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Standardization and preliminary characterization of an ayurvedic stress-relieving head massage oil of *Nardostachys jatamansi* DC

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ABSTRACT

Background: Jatamansi/Nardostachys jatamansi (NJ) is an important aromatic shrub widely used by Ayurvedic practitioners for centuries due to its usefulness in intellect-enhancing (*Medhya*), strengthening (*Balya*), and skin disorders. Several classical dosage forms like hot or cold infusion, decoction, distillate, powders, etc. have been mentioned for NJ. Clinical trials of Jatamansi Oil (JO) as a head massage conducted by clinicians and therapists have shown encouraging results in de-stressing/stress management of cancer patients through head anointing treatment.

Objective: Such effective proprietary formulation needs assessment of its characteristics using modern analytical technologies to comprehend the Ayurvedic concept of dermal pharmacology.

Materials and methods: Triplicate batches of JO were prepared by evaporating its decoction in sesame oil (SO). Basic physicochemical analysis of the raw material, in-process samples, and finished products was carried out to develop a monograph. Further, raw SO and finished product JO were subjected to TLC, and extracted in hexane and dichloromethane separately for Gas Chromatography-Mass Spectrometry (GC-MS) analysis to profile several bioactive molecules from NJ in the final product, JO.

Results: Standard Operating Procedure was developed and a basic monograph was prepared for JO. GC-MS analysis revealed several phytocompounds dissolved/dispersed in SO after processing, while 18 additional distinct peaks were observed in JO as compared to SO.

Conclusion: This preliminary analysis supports the Ayurvedic concept of lipid-based formulations. The plausible phytocompounds anticipated based on retention times can be further quantified and studied for their probable action as anointing treatment. A detailed experimental strategy for understanding the phytochemical changes during the entire process needs to be planned and performed.

1. Introduction

Jatamansi vis'-a-vis' *Nardostachys jatamansi* (NJ) having natural habitat in Alpine Himalayas at 3000–6000 m altitude is a widely used herb by Ayurvedic practitioners. It has been ascribed with bitter (*tikta*), astringent (*kashaya*), sweet (*madhur*) tastes; cool (*shita*) potency; sweet (*madhur*) after-digestion effect; and light (*laghu*), unctuous (*snigdha*), aromatic (*sugandhi*) as properties. It pacifies *Tridosha*, has memory (*medhya*), complexion (*kanti/varna*), strength (*bala*) enhancing while burning sensation (*daha*), inflammation (*shopha*), pain (*ruja*) and toxin (*visha*) relieving actions. It is recommended in erysipelas (*visarpa*), skin diseases (*kushtha*), throat diseases (*kantharog*), hemorrhagic disorders (*rakta pitta*), wounds (*vrana*), and sinuses-fistula (*nadivrana*) [1].

Charaka Samhita has mentioned NJ in groups of herbs used for anti-pruritis (*kandughna*), restoring consciousness (*sangya sthapana*), medicated smoking (*dhumapana*), bitter tonics (*tikta skandha*) and urinary calculi (*ashmari*) [2]. In Sushrut Samhita, it is cited for fumigating infected wounds [3]. Various dosage forms of NJ like *Mansi Taila* [4] for skin disorders, *Mansyadi Ghrut* [5] for orchiditis, *Dashanga Lepa* for inflammations, *Mansyadi Lepa* for skin diseases [6], *Mansyadi Kwath* [7] for nervous diseases, *Jatamansyarka* [8], etc. have been stated in classical texts. Ayurvedic Pharmacopeia of India (API) has mentioned it as a

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sedative [8] while Indian Materia Medica has described NJ mixed with sesame oil to be rubbed on the head as a nerve sedative [9].

Jatamansi Taila (manufactured by Atharva Nature Healthcare Pvt Ltd; Food and Drugs Administration Department, Maharashtra State approved product no.- 417809) is available in the market and has been reported to be studied as a head massage oil for de-stressing []. In Ayurveda, four types of head anointing techniques (*Murdha/Murdhni Taila*) viz., smearing and massaging (*shiro-abhyang*); pouring oil (*shiro-dhara*); placing oil-soaked cloth (*shiro-pichu*) and making oil to stand (*shiro-basti*) have been mentioned [10]. In a recent study, Triple Negative Breast Cancer (TNBC) patients were treated with *Jatamansi Taila*/Oil (JO) as adjunct Ayurvedic treatment- *Shirodhara* therapy against psychological triggers as aggravating factors. JO used as relaxation therapy showed better psychological outcomes leading to one of the important parameters for improved quality of life in TNBC patients [11]. In another ongoing registered clinical trial, JO *Shiropichu* has been included in the TNBC patient treatment protocol as a stress reliever [12]. Such medicated oils have a supreme penetration potency through the scalp thereby carrying the dispersed/dissolved phytocompounds in the head and neck blood circulation. However, such medicated oils need to be studied for the presence of bioactive phytocompounds from their respective plant.

Several phytochemical studies on NJ using GS-MS have been conducted to date. Petroleum ether, chloroform, and ethanol extracts exhibited 61 compounds such as actinidine, indane, aristolene, gurjuenen, valencene, globulol, betapatachoulene, etc. [13]. Aqueous methanolic extract showed the presence of Seychellene, Acenaphthylene, Patchouli alcohol, Jatamansone, 1-methyl-4-chloro-3,5-dimethoxy-1H--pyrazole, Illudol, and 3,6,6,7b-tetramethyl decahydro-1H-cyclobuta[e] endene-3-ol [14]. Metabolites viz., Calarene, Vardiflorene, α- Panasinsen, α- Santalene, γ-Himachelene, Jatamansone, Ionol 4, 2,2,7,7-Tetramethyl tricyclo[6,2,1,0 (1,6)] undec-4-ene-3-one, Epiglobulol, and Resibufogenin were identified in volatile oil of NJ [15]. GC-MS has been widely used for the characterization of Ayurvedic and Siddha formulations such as oils/ghee [16,17], *guggulu* (*Commiphora wightii* resin formulations) [18], and *asava-arishta* (hydroalcoholic formulations) [19]. The importance of medicated oils as prepared by Ayurvedic methods has been previously studied indicating high stability and recognizable changes in the finished products containing specific chromatographic changes [20]. This highlights the importance of standardization of medicated Ayurvedic oils essential for their bioactivity and wider acceptance [21].

A preliminary attempt was, therefore, made in the present study wherein JO was prepared by a Standard Operating Procedure, quality tested, and analyzed by TLC and Gas Chromatography-Mass Spectrometry (GC-MS) to trace out the probable phytocompounds of NJ dispersed/dissolved in sesame oil. The outcomes of this primary report will help in conducting further mechanistic studies targeting the probable phytometabolites of NJ responsible for therapeutic use and detailed experimental design may warrant more interesting findings.

2. Material and methods

2.1. Raw materials

Dried rhizomes of NJ were obtained from Om Sai Traders, Delhi, India. Authentication was done by the Indian Drug Research Association and Laboratory, Pune to comply with the Ayurvedic Pharmacopoeia of India (API), and the specimen was deposited at the institutional repository (Voucher No.: BSDT/V-31). Edible Sesame Oil (SO) was purchased from Pitambari Products Pvt Ltd, Thane, India.

2.2. Instruments, standards, and reagents

The decoction and oil were assessed for temperature by a non-contact digital infrared thermometer having a range between room temperature to 1000 °C with an accuracy of ±3 °C (Gain Express Holding Ltd., Hong Kong).

2.3. Preparation of formulation

A standard procedure [6] for JO preparation was adopted with minor modifications (Fig. 1) encompassing three major steps viz., soaking, decoction, and oil simmering, completed within a period of three days. These were commercial-sized batches (n=3) manufactured in the R&D section of Atharva Nature Healthcare Pvt Ltd, Pune.

2.3.1. Decoction of JO

NJ coarse powder was soaked in potable water in a ratio of 1:8, respectively; for 16 h (i.e., 5 Kg NJ in 40 L water). The next day, it was boiled in a stainless steel (316 SS) vessel by maintaining 90–100 °C of temperature to reduce water to half (20 L out of 40 L. It was filtered immediately through sieve no. 40 (355 μm) to get JO decoction (n = 3).

2.3.2. Preparation of JO

A tin-coated brass vessel of 70 L capacity was used to prepare JO. Equal parts of SO (v/v) were added in JO decoction i.e., 20 L NJ decoction with 20 L SO, and heated at 90–100 °C till complete evaporation of water content. A duration of 6 h was required to attain the endpoint of the finished product. It was judged by the classical tests of *sneha siddhi* and *madhyam paka* [6] mainly by conforming the moderately hard consistency residue of the undissolved/undispersed matter from NJ decoction. Finally, JO was filtered in hot conditions (80 °C) by using a two-layered muslin cloth and cooled to room temperature (n = 3).

2.4. Basic physicochemical analysis

Raw material, intermediate and finished products were tested by organoleptic characters [6]; physicochemical tests like loss on drying (LOD), pH, specific gravity, refractive index, extractive values; ash, and acid insoluble ash values; as well as lipid parameters like saponification, iodine (iodine monochloride method), acid, peroxide, and un-saponifiable values as per the guidelines for oil preparation in 'The Ayurvedic Pharmacopoeia of India' [22,23].

2.5. Chromatographic analysis

2.5.1. Thin Layer Chromatography

Alcoholic extracts of raw NJ and NJ decoction, while ethanolic extract of JO re-extracted in petroleum ether (80:20), all were spotted on a pre-coated plate of Silica Gel $G60F_{254}$ and Thin Layer Chromatography (TLC) was carried on by adding mobile phase toluene: ethyl acetate (9:1), under standard conditions [23]. Chromatograms were visualized under short UV (254 nm), and long UV (365 nm) wavelengths pre- and post-spraying with anisaldehyde sulphuric acid reagent or treating with iodine vapors.

2.5.2. Gas Chromatography-Mass Spectrometry analysis

GC-MS analysis of JO and SO was performed on a Gas Chromatography system (7890B) attached with Agilent Mass Selective Detector-5977A (Agilent Technologies, CA, USA). Extraction was done by two methods viz., in dichloromethane (DCM) and hexane solvent systems, separately. For extraction, 1 ml sample of each was mixed with 5 ml of DCM/hexane for 1 h at room temperature (~25 °C), then dehydrated by using anhydrous Na_2SO_4 and finally concentrated to 1 ml. Extracts were incubated overnight at −80 °C and then centrifuged (10,000 rpm) at 4 °C for 15 min to extrude lipids with high molecular weight. GsBP-5MS column (General Separation Technologies, Newark, DE) with dimensions 30 m*0.32 mm i.d.*0.25 μm film thickness was used. For DCM extract, carrier gas Helium (He) with 1 ml/min, and for hexane extract, He with 1.22 ml/min flow rates were used. For DCM extract, oven temperatures were 40 °C for 5 min, raised to 180 °C at 5 °C/min

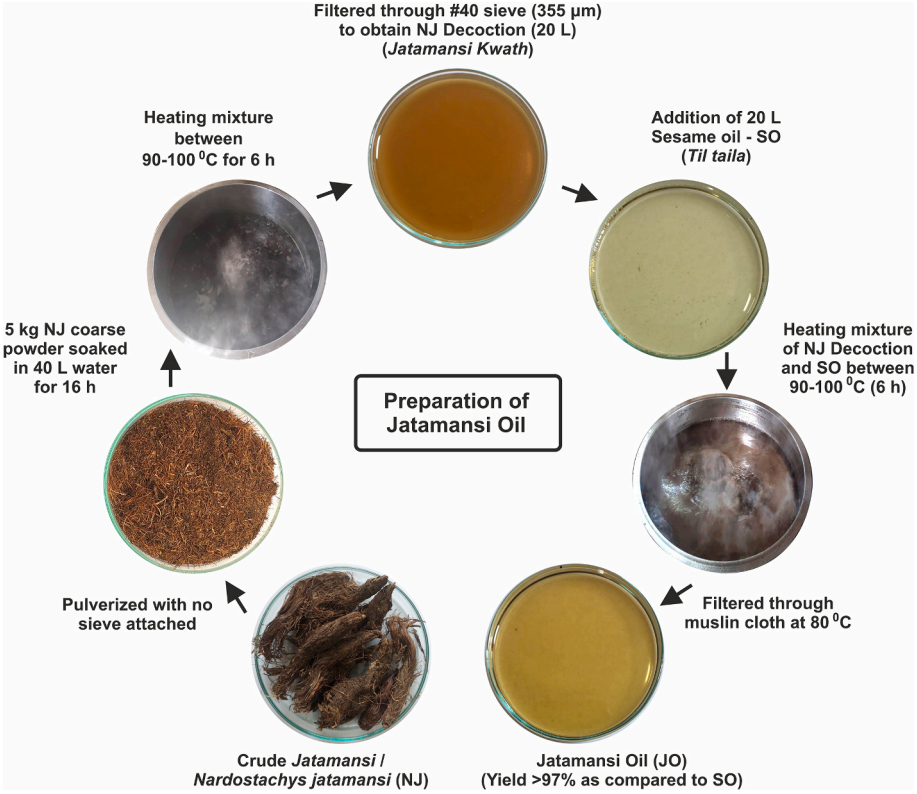

Fig. 1. Steps involved during the traditional manufacturing process of Jatamansi Oil (JO) with pharmaceutical details such as ratio, temperature, duration, sieve size and yield for quality control and assurance.

followed by an increase to 280 °C at the rate of 20 °C/min and held at 280 °C for 5 min; with injector temperature at 200 °C, and detector temperature at 250 °C. Similarly, for hexane extract, the oven temperature was 50 °C (5 min hold), the temperature was ramped to 100 °C with a ramp rate of 50/min, then increased up to 220 °C at a 20/min ramp rate and finally held for 10 min at 220 °C with injector temperature of 230 °C and detector temperature at 220 °C. In both cases, a 2 µl sample per run was injected. The spectra of compounds detected were matched with the National Institute of Standards and Technology (NIST) 2011 and Wiley (10th edition) GC-MS mass spectral libraries for identification.

2.6. Safety parameters

Microbial, fungal, and specific pathogens load, as well as heavy metals viz., Hg, As, Cd, and Pb were assessed using standard protocol [23].

2.7. Statistical analysis

The data of physicochemical parameters were processed for deriving mean ± SD (standard deviation) or mean ± SEM (Standard Error of the Mean) in Microsoft Excel 2010.

3. Results

3.1. Raw material analysis

Raw *Jatamansi* was authenticated as *Nardostachys jatamansi* (D.Don) DC. from the family Caprifoliaceae. The dried rhizome of NJ was externally black, internally dark brown, cylindrical, covered with black fibers forming a network of skeletons of leafy bases, fractured brittle, and strongly aromatic. It exhibited physicochemical parameters as a loss on drying at 105 °C- 10.15, total ash- 8.60, acid insoluble ash value - 2.16, water-soluble extractive value - 6.75, and alcohol soluble extractive value - 4.35 %w/w. Thin layer chromatography showed seven spots at *Rf*: 0.15 (fluorescent blue), 0.32, 0.39 (both yellow), 0.47 (fluorescent blue), 0.67 (yellow), 0.78 (pink), 0.82 (blue) under UV-light at 365 nm while eight spots at *Rf*: 0.13 (brown), 0.32 (blue) 0.42, 0.47, 0.49, 0.54 (all pink violet), 0.80, 0.88 (both blue) after spraying anisaldehyde sulphuric acid reagent.

Raw material SO was a slightly pale yellow colored viscous oil with a refractive index of 1.546, a specific gravity of 0.9148, iodine value - 63.82, acid value - 3.17, saponification value - 222.89, while unsaponifiable matter- 1.73 and moisture content as 0.49 %w/w. Cotton seed oil was absent. Water used for decoction had physical properties as color, 1 Hazen unit; agreeable odor; turbidity, 1 Nephelometric Turbidity Unit; pH, 7.8 and conductivity, 0.054 while on chemical analysis had total hardness as $CaCO_3$, 10; calcium hardness, 2; magnesium as Mg, 1; total solids, 40; total dissolved solids, 40; total suspended solids, nil; chlorides (Cl^-), 10; sulfate (SO_4^{-2}), 5; P-alkalinity, nil and M-alkalinity, 5 (all mg/L). Heavy metals were lead, <0.005; arsenic, <0.01; mercury, <0.0005 and chromium (Cr^{+6}), <0.04 (all mg/L), while total coliforms (most probable number) and *Escherichia coli*, both were absent, (0 cfu/100 ml).

3.2. In-process analysis

One part of coarsely powdered raw NJ was soaked in 8 parts of potable water and decoctioned as one-half (*ardhavashishta*) i.e., 4 parts were prepared [6]. Four parts of SO were added to this decoction (4 parts) to obtain JO. Hence, for a commercial batch of JO, NJ- 5 kg, water- 40 L, decoction- 20 L and SO- 20 L were used. Decoctions were light brown colored, opaque, watery liquids with a characteristic smell of NJ. The readings of pH, specific gravity, and TLC of NJ decoction as an intermediate step are illustrated in Table 1.

Table 1
Physicochemical and chromatographic analysis of *Nardostachys jatamansi* (NJ) decoction (NJ coarse powder 1 part + water 8 parts = boiled to reduce half) used for manufacturing of Jatamansi Oil (JO).

Decoction	pH at 25 °C	Specific gravity	Thin Layer Chromatography (TLC)	
			Observation under UV visible light at 365 nm	Observation after anisaldehyde sulphuric acid reagent spraying
NJ-D1	4.73	1.0040	03 spots at R_f: 0.18, 0.32 (both yellow), 0.41 (fluorescent blue)	08 spots at R_f: 0.051, 0.09, 0.17, 0.32 (all violet), 0.41 (orange), 0.45, 0.65 (both blue), 0.80 (brown)
NJ-D2	4.70	1.0037	03 spots at R_f: 0.17, 0.32 (both yellow), 0.44 (fluorescent blue)	08 spots at R_f: 0.049, 0.08, 0.18, 0.32 (all violet), 0.43 (orange), 0.47, 0.67 (blue), 0.78 (brown)
NJ-D3	4.68	1.0041	03 spots at R_f: 0.18, 0.33 (both yellow), 0.40 (fluorescent blue)	08 spots at R_f: 0.052, 0.09, 0.18, 0.33 (all violet), 0.41 (orange), 0.46, 0.66 (both blue), 0.77 (brown)
Mean ± SD	4.703 ± 0.025	1.0039± 0.0002	03 spots at *Rf*: 0.18 (0.17–0.18), 0.32 (0.32–0.33)- both yellow, 0.42 (0.40–0.44)- fluorescent blue	08 spots at R_f: 0.05 (0.049–0.052), 0.09 (0.08–0.09), 0.18 (0.17–0.18), 0.32 (0.32–0.33)- all violet, 0.42 (0.41–0.43)- orange, 0.46 (0.45–0.47), 0.66 (0.65–0.67)- both blue, 0.78 (0.77–0.80)- brown

Ayurvedic assessment for the endpoint in the JO manufacturing was done by the appearance of froth (*phenodgama*), making a wick out of the decoction residue by rolling between the finger (*varti pariksha*) and lightning the oil-soaked wick to assure no cracking sound generated (*shabdahinata*) to confirm complete moisture evaporation at the end of the process [6]. JO was a thick, yellow, viscous liquid with a characteristic odor. The average percent pharmaceutical yield of JO was 98.1 ± 0.5 % while the loss was 1.92 ± 0.52% v/v as detailed in Table 2.

3.3. Finished product analysis

Basic physicochemical, safety, and TLC analysis for $n = 3$ batches, while GC-MS analysis (n = 1) of a representative batch of finished product JO was carried out. The results of basic physicochemical and safety parameters are given in Table 3 while that of TLC of JO are displayed in Table 4. All the parameters were very close to each other for all the batches. Some within-limit variation was noticed only in peroxide and iodine values in JO, which could be considered as a range for this oil formulation [23].

The details of peaks obtained in GC-MS analysis of JO and SO done using two solvent systems are exhibited in Supplementary File Table S1. A total of 25 peaks were detected in the hexane extract of JO as compared to eight in SO. Similarly, a total of 34 peaks were detected in the DCM extract of JO as compared to 17 in SO. The retention time of compounds detected in JO and SO were compared and the segregation of data was carried out as demonstrated in Table 5.

Peaks likely originate from sesame oil if they are present in both samples (JO and SO) and both solvents (A), or if they are present only in the SO sample but detected in both solvents (partially in B). while those present in only JO in both the solvents but not in SO are from NJ raw material (partly in B). Similarly, those present only in the SO samples but in any of the solvents (C) may have been from sesame seeds or due to the chemical reaction of sesame compounds with either of the solvents during the preparation process. However, a total of 18 peaks i.e., 12 from both the solvents (D) and 6 from DCM (E) only in JO samples clearly represent phytocompounds dispersed/dissolved from the raw

Table 2
The pharmaceutical yield of commercial sized batches of *Jatamansi* Oil (JO) with respect to Sesame oil used as base.

Batch	Quantity of NJ	Quantity of SO	Yield	% Yield	Loss	% Loss
	Kg	L	L	v/v	mL	v/v
JO1	5.0	20.0	19.50	97.5	500	2.50
JO2	5.0	20.0	19.65	98.25	350	1.75
JO3	5.0	20.0	19.70	98.5	300	1.50
Mean ±SD			19.62 ± 0.10		383 ± 104	

NJ: *Nardostachys jatamansi* coarse powder, SO: Sesame oil.

Table 3
Basic physicochemical and safety parameter testing of Jatamansi Oil (JO) as per Ayurvedic Pharmacopoeia of India.

Batch	JO1	JO2	JO3	Mean ± SEM
Description	Slightly yellowish colored thick viscous liquid			
Specific gravity	0.9186	0.9213	0.9183	0.9194 ± 0.001
Refractive index	1.546	1.5455	1.547	1.546 ± 0.0004
Acid value	2.851	2.996	2.879	2.909 ± 0.04
Saponification value	134.84	149.93	147.83	144.2 ± 4.72
Ester value[a]	131.99	146.93	144.95	141.30 ± 4.69
Iodine value	92.07	74.68	138.72	101.82 ± 19.12
Peroxide value	0.57	1.44	2.03	1.34 ± 0.42
Microbial count	NMT 10^5			
Fungal count	NMT 10^3			
Heavy metals (ppm)	Hg- Nil, Cd- Nil, As- Nil			
	Pb- 1.404	Pb- 1.404	Pb- 1.119	1.309 ± 0.095
Specific pathogens	Absent	Absent	Absent	
Aflatoxins	Absent	Absent	Absent	

[a] Ester value = Saponification value - Acid value; NMT: Not More Than; Hg- Mercury, Cd- Cadmium, As- Arsenic, Pb- Lead.

Table 4
Thin Layer Chromatographic analysis of ethanolic extract of Jatamansi Oil (JO) re-extracted in Pet-ether

Visualization	JO1	JO2	JO3
Under UV 254 nm	02 spots R_f: 0.32, 0.62 (both blue)	02 spots R_f: 0.33, 0.65 (both blue)	02 spot R_f: 0.35, 0.59 (both blue)
After iodine vapors treatment	04 spots R_f: 0.27, 0.32, 0.40, 0.62 (all yellow)	04 spots R_f: 0.24, 0.34, 0.40, 0.64 (all yellow)	04 spots R_f: 0.29, 0.35, 0.40, 0.63 (all yellow)
After anisaldehyde sulphuric acid spraying	04 spots R_f: 0.27, 0.32, 0.41, 0.87 (all light blue)	04 spots R_f: 0.24, 0.34, 0.40, 0.88 (all light blue)	04 spots R_f: 0.29, 0.37, 0.40, 0.92 (all light blue)

Table 5
The outcome of Gas Chromatography-Mass Spectrometry analysis based on peaks detected at various retention times for Jatamansi Oil (JO) and Sesame Oil (SO) extracted in hexane and dichloromethane (DCM)

Sr. No.	Analysis	Retention times (min)	No. of peaks (Group)
1	Peaks present in both JO and SO samples in both hexane and DCM	8.8, 8.99, 9.0, 9.4, 10.8, 11.2, 10.79, 10.87, 12.4, 12.1, 12.44, 12.51	12 (A)
2	Peaks present either in JO or SO but in both the solvents	3.74, 10.23, 16.85, 18.67, 19.55, 21.2, 22.18, 24.05, 27.02	9 (B)
3	Peaks present only in SO in either of the solvents	16.87, 17.9, 19.2, 25.4	4 (C)
4	Peaks present only in JO in both the solvents	10.5, 13.7, 14.0, 16.6, 18.5, 20.6, 20.85, 21.0, 21.4, 21.7, 22.3, 24.4	12 (D)
5	Peaks present only in JO in DCM	18.26, 21.83, 22.5, 30.63, 30.81, 30.93	6 (E)

material of NJ directly or after processing it with SO into the final product of JO.

The identified compounds based on NIST2011 and Wiley mass spectral libraries are detailed in Supplementary File Tables S1 and S2. Almost, 16 compounds were identified in JO as well as SO in common while another 16 were identified only in JO, and only one was found in SO but not in JO.

4. Discussion

Jatamansi has been included in many classical oil monographs such as *Arimedadi Taila, Bala Taila, Mahanarayan Taila, Prabhanjan Vimardan Taila* [24], *Durvadi Taila, Palankashadya Taila, Maharudra Guduchi Taila* [25], *Kubjaprasarani Taila, Guduchyadi Taila, Bruhanmarichyadi Taila, Madhyam Narayan Taila* and *Marichyadi Taila* [26] to be used in several ailments like insanity, epilepsy, head and neck diseases, syncope, gout, skin diseases, and many Vata diseases. Ayurvedic medicated lipids can be considered as an effective Lipid-Based Drug Delivery System wherein fat and water-soluble phytoconstituents get evenly distributed and absorbed [27]. They are administered by oral, nasal, dermal, rectal, per vaginal, and external routes like head massage [28]. Bi-molecular lipid matrix-made cell membranes determine membrane permeability for specific molecules, hence lipid-based formulations can be readily absorbed through the cell membrane via passive diffusion [29] even for poorly water-soluble drugs. This hydrophilic system in lipids as seen in *Taila* and *Ghruta* formulations in Ayurveda is advantageous due to their high degree of biocompatibility and versatility useful as target action in diverse disease conditions and routes of drug administration [30]. Sesame oil has properties such as heavy, cleansing, subtle, cool in touch, and augmenting; enhances stability, strength, complexion, nourishment, intelligence, and memory; is good for skin, hair, eyes, blood, and bones; heals wounds, pain, fractures and can be used as multiple modes of administration like oral, massage, enema, nasal-ear-eye instillation, etc., hence was selected for this study [1].

JO, a sesame oil-based formulation used as a topical treatment, needs to be standardized and characterized based on phytocompounds responsible for therapeutic action. In the present study, primary physicochemical, chromatographic, and safety parameters were tested for JO and SO. The monograph developed in the present study will help in undertaking further research on single herb-treated lipid-based drugs for various ailments.

Being a fibrous material, NJ was pulverized in a pulverizer by purposefully not attaching the sieve to avoid unnecessary elimination of fibers thereby controlling the attrition of aromatic compounds. For this reason, sufficient soaking time (16 h) was also given. JO being a proprietary product, the ratios mentioned were based on pilot experiments taking into account the volume and aromatic nature of *Jatamansi* rhizomes. Eight times water was used so that crude *Jatamansi* remained submerged in water throughout the decoction process and reduced to half to avoid unnecessary heat to this soft and aromatic herb. The whole manufacturing procedure was done at room temperature and in non-interacting SS vessels for NJ decoction and tin-coated brass vessels for JO preparation. The temperature of boiling mixtures was checked intermittently to ensure uniform heating while the mixtures were stirred repeatedly to avoid settling of particles to the bottom of vessels leading to charring. Sieves used for decoction filtration and two-layered muslin cloth facilitated the least suspended particles in the filtrate.

Raw NJ was found to comply with the pharmacopeial standards. It is an aromatic herb of a soft nature (*mrudu dravya*) and hence, it was heated moderately with respect to time and temperature [6]. Low pH value showed the acidic attribute of NJ decoction and specific gravity indicated moderately dense liquid probably by virtue of soluble matter. The finished product, being of the proprietary medicine category, a monograph for JO was developed as per the API parameters [31].

Final therapeutic product JO showed variations in some parameters as compared to raw SO like specific gravity increase, iodine value increase, acid value decrease, and saponification value decrease while the refractive index, un-saponifiable matter, peroxide value, and moisture content remained unaltered. These can be considered unique for JO formulation and further studies can be undertaken to assess their impact on product stability. Microbial and fungal counts within permissible limits; absence of specific pathogens and aflatoxins as well as lack of heavy metals in the finished products clearly indicated Good Manufacturing Practices were followed during the preparation [32].

TLC is a simple and widely accepted technique for the identification of the phytocompounds in plants as well as finished products and hence was used to understand the possible changes during JO preparation [33, 34]. Basic physicochemical analysis of NJ raw material complied with the values as per API. TLC, though not given in API, exhibited several prominent spots under UV 365 nm and after derivatization, indicating the presence of several phytocompounds in the raw material. However, in the NJ decoction, the pattern of TLC spots altered. Spots at R_f values 0.32 (yellow) and 0.41 or 0.47 (fluorescent blue) were retained as seen under UV at 365 nm while other spots from raw NJ were not seen. Similarly, 6 out of 8 spots at R_f values 0.13 or 0.18, 0.32 (blue/violet), 0.42 or 0.41 (pink-violet/orange), 0.47 (pink-violet/blue), 0.54 or 0.67 and 0.80 or 0.78 (all either pink violet, blue or brown) were retained in NJ decoction as compared to raw NJ while others were not seen after derivatization. Interestingly, two new spots at R_f values 0.051 and 0.09 (both violet) were additionally found in NJ decoction. Thus, simple TLC indicated that several phytocompounds were present as it is in the decoction form while few were detectable newly, probably due to certain thermal and chemical reactions during the boiling process. In another aspect of the finished product JO, wherein SO was used as a liquid medium for the extraction of NJ phytocompounds from this decoction; no spots were seen under UV at 365 nm. However, only two spots at R_f 0.34 and 0.40 matching with NJ decoction were seen in JO while two additional spots at R_f 0.27 and 0.88 were also observed. Interestingly, new two spots at R_f 0.32 and 0.63 (both blue) under UV at 254 nm, as well as four spots at R_f 0.26, 0.33, 0.40, and 0.63, were noticed after derivatization with iodine vapors. This designates that during the evaporation process of NJ decoction in SO, retention of existing while the formation of new stable phytocompounds is quite possible.

GC-MS analysis of JO *vs* SO clearly indicated that JO contained certain phytometabolites from NJ since the number of peaks was more in JO as compared to SO, in both the solvent systems. The variations in the retention times of the compounds for the hexane and DCM extracts of JO and SO (ranging between 0.01 and 0.6 min) as well as between JO and SO in both extracts (ranging between 0.1 and 0.4 min) can be considered as an acceptable experimental variation for this GC-MS analysis. The probable phytoconstituents from hexane fraction of NJ as reported earlier [35] can be listed as- Dodecane (9.62); 2-Furanmethanol, tetrahydro-5-methyl-*trans* (11.22); Linalool (13.98); L-calamenene (16.69); Neoisolongifolene,8,9-dehydro (18.13); Cyclolongifolene oxide, dehydro (20.2); 1(2H)-Naphthalenone,octahydro-4a,8a-dimethyl-7-(1-ethylethyl)-, (4aR-(4aa,7a,8aa)) (21.51); 2-tetradecenal (22.05) and Palmitic acid (30.1) against the peaks observed at RT's 10.5, 13.7, 16.6, 18.26, 20.6/20.85, 21.0/21.4/21.7/21.83, 22.3/22.5 and 30.63/30.81/30.93 min in JO (Table 4). These may be considered as the unaltered phytocompounds dispersed/dissolved in SO. Furthermore, the alterations/derivatizations of certain compounds as observed in the present study need to be studied in more detail to understand thermal and chemical modifications. Such changes, whether, bring better absorption and efficacy of the product prepared by Ayurvedic protocol would make another interesting and useful investigation. The identification analysis from mass spectral libraries (Table S2) also highlights the same possibilities that need to be further investigated by running the experiments with respective phytochemical reference standards.

Previous studies on *Shirodhara* oil prepared out of *Centella asiatica* (*Brahmi*), *Nardostachys jatamansi* (*Jatamansi*), and *Withania somnifera* (*Ashwagandha*) have shown significant relief in mood scores and stress

levels ($P < 0.001$) along with a decreased breathing rate, diastolic blood pressure, and heart rate. The relaxed alert state post-*Shirodhara* treatment was denoted by an elevation in the alfa rhythm of the Electroencephalogram [36]. Similarly, *Shirodhara* with *Brahmi* oil studied in a case series proposed it to be beneficial for moderate to severe insomnia [37]. Even, *Shirodhara* with *Ksheer Bala* Oil in generalized anxiety disorder patients depicted changes in Hamilton Anxiety Scale [38]. Another case report of a 35-year-old female suffering from sleep deprivation, loss of concentration, and irritable mood symptoms subjected to sesame oil *Shirodhara* showed significant improvement in the above-stated complaints as well as in the Profile of Mood Score and Stress biomarkers [39]. Thus, head anointing treatments like *Shirodhara* exert a state of alert calmness and can be clinically beneficial in anxiety, hypertension, and stress due to physiological as well as pharmacological effects in chronic degenerative diseases.

NJ is a well-studied herb and explored well with respect to ethnobotany, ethnomedicine, phytochemistry, and pharmacology [40]. Phytocompounds from NJ have been studied for their absorption and action in the scalp with respect to hair growth [41]. This supports the clinical use of NJ medicated sesame oil i.e., JO as head anointing treatment. The probability of certain metabolites from JO reported in this study being absorbed through the scalp and exerting the benefit as a stress reliever in cancer patients is quite high. Analytical studies on Ayurvedic lipid-based formulations have shown that the Ayurvedic method of preparation imparts pharmaceutical separation of phytocompounds like de-glycyrrization occurs when *Glycyrrhiza glabra* is similarly heated with ghee or oil [42]. Thorough pharmacokinetic and dynamic studies will help to understand the clinical effects of these phytocompounds.

This is a preliminary study probing the concept of using aromatic herbs like *Jatamansi* for making Ayurvedic lipid-based formulations. As prospects, extensive characterization needs to be done with a comprehensive experimental model along with temperature-dependent comparative analyses for complete justification of the formulation. Raw NJ, the decoction of NJ, the residue of strained NJ, and the residue of strained JO along with validation studies could be done to understand the conversions or partitioning. Similarly, primary Skin Irritation Tests, Sensitivity Tests, and biological screening like analgesic activity could be done to explore its safety and therapeutic potential.

5. Conclusion

Jatamansi/Nardostachys jatamansi is a promising herb and has been mentioned in several classical lipid-based formulations. JO is a unique single herb sesame oil-based formulation being used for head massage procedures for several ailments including cancers. The present study shows that JO exhibits certain lipid-soluble/dispersible phytocompounds in the finished product when prepared by the Ayurvedic pharmaceutical technique of *Taila Siddhi* i.e., decoction evaporated in sesame oil. Further, a comprehensive experimental design for confirmation using phytochemical reference standards of NJ could be conducted. Moreover, several *in-vivo*/clinical studies can be undertaken to understand the mechanism of JO in ailments like cancer.

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Author contribution

SBC, VSG, VVD, SSS, and SPS: Conceptualized, developed Methodology, and Visualized the data, SBC, VSG, SSS: Conducted the pharmaceutical and chromatographic experiments, curated and analyzed the data; VSG, VVD, SPS: Validated the data; SBC, SSS: Wrote the first draft of the manuscript while all the authors reviewed and edited the final manuscript.

Declaration of competing interest

JO has been partly included in a patent application, “A Herbo-Mineral Metallic Pharmaceutical Kit” application no. 202121011657 for an Indian patent and published as well as in PCT application no. PCT/IB2022/052192 and US Patent application no. 18/281,545 for Triple Negative Breast Cancer patients.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaim.2024.100900.

References

[1] Chunekar KC, Pandey GS, editors. Bhavprakash Nighantu of Bhavmishra, vol. 229. Varanasi: Chaukhamba Bharati Academy; 2013. p. 763. Karpuradi Varga.
[2] Tripathi B. In: Reprint, editor. Charaka Samhita of Agnivesha. Delhi: Chaukhamba Surbharati Prakashan; 2004. p. 82. 96, 118, 782, 634.
[3] Shastri A. In: Reprint, editor. Sushrut Samhita of Sushrutacharya, Vranitopasaniya Adhyaya: Chapter 19, Verse 29. New Delhi: Chaukhamba Publications; 2005. p. 81.
[4] Shah NC, editor. Bharat Bhaishajya Ratnakar, vol. 4. New Delhi: B Jain Publishers Pvt Ltd; 1999. p. 120.
[5] Shastri L, Shastri B, editors. Yogratnakar of Anonymous, Uttarardha; Mushka Antravruddhi Vardhma Nidan Chikitsa Adhyaya. seventh ed. New Delhi: Chaukhamba Publications; 1999. p. 139.
[6] Shastri P. In: Sharangdhar Samhita of Sharagdharacharya. fourth ed. Varanasi: Chaukhambha Orientalia; 2000. p. 144. 212, 356, 361.
[7] Yadavji T. Siddhayog Sangraha. In: Bhrama anidra Unmada Adhikar. first ed. Kolkata: Shri Baidyanath Ayurved Bhavan Ltd; 1954. p. 108.
[8] Anonymous. In: The Ayurvedic Pharmacopoeia India, Part I. first ed., vol. I. New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 1999. p. 51.
[9] Nadkarni AK, editor. Indian Materia Medica of Nadkarni KM, vol. 1. Mumbai: Popular Prakashan Private Ltd; 1995. p. 840–2.
[10] Tripati Tripathi B, editor. Ashtang Hridayam of Vagbhata, Sutrasthana; Gandushadi Vidhi Adhyaya: Chapter 22, Verse 23-26. Delhi: Chaukhamba Sanskrit Pratishthan; 2012. p. 260–1.
[11] Pathrikar A. A Clinical assessment of mind relaxation effect of Jatamansi oil Shirodhara on Chittodvega psychological distress in TNBC triple-negative breast cancer patients (PhD thesis). Pune: Tilak Maharashtra Vidyapeeth; 2019. http://hdl.handle.net/10603/294529.
[12] Clinical Trial Registry of India, New Delhi. https://ctri.nic.in/Clinicaltrials/pdf_generate.php?trialid=14318&EncHid=&modid=&compid=%27,%2714318det%27;3 (accessed on 18/May/2023).
[13] Me V, Niranjana P, Achur RN. Gas Chromatography-Mass Spectrometry analysis of bioactive components from the rhizome extract of *Nardostachys Jatamansi* DC. Asian J Pharm Clin Res 2016;9(3):115–8.
[14] Singh M, Khan MA, Kamal YT, Ahmad J, Fahmy UA, Kotta S, Alhakamy NA, Ahmad S. Effect of *Nardostachys jatamansi* DC. on apoptosis, inflammation and oxidative stress induced by doxorubicin in wistar rats. Plants 2020;9:1579. https://doi.org/10.3390/plants9111579.
[15] Singh V, Rana N, Ali M. GC-MS analysis and anti-microbial activities of volatile oil of *Nardostachys jatamansi* D.C. rhizomes obtained from Haridwar region, Uttrakhand. Pharmacog J 2018;10(2):230–4. https://doi.org/10.5530/pj.2018.2.40.
[16] Deepak M, Kumar PU, Nitha B, Balachandran I, Rema SAB. Quality parameters, fatty acid profiling and estimation of umbelliferone in grahaṇimihira tailam: an ayurvedic oil preparation. Anc Sci Life 2013;33(1):10–4. https://doi.org/10.4103/0257-7941.134557.
[17] Arunadevi R, Susila R, Murugammal S, Divya S. Preparation and standardization of Mathan Tailam: a classical Siddha formulation for diabetic ulcerative wound healing. J Ayurveda Integr Med 2020;11(1):10–5. https://doi.org/10.1016/j.jaim.2017.08.011.
[18] Balkrishna A, Tomer M, Joshi M, Gujral S, Kumar Mishra R, et al. Standardization and validation of phytometabolites by UHPLC and high-performance thin layer

chromatography for rapid quality assessment of ancient ayurvedic medicine. Mahayograj Guggul. J Sep Sci 2022;45(10):1616–35. https://doi.org/10.1002/jssc.202100935.
[19] Vinothkanna A, Sekar S. Influence of intrinsic microbes on phytochemical changes and antioxidant activity of the Ayurvedic fermented medicines: balarishta and Chandanasava. Ayu 2018;39(3):169–81. https://www.ayujournal.org/text.asp?2018/39/3/169/255252.
[20] Lahorkar P, Ramitha K, Bansal V, Anantha NDB. A comparative evaluation of medicated oils prepared using ayurvedic and modified processes. Indian J Pharm Sci 2009;71(6):656–62. 10.4103%2F0250-474X.59548.
[21] Shailajan S, Menon SN, Tiwari BR, Singh AS. Standardization of shadbindu Taila: an ayurvedic oil based medicine. Ayu 2013;34(1):103–7. https://doi.org/10.4103/0974-8520.115442.
[22] Anonymous. The Ayurvedic Pharmacopoeia India, Part I. first ed.vol. 6. New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 2008. p. 208. 224.
[23] Anonymous. The Ayurvedic Pharmacopoeia India, Part II. first ed.vol. 1. New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 2007. p. 144.
[24] Anonymous. The Ayurvedic Pharmacopoeia of India, Part II. first ed.vol. 3. New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 2010. p. 60–94.
[25] Anonymous. The Ayurvedic formulary of India. Part 3. first ed. New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 2011. p. 191–9.
[26] Anonymous. The Ayurvedic formulary of India, Part 2, first ed., New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 200. p. 138-151..
[27] Shrestha H, Bala R, Arora S. Lipid-based drug Delivery systems. J Pharm (Lahore) 2014;2014:1–10. https://doi.org/10.1155/2014/801820.
[28] Bali S, Prasad S, Saini V. Ayurvedic lipid based rasayans - a perspective on the preparation and pharmacological significance of lipids on the bioavailability of phytoconstituents. J Ayurveda Integr Med 2022;13(2):100526. https://doi.org/10.1016/j.jaim.2021.09.004.
[29] Tripathi KD. Essential of medical pharmacology. seventh ed. New Delhi: Jaypee Brothers Medical Publishers (Pvt.) Ltd.; 2013.
[30] Duraipandi S, Selvakumar V, Er NY. Reverse engineering of Ayurvedic lipid based formulation, ghrita by combined column chromatography, normal and reverse phase HPTLC analysis. BMC Complement Altern Med 2015;15:62. https://doi.org/10.1186/s12906-015-0568-9.
[31] Ministry of AYUSH, New Delhi. https://www.ayush.gov.in/docs/guideline-drug-development.pdf (accessed on 18/May/2023)..
[32] Deshpande SW, Gandhi N. The drugs and Cosmetics Act. commentators. eighth ed. 1940. p. 705–24. and Rules 1945; 2015.
[33] Hiremath SG, Joshi D, Ray AB. TLC as a tool for standardization of Ayurvedic formulations with special reference to Kutajarishta. Anc Sci Life 1993;12(3–4):358–62.
[34] Pyka A. Detection progress of selected drugs in TLC. BioMed Res Int 2014;2014:732078. https://doi.org/10.1155/2014/732078.
[35] Razack S, Kumar KH, Nallamuthu I, Naika M, Khanum F. Antioxidant, biomolecule oxidation protective activities of *Nardostachys jatamansi* DC and its phytochemical analysis by RP-HPLC and GC-MS. Antioxidants 2015;4:185–203. https://doi.org/10.3390/antiox4010185.
[36] Dhuri KD, Bodhe PV, Vaidya AB. Shirodhara: a psycho-physiological profile in healthy volunteers. J Ayurveda Integr Med 2013;4:40–4. https://doi.org/10.4103/0975-9476.109550.
[37] Vinjamury SP, Vinjamury M, Martirosian C, Miller J. Ayurvedic therapy (shirodhara) for insomnia: a case series. Global Adv Health Med 2014;3(1):75–80. https://doi.org/10.7453/gahmj.2012.086.
[38] Rastogi S, Baiswar A, Nischal A, Srivastava PS, Nischal A. Effects of shirodhara in generalized anxiety disorder. TANG [Humanitas Medicine 2016;6(4):e27. https://doi.org/10.5667/tang.2016.0016.
[39] Rajan S, Shamkuwar MK, Tanwar AK. Impact of Shirodhara on biological markers of stress: a case study. J Ayurveda Integr Med 2021;12(1):178–81. https://doi.org/10.1016/j.jaim.2021.01.008.
[40] Kaura H, Lekhak MM, Chahala S, Goutam U, Jha P, Naidoo D, et al. Nardostachys jatamansi (D.Don) DC.: an invaluable and constantly dwindling resource of the Himalayas. South Afr J Bot 2020;135:252–67. https://doi.org/10.1016/j.sajb.2020.08.010.
[41] Gottumukkala VR, Annamalai T, Mukhopadhyay T. Phytochemical investigation and hair growth studies on the rhizomes of *Nardostachys jatamansi* DC. Phcog Mag 2011;7:146–50. https://doi.org/10.4103/0973-1296.80674.
[42] Kumbhalkar B, Walunj T, Chavan S, Gupta V, Deshmukh V, Sardeshmukh S. Simultaneous identification and estimation of glycyrrhizin, glabridin, and 18β-glycyrrhetinic acid in de-glycyrrhized Ayurvedic lipid-based formulation of *Glycyrrhiza glabra* using dual wavelength reverse phase-high-performance liquid chromatography. Sep Sci Plus 2020;3:460–71. https://doi.org/10.1002/sscp.202000015.