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A COMPARATIVE PHARMACOGNOSTIC EVALUATION OF ANATOMICAL AND CHEMICAL CHARACTERS OF *NARDOSTACHYS JATAMANSI* (D.DON) DC. (JATAMANSA) AND *VALERIANA MOONI* ARN. EX C.B.CLARKE (LANKA THUWARALA)



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Abstract

Nardostachys jatamansi (Caprifoliaceae), known as Jatamansa, is an important plant in Ayurveda and traditional medicine in Sri Lanka. The dried rhizome of the plant is an important ingredient in many drugs. This plant does not grow in Sri Lanka. The practice has been to import it from India However, currently its export has been banned as it has become an endangered species in India . An island wide market survey carried out by authors revealed, that the material sold as Jatamansa is substituted by materials other than its recommended substitute, *Valeriana wallichii* to an extent ranging from 66% to 100%. Substitution with materials other than recommended substitute might adversely affect the efficacy and safety of the drugs manufactured. As *V. wallichii* also does not grow naturally in Sri Lanka, the present study was carried out to compare pharmacognostic properties of *V. mooni* the only species of *Valeriana* found in Sri Lanka with *N. jatamansi* to evaluate the possibility of replacing *N. jatamansi* with *V. mooni* and to establish comparative quality standards of the two plant species which will enable to identify raw material at the point of purchasing.

Preliminary phytochemical screening indicated the presence of alkaloids, flavonoids and hydrolysable tannins in both species. The TLC analysis of the ethanol extract of the rhizomes, and GCMS analysis of their essential oils indicated that both species possess similar chemical compounds. The pharmacologically important principal sesquiterpene Jatamansone, was found even in higher amount in *V. mooni* (25.6%) than in *N. jatamansi* (8.9%). However clinical trials are needed to decide on the possibility of substituting Indian *N. jatamansi* with local *V. mooni*. The findings of the present study have added a new member to the potential list of medicinal plants and identified as an important endangered plant with the need for conservation, hence develop suitable propagation systems and cultivation.

Key words: *Valeriana mooni, Nardostachys jatamansi,* pharmacognosy, chemical analysis, jatamansone

1. Introduction

Herbal medicine is getting popular among people around the world. The use of accurate raw material is essential to produce high quality drugs with expected therapeutic values. With respect to quality control, correct identification of raw material in fresh, dry or powder state is of prime importance (Springfield et al., 2005). Misidentification and subsequent substitution with incorrect material might lead to severe health problems or even to death (Chaudhury & Rafei, 2002). Therefore, implementation of rigorous standardization procedure for material identification botanical and chemical features will ensure high standards of the product with desired therapeutic values.

of Nardostachys jatamansi family Caprifoliaceae is an important medicinal herb in Avurveda which is heavily used in Sri Lanka (Pandey, 1991), (Jadhav et al., 2009). This species is not recorded in Sri Lanka and importing of the material from India has been also banned since 1997as it has become endangered in India. In Ayurveda practice, many exotics are being used mistakenly or as substitute when the original plant recommended does not grow in the country or when the amount of locally available raw materials is insufficient to meet the increasing daily demand. A survey carried out by authors among drug raw material dealers in five representative provinces some Ayurveda and some and traditional physicians in the country revealed that majority of them use subterranean part of a wild plant called Balanophora fungosa (Balanophoraceae) instead of iatamansi. This is plant not recommended as a substitute of N.jatamansi in any Ayurveda pharmacopeia. Therefore finding a possible substitutes for such rare and expensive raw material is therapeutically as well as economically.

Valeriana mooni (Caprifoliaceae) is an erect perennial critically endangered

herb, growing in high altitude areas of Sri Lanka and Tamil Nadu, India. Being globally threatened, V. mooni is an untouched medicinal plant. If V. mooni could be used as a substitute for N. jatamansi, it would minimize the unwanted bad effects which might be present in manufactured drugs due to the use of wrong raw material such as fungosa Balanophora (Balanophoraceae). However no reports available regarding pharmacognostic properties of *V. mooni*. Even though *V. mooni* appears to be a safe substitute and possibly effective, it is not available in the market. Lack of investigations and documentation on pharmacognostic properties of *V. mooni* might be the reason for its unavailability in the crude drug market of the country. Therefore this species has hardly earned attention of conservationists and other researchers.

2. Material and Methods

2.1 Material

Raw, dried rhizomes of V. mooni collected from Pattipola herbal garden and market samples (dried rhizomes) of N. jatamansi were used in the present study. Collected samples were identified using their macro morphological and organoleptic characteristics, following standard methods (WHO, 1998). Then the identification was confirmed by comparing samples collected with the authenticated specimens deposited at drug museum of the Bandaranayake Ayurvedic Research Institute. Air dried plant materials were used anatomical and chemical studies.

2.2 *Microscopic studies*

Microscopic studies (including powder microscopy) of raw material samples were carried out using standard methods (Trease & Evans, 1983; Prasad & Prasad, 1986 & Khandelwal, 2005).

2.2.1 Anatomical studies

Free hand sections of dried rhizomes of *N. jatamansi* and *V. mooni* soaked in water for ten minutes were taken using a sharp razor blade. Subsequently, sections were stained by double stained permanent slide method (Prasad & Prasad 1986). Anatomical features of the **s**tained sections were studied using compound light microscope (model Kyowa, Biolux-12) and photomicrographs were taken (Sony cyber-shot digital camera).

2.2.2 Preparation of counter stained permanent slides of freehand sections

Selected sections were successively soaked in 30%, and 50% alcohol (for 5 minutes each) and subsequently stained with 1% safranin in 50% alcohol. Excess stain was washed off using 50% alcohol and subsequently, sections dehydrated by treating successively with 70%, 90% alcohol (for 1 minute each), followed by 3 changes of absolute alcohol, keeping 1 minute interval at each step. Counterstaining was done using light green SF in clove oil for 2 minutes. Excessive stain was removed using absolute alcohol immediately. Sections were washed with clearing solution (clove oil) for 2 minutes. Clearing solution was removed by dipping sections for few minutes in xylene. After clearing in xylene for two changes, they were mounted on slides with a drop of Canada balsam (Prasad & Prasad 1986).

2.2.3 Powder Microscopy

Dried rhizomes were powdered using a grinder (Kinematica - CH-6014, Switzerland) and passed through steel sieve mesh No 355 (Retsch®, AS 200, Germany). Powder microscopic studies were carried out following standard method (Trease & Evans, 1983; Khandelwal, 2005). Small amount of powder of both plants were separately transferred to a drop of Chloral hydrate on cleaned glass slide. After mixing and placing a cover glass, the slide was

heated carefully over a small flame of micro burner until the air bubbles were completely removed. Ten microscopic fields (10x10 each) per sample were examined under compound light microscope and the relevant parameters were recorded. Photomicrographs of xylem components in the sample were taken using Sony cyber-shot digital camera.

2.3 Chemical Studies

2.3.1 Preliminary phytochemical screening

Qualitative chemical tests for identifying various phyto-constituents present were carried out following standard techniques (Trease & Evans 1983; WHO 1998; Khandelwal, 2005). Partially ground plant material (10 g) was kept in contact with 100 mL of 70% ethanol in a stoppard conical flask at room temperature for a period of 36 hours with frequent agitation (Tiwari *et al.*, 2011).

The extracts of plant materials prepared as above were subjected to a preliminary phytochemical analysis to detect the different chemical compounds present (Fong *et al.*, 1986).

2.3.2 Thin Layer Chromatography (TLC)

The powdered rhizome (20 g) was extracted sequentially with petroleum ether (60°-80 °C), ethyl acetate, and ethanol (80%) respectively in a Soxhlet apparatus (250 mL- YO-99036-50) for 6 hours. The ethanol extracts were evaporated in a rotary evaporator (Büchi® B-480 rotary evaporator) under reduced pressure at 40 °C. Resulted residue was stored in air tight dark containers at 4 °C until use.

Commercially available pre-coated (Merck) analytical high performance silica plates (Silica gel 60A, 20x20 cm, thickness of 0.25 mm) were used after prewashing them by eluting methanol once. Subsequently, plates were air dried and activated at 110 °C for 30 minutes prior to use. The samples were

applied to plates, and the plates were developed separately using peroleum ether (PE): ethyl acetate (EAc) 1:1; PE:EAc 4:1; EAc:Acetone 4:1 and dichloromethane (DCM) as solvent systems. The chromatograms were visualized under UV (366 nm) and by spraying with anisaldehyde sulphuric reagent.

2.3.3 Detection of valepotriates

The powdered rhizomes were extracted in a soxhlet apparatus using Dichloromethane. The sample was spotted on TLC plates and developed using toluene:ethyl acetate (3:1) as the mobile phase(Wagner et al, 1984). Chromatograms were visualized by spraying with HCl – glacial acetic acid(GAA) reagent followed by heating at 110°C.

2.4 GCMS analysis

2.4.1 Extraction of oil using hydro distillation

Ground rhizomes were subjected to hydro-distillation using a Clevenger apparatus (3340024-Borosil®) for 6 hours. The heating was stopped and the volume of oil collected in the graduated tube was read after 10 min. The yield of the oil was calculated as milliliters per kilogram of drug (v/w). The total volume of oil and water containing any dissolved oil in the clevenger arm was extracted with hexane in a separating funnel. The hexane layer was dried over anhvdrous sodium sulphate evaporated in a rotary evaporator under vacuum at 40 °C and the last traces of hexane were removed by stream of nitrogen. The oil was stored at 4°C in

dark coloured air-tight containers and used within one week for Gas chromatography/ Mass spectrometry (GC/MS) analysis.

The oil (0.1 mL) was dissolved in 2 mL of hexane and 2µl sample was injected to the GC/MS. The detected compounds were identified by comparing with National Institute of Standard and Technology (NIST), USA mass spectra data base and from retention times and mass spectra of standard compounds. The analysis of essential oils was carried GC/MS of using Agilent Technologies, Model 7890A operating in the EI (Electron Impact) mode with an ionization energy of 70ev equipped with a split injector. Helium used as a carrier gas at the flow rate of 0.8 mL/min, while $HP - 5 MS (30 m \times 0.25 mm, 0.25 \mu m)$ capillary volume was used. The initial temperature was programmed at 40 $^{\circ}$ C at the rate of 5 $^{\circ}$ C/min and 250 $^{\circ}$ C at the rate of 2 °C/min The injector temperature was 275 °C.

3. Results

3.1 Macroscopic and organoleptic characteristics of two raw materials

Rhizomes of *V. mooni* (Figure1a) are dull yellowish brown in color slightly curved unbranched, sub cylindrical, dorsi ventrally somewhat flattened and marked with transverse ridges while those of *N. jatamansi* (Figure 1b) are externally reddish brown. Both are fibrous and have bitter taste with an aromatic smell.

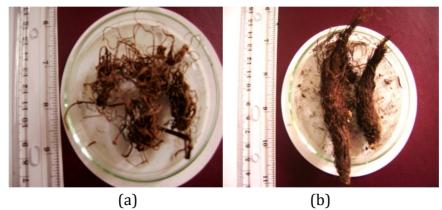


Figure 1. Dried rhizomes of study plants : (a) *V. mooni*, (b) *N. jatamansi*

3.2 Anatomical and powder characteristics

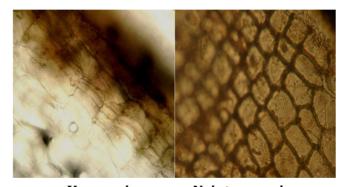
In transverse section, the outer most tissue is made up of cork and it is specified as 4-7 layers of compact, radially elongated, rectangular to polygonal brownish cells in both species (Figure 2). Cortex is parenchymatouse, moderately thick and secondary cortex (tissue just beneath the cork) consists of parenchyma cells with sinuous walls. Other part consists of parenchyma with moderately thick walls, numerous starch grains and oil globules (Figure 3).

Presence of inter xylary cork, stellate shaped medullary cork and schizogenous cavities is obvious in *N. jatamansi* (Figure 4) while they are absent in *V. mooni*. Collateral type 10-12 vascular bundles encircled by cork rings are prominent in *N. jatamansi* while

similar type of vascular bundles are organized in a ring in vascular region of *V. mooni* (Figure 5).

In both species, ray parenchyma are clear in some areas and phloem appears as patches of small cells. In *V. mooni* circular shape comparatively large pith remains alive with compact parenchymatouse cells (Figure 6) while it becomes stellete shape, necrotic and is margined by medullary cork layer in *N. jatamansi* (Figure 5).

V. mooni is comparable with *N. jatamansi* in many powder characters. Both powders are yellowish brown, fibrous, aromatic and bitter in taste. Starch granules, sclerides and fibers are abundant in both species (Figures 7 and 8). Special features are summarized in Table 1.



V. mooni N. jatamansi

Figure 2. T.S. of rhizome part showing cork cells (x400)

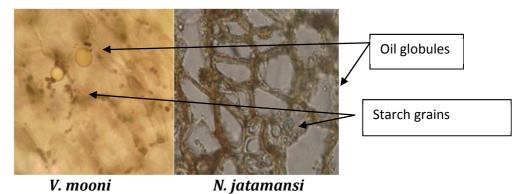


Figure 3. T.S. of cortex showing numerous oil globules and starch grains(x 400)

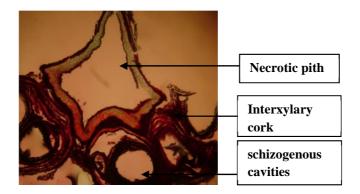


Figure 4. T.S. of interxylary and medullary cork with schizogenous cavities in *N. jatamansi* (X 400)

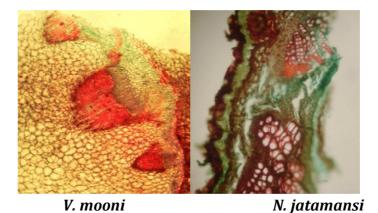


Figure 5. Vascular bundles (x400)

V. mooni – regular arrangement of collateral vascular bundles alternating with large and small bundles. *N. jatamansi* – collateral vascular bundle present in regular manner



Figure 6. T.S. of rhizome of *V. mooni* showing circular compact pith

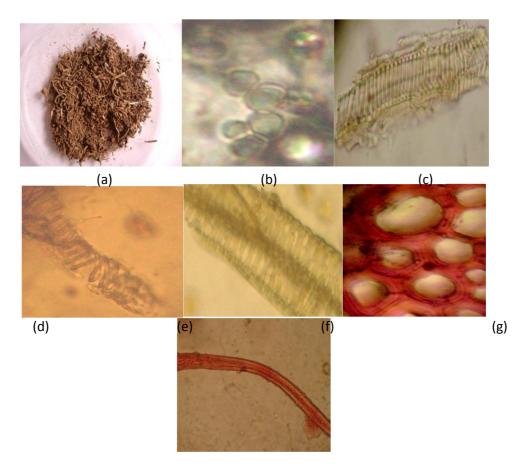


Figure 7. Powder microscopic view of *V. mooni* (x100) (a) Powdered material (unmagnified), (b) Starch grains, (c) Group of annular vessels, (d) Part of a spiral vessel, (e) Group of reticulate vessels, (f) Group of sclerides, (g) Part of a fiber

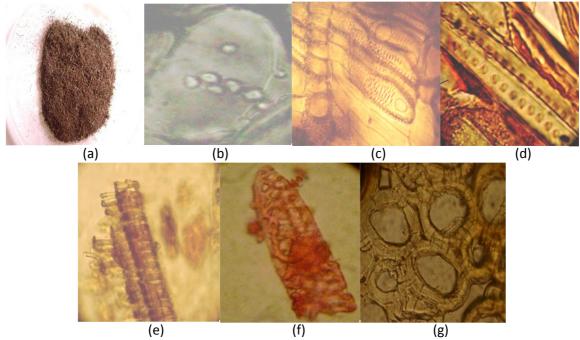


Figure 8. Powder microscopic view of *N. jatamansi* (x100)

(a) Powdered matereal (unmagnified), (b) Starch grains, (c) Group of scallaryform vessels with circular simple perforations, (d) Pitted vessels, (e) Spiral vessels, (f) Reticulate vessel, (g) T.S. of sclerides/ fiber

3.3 Chemical Studies

3.3.1 Preliminary phytochemical screening

The results of the phytochemical screening of the two species are given in the Table 2.

The results for both plants were similar with positive results for alkaloids, flavonoids and hydrolysable tannins.

3.3.2 Thin layer chromatography

The sequential ethanol extract gave (under uv 366nm) a single fluorescent blue spot and Rf = 0.42 in both samples (Figure9a) and an almost identical

pattern of purple gray spots (Rf =0.78, 0.67, 0.53, 0.33, 0.29, 0.22) were found with anisaldehyde sulphuric acid reagent (Figure 9b).

When considering the TLC profiles which were used to determine the valepotriates in two plant species, both species exhibited four fluorescent bands at Rf = 0.8, 0.7, 0.5 and 0.3 under UV 366 nm and five common bands having Rf =0.82, 0.8, 0.65, 0.62, 0.37 (pinkish brown) with HCL/GAA spray reagent (Figure 10).

As evident from the TLC finger prints, it could be suggested that the chemical constituents present in *V. mooni* and *N. jatamansi* are comparable.

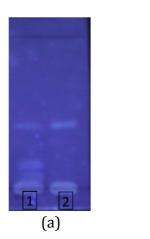
Table 1. Characteristics of powdered dried rhizome of *V. mooni* and *N. jatamansi*

Character	V. mooni	N. jatamansi		
Starch grains Shape	Numerous, found in masses, mainly compound with,3, or 4 components	Numerous, simple or compound with 2,3,4 or occasionally up to 6 components		
Diameter	Spherical to ovoid	Sub spherical to polyhedral		
Hilum	5-30 μm	2-30µm		
Striations	Rather indistinct small cleft or faintly visible radiate hilum	Fairly distinct radiate hilum or cleft with 3 -5 rays		
	Clearly visible	Clearly visible		
Vessels	Singly or in small groups	Singly or in small groups		
Thickenings	Annular Spiral	Scalariform Bordered Pitted Spiral		
Perforation	Not detected	Circular simple		
Tracheids	Found in groups of interlocking cells with annular thickenings	Rare		
Sclerides	Common, found in groups	Common, found in small groups Isodiametric		
Shape	Usually polyhedral	Evenly thickened, strongly striated, clearly visible		
Wall	Heavily thickened, distinct striations,	simple or branched pits		
Lumen	pits rarely found or	Relatively broad and empty		
Size	undetectable	Vary		
	Relatively broad and empty Vary			
Fibers	Abundant, found scattered or associated with xylem	Abundant, found in groups or as isolated fragments		
Shape	Moderately narrow with sharply pointed ends	Long ,narrow, frequently twisted, irregular in out line with blunt or		
Walls	Moderately thin with simple slit shaped pits	pointed ends Partially lignified with few pits		
Lumen	Relatively broad and empty, aseptate	Narrow, uneven, and often discontinuous, filled with contents , aseptate		

Table 2. Classes of compounds found in *V. mooni* and *N. jatamansi*

Phytoconstituent	V. mooni	N. jatamansi
Alkaloids	+	+
Fixed oil	nd	nd
Flavonoids	+	+
Reducing sugars	nd	nd
Saponins	nd	nd
Tannins (Condensed)	nd	nd
Tannins (Hydrolysable)	+	+
Unsaturated sterols	nd	nd

(+ detected; nd- not detected)



1 2 (b)

Figure 9. TLC of ethanol (80%) extracts of rhizomes developed with PE:EAc 4:1 (a) visualized under UV 366, (b) visualized with anisaldehyde sulphuric acid reagent. Track 1 – *V. mooni*; Track 2 – *N. jatamansi*



Figure 10. TLC profiles of Valepotriates (DCM extracts of rhizomes of *V. mooni* and *N. jatamansi* developed in Toluene: EAc 3:1 solvent system) with HCL/GAA spray reagent. Track1 *V. mooni;* Track 2 - *N. jatamansi*

3.3.3 Comparative analysis of the essential oils of two plant material

The oil extracted using hydrodistilation of the rhizomes exhibited a variation in their oraganolepticity (Table 3).

3.3.5 GCMS analysis

The oils, hydro distilled from the two plant materials were used for the GC/MS analysis.

Only a small number of peaks could be identified by comparing their mass spectrum with those in NIST library data bases (NIST 2011) suggesting that GC condition need to be optimized for better resolution. Table 4 gives the formulae, retention time (RT) and the relative concentrations (in percentage) of the compounds identified in GCMS analysis with a matching factor >90%.

The essential oils of both species are rich in sesquiterpenes. The main components detected in oil of *N. jatamansi* were patchouli alcohol

(16.66%), Jatamansone (8.9%), epialloaromadendrene (4.9%) and azulene (2.12%). Those in oil of *V. mooni* were Jatamansone (25.65%),1H-Cyclopropa(a)naphthalene, decahydro-1,1,3a trimethyl -7- methylene- [1a.S-(1a- alpha,3a. alpha, 7 a beta, 7b, alpha (15.15%), γ-Gurjunene (8.81%) and Bornyl acetate (7.62%).

Further 1H-Cyclopropa(a)naphthalene, decahydro-1,1,3a trimethyl -7-methylene- [1a.S-(1a- alpha,3a. alpha, 7 a beta, 7b, alpha (15.15%), γ-gurjunene (8.81%) and Bornyl acetate (7.62%) were most abundant components among identified compounds in *V. mooni.* According to literature, most of these compounds are used in perfumery industry and possess sedative effects as in *N. jatamansi* (Long, 2000).

Table 3. Organoleptic properties and percentage yield of the essential oil of *V. mooni* and *N. jatamansi*

Property	V. mooni	N. jatamansi		
Appearance	Fluid to slightly viscous liquid	Fluid to slightly viscous liquid		
Colour	Pale yellow	Greenish blue		
Aroma	Pleasant smell	Sweet woody and spicy- animal odor		
* % of yield	0.47 ± 0.04	0.55 ± 0.11		

^{*}The values given are mean ± standard deviation (n=3, separate distillations)

 $\textbf{Table 4.} \ \textbf{Compounds identified from GCMS analysis of the essential oils of the two plant species}$

compound	RT (Min)	V. mooni	N. jatamansi
Camphene *			
2,2 Dimethyl3MethyleneBicyclo[2.2.1]Heptane ($C_{10}H_{16}$)	6.27	0.77	nd
Sabinene *:			
Bicyclo[3,1,0]hexane, 4- methylene-1- (1- methylethyl) ($C_{10}H_{16}$)	6.96	0.2	nd
Borneol *			
$(C_{10}H_{18}O)$	12.30	0.76	nd
Bornyl acetate *:			
Bicyclo[2,2,1] heptan-2-ol, 1,7,7 – trimethyl Acetate (1 S – endo) ($C_{12}H_{20}O_2$)	17.86	7.62	nd
β-Patchoulene *:			
4,7 Methanoazulene,1,2,3,4,5,6,7,8- octahydro-1,4,9,9-tetramethyl-, [1S – (1, α , 4, alpha, 7 alpha)] ($C_{15}H_{24}$)	23.9	nd	0.72
alpha Gurujene *:			
1H Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b- octahydro-1,1,7,7a,- terta methyl- [1aR- (1a,alpha,7alpha,7b alpha)] (C ₁₅ H ₂₄)	27.62	nd	1.46
Azulene :			
β guaiene (C ₁₅ H ₂₄)	28.6	nd	2.12
1,2,4,8, Tetramethyl bicyclo [6,3,0] undeca-2,4-diene* (C ₁₅ H ₂₄)	29.96	nd	1.76
γ-Gurjunene *:			
Azulene, 1,2,3,3a,4,5,6,7octahydro- 1,4- dimethyl-7- (1-methylethyl)- [1R-(1a alpha, 3a beta, 4, alpha,7 beta) ($C_{15}H_{24}$)	41.18	8.81	nd
(α-Gurgujene) *:			_
1HCycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro1,1,4,7-tetramethyl-[1aR- (1a. alpha, 4 .alpha,4a. beta, 7b. alpha)]- $(C_{15}H_{24})$	42.73	1.49	nd
Epialloaromadendrene *:			
H- Cycloprop[e]azulene ,decahydro-1,1,7-trimethyl-4- methylene - [1aR-(1a alpha, 4a beta , 7 alpha, 7a beta,7b alpha)] ($C_{15}H_{24}$)	43.92	nd	4.95
α Maliene			
1H-Cyclopropa(a)naphthalene, decahydro-1,1,3a trimethyl -7- methylene- [1a.S-(1a- alpha,3a. alpha, 7 a beta, 7b, alpha* ($C_{15}H_{24}$)	44.25	15.15	nd -

Patchouli alcohol* (C ₁₅ H ₂₆ O)	44.47	2.48	16.6
Jatamansone *:			
1(2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a α ,7 β ,8a α)] (C ₁₅ H ₂₆ O)	44.77	25.65	8.93
Cyclohexene- 6-ethyl-6-methyl-1-(1-methylethyl)3(1methylethyledene), (S) * $(C_{15}H_{24})$	45.49	0.589	nd
Megastigma-4,6(E),8(z)-triene ($C_{13}H_{20}$)	45.63	0.104	nd
Total		63.62	36.54

Names in bold letters – common names for compounds

4. Discussion

Results of the present study show that *N. jatamansi* and *V. mooni* which belong to the same family exhibit many similar features in their pharmacognostic characters. Therefore it could be suggested that there is a possibility of using *V. mooni* as a substitute for *N. jatamansi*.

In the TLC profiles, extracts of *N. jatamansi* and *V. mooni* indicated a close resemblance exhibiting similar bands in both species.

In GCMS analysis of *N. jatamansi* and *V.* mooni demonstrated a similarity in the chemical constitution and their essential oils. Both species had substantial quantities of Jatamansone, (V. mooni -25.65%, *N. jatamansi*-8.93%) which has number of pharmacological actions, was recorded as the principal sesquiterpene and as the marker compound in jatamansa oil (Jha et al., 2012). The presence of even a higher percentage (25.65% when compared to 8.93% in N. *jatamansi*) of this key compound in oil extracts of V. mooni provide evidence for its suitability to use as a substitute for N. jatamansi. In addition to Jatamansone, patchouli alcohol which is used in some drugs and also in perfumery industry was also detected in N. jatamansi (Long, 2000).

Moreover, macroscopic and microscopic characters (organoleptic, anatomical and powder microscopic characters) together with banding pattern in TLC of *N. jatamansi* and *V. mooni* presented in this study could be used in raw material identification at the time of purchase and this will minimize the risk of unintentional use of incorrect plant species in drug industry.

5. Conclusion

Phytochemical screening, TLC and GCMS analyses indicate that the chemistry of two species is similar but not identical. Therefore, it could be recommended that *V. mooni* be considered as a substitute for *N. jatamansi* with further clinical studies.

Moreover, *V. mooni* could be considered as a new addition to the list of medicinal plants in Sri Lanka. Findings of this study will support the possible medicinal usage of this plant which in turn will ensure that it will be propagated, cultivated and conserved. The data produced in the present investigation will also be helpful in the preparation of monograph of the herb inclusion for the in various pharmacopeias.

^{*}compounds with matching factor > 90%; nd- not detected

6. Acknowledgements

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