IJRD International Journal of Research & Development Organisation

ISSN: 2455-7676

Journal of Biological Science

Extraction and Qualitative Analysis of Floral Volatiles of *Alstonia scholaris*

Kevin J. D'cruz[a] and Mugdha V. Ambatkar[a]

[a] Department of Botany, St. Xavier's College, 5, Mahapalika Marg,

Mumbai-400 001, INDIA

Email addresses: kjdcruz@gmail.com and mugdhaambatkar@gmail.com

Telephone numbers: +91-932221308 and +91-9324385275

Abstract:

The strong fragrance of the flowers of *Alstonia scholaris* indicates the presence of volatile compounds. The pharmacological properties of the flowers of *A. scholaris* have been explored to a lesser extent than those of the bark and leaves. In the present study, the floral volatiles were extracted by steam distillation, and the major components were identified using GC–MS analysis. The cooling system in the distillation set-up was modified to significantly reduce the wastage of water. The distillate contained terpenes, such as eucalyptol (1,8-cineole) and linalool, which have well established medicinal properties. The present study qualitatively identified those volatiles that can be extracted from the flowers by through steam distillation, without the use of an organic solvent. The study explores the potential of the flowers of *A. scholaris* as a source of fragrant compounds with biologically activity.

Keywords: *Alstonia scholaris*, floral volatile oils, terpenes, steam distillation, recycling cooling water

1. Introduction:

The tree *Alstonia scholaris* has been used in traditional medicine for the treatment of debility, ear-ache, asthama, hepatitis and malaria in humans as well as for the treatment of fever in cattle (Dey, 2011). A list of some of those biological activities of the different parts of *A. scholaris*, which have been scientifically proven, has been given in Table 1.

Table 1: Biological activities of *Alstonia scholaris*

Activity	Plant part used	Type of extract	References
Antidiarrhoeal	Bark	Ethanolic	Patil et al, (1999)
Antimicrobial	Leaf, stem and root bark	Butanolic	Goyal et al, (1995)
Anticancer	Root bark	Methanolic	Keawpradub et al, (1997)
Antiasthamatic	Leaves	Ethanolic	Channa et al, (2005)
Analgesic and anti-inflammatory	Leaves	Ethanolic	Arulmozhi et al, (2012)
Anti-ulcer	Leaves	Ethanolic	Arulmozhi et al, (2012)

The tree *A. scholaris* belongs to family Apocynacea and is commonly planted as an avenue tree in Indian cities and towns. The flowers of *A. scholaris* are small, white or off-white and fragrant. The

characteristic fragrance of its flowers of indicates the presence of volatile compounds in them. The floral fragrance of *A. scholaris* is most conspicuous after sunset, which indicates that the flowers may be pollinated by nocturnal insects or animals (Raguso, Levin, Foose, Holmberg & McDade, 2003).

The role of volatile compounds in plants is important and interesting. Extensive research on plant terpenes in the 1970s resulted in the attribution of several roles to this complex group of chemicals, the terpenes. Terpenes were demonstrated to be toxins, attractants and repellents. The role of terpenes has been indicated in ecological interaction, such as mutualism (Gershen & Dudareva, 2007). Plants can attract the appropriate pollinator with the help of their odorous compounds (*Mutualistic Networks*, 2014). It is interesting to note that, in many cases, strongly fragrant flowers lack bright colors. In addition, nocturnal animals often pollinate fragrant and dull-colored flowers (Raven, Evert & Eichhorn, 1999). Many studies on the coevolution plants and their insect pollinators have emphasized the importance of volatile compounds in influencing the course of evolution in insects.

The role of odorous compounds or fragrances in human life is also remarkable. The tremendous pace (Global Flavor and Fragrance Market Report, 2015) of growth of the perfumery industry further endorses the importance of fragrance in human life. In India, the Fragrance and Flavour Association of India (FAFAI) is concerned with the trade of essential oils, spices and concretes. Perfumers are constantly exploring new and unconventional sources of fragrances in a bid to produce the unique and alluring fragrance. The flowers of *A. scholaris* offer an easily available source of fragrant compounds whose commercial potential has yet to be explored.

In a previous study by Dung et al. (2001) on the floral volatiles of *A. scholaris* was performed using distillation of hexane extract of the flowers as the extraction method. In the present study, the floral volatiles were extracted using steam distillation to avoid the use of an organic solvent in the extraction process. The floral distillate was qualitatively analysed using GC–MS to identify the components.

A majority of the scientific literature available on *A. scholaris* focuses mainly on the medicinal and pharmacological properties of the bark and leaves. There are relatively few reports on the use of the flowers of *A. scholaris*. An analysis of the extracted floral volatiles can provide an insight into the potential uses of the flowers as antibacterial or antifungal agents in addition to being a source of fragrant compounds.

2. Material and Methods:

2.1 Collection and authentication of plant material

Flowers of *Alstonia scholaris* were collected from trees in the Dhobi Talao locality of South Mumbai. The authentication of the source plants was performed at Blatter Herbarium, Mumbai (specimen accession no. 41427). The flowers were separated from the stalks, packed in air tight boxes and refrigerated until further use.

2.2 Steam distillation

A biomass flask (1000 mL) was filled with approximately 150 g of fresh flowers of *Alstonia scholaris*. This biomass flask was connected to round-bottom flask (250 mL) containing 100 mL distilled water (at 30°C) and 4–6 glass beads. The assembly used for steam distillation has been shown in Figure 1. All the joints of the distillation apparatus were washed, wiped and coated with a thin film of silicone grease. The cooling system of the distillation set up consists of a reservoir of water, in which coolant gel packs are periodically introduced. Water from this reservoir is circulated in the outer jacket of the condenser using an aquarium pump. When hot water returns to the reservoir, the cooling gel packs reduce the temperature of water, and the cooled water can be reused to cool the condenser. The stages of heating and their duration are shown below in Table 2.

Figure 1: Steam distillation assembly

(Heated water in the condenser jacket exits from the upper outlet of the condenser goes to the cooling water reservoir and cooler water enters the condenser jacket from the inlet at the bottom)

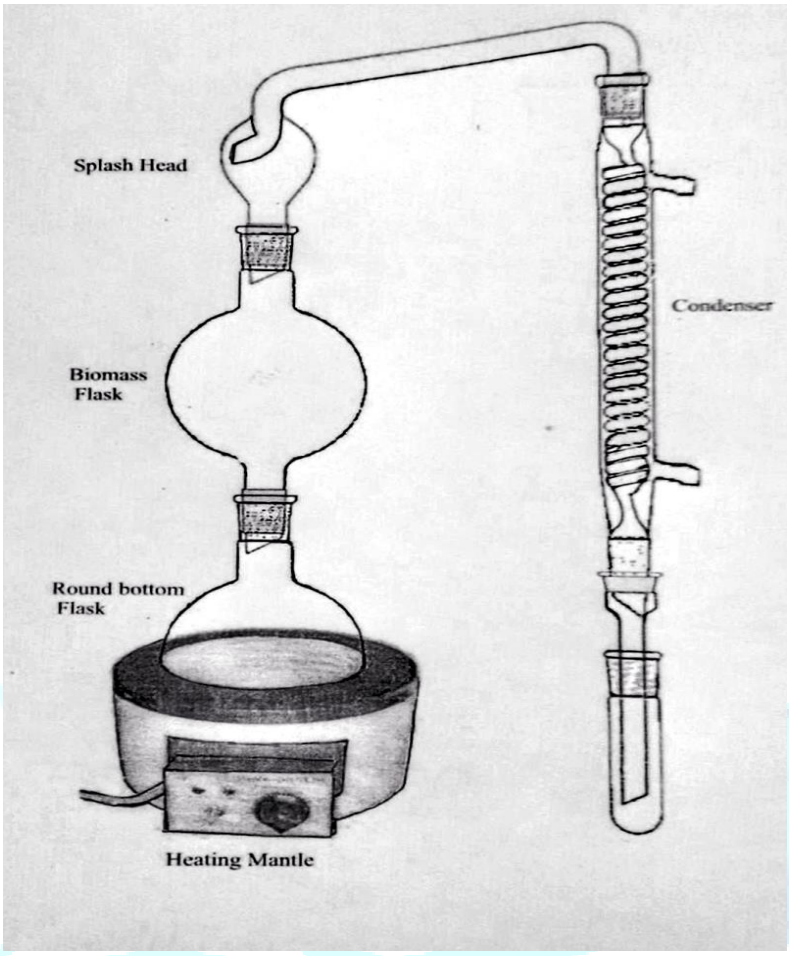

Table 2: Stages of heating during distillation

Temperature (°C)	Duration (min)
55	20
64	10
82	10

As the vapours moved to the splash-head, cold water was circulated in the outer jacket of the condenser, using a water pump immersed in a reservoir. This cold water circulation system significantly reduces the wastage of water for cooling. The distillate was collected in an air-tight glass tube and refrigerated till further use. The duration of the entire extraction process is approximately 70 min.

2.3 GC–MS Analysis of distillates

The distillate obtained from the steam distillation was mixed with analytical grade n-hexane (Merck, India) in the proportion 1:1. The mixture was shaken vigorously. The n-hexane layer was then extracted and stored in a sealed glass vial until use in GC–MS analysis of the n-hexane layer was performed on CLARUS 500 GC–MS at Perkin–Elmer Laboratory. The details were as follows—Oven: Initial temperature 40°C for 10 min, ramp 5°C/min to 220°C, hold 15 min, Injection temperature = 230°C, injection volume = 0.01 µL, split = 20:1, carrier gas—He, transfer temperature = 200°C, source temperature = 200°C, column 30.0 m × 0.25 mm fused silica capillary column, scan range for MS = 20–400 Da.

The retention times of the components recorded during GC and the molecular structure data from MS were compared using the data from the Perkin–Elmer database NIST. The volatile components of the distillate were identified based on the similarities they shared with the molecular structures available in the database.

3. Results and Discussion

Eight peaks were identified in the chromatogram (Figure 2). The peaks of the components were identified on the basis of a combination of the retention time (RT) and MS data made available from the Perkin–Elmer database. The components of the floral distillate have been listed in Table 3. Since the components of the floral distillate were extracted in n-hexane, they are likely to be non-polar in nature. The distillate contained oxygenated terpenes (Dung et al., 2001).

Figure 2: Gas chromatogram of floral distillate of *Alstonia scholaris*

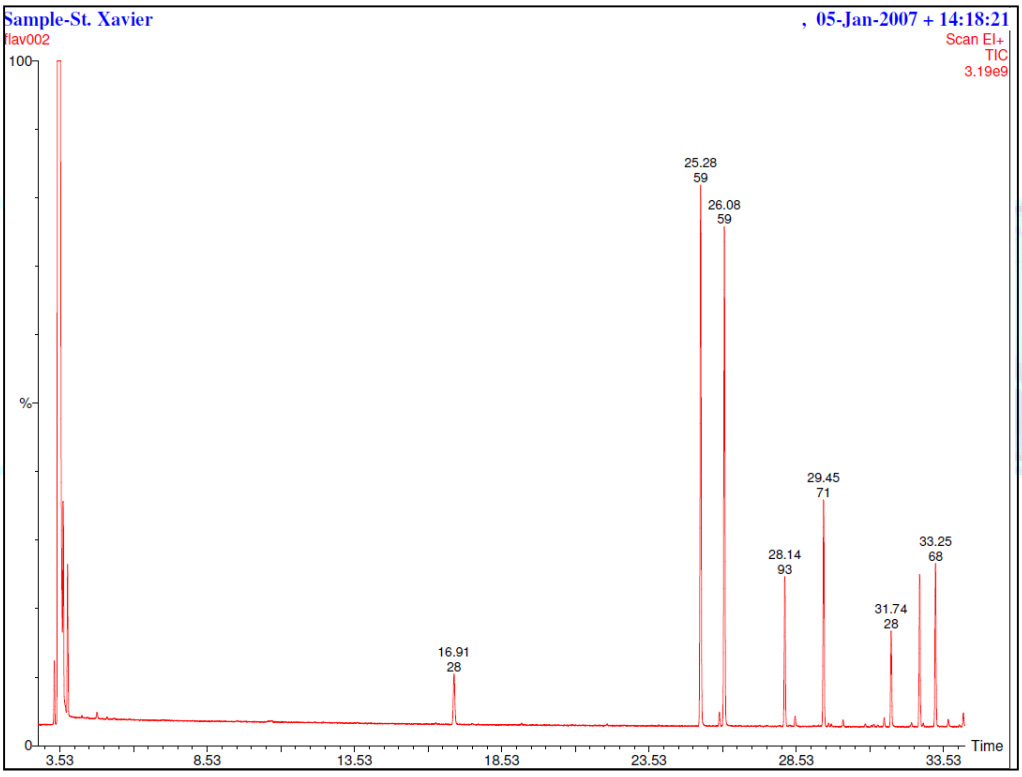

Table 3: Components of the floral distillate

Peak no	Retention time (min)	Volatile component
1	16.91	Eucalyptol (1,8- cineole)
2	25.82	Linalool oxide
3	26.08	Linalool oxide
4	28.14	Linalool
5	29.45	Sabinene hydrate
6	31.74	Linalyl propionate
7	32.72	Epoxy linalool linalool
8	33.25	Epoxy linalool linalool

As seen from Table 2, the peaks 2 and 3 both represent Linalool oxide, while peaks 7 and 8 both represent Epoxy linalool. There are two possible explanations for this observation. Since both Linalool oxide and Epoxy linalool contain double bonds, the peaks 2 and 3 may represent cis- and trans-isomers of Linalool oxide, while peaks 7 and 8 represent cis- and trans-isomers of Epoxy linalool. The MS database, however, identifies the compounds with similar fragmentation patterns as the same compound. Hence the identities assigned to peaks 2 and 3 are both Linalool oxide and those assigned to peaks 7 and 8 are both Epoxy linalool. Alternatively, peaks 2 and 3 may represent two different compounds, both of which produce molecular fragments that closely match the fragments produced by Linalool oxide (according to the Perkin–Elmer database). A similar scenario can be considered in the case of peaks 7 and 8. Both cis- and trans-isomers of linalool oxide have been previously reported in the volatile floral oil of *A. scholaris* extracted by distillation using hexane (Dung et al, 2001). Linalool oxide is an important fragrant compound in pollination (Pichersky, Raguso, Lewinsohn & Croteau, 1994; Karin, Karlson, Unelius, Valterová & Nilsson, 1996) and in the

management of malodors using perfumes (Pinney, 2007). The present study used steam distillation as an extraction method. Hence the use of a solvent during the extraction process was avoided.

The distillate showed the presence of terpenes such as eucalyptol which has reported to show antibacterial and antifungal activity (Delmare, Moschen-Pistorello, Artico, Atti-Serafini, & Echeverrigaray, 2007; Morcia, Malnati & Terzi, 2012) and linalool that has been reported to show anti-inflammatory activity (Peana et al, 2002). An analysis of the hydrodistillate of the flowers of *A. scholaris* has also shown the presence of linalool (2.2%) in the distillate (Islam, Islam, Nandi & Satter, 2013). The presence of linalool as a major constituent (35.7%) has been reported in a hexane distillate (Dung et al., 2001). Linalool is a medicinally important terpene. The flowers of *A. scholaris* have the potential to become an easily available source of medicinally important compounds like linalool, linalool oxide and eucalyptol.

The present study describes the distillation of floral volatile compounds without the use of an organic solvent, such as hexane, and may, therefore, be considered as a step towards green chemistry. The cooling system of the distillation set up consists of a reservoir of water, in which coolant gel packs are periodically introduced to maintain the low temperature of the cooling water. The water is circulated through the condenser and reservoir by means of an easily available aquarium pump. This simple modification, which enables the recycling of cooling water, saves a significant amount of water when compared to the conventional cooling set up that uses flowing tap water. In addition, the use of cooling gel packs helps to save more water than the use of ice blocks. The total duration of distillation is approximately 70 min of which the actual heating time is about 45 min. Hence, the distillation method followed is an economical means of obtaining the floral distillates of *A. scholaris*. This method may be refined further to ensure maximum extraction from the biomass used. Quantification of the components of the distillates will enable modification of the distillation method to obtain the maximum yield of those components that are medicinally and commercially most important.

4. Conclusion

The present study provides data about the composition of the steam distillate of the flowers of *A. scholaris* and indicates that commercially and medicinally important compounds like linalool and eucalyptol can be extracted by steam distillation, without the use of a solvent. The floral volatiles of *A. scholaris* are potentially a source of fragrant compounds.

5. Acknowledgement

The authors would like to thank Dr. U.C. Bapat of the Department of Botany for her guidance during the completion of the work detailed in the manuscript.

6. References

1. Arulmozhi, S., Mazumder, P. M., Narayan, L. S. & Thakurdesai, P. A. (2012). Analgesic, anti-inflammatory and anti-ulcerogenic activities of fractions from *Alstonia scholaris*. Pharmacologia, 3(5), 132–135.
2. Biodiversity and Plant-Animal Coevolution (2014). Biodiversity and Plant-Animal Coevolution. In *Mutualistic Networks* [E-reader version] (pp. 1–14). Retrieved from http://www.jstor.org/stable/j.ctt5hhnpq.4
3. Channa, S., Dar, A., Ahmed, S., & Rahman, A. U. (2005). Evaluation of *Alstonia scholaris* leaves for broncho-vasodilatory activity. Journal of Ethanopharmacology, 97 (3), 469–476.
4. Delamare, A. P. L., Moschen-Pistorello, I. T., Artico, L., Atti-Serafini, L., & Echeverrigaray, S. (2007). Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chemistry, 100(2), 603–608.
5. Dey, A. (2011). *Alstonia scholaris* R. Br. (Apocynacea): Phytochemistry and pharmacology: A concise review. Journal of Applied Pharmaceutical Science, 1, 51-57.
6. Dung, N. X., Ngoc, P. H., Rang, D. D., Nhan, N. T., Klinkby, N. & Leclercq, P. (2001) Chemical Composition of the Volatile Concentrate from the Flowers of Vietnamese *Alstonia scholaris* (L.) R.Br., Apocynaceae. Journal of Essential Oil Research, 13(6), 424–426.
7. Gershenzon, J. & Dudareva, N. (2007). The function of terpene natural products in the natural world. Nature Chemical Biology, *3*(7), 408–414.
8. Global Flavor and Fragrance Market Report: 2015 Edition (2015 November 27).Retrieved from http://www.reuters.com/article/research-and-markets-idUSnBw275323a+100+BSW20151127
9. Goyal, M. M. & Varshney, A. (1995). Effects of natural products isolated from three species of *Alstonia* on some gram-positive and gram-negative bacteria. Indian Drugs, 32(2), 69-72.
10. Islam, F., Islam, S., Nandi, N. C. & Satter, M. A. (2013). Essential oil composition from the flowers of *Alstonia scholaris* of Bangladesh International Food Research Journal, 20(6), 3185-3188.
11. Karin, A., Karlson, B., Unelius, C. R., Valterová I. & Nilsson ,L. A. (1996). Floral fragrance chemistry in the early flowering shrub *Daphne mezereum*. Phytochemistry, 41(6), 1477–1483.
12. Kewapradub, N., Houghton, P. J., Eno-Amooquaye, E. & Burke, P. J. (1997). Activity of extracts and alkaloids of Thai *Alstonia scholaris* against human lung cancer cells. Planta Medica, 63(2), 97–101.
13. Khan, M. R., Omoloso, A. D. & Kihara, M. (2003). Antibacterial Activity of *Alstonia scholaris* and *Leea tetramera*. Fitoterapia, 74 (7–8), 736–740.
14. Morcia C, Malnati M, & Terzi V. (2012) In vitro antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Additives and Contaminants, pp.1. DOI: 10.1080/19440049.2011.643458.
15. Patil, R. S., Juvekar, A. R., Joglekar, S. N., Shamkuwar, P. B. & Nimbkar, S. R. (1999). Study of antidiarrhoeal activity of *Alstonia scholaris* bark. Indian Drugs, 36, 463–465.
16. Peana, A. T., D'Aquila, P. S., Panin, F., Serra, G., Pippia, P. & Moretti, M. D. L. (2002). Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine, 9(8), 721–726.

17. Pichersky, E, Raguso, R. A., Lewinsohn, E., & Croteau, R. (1994). Floral Scent Production in Ciarkia (Onagraceae) Localization and Developmental Modulation of Monoterpene Emission and Linalool Synthase Activity. Plant Physiology, 106, 1533–1540.
18. Pinney, V. R. (2007) U.S. Patent No. 20070065394 A1. U.S. Patent Office.
19. Raguso, R. A., Levin, R. A., Foose, S. A., Holmberg, M. W. & McDade, L. A. (2003). Fragrance chemistry, nocturnal rhythms and pollination ''syndromes'' in Nicotiana. Phytochemistry, 63, 265–284.
20. Raven, P., Evert, R., & Eichhorn, S. (1999). *Biology of plants*. New York: W.H. Freeman and Company.
21. Santos, F. A. & Rao, V. S. N. (2000). Antiinflammatory and antinociceptive effects of 1, 8-cineole a terpenoid oxide present in many plant essential oils. Phytotherapy Research, 14(4), 240–244.