue Scale (VAS) and Insomnia Severity Index (ISI). The continuous variables within the group at baseline and at the end of the treatment were compared using student's *t*-test (paired). The p-value <0.05 was considered to be significant.

Results

Physicochemical analysis of *Roghan Banāfshā'*

The physicochemical findings of *Roghan Banāfshā'* are depicted in (Table 1).

Table 1 — Physicochemical standardization of *Roghan Banāfshā'*

Physicochemical parameters	Results	Permissible limits
Colour	Dark yellow	-
Appearance	Reduced translucent	-
Touch	Smooth	-
Consistency	Slight viscid	-
Odour	Typical sesame oil	-
Apparent density (Weight/ mL	0.919	0.915 - 0.924
Saponification value	188.82	186 - 195
Acid value	1.91	2
Peroxide value	2.50	<10
pH	6.8	-
Refractive index at 25°C	1.475	1.465–1.469

Phytochemical analysis of *Roghan Banāfshā'*

The secondary metabolites found in the drug are detailed in (Table 2).

Heavy metal analysis of *Roghan Banāfshā'*

The findings of heavy metal analysis have revealed that no arsenic content was detected, however cadmium (0.0303 ppm), lead (0.0409 ppm), and mercury (0.6378 ppm) were found in *Roghan Banāfshā'* within permissible limits as per the WHO standards.

Microbial load and aflatoxin analysis of *Roghan Banāfshā'*

The TBC and TFC were found to be within permissible limits, and individual microorganisms were found to be absent (Table 3).

HPTLC of *Roghan Banāfshā'*

HPTLC fingerprinting conducted under Short UV scanning identified seven distinct bands with the following R_f values and areas: 0.01 (0.88%), 0.29 (26.12%), 0.34 (14.13%), 0.43 (24.44%), 0.81 (4.86%), 0.83 (4.12%), and 0.94 (25.45%). In contrast, Long UV scanning revealed nine bands with R_f values and areas as follows: 0.20 (3.67%), 0.30 (3.77%), 0.35 (18.73%), 0.42 (18.86%), 0.48 (8.32%),

Table 2 — Phytochemical analysis of n-hexane extract of *Roghan Banāfshā'*['+'- present '-'-absent]

Phytochemical components	Results
Alkaloid	-
Carbohydrate	+
Reducing sugar	-
Flavonoid	+
Phenol	+
Cardiac glycoside	-
Terpenoid	+
Triterpenoid	-
Saponin	-
Tannin	+
Phytosterol	-
Anthraquinone	-
Lignin	-

Table 3 — Microbial load of *Roghan Banāfshā'*

Parameters	Results	Remarks (WHO Standards)
Total Bacterial Count (TBC)	$1x10^3$cfu/mL	Within permissible limits
Total Fungal Count (TFC)	Less than 3 cfu/mL	
Enterobacteriaceae	Absent	
Escherichia coli	Absent	
Salmonella Spp	Absent	
Staphylococcus aureus	Absent	
Pseudomonas aeruginosa	Absent	
Total Aflatoxin (B1+B2+G1+G2)	Below Detection Limit	

0.82 (9.24%), 0.86 (5.64%), 0.91 (20.68%), and 0.97 (11.08%). Additionally, fingerprinting of the post-derivatized plate under white light displayed ten bands with R_f values and areas: 0.01 (0.32%), 0.04 (0.43%), 0.12 (1.64%), 0.24 (4.31%), 0.30 (11.09%), 0.35 (10.10%), 0.43 (10.62%), 0.65 (3.17%), 0.86 (52.46%), and 0.97 (5.87%). In HPTLC analysis, there is an inverse relationship between the polarity of the separated bands, or compounds, and their R_f values. The band with the highest R_f value corresponds to the least polar compound, while the band with the lowest R_f value indicates the highest polarity. The post-derivatized plate treated with vanillin-sulfuric acid reagent displayed violet-coloured bands, confirming the presence of terpenoids and essential oils (Fig. 1).

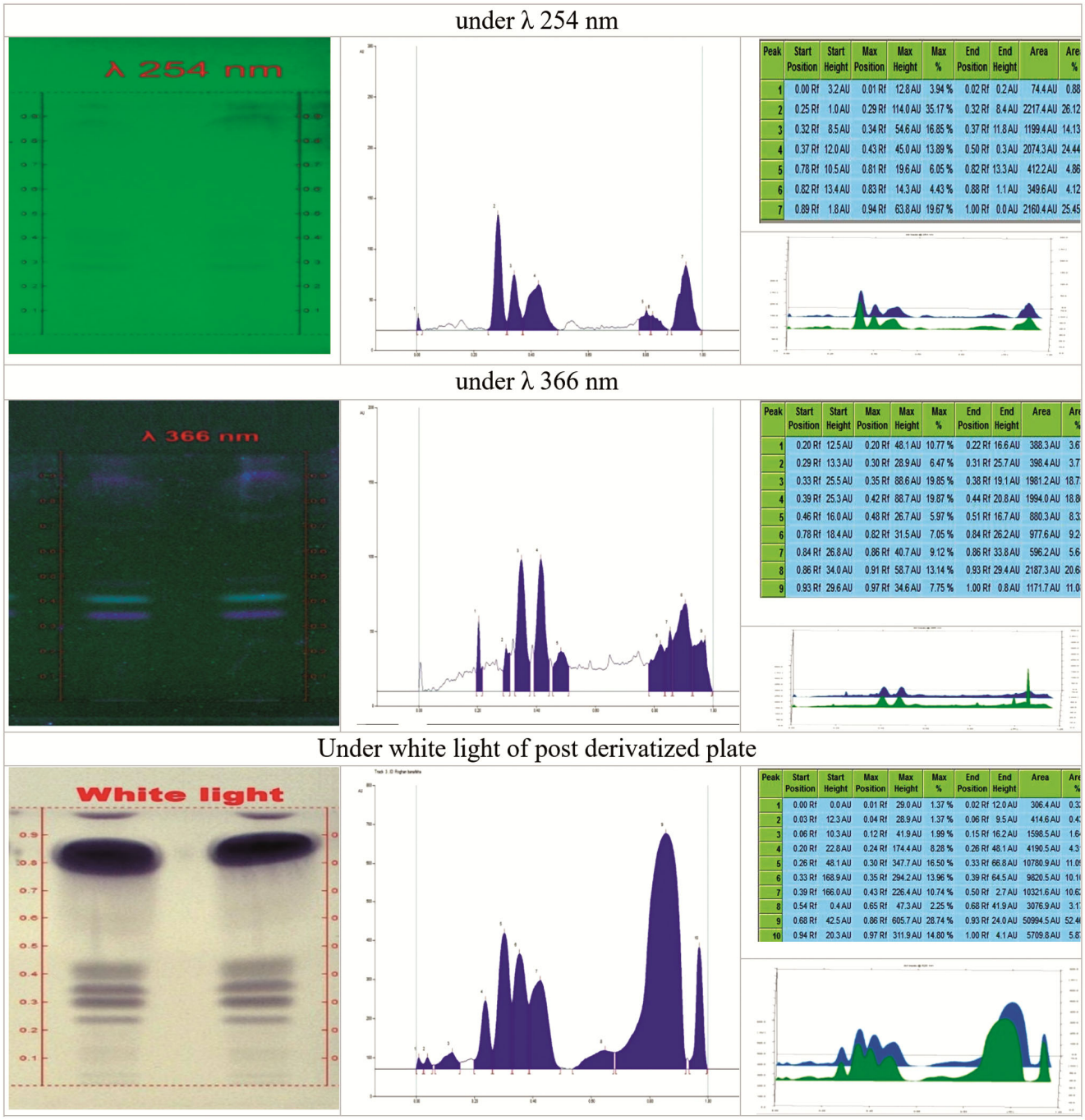


Fig. 1 — HPTLC fingerprint profile of *Roghan Banāfshā'*

GC-MS of *Roghan Banāfshā'*

A total of 10 peaks were detected in the oil sample with each peak representing specific bioactive compounds. The identification of these compounds was achieved by correlating their similarity index, peak retention times, molecular weights, and molecular formulas with those of established compounds found in the National Institute of Standards and Technology and WILEY 8 libraries. According to the peak area and retention time, the major compounds were found to be 9-octadecenoic acid, 1,2,3-propanetriyl ester (32.20%) followed by oleic acid (14.83%), bicyclo tridec-1-ene (13.71%), Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (10.76%), 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (8.33%), l-(+)-Ascorbic acid 2,6-dihexadecanoate (7.23%), 3-carene (4.39%), glycidol stearate (3.34%), Butyl 9,12-octadecadienoate (2.81%), and (7R,8S)-cis-anti-cis-7,8-Epoxytricyclo[7.3.0.0(2,6)]dodecane (2.41%). The results of the GC-MS analysis are illustrated in (Table 4 & Fig. 2).

Demographic profile of patients with primary insomnia (*n= 112*)

The cases of primary insomnia screened, registered, dropped out, completed, and the incidence according to the age are depicted in (Fig. 3 & Fig. 4).

Effects of *Roghan Banāfshā'* in the improvement of clinical signs and symptoms in patients with primary insomnia

All the clinical parameters were significantly reduced at the end of the treatment (Table 5).

Table 4 — GC-MS analysis of *Roghan Banāfshā'*

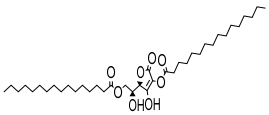
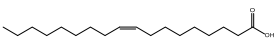
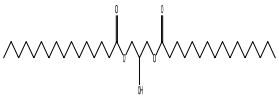
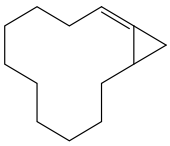
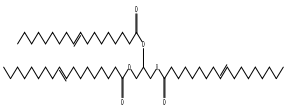
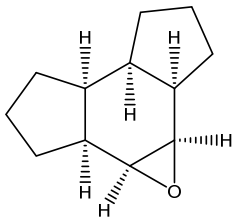
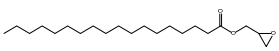
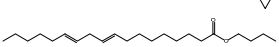
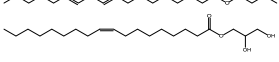
R.Time	Molecular formula	Area %	Chemical structure	Name of the compound	Pharmacological activity/ Therapeutic uses
3.502	$C_{10}H_{16}$	4.39		3-carene	Anti-inflammatory, Antimicrobial, Anxiolytic, Sleep enhancing effect[19]
33.237	$C_{38}H_{68}O_8$	7.23		l-(+)-Ascorbic acid 2,6-dihexadecanoate	Antioxidant, Antiproliferative[20]
38.262	$C_{18}H_{34}O_2$	14.83		Oleic Acid	Stress resistance, Antioxidant, Anti-inflammatory[21]
41.394	$C_{35}H_{68}O_5$	10.76		Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	-
44.237	$C_{13}H_{22}$	13.71		Bicyclo[10.1.0]tridec-1-ene	-
44.327	$C_{57}H_{104}O_6$	32.20		9-Octadecenoic acid, 1,2,3-propanetriyl ester	Anti-spasmodic, Immunomodulation[22]
44.445	$C_{12}H_{18}O$	2.41		(7R,8S)-cis-anti-cis-7,8-Epoxytricyclo[7.3.0.0(2,6)]dodecane	-
44.735	$C_{21}H_{40}O_3$	3.34		Glycidyl stearate	-
47.345	$C_{22}H_{40}O_2$	2.81		Butyl 9,12-octadecadienoate	-
47.418	$C_{21}H_{40}O_4$	8.33		9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Surfactant, Emulsifying properties, Drug delivery enhancer[23-25]

Table 5 — Response to the treatment on VAS and total sleeping time (*n= 112*). Calculated Cohen's d was 0.70, indicating a moderate to large effect size, which suggests that the intervention produced a clinically meaningful improvement in subjective symptoms. According to Cohen's interpretation, values around 0.2 indicate a small effect, 0.5 indicates a moderate effect, and 0.8 or above indicates a large effect. Therefore, a value of 0.70 reflects substantial symptomatic improvement.

Parameters	Base Line (VAS) Score (Mean ± SD)	At the end of the treatment (VAS) Score (Mean ± SD)	P-value	Mean difference	MCID score	Clinical significance
General tiredness	5.14±1.63	1.32±1.57	<0.05	3.8215	0.82	Yes
Irritability	3.37±1.69	0.74±1.16	<0.05	2.63393	0.85	Yes
Problem in concentration	2.43±2.24	0.66±1.30	<0.05	1.7679	1.12	Yes
Total sleeping time	3.3±1.06	6.07±1.59	<0.05	-2.7714	0.53	Yes

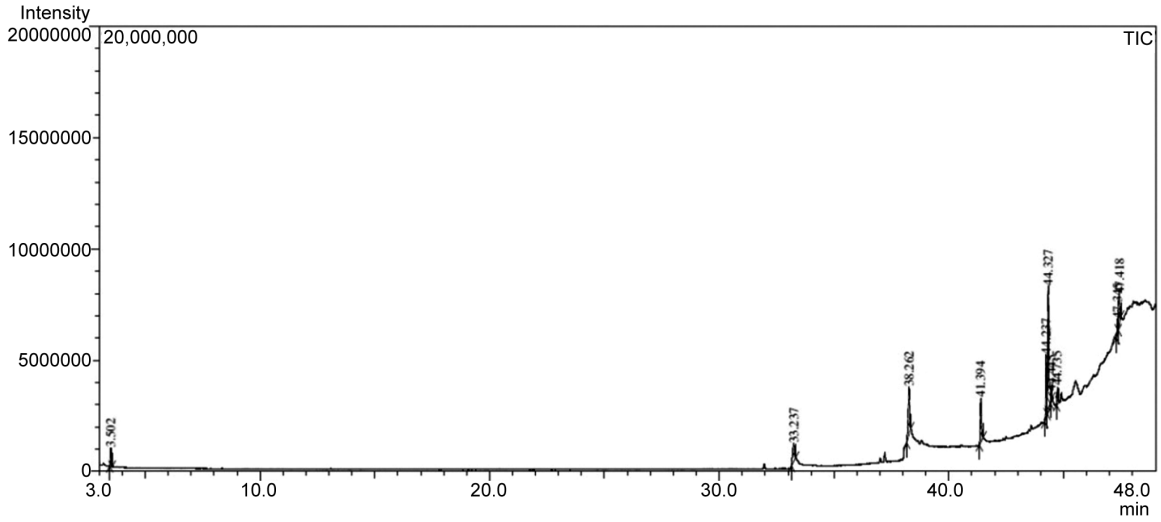


Fig. 2 — GC-MS analysis of *Roghan Banāfshā'*

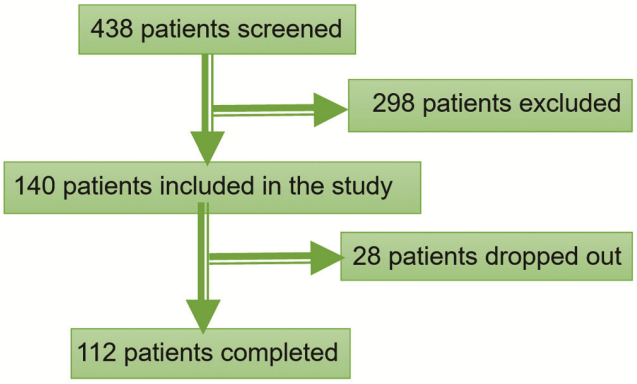


Fig. 3 — Overview of screened, registered, dropped out, and completed cases of primary insomnia

Effects of *Roghan Banāfshā'* on Insomnia Severity Index (ISI)

The significant changes were observed in Insomnia Severity Index (ISI) at the end of the treatment (Table 6).

Safety evaluation of *Roghan Banāfshā'*

No local adverse effects, including allergic reactions, irritations, oedema, redness, pruritus, scaling, stinging, etc., were observed in any patients during the whole clinical trial. The haematological and biochemical indices also didn't show any significant changes at the end of the treatment.

Discussion

In the present scientific era, the assessment of the safety, efficacy, stability, and quality control of herbal products by employing modern methodologies is mandatory[26]. In the past few decades, the trend has moved toward searching for cost-effective, eco-friendly, bio-friendly, and relatively safe herbal medicines for preventing and treating various bodily ailments. In this regard, Unani and other traditional medicines have played a significant role in mainstreaming the health care needs. The organoleptic evaluation of *Roghan Banāfshā'* has revealed that its colour and odour were found to be brownish and pleasant, respectively. The saponification value of an oil or fat is the amount of potassium hydroxide (KOH) in milligrams required to saponify one gram of the oil or fat[27]. This value suggests that *Roghan Banāfshā'* consists mainly of long-chain fatty acids, which require less KOH for saponification compared to oils with shorter chains. Most of the commonly used oils and fats generally yield long-chain fatty acids[28]. Acid value

Table 6 — Response to the treatment on Insomnia Severity Index (ISI) Score (*n= 112*). Calculated Cohen's d was 2.21, indicating a very large effect size, which demonstrates a significant reduction in ISI.

Parameters	Base Line (ISI) Score (Mean ± SD)	At the end of the treatment (ISI) Score (Mean ± SD)	P-value	Mean difference	MCID score	Clinical significance
Difficulty falling asleep	2.53±0.73	0.64±0.81	<0.05	1.8928	0.367	Yes
Difficulty staying asleep	2.07±0.84	0.37±0.72	<0.05	1.6964	0.423	Yes
Problems walking up too early	1.30±0.89	0.27±0.55	<0.05	1.0268	0.449	Yes
How satisfied/ dissatisfied are you with your current sleep pattern?	2.76±0.60	0.81±0.87	<0.05	1.9554	0.3	Yes
How noticeable to others do you think your sleep problem is in terms of impairing the quality of your life?	2.41±0.66	0.59±0.79	<0.05	1.8214	0.333	Yes
How worried/ distressed are you about your current sleep problem?	2.95±0.94	0.83±0.95	<0.05	2.125	0.471	Yes
To what extent do you consider your sleep problem to interfere with your daily functioning (e. g. daytime fatigue, mood, ability to function at work/ daily chores, concentration, memory, etc.) Currently?	2.50±0.73	0.71±0.85	<0.05	1.7946	0.3676	Yes

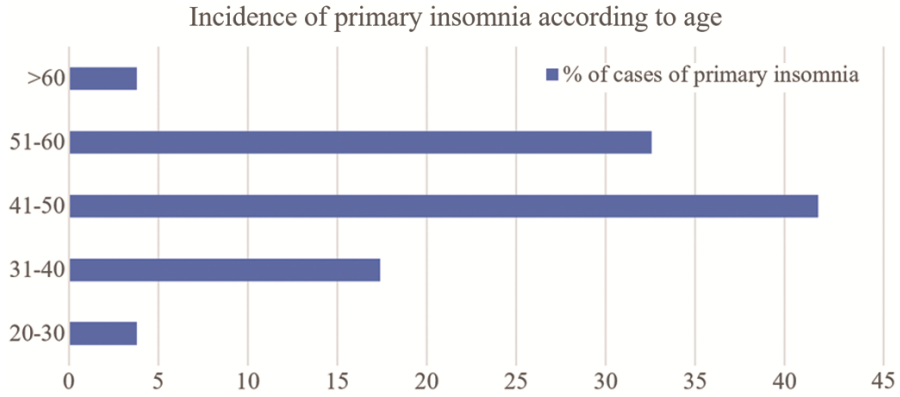


Fig. 4 — Incidence of primary insomnia according to age amongst completed cases (*n= 112*), shows the higher incidence in middle and old age groups

is estimated to evaluate the presence of free fatty acids and is an authoritative quality control parameter to determine the refining degree and quality variations of fats and oils during storage. The formation of free fatty acids depends on triglycerides hydrolysis and is further aggravated by reaction of the oil with moisture[29]. The observed acid value in *Roghan Banāfshā'* was found to be within the normal range[30]. The peroxide value indicates the presence of oxidative levels in oils and consequently suggests the rancidity. The peroxide value of *Roghan Banāfshā'* was observed to be within the normal range[30]. The pH of a drug affects its absorption, distribution, metabolism, excretion, and toxicity[31]. The pH of *Roghan Banāfshā'* was observed to be weakly acidic in nature, which absorption is pH dependent[32]. Essential oils typically have higher refractive indices than carrier oils like sesame oil due to their aromatic compounds and volatile components. The refractive index of the tested oil was observed to be within the range[30]. Phytochemical analysis of *Roghan Banāfshā'* identified the presence of carbohydrate, phenol, flavonoid, tannins, and terpenoids. The phytochemical analysis of *Viola odorata* flowers has shown that it yields a good concentration of an important flavonoid, namely rutin, which produces remarkable anxiolytic and sedative effects through GABAergic neurotransmission[33,34].

Qualitative HPTLC analysis identified 7, 9, and 10 distinct bands under short UV, long UV, and white light scanning of the post-derivatized plate, respectively. The presence of violet-colored bands confirmed the presence of terpenoids and essential oils, which is consistent with the findings from phytochemical analysis and the compounds identified in the GC-MS analysis[35]. GC-MS analysis of *Roghan Banāfshā* identifies various constituents that have been shown in the literature to have anti-inflammatory, antibacterial, anxiolytic, antioxidant,

and antiproliferative properties. The suggested sedative effect of *Roghan Banāfshā* may be attributed to the presence of 3-carene and its absorption enhancing property. The study on hypnotic effects of 3-carene shows that oral administration of 3-carene increases sleep duration and reduces sleep latency in a pentobarbital-induced sleep test, which is possibly due to positive regulation of GABAA receptors interacting at the benzodiazepine-binding site[19]. Oleic acid has been detected in substantial quantities sesame oil-based *Roghan Banāfshā'*, activates SIRT1 and boosts antioxidant enzymes, promoting longevity, stress resistance and anti-inflammatory effect. Oleoylethanolamide, a derivative of oleic acid is a recognized PPARα ligand involved in energy control and lipid metabolism[21]. Similar reports of anti-spasmodic, immunomodulatory, antioxidant, and antiproliferative pharmacological effects have been made for other detected components, such as fatty acid esters 9-octadecenoic acid, 1,2,3-propanetriyl ester and derivatives of ascorbic acid l-(+)-Ascorbic acid 2,6-dihexadecanoate[20,22]. Compound 9-octadecenoic acid, (Z)-2,3-dihydroxypropyl ester (monoolein), often referred to as a "magic lipid," is acknowledged for its surfactant and emulsifying properties, and it serves as an enhancer for drug delivery[22,25]. The tested sample didn't show the presence of Enterobacteriaceae, *Escherichia coli*, Salmonella Spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total bacterial count, total fungal count, total aflatoxins, and heavy metals, such as arsenic, cadmium, lead and mercury were found to be within the permissible limits as mentioned[30].

In the clinical study, the demographic data has shown that 42% of patients with primary insomnia belonged to the middle age group, while 33% of patients belonged to the old age group, which suggested that primary insomnia is prevalent in the middle and old age groups. The data also revealed that the female gender was found to be commonly affected with primary insomnia. The gentle massage with *Roghan Banāfshā'* over the scalp and forehead significantly increased the total sleeping duration (<0.05). The associated features with insomnia, such as tiredness, irritability, and problems in concentration, were also observed to be significantly reduced (<0.05). The insomnia severity index was found to be markedly decreased (<0.05). The overall findings revealed that Roghan *Banāfshā'* along with lifestyle and dietary modifications significantly improved primary insomnia.

A randomized controlled clinical trial has revealed that the irrigation with *Roghan Banāfshā'* over the forehead and scalp for a few minutes significantly relieved insomnia as compared to the comparator[34]. Some scientific studies carried out on human beings and animals suggested that either *Viola odorata* flowers' extract or its oil significantly induced sleep and improved the total sleep duration and its quality as compared to conventional drugs[35-38]. Gamma-amino butyric acid (GABA) is an important inhibitory neurotransmitter found in the tissues of the central nervous system. The ligands that stimulate GABA receptors exert anxiolytic, sedative, and hypnotic effects. Studies have confirmed that many flavonoids found in plants have an affinity to bind with GABA receptors. The phytochemical analysis of *Viola odorata* flowers has shown that an important flavonoid, rutin produces remarkable anxiolytic and sedative effects through GABAergic neurotransmission[39,40]. In the present report, the bioactive compounds identified in *Roghan Banāfshā'*, such as 3-carene and dodecahydro-as-indaceno [4,5-b] oxirene exhibited sedative, anxiolytic, hypnotic, and anaesthetic properties[20]. It is pertinent to mention that the study was conducted on a single group of patients, hence the findings may be considered as preliminary evidence. Further, the clinical efficacy may be evaluated in a large sample size with taking comparator groups like modern drugs, placebo, etc., for drawing definite inferences.

Conclusion

All quality control parameters of the Unani oil were found to be within permissible limits, confirming the quality. The fingerprinting profile of HPTLC and GC-MS of *Roghan Banāfshā'* may serve as reference standards for its future authentication. The preliminary clinical outcomes suggested that further randomized clinical trials with including comparators may give some productive conclusions.

Limitations and future prospects

The standardization and quality control of a single batch of *Roghan Banāfshā* were carried out, hence the data is lacking in inter-batch variability, it is therefore suggested that the same may be conducted with taking minimum three batches of *Roghan Banāfshā* in future. The significant clinical outcomes of the

present study based on preliminary evidence provide a substantial lead for further clinical exploration with including comparator groups and objective parameters like actigraphy, polysomnography, or sleep diaries with standardized metrics for drawing better scientific inferences.

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Author Contributions

APA: Carried out the whole clinical trial; Writing original manuscript; Data curation; Data analysis; Investigation; Conceptualization. NZA: Writing, review and editing. MSA: Performed quality control, HPTLC and GC-MS; Writing original manuscript. AKM: Performed quality control and HPTLC; Writing original manuscript. HMA: Review and editing.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

The ethical approval for the clinical study was obtained from the Institutional Ethics Committee of Regional Research Institute of Unani Medicine, Chennai, vide reference 2-21/IEC/Tech/RRIUM/ 2020-21/740.

Informed Consent

The written informed consent was obtained from all the participants before their enrollment in the study. All the participants were well informed about the nature, aims, and procedures of the clinical trial. The confidentiality of all the participants was maintained.

Use of artificial intelligence (AI)

Artificial intelligence (AI) was not used while writing the manuscript.

Data Availability

The raw data related to the study will be providing on request.

References

1 Muyumba N W, Mutombo S C, Sheridan H, Nachtergael A & Duez P, Quality control of herbal drugs and preparations: The methods of analysis, their relevance and applications, *Talanta Open*, 4 (2021). 100070. https://doi.org/10.1016/ j.talo.2021.100070.

2 Anonymous, *National formulary of Unani medicine, Part 1*, New Delhi: Dept. of AYUSH, Ministry of H & FW, Govt. of India, (2006) 192.

3 Benjamins J S, Migliorati F, Dekker K, Wassing R, Moens S, *et al*., Insomnia heterogeneity: Characteristics to consider for data-driven multivariate subtyping, *Sleep Med Rev*, 36 (2017) 71-81. http://dx.doi.org/10.1016/j.smrv.2016..005.

4 Billiard M & Bentley A, Is insomnia best categorized as a symptom or a disease, *Sleep Med*, 5 (2004) S35-S40. DOI: 10.1016/j.sleep.2004.04.000.

5 Reeve K & Bailes B, Insomnia in adults: Etiology and management, *J Nurse Pract*, 6 (1) (2010) 53-60. https://doi.org/10.1016/j.nurpra.2009.09.013.

6 Monti J M, Primary and secondary insomnia: Prevalence, causes and current therapeutics, *Curr Med Chem - Central Nervous System Agents*, 4 (2004) 119-137.

7 Lancel M, Boersma G J & Kamphuis J, Insomnia disorder and its reciprocal relation with psychopathology, *Curr Opin Psychol*, 41 (2021) 34-39. https://doi.org/10.1016/ j.copsyc.2021.02.001.

8 Sanjay T V, Thejaswini P, Vinay J, Nandin R C, Kavya U, *et al*., Prevalence of insomnia and its associated factors among aged population in an urban locality of Bengaluru, Karnataka, India- A Cross-sectional study, *J Clin Diagn Res*, 17 (2023) 6-10. DOI: 10.7860/JCDR/2023/58991.17639.

9 Lee K H, Lee H G, Kwon S, Park S U, Jung W S, *et al*., Effect of Kami Guibi-tang (KGT) in elderly subjects with insomnia: a study protocol from a single center, randomized, double-blind, placebo-controlled trial, *BMC Complement Med Ther*, 23 (2023). DOI: 10.1186/s12906-023-04199-y.

10 Shergis J L, Hyde A, Meaklim H, Varma P, Da Costa C, *et al*., Medicinal seeds *Ziziphus spinosa* for insomnia: A randomized, placebo-controlled, cross-over, feasibility clinical trial, *Complementary Ther Med,* 57 (2021). DOI: 10.1016/j.ctim.2020.102657.

11 Ismail B A, Nawab M, Fatima S H, Minhajuddin A & Naikodi M A R, Evaluation of the efficacy and safety of Unani Formulations in Pityriasis Versicolor: A randomized controlled trial, *J Ethnopharmacol*, 298 (2022). https://doi.org/10.1016/j.jep.2022.115603.

12 Harborne J B, *Phytochemical Methods, ed 3rd*, London: Chapman and Hall, (1998) 4-278.

13 Arokiarajan M S, John Christopher J, Akram S A W, Ahmed Z N, Meena R, *et al*., Phyto-chemical, analytical evaluation and biological attributes of rare medicinal species of flowering shrub *Bauhinia racemosa* L., *Adv Tradit Med*, 24 (2024) 1181-1203. https://doi.org/10.1007/s13596-024-00761-2.

14 Anonymous, *The Unani Pharmacopeia of India, Part II, Vol. III*, New Delhi: Ministry of Ayush, Govt. of India, (2016) 124-135.

15 World Health Organization, *WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues*, Geneva: WHO Press, (2007) 1-118.

16 Feng P, Weagant S D & Jinneman K, BAM Chapter 4A: Diarrheagenic *Escherichia coli,* Bacteriological analytical manual, U. S. Food & Drug Administration (2009), Available at: https://www.fda.gov/food/laboratory-methods-

food/bam-chapter-4a-diarrheagenic-escherichia-coli. [Accessed on 22nd July 2024]

17 Kancherla M, Shamya M A, Parvez A A, Ahmed N Z, Meena R, *et al*., Physicochemical, heavy metal analysis, HPTLC, Gas Chromatography-Mass spectrometry, antibacterial and antioxidant activity of an ethanol extract of *Viola pilosa* Blume whole plant, *J Herb Med*, 42 (2023). https://doi.org/10.1016/j.hermed.2023.100812.

18 Vasantharaja R, Abraham L S, Inbakandan D, Thirrugnasambandam R, Senthilvelan T, *et al*., Influence of seaweed extracts on growth, phytochemical contents and antioxidant capacity of Cowpea (*Vigna unguiculate* L. Walp), *Biocatal Agric Biotechnol*, 1 (2019) 589-594.

19 Garg J, Ghoshal G, Bhadada S K & Katare O P, Derivatisation mechanistic-guided identification of phytoconstituents of different extracts of *Cissus quadrangularis* by TLC and standardization by HPTLC, *Phytomed Plus*, 4 (3) (2024) 1-11. https://doi.org/10.1016/j.phyplu.2024.100601.

20 Woo J, Yang H, Yoon M, Gadhe C G, Pae A N, *et al*., 3-Carene, a phytoncide from pine tree has a sleep-enhancing effect by targeting the GABAA-benzodiazepine receptors, *Exp Neurobiol*, 28 (2019) 593-401. DOI: 10.5607/en.2019.28.5.593.

21 Martínez-Lobos M, Silva V, Villena J, Jara-Gutiérrez C, Vera Quezada W E, *et al*., Phytoconstituents, antioxidant activity and cytotoxicity of *Puya chilensis* Mol. Extracts in Colon Cell Lines, *Plants*, 13 (2024). DOI.org/10.3390/plants13212989.

22 Santa-María C, López-Enríquez S, Montserrat-de la Paz S, Geniz I, Reyes-Quiroz M E, *et al*., Update on anti-inflammatory molecular mechanisms induced by oleic acid, *Nutrients*, 15 (1) (2023). DOI: 10.3390/nu15010224.

23 Al-Marzoqi A H, Hadi M Y & Hameed I H, Determination of metabolites products by *Cassia angustifolia* and evaluate antimicrobial activity, *J Pharmacognosy Phytother*, 8 (2) (2016) 25-48. DOI: 10.5897/JPP2015.0367.

24 Yomica L, George P & Mohammed F K, GC-MS analysis of the bioactive phytoconstituents of various organic crude extracts from the seed kernels of *Manilkara bidentata* (balata) collected in Trinidad, W.I, *Nat Prod Res*, 32 (3) (2018) 358-361. https://doi.org/10.1080/14786419.2017.1354192.

25 Ganem-Quintanar A, Quintanar-Guerrero D & Buri P, Monoolein: a review of the pharmaceutical applications, *Drug Dev Ind Pharm* 26 (8) (2000) 809-820. DOI: 10.1081/ddc-100101304.

26 Kulkarni C V, Wachter W, Iglesias-Salto G, Engelskirchen S & Ahualli S, Monoolein: a magic lipid, *Phys Chem Chem Phys*, 13 (8) (2011) 3004-3021. DOI: 10.1039/c0cp01539c.

27 Neethu S, Veena S K, Indulekha V C, Eapen J & Radhakrishnan K V, Phytoconstituents assessment and development of standardization protocol for 'Nayopayam Kwatha', a polyherbal Ayurvedic formulation, *J Ayurveda Integr Med*, 12 (2021) 489e499. https://doi.org/10.1016/j.jaim.2021.05.002.

28 Sajjadi B, Raman A A & Arandiyan H, A comprehensive review on properties of edible and non-edible vegetable oil-based biodiesel: Composition, specifications and prediction models, *Renew Sustain Energy Rev*, 63 (2016) 62-92. http://dx.doi.org/10.1016/j.rser.2016.05.035.

29 Lvanova M, Hanganu A, Dumitriu R, Tociu M, Lvanov G, *et al*., Saponification value of fats and oils as determined from 1 H-NMR data: The case of dairy fats, *Foods*, 11 (2022). https://doi.org/10.3390/foods11101466.

30 Sruthi N U, Premjit Y R, Pandiselvam, Kothakota A & Ramesh S V, An overview of conventional and emerging techniques of roasting: Effect on food bioactive signatures, *Food Chemistry*, 348 (2021). https://doi.org/10.1016/j.foodchem.2021.129088.

31 Anonymous, *The Unani Pharmacopeia of India, Part II, Vol. I,* New Delhi: Ministry of Ayush, Govt. of India, (2009), 120-121, 169, 184, 197.

32 Manallack D T, Prankerd R J, Yuriev E, Oprea T I & Chalmers D K, The significance of acid/ base properties in drug discovery, *Chem Soc Rev*, 42 (2013) 485-496. DOI:10.1039/c2cs35348b.

33 Akula P & Lakshmi P K, Effect of pH on weakly acidic and basic model drugs and determination of their *ex vivo* transdermal permeation routes, *Braz J Pharm Sci*, 54 (2018). http://dx.doi.org/10.1590/s2175-97902018000200070.

34 Taherzadeh Z, Khaluyan H, Iranshahy M, Rezaeitalab F, Ghalibaf M H, *et al*., Evaluation of sedative effects of an intranasal dosage form containing saffron, lettuce seeds and sweet violet in primary chronic insomnia: a randomized, double-dummy, double-blind placebo controlled clinical trial, *J Ethnopharmacol*, 15 (262) (2020). DOI: 10.1016/j.jep.2020.113116.

35 Huang S, Huang Q, Zhou Z, Zhang J, Zhan Y, *et al*., The efficacy of *Viola odorata* extract in the treatment of insomnia: A systematic review and meta-analysis, *Front Neurol*, 13 (2022). https://doi.org/10.3389/fneur.2022.730311.

36 Feyzabadi Z, Ghorbani F, Vazani Y & Zarshenas M M, A critical review on phytochemistry, pharmacology of *Viola odorata* L., and related multipotential products in traditional Persian medicine, *Phytother Res*, 31 (11) (2017) 1669-1675. DOI: 10.1002/ptr.5909.

37 Feyzabadi Z, Jafari F, Kamali S H, Ashayeri H, Aval S B, *et al*., Efficacy of *Viola odorata* in treatment of chronic insomnia, *Iranian Red Crescent Med J*, 16 (12) (2014) e17511. DOI: 10.5812/ircmj.17511.

38 Rezaie A, Issabeaglou E, Zakhireh S & Hosseinpour S, Evaluation of sedative and pre-anesthetic effects of *Viola odorata* Linn. extract compared with diazepam in rats, *Life Sci J*, 19 (8) (2022) 30-37.

39 Taherzadeh Z, Khaluyan H, Iranshahy M, Rezaeitalab F, Ghalibaf M H, *et al*., Evaluation of sedative effects of an intranasal dosage form containing saffron, lettuce seeds and sweet violet in primary chronic insomnia: a randomized, double-dummy, double-blind placebo controlled clinical trial, *J Ethnopharmacol*, 15 (262) (2020). DOI: 10.1016/j.jep.2020.113116.

40 Huang S, Huang Q, Zhou Z, Zhang J, Zhan Y, *et al*., The efficacy of *Viola odorata* extract in the treatment of insomnia: A systematic review and meta-analysis, *Front Neurol*, 13 (2022). https://doi.org/10.3389/fneur.2022.730311