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ORIGINAL RESEARCH ARTICLE

Chemical composition and antibacterial activity of floral volatile oil of *Alstonia scholaris* (L.) R. Br. from India

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Key Words

Apocynaceae
Flower volatiles
GC-MS
Linalool
Linalool oxides
Saptaparna

ABSTRACT

Alstonia scholaris (L.) R. Br, commonly known as saptaparna is an evergreen tropical tree of medicinal value. In this study, the floral volatile oil composition and antibacterial activity of A. scholaris collected in the morning and evening times were investigated. A total of thirty-six constituents, forming 81.1-83.7 % of total composition were identified. The oil was characterised by the presence of a higher amount of oxygenated monoterpenes (60.3-66.7 %), followed by benzenoids (9.2-9.9 %). Major components of the oil were linalool (13.6-14.3%), cis-linalool oxide (furanoid) (7.3-12.2 %), trans-linalool oxide (furanoid) (7.0-7.5 %), cis-linalool oxide (pyranoid) (3.3-7.2 %), trans-linalool oxide (pyranoid) (3.8-4.7 %), terpinen-4-ol (7.9-10.6 %), and α-terpineol (6.3-6.6 %). The oil showed moderate to good activity against Salmonella typhimurium (MTCC 98), while it showed moderate activity against Escherichia coli (MTCC 739). It can be concluded that the collection time influenced the yield and chemical composition of A. scholaris floral volatiles.

INTRODUCTION

Alstonia scholaris (L.) R. Br (Apocynaceae), popularly known as 'saptaparna' is an evergreen tropical tree native to the Indian sub-continent and Southeast Asia. The plant is distributed throughout moist regions of India, especially in West Bengal and west-coast forests of southern India. In the Indian system of medicine (Ayurvedic, Unani, and Siddha), the bark of the plant is used as a stimulant, febrifuge, spasmolytic, antidysenteric, uterine, antiperiodic, hypotensive, and for internal fevers (Khare, 2007). It is used to cure asthma, pneumonia, tuberculosis, cholera, diarrhea, cancer, hepatitis, and malaria (Jain, 1991; Mollik et al., 2010; Kaushik et al., 2011). Phytochemical studies of the leaf, root, and bark of *A. schohris* have resulted in the isolation of various bioactive molecules of diverse chemical classes like alkaloids, terpenoids, phlobatannins, steroids, and saponins that exhibited various pharmacological activities (Khyade et al., 2014). Several indole alkaloids, including echitamine, echitamidine,

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akuammiginone, scholaricine, 19-*epi*-scholaricine, vallesamine, picrinine have been isolated and characterised from the plant (Keawpradub et al., 1997; Yamauchi et al., 1990a; 1990b; Salim et al., 2004; Zhao et al., 2020). Echitamine is reported as a lipase inhibitor, antiproliferative agent, and targeted for cancer chemotherapy (Saraswathi et al., 1999; Jahan et al., 2009; Singh et al., 2015; George et al., 2019). Besides, *A. scholaris* possesses a pleasant-smelling fragrance in its beautiful flowers. The floral volatile oil composition of the plant has been studied in the past from Vietnam (Dung et al., 2001). However, studies dealing with the chemical composition of the floral volatile oil of *A. scholaris* with reference to collection time are meagre. Therefore, it was planned to examine the floral volatile oil composition and antibacterial activity of *A. scholaris* collected early in the morning and evening time from India.

MATERIALS AND METHODS

Plant material and isolation of floral volatile oil

Fresh flowers of *A. scholaris* were collected in full bloom in the morning (6 am) and evening (6 pm) from Lucknow, Uttar Pradesh in October 2019 (voucher specimen no: CIMPANT1005). The freshly collected flowers (1.0 kg) were immediately subjected to hydrodistillation in duplicates in a Clevenger's type apparatus and floral distillates (1 liter) were collected. The floral distillate was saturated with sodium chloride and then extracted successively with hexane (200 ml) and dichloromethane (200 ml). The organic layers were mixed, dehydrated over anhydrous sodium sulphate, and concentrated under vacuum at 35 ˚C. The concentrated floral volatiles were collected and stored in the refrigerator (3°C) until further analysis.

GC-FID and GC-MS analysis

The floral volatile oil analysis was carried out using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques as reported earlier (Nogueira et al., 2001; Adams, 2007; Andriamaharavo, 2014; Verma et al., 2020).

Antibacterial assay

Antibacterial activity of floral volatile oils of *A. scholaris* was performed by disc diffusion method against Gram-positive bacteria [*Staphylococcus aureus* (MTCC-96), *Streptococcus epidermidis* (MTCC-435), *Streptococcus mutans* (MTCC-890)], and Gram-negative bacteria [*Salmonella typhimurium* (MTCC-98), *Klebsiella pneumoniae* (MTCC-109), *Escherichia coli* (DH5á), *Escherichia coli* (EC739)] as per CLSI guidelines (Verma et al., 2016).

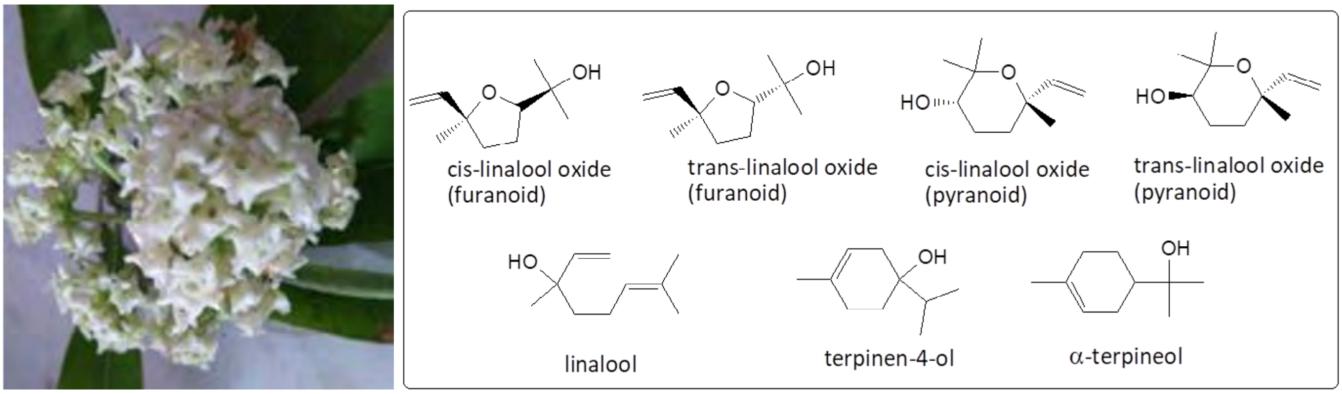
RESULTS AND DISCUSSION

The yield (w/w) of the floral volatile oil of *A. scholaris* collected in the morning and evening time was 0.01 % and 0.02 %, respectively. Previously, 0.03% yield of volatile concentrate was observed from the flowers of *A. scholaris* (Dung et al., 2001). These variations are possible due to the location, variation of extraction methods adopted, etc (Barra, 2009). The resulting floral volatile oils were analysed by GC-FID and GC-MS. A total of thirty-six constituents, forming 81.1-83.7 % of total composition were identified (Table 1). The oils were characterised by higher amount of oxygenated monoterpenes (66.7 % at 6 am and 60.3 % at 6 pm), followed by benzenoids (9.2% at 6 am and 9.9 % at 6 pm). Major components of the oils were linalool (13.6 % at 6 am and 14.3 % at 6 pm), *cis*-linalool oxide (furanoid) (12.2 % at 6 am and 7.3 % at 6 pm), *trans*-linalool oxide (furanoid) (7.0 % at 6 am and 7.5 % at 6 pm), *cis*-linalool oxide (pyranoid) (7.2 % at 6 am and 3.3 % at 6 pm), *trans*-linalool oxide (pyranoid) (4.7 % at 6 am and 3.8 % at 6 pm), terpinen-4-ol (7.9 % at 6 am and 10.6 % at 6 pm), α-terpineol (6.3 % ant 6 am and 6.6 % at 6 pm), and phenyl ethyl alcohol (4.5 % at 6 am and 6.5 % at 6 pm). The structures of the major floral volatiles and a representative chromatogram of floral volatile oil of *A. scholaris* are shown in Figure 1 and Figure 2, respectively. The floral volatile oils extracted from the morning and evening collected flowers showed the presence of similar components, but in different relative compositions. The amount of *cis*-linalool oxides (furanoid and pyranoid), *trans*-linalool oxide (pyranoid), and *p*-vinyl guaiacol were higher in the morning than evening. However, linalool, *trans*-linalool oxide (furanoid), phenyl ethyl alcohol, terpinen-4-ol, α-terpineol, and hexahydrofarnesyl acetone were relatively higher in the evening than morning. Diurnal variability studies of aromatic plants showed that the volatile oil composition changes during the day hours. The content of the essential oil is observed to be varying during different hours of day time. Generally, it is increases from the morning to afternoon and afterward decreases in

Table 1: Chemical constituents of the floral volatile oil of *Alstonia scholaris*

Compound	RI[a]	RI[b]	Content (%)[c]	
			6.00 am	6.00 pm
Dehydro-1,8-cineole	990	988	3.1	1.8
1,8-Cineole	1031	1026	0.9	0.4
Benzyl alcohol	1037	1026	0.4	0.5
γ-Terpinene	1057	1054	0.1	0.1
cis-Linalool oxide (furanoid)	1072	1067	12.2	7.3
trans-Linalool oxide (furanoid)	1088	1084	7.0	7.5
Linalool	1098	1095	13.6	14.3
trans-Sabinene hydrate	1102	1098	t	0.1
Phenyl ethyl alcohol	1112	1107	4.5	6.5
cis-p-Menth-2-en-1-ol	1121	1118	1.0	1.2
trans-p-Menth-2-en-1-ol	1137	1136	0.5	0.5
trans-Verbenol	1148	1140	0.6	0.4
cis-Linalool oxide (pyranoid)	1168	1170	7.2	3.3
trans-Linalool oxide (pyranoid)	1172	1173	4.7	3.8
Terpinen-4-ol	1179	1174	7.9	10.6
p-Cymen-8-ol	1184	1179	0.7	0.5
α-Terpineol	1191	1186	6.3	6.6
Geraniol	1250	1249	0.9	1.3
p-Vinyl guaiacol	1312	1309	2.0	0.7
Eugenol	1356	1356	0.1	0.1
1-Tetradecene	1388	1388	0.4	0.5
Geranyl acetone	1450	1453	0.8	1.2
2,4-Di-tert-butylphenol	1509	1512	1.5	1.6
1-Hexadecene	1589	1588	0.5	0.6
1-Octadecene	1789	1789	0.5	0.5
(*E*,*E*)-Farnesyl acetate	1838	1845	0.2	0.3
Hexahydrofarnesyl acetone	1843	1846	1.7	3.4
Methyl hexanoate	1923	1921	0.3	0.4
1-Eicosene	1990	1987	0.4	0.5
n-Heneicosane	2097	2100	0.9	0.8
(*E*)-Phytol	2112	2113	0.7	0.9
1-Docasene	2190	2189	0.3	0.4
n-Docasane	2196	2200	0.2	0.2
n-Tricosane	2296	2300	1.0	1.5
n-Tetracosane	2396	2400	0.2	0.2
n-Pentacosane	2496	2500	0.4	0.6
Class composition				
Monoterpene hydrocarbons			0.1	0.1
Oxygenated monoterpenes			66.7	60.3
Oxygenated sesquiterpenes			1.9	3.7
Oxygenated diterpenes			0.7	0.9
Benzenoids			9.2	9.9
Alkanes			2.7	3.3
Alkenes			2.1	2.5
Fatty acid ester			0.3	0.4
Total			83.7	81.1

[a]Retention Index determined on BP-5 gas chromatography column (30 m × 0.25 mm) using *n*-alkanes; [b]retention index from literature; [c]mean of two samples; t: trace (<0.05%).



The flower — Floral volatile constituents

Figure 1: Major floral volatiles of *Alstonia scholaris*

the evening times in the aromatic plants (Toncer et al., 2016; Karik et al., 2019). It is reported that the decrease of essential oil content of flowers had a relationship with the increase in atmospheric temperature. In a study on *Rosa damascena* maximum content of the essential oil was obtained when the flowers picked up early morning (Kumar et al., 2013). Previously, the floral volatile oil composition of *A. scholaris* has been studied from Vietnam. The study reported linalool, *cis*-and *trans*-linalool oxides, α-terpineol, 2-phenylethyl acetate, and terpinen-4-ol as the major components of the volatile concentrate of *A. scholaris* (Dung et al., 2001). Besides, chemical resemblance, the amounts of *cis*-and *trans*-linalool oxides (31.1 % at 6 am and 21.9 % at 6 pm), terpinen-4-ol (7.9 % at 6 am and 10.6 % at 6 pm), and phenyl ethyl alcohol (4.5 % at 6 am and 6.5 % at 6 pm) was higher in the present study than Vietnam. However, the amount of linalool (13.6 % at 6 am and 14.3 % at 6 pm) and α-terpineol (6.3 % ant 6 am and 6.6 % at 6 pm) was relatively lower in the present study than Vietnam. One of the major components in the Vietnam study, 2-phenyl ethyl acetate was not found in the present study. Despite the presence of several common components, the examined floral volatile oil of *A. scholaris* was quite different from those reported in the literature. These variations are might be a reflection of variation in various exogenous and endogenous factors (Barra, 2009). Further, this study identified four forms of linalool oxide (*cis*- and *trans*- forms of furanoid and pyranoid linalool oxide) in *A. scholaris* floral volatiles that collectively made up to 31.1 % of total oil composition. The occurrence of furanoid linalool oxide is quite common in nature, while the prevalence of pyranoid linalool oxide is less (Kumar et al., 2013). Furanoid linalool oxide plays a major role in the smell of linalool oxide, which has leafy, earthy, sweet, floral, and creamy odour. Pyranoid linalool oxide has a sweet, floral, creamy, and earthy odour. They are of high commercial importance in perfumery (Wang et al., 1994; Qin and Yang, 2016). The linalool is a much sought-after compound in the flavour and fragrance industry. This compound is also present in the floral fragrance of many plant families such as Lamiaceae, Lauraceae, Verbenaceae, etc. and is attractive to a broad spectrum of pollinators, herbivores, and parasitoids (Kamatou and Viljoen, 2008).

The floral volatile oils of *A. scholaris* were screened for antibacterial activity against Gram-positive and Gram-negative bacterial strains. The Gram-negative bacterial strains, namely *S. typhimurium* (MTCC-98) and *E. coli* (MTCC-739) were found to be susceptible to the essential oils (ZOI: 10-11 mm and 7 mm, respectively). However, the oils exhibited low antibacterial activity against Gram positive bacterial species tested (ZOI: nil-2.0 mm). In general, morning and evening collected floral volatile oils exhibited comparable antibacterial activity. Slight variation in the activity may be due to compositional variation in these oils. The observed antibacterial activity might be due to the presence of a higher amount of oxygenated monoterpenes (60.3-66.7 %) in the oil. Other major or minor

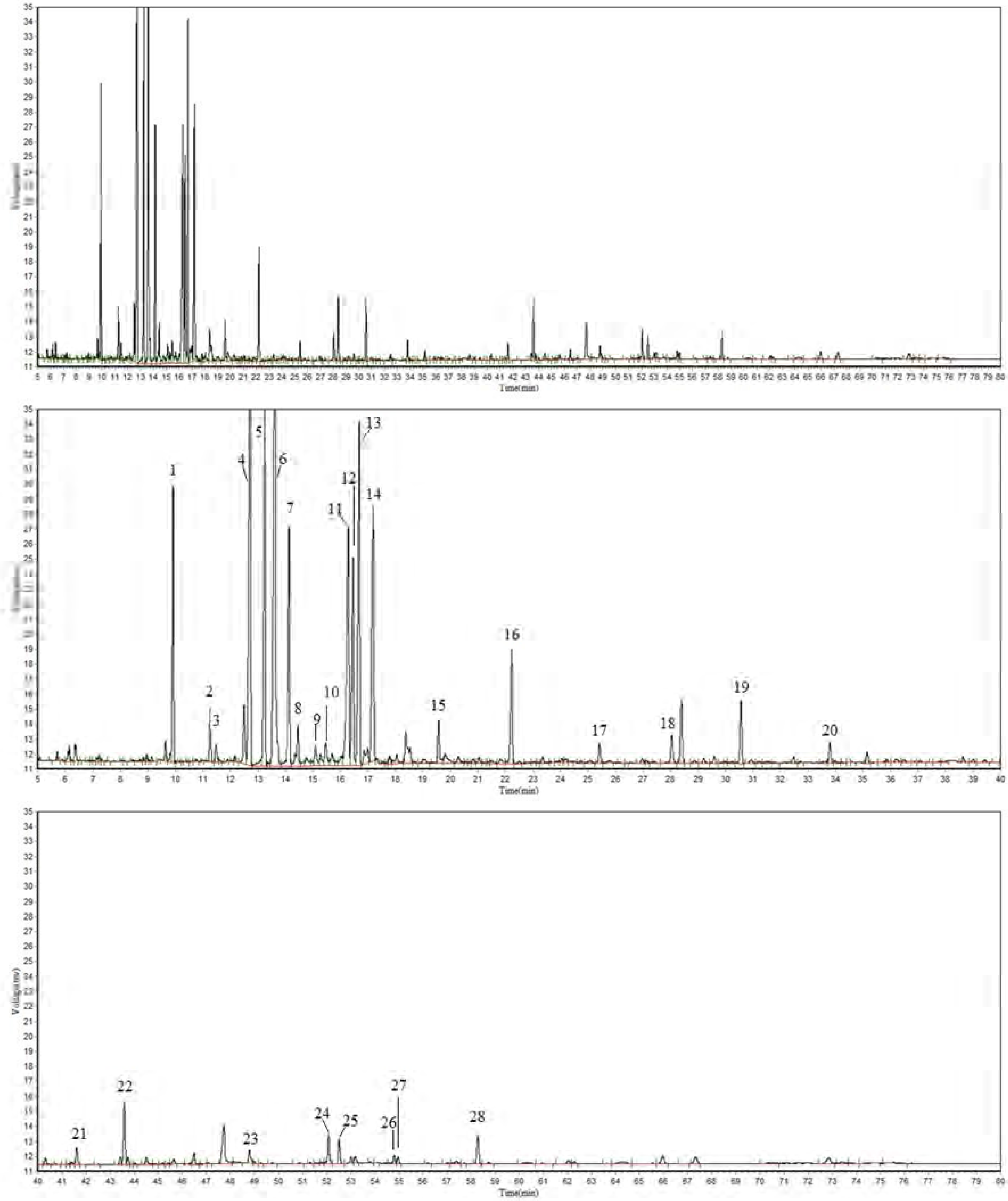


Figure 2: Gas chromatogram (GC-FID) of the floral volatile oil of *Alstonia scholaris*. Peak 1: dehydro-1,8-cineole; 2: 1,8-cineole; 3: benzyl alcohol; 4: *cis*-linalool oxide (furanoid); 5: *trans*-linalool oxide (furanoid); 6: linalool; 7: phenyl ethyl alcohol; 8: *cis*-p-menth-2-en-1-ol; 9: *trans*-p-menth-2-en-1-ol; 10: *trans*-verbenol; 11: *cis*-linalool oxide (pyranoid); 12: *trans*-linalool oxide (pyranoid); 13: terpinen-4-ol; 14: α-terpineol; 15: geraniol; 16: *p*-vinyl guaiacol; 17: 1-tetradecene; 18: geranyl acetone; 19: 2,4-di-tert-butylphenol; 20: 1-hexadecene; 21: 1-octadecene; 22: hexahydrofarnesyl acetone; 23: 1-eicosene; 24: *n*-heneicosane; 25: (*E*)-phytol; 26: 1-docasene; 27: *n*-docasane; 28: *n*-tricosane.

constituents present in the oils might be also responsible for the observed antimicrobial activity (Kamatou and Viljoen, 2008; Guimaraes et al., 2019).

CONCLUSION

It can be concluded that the collection time influenced the yield and chemical composition of *A. scholaris* floral volatiles. Evening (6 pm) collection of flowers can be beneficial for getting a higher yield of floral volatile oil. However, morning (6 am) collection can be beneficial for getting a linalool oxides rich floral volatile oil.

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REFERENCES

Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation Carol Stream, IL, USA (ISBN-13: 978-1932633214).

Andriamaharavo NR. 2014. Retention Data. NIST Mass Spectrometry Data Center, NIST Mass Spectrometry Data Center. (https://webbook.nist.gov/cgi/cbook.cgi?ID=C502692&Mask=2000#ref-1).

Barra A. 2009.Factors affecting chemical variability of essential oils: A review of recent developments. *Nat Prod Commun* **4**: 1147-1154.

Dung NX, Ngoc PH, Rang DD, Nhan NT, Klinkby N, Leclercq P. 2001.Chemical composition of the volatile concentrate from the flowers of Vietnamese *Alstonia scholaris* (L.) R.Br., Apocynaceae. *J Essent Oil Res* **13**: 424-426.

George G, Dileep PS, Paul AT. 2019. Development and validation of a new HPTLC-HRMS method for the quantification of a potent pancreatic lipase inhibitory lead Echitamine from *Alstonia scholaris*. *Nat Prod Res* pp 1-5 (doi: 10.1080/14786419.2019.1705817).

Guimaraes AC, Meireles LM, Lemos MF, Guimaraes MCC, Endringer DC, Fronza M, Scherer R. 2019. Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules* **24**: 2471 (doi: 10.3390/molecules24132471).

Jahan S, Chaudhary R, Goyal PK. 2009. Anticancer activity of an Indian medicinal plant, *Alstonia scholaris*, on skin carcinogenesis in mice. *Integr Cancer Ther* **8**: 273-279.

Jain SK. 1991. Dictionary of Indian folk medicine and ethnobotany, Deep Publications, New Delhi, India, (ISBN 8185622000, 9788185622002).

Kamatou GPP, Viljoen AM. 2008. Linalool-A review of a biologically active compound of commercial importance. *Nat Prod Commun* **3**: 1183-1192.

Karik U, Cinar O, Tuncturk M, Sekeroglu N. 2019.Morphological and diurnal variability of essential oil in Lemon Verbena (*Lippia citriodora* HBK.). *ANADOLU J Aegan Agri Res Inst* **29:** 114-120.

Kaushik P, Kaushik D, Sharma N, Rana AC. 2011. *Alstonia scholaris* - It's phytochemistry and pharmacology. *Chron Young Sci* **2**: 71-78.

Keawpradub N, Houghton PJ, Eno-Arnoquaye E, Burke PJ.1997. Activity of extract and alkaloids of Thai *Alstonia* species against human cancer. *Pl Med* **63**: 97-101.

Khare CP. 2007. Indian medicinal plants, an illustrated dictionary, Springer-Verlag Berlin/Heidelberg, pp 38.

Khyade MS, Kasote DM, Vaikos NP. 2014. *Alstonia scholaris* (L.) R. Br. and *Alstonia macrophylla* Wall. ex G. Don: A comparative review on traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* **153**: 1–18.

Kumar R, Sharma S, Sood S, Agnihotri VK, Singh B. 2013.Effect of diurnal variability and storage conditions on essential oil content and quality of damask rose (*Rosa damascena* Mill.) flowers in north western Himalayas. *Sci Hortic* **154**: 102-108.

Mollik MAH, Hossan MS, Paul AK, Taufiq-Ur-Rahman M, Jahan R, Rahmatullah M. 2010.A comparative analysis of medicinal plants used by folk medicinal healers in three districts of Bangladesh and inquiry as to mode of selection of medicinal plants. *Ethnobot Res App* **8**: 195–218.

Nogueira PCL, Bittrich V, Shepherd GJ, Lopes AV, Marsaioli AJ. 2001.The ecological and taxonomic importance of flower volatiles of *Clusia* species (Guttiferae). *Phytochem* **56**: 443-452.

Qin T, Yang S. 2016. A new and simple synthetic route to furaniod linalool oxide from citral.3rd International Conference on Engineering Technology and Application (ICETA-2016), 1162-1167.

Salim AA, Garson MJ, Craik DJ, 2004.New indole alkaloids from the bark of *Alstonia scholaris*. *J Nat Prod* **67**: 1591–1594.

Saraswathi V, Mathuram V, Subramanian S, Govindasamy S. 1999.Modulation of the impaired drug metabolism in sarcoma-180-bearing mice by echitamine chloride. *Cancer Biochem Biophys* **17**: 79–88.

Singh SK, Joshi T, Kanojiya S, Tripathi V, Mishra DK. 2015.Callus culture and *in vitro* biosynthesis of echitamine from *Alstonia scholaris* (L.) R. Br. *Pl Cell Tissue Organ Cul* **120**: 367–372.

Toncer O, Karaman S, Diraz E, Sogut T, Kizil S. 2016. Diurnal variation effects in essential oils of wild thyme (*Thymbra spicata* var. *spicata* L.) under cultivation conditions.*J.Essent Oil Bear Pl* **19**: 2037-2048.

Verma RS, Verma SK, Tandon S, Padalia RC, Darokar MP. 2020.Chemical composition and antimicrobial activity of Java citronella (*Cymbopogonwinterianus*Jowitt ex Bor) essential oil extracted by different methods. *J Essent Oil Res* **32**: 449-455.

Verma SK, Goswami P, Verma RS, Padalia RC, Chauhan A, Singh VR, Darokar MP. 2016. Chemical composition and antimicrobial activity of bergamot-mint (*Mentha citrata* Ehrh.) essential oils isolated from the herbage and aqueous distillate using different methods. *IndCrop Prod* **91**: 152-160.

Wang D, Ando K, Morita K, Kubota K, Kobayashi A. 1994. Optical isomers of linalool and linalool oxides in tea aroma. *Biosci Biotechnol Biochem* **58**: 2050-2053.

Yamauchi T, Abe F, Chen RF, Nonaka GI, Santisuk T, Padolina WG.1990.Alkaloids from the leaves of *Alstonia scholaris* in Taiwan, Thailand, Indonesia and Philippines. *Phytochem* **29**: 3547–3552.

Yamauchi T, Abe F, Padolina WG, Dayrit FM.1990.Alkaloids from the leaves and bark of *Alstoniascholaris* in the Philiippines. *Phytochem* **29**: 3321-3325.

Zhao YL, Yang ZF, Wu BF, Shang JH, Liu YP, Wang XH, Luo XD. 2020. Indole alkaloids from leaves of *Alstonia scholaris* (L.) R. Br. protect against emphysema in mice. *J Ethnopharmacol* **259**: 112949.