Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-11, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

Comparison of phytochemicals present in *Aquilaria malaccensis* Lam. (Agarwood) and *Gyrinops walla* Gaertn.

W.T.P.S.K. Senarath[1], D.T. Jayalath[1] & A.N. Buddhapriya[1]
[1]Department of Botany, University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250, Sri Lanka.

Abstract: *Aquilaria malaccensis Lam. and Gyrinops walla Gaertn. are large evergreen trees (Family Thymelaeaceae). A. malaccensis produces resin called agarwood that has a high economic value in perfumery industry. G. walla also produces a resin similar to agarwood. Thus, screening of phytochemicals present in A. malaccensis and G. walla would be important. Stem samples of test species were collected and air dried. After that they were size reduced and phytochemicals were extracted using soxhlet extractor with two different solvents. Extracts were concentrated using rotary evaporator and concentrated samples were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Obtained chromatograms were used to identify and compare the phytochemical constituents. Dichloromethane found to be better solvent than acetone to extract phytochemicals. Phytochemical composition of A. malaccensis and G. walla are not similar even though they share some phytochemicals in common. Major compounds that are responsible for the fragrance of agarwood are absent in both stem samples when collected in immature stages. This study could be extended to further optimize phytochemical extraction procedure, screening protocol and GC-MS programme.*

1. Introduction

Aquilaria malaccensis Lam. is one of 15 tree species in family Thymelaeaceae. It is a large evergreen tree growing up to 40 m tall. The species has a wide distribution, in South and Southeast Asia. *A. malaccensis* and other *Aquilaria* sp. produce a resin called Agarwood that is fragrant and highly valuable [1]. *Gyrinops walla* Gaertn. also belongs to the same family, which is a medium tall tree that grows up to 15 m in height with straight, slender trunk with a small rounded crown [2]. *G. walla* is found in the wet zone of southern part of Sri Lanka and Southeast regions in India. These plants have been utilized as a natural source of medicinal compounds since thousands of years [3]. Agarwood is a resinous heartwood that forms in *Aquilaria* sp. and *G. walla* trees when they are infected with a type of mould or damaged mechanically. Normally, the heartwood is relatively light and pale yellow in colour; however, as the infection progresses, the tree produces a dark coloured resinous substance in response to the attack, which results in dense, dark, embedded heartwood [4]. The resin embedded wood values in many cultures for its distinctive fragrance and thus is used for incense and perfumes [3]. Tropical Agarwood (*Aquilaria* sp.) is in danger of extinction in the wild due to illegal logging. In 2002 IUCN Red List classifies this species as vulnerable [5]. Moreover indiscriminate felling of *G. walla* trees, would cause a severe environmental damage in Sri Lanka.

All plants in nature do not produce the fragrant resinous substance. However, almost 75 percent of the *G. walla* plants grown in nature are cut down by people with the intention of making a fast cash, but have no economic value. Qualitative and quantitative analysis of phytochemicals and other volatile organic compounds may help to identify the resinous individuals and on the other hand to rescue the natural populations of *A. malaccensis* and *G. walla*. GC-MS is widely applied technique, which is used in phytochemical screening. Individual chemical components could obtain by the separation of mixtures from Gas chromatography (GC) using a temperature-controlled chromatography column. Smaller molecules with lower boiling points are travelling down the column more quickly than larger molecules which has a higher boiling point. Mass spectrometry (MS) is used to identify various components from their mass spectra [6]. Each compound in the mixture has a unique mass spectrum that can be compared with mass spectral databases and thus could be identified. In the present study, phytochemicals present in *A. malaccensis* and *G. walla* wood have been compared.

2. Materials and methods

A. malaccensis and *G. walla* stem samples were collected from the trees with 30.0-40.0 cm GBH that

Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-11, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

were pruned, damaged due to wind or injured in termite attacks without felling trees. Three *A. malaccensis* stem samples and three *G. walla* stem samples were used for the analysis. Samples were size reduced manually and air dried at room temperature. Then the dried samples were ground into a coarse powder using a kitchen grinder. Phytochemicals were extracted using the Soxhlet extractor. Ten grams (10.0 g) of each sample were extracted at a temperature of 70 °C for 20-30 extraction cycles over a period of 3 hours either in dichloromethane (250.0 ml) or acetone (250.0 ml) as the solvent. After extraction the solvent was evaporated, using a rotary evaporator (BUCHI–R124), yielding the 1.0 ml concentrated solution under reduced pressure at 40 °C temperature. Then the concentrated sample was dissolved in 50.0 ml of the solvent. Following soxhelt extraction, 2.0 µl aliquots were fractionated on the GC-MS machine (Agilent 7890A), using following conditions (Table 1). An external standard method was used by GC-MS machine to identify peaks and to find out the relationship between peak areas (or height) and analyte concentration in the chromatogram. Each chromatogram obtained were compared. Conditions of the GC-MS are as follows; Column-Agilent 7890A GC (30 m × 0.25 mm, thickness 0.25 µm) (5% Phenyl Methyl Siloxane), Injector temperature-280 °C, Maximum temperature-350 °C, Column flow-1 ml/min, Column pressure-11.567 psi, Film thickness-0.25 µm, Average velocity-25.028 cms^{-1}, Detector-5975C inert XL EI/C1 MS detector and Hold up time-1.0539 min.

Table 1. System conditions of GC-MS analysis

	Rate (^{0}C/min)	Value (^{0}C)	Hold time (min)	Run time (min)
Initial		70	4	4
Ramp	10	280	4	30

3. Results and discussion

Phytochemicals present in two species by means of percentage similarity in each solvent is given in Table 2. Both dichloromethane and acetone have been used in previous studies to extract phytochemicals from *A. malaccensis* and *G. walla* [7], dichloromethane proven to be a better solvent than acetone for the extraction of phytochemicals from both tested plant species. Sixteen (16) phytochemicals were identified in *A. malaccensis* and only thirteen (13) were found in *G. walla*. Out of those, only nine (09) compounds found to be common in both species.

Chromatograms obtained from GC-MS analysis (Figure 1-4) reveals that higher number of economically important phytochemicals with higher abundance, found in *A. malaccensis* than *G. walla* with dichloromethane solvent.

Table 2. Comparison of phytochemicals present in stem samples of *A. malaccensis* and *G. walla* extracted in dichloromethane (Dm) and acetone (Ac)

	Compound	Percentage Similarity			
		Aquilaria		*Gyrinops*	
		Dm	Ac	Dm	Ac
1	2-methoxy-4-vinylphenol	93	93	-	95
2	2,6-dimethoxy phenol (Syringol)	97	97	95	97
3	3,4,5-trimethoxy-phenol	97	94	94	97
4	4-hydroxy-3,5-dimethoxy-benzaldehyde	95	90	90	96
5	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	96	97	97	96
6	Tetradecanoic acid (Myristic acid)	99	-	-	94
7	n-Hexadecanoic acid (Palmitinic acid)	99	-	99	98
8	Hexanedioic acid, bis(2-ethylhexyl)ester	98	-	95	-
9	Heneicosane	98	-	-	-
10	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	91	-	91	-
11	Dodecanoic acid (Lauric acid)	99	-	97	-
12	Octadec-9-enoic acid (Oleic acid)	99	-	-	-
13	Pentadecanoic acid	98	-	-	-
14	Octadecanoic acid (Stearic acid)	99	-	-	-
15	Benzaldehyde	94	95	96	90
16	Vanillin	93	95	-	91
17	Hexadecanoic acid ethyl ester (Ethyl palmitate)	-	-	94	-
18	Docosane	-	-	90	-
19	Benzylacetone	-	-	96	-
20	ɤ - sitosterol	-	-	93	-

Khalil *et al.* (2013) [8] reported that, GC-MS analysis of the plant extracts led to the identification of 14 components from leaf extracts of two *Aquilaria* sp. Hexadecanoic acid found to be one of the major compounds in methanolic extracts. Other compounds were 1,4,7,10,13-pentaoxacyclopentadecane, acetic acid, 1,4,7,10,13,16-hexaaoxacyclooctadecane, hexaethylene glycol monododecyl ether, 1,4,7,10,13-pentaoxacyclopentadecane, 2,6,10,14,18,22-tetracosahexaen and 2,6,10,15,19,23-hexamethyl. In the present study, wood samples of *A. malaccensis* reported the presence of pentadecanoic acid and octadecanoic acid, however those compounds could

Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-11, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

not see in tested samples of G. walla. The presence of some volatile aromatic compounds that had been identified by Khalil *et al.* (2013) [8] also found in tested *A. malaccensis* and *G. walla* samples (in both acetone and dichloromethane extracts). Gas chromatography analysis revealed that the resin of *G. walla* contained aroma compounds such as sesquiterpenes of guaine and eudesmane which commonly found in commercially available agarwood [7]. In the present study, 2-methoxy-4-vinylphenol - an aromatic substance used as a flavouring agent and also the natural aroma of buckwheat, 2,6-dimethoxy phenol (Syringol) - a naturally occurring dimethyl ether of pyrogallol, dodecanoic acid (lauric acid) which gives a faint odour of bay oil and ethyl hexadecanoate - a volatile ethyl ester which gives the characteristic fragrance for vine was observed in both *A. malaccensis* and *G. walla*. However, hexadecanoic acid ethyl ester (ethyl palmitate), benzylacetone with a sweet, flowery smell that is considered to be the most prominent compound in flowers, which responsible for attractiveness and one of volatile components of cocoa was only found in *G. walla*.

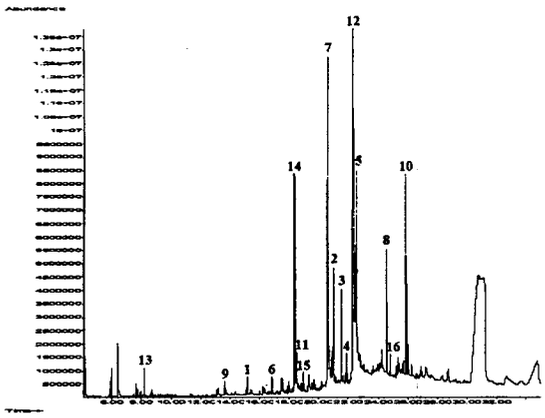

Figure 1. Chromatogram of *A. malaccensis* by GC-MS (Dichloromethane solvent)

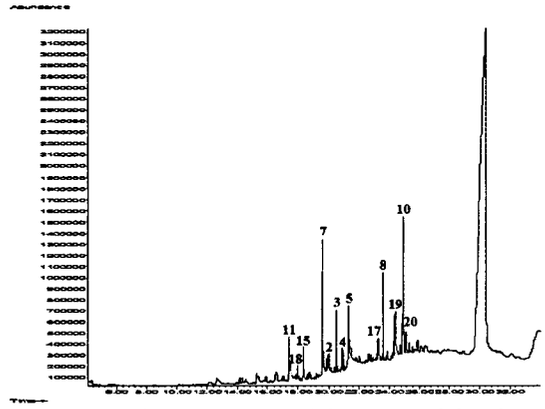

Figure 2. Chromatogram of *G. walla* by GC-MS (Dichloromethane solvent)

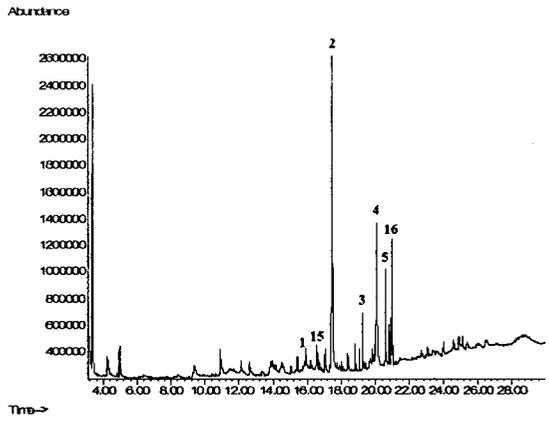

Figure 3. Chromatogram of *A. malaccensis* by GC-MS (Acetone solvent)

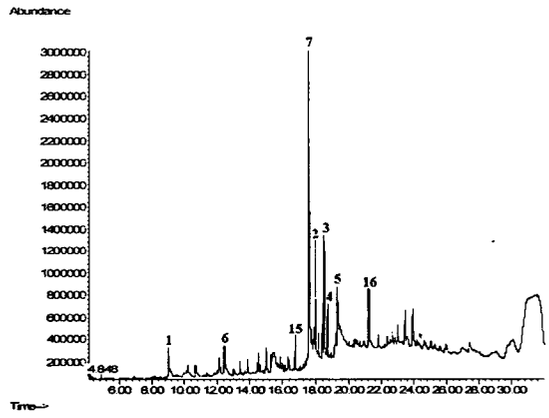

Figure 4. Chromatogram of *G. walla* by GC-MS (Acetone solvent)

Several fatty acids (tetradecanoic acid, pentadecanoic acid, etc.) and alkanes (heneicosane, heptacosane etc.) that are reported to be present in commercially available agarwood [9], [10] also found in both *A. malaccensis* and *G. walla* samples analysed in the present study.

Benzaldehyde and vanillin were common in samples collected from undamaged *A. malaccensis* and *G. walla* plants, revealing that those compounds are not only present in resin produced, but also in the natural heartwood too.

Presence of ϒ sitosterol, which is a phytochemical present in commercially available agarwood oil [11] was observed in tested *G. walla* wood samples, but not detected in *A. malaccensis* samples.

The present study revealed that although there are some common fragrance compounds present in *A. malaccensis* and *G. walla*, they do not share all fragrant compounds present in commercial agarwood samples. So, felling *G. walla* trees to extract agarwood without knowing the exact mechanism behind resin induction is a waste of the natural resources of Sri Lanka, and the authorities need to

Imperial Journal of Interdisciplinary Research (IJIR)
Vol-2, Issue-11, 2016
ISSN: 2454-1362, http://www.onlinejournal.in

make appropriate actions to control the felling of young *G. walla* plants for commercial purposes.

4. Conclusions and recommendations

Dichloromethane found to be better solvent than acetone to extract phytochemicals present in *A. malaccensis* and *G. walla*. Phytochemical composition of *A. malaccensis* and *G. walla* are not similar even though they share some phytochemicals in common. Major compounds that are responsible for the fragrance of agarwood (e.g. agarospirol, agarol, alpha agarofuran, beta agarofuran and eudesmol, etc.) are absent in *A. malaccensis* and *G. walla* stem samples when collected in immature stages. Even though there are several claims that *A. malaccensis* and *G. walla* have similar phytochemicals, present study revealed that they are not similar in the wood. However, presence of some important phytochemicals in both *A. malaccensis* and *G. walla* have greater economic importance in many fields. For this reason, optimizing phytochemical screening procedure and separation of them may benefit for Sri Lankan economy.

5. Acknowledgements

This research was supported by University of Sri Jayewardenepura (USJP), Sri Lanka. We are grateful to Ms. Rajitha, Department of Chemistry, USJP for assistance with GC-MS.

6. References

[1] A. Barden, N.A. Anak, T. Mulliken and M. Song, Heart of the matter: agarwood use and trade and CITES implementation for *Aquilaria malaccensis*, portals.iucn.org, 2000.

[2] Dassanayake, M.D., Fosberg, F.R. and Trimen, H., 1981. *A revised handbook to the flora of Ceylon,* Vol. 2. Amerind Publishing, New Delhi. Pp 501-511.

[3] Tintumol K.S., Shana Sherin N., Farisha Karuvally, Sudha Das P. K., Shafi K. M.2 and Hashim K.M., "Phytochemical investigation and in-vitro anti-oxidant activity of the non- polar extract of agarwood", *Asian Journal of Plant Science and Research*, 2013, 3(4):38-41.

[4] Y. Okudera and M. Ito, "Production of agarwood fragrant constituents in *Aquilaria* calli and cell suspension cultures", *Plant Biotechnology,* Vol. 26 (2009) No. 3: pp 307-315.

[5] The IUCN Red List of Threatened Species. Version 2015-4.

[6] F.G. Kitson, B.S. Larsen and C.N. McEwen, *Gas Chromatography and Mass Spectrometry: A Practical Guide*, Academic Press, 1996.

[7] S.M.C.U.P. Subasinghe, D.S. Hettiarachchi, E. Rathnamalala, "Agarwood-type Resin from *Gyrinops walla* Gaertn: A New Discovery", *Journal of Tropical Forestry and Environment*, 2012, Vol. 2, No. 02, pp 43-48.

[8] Khalil, A.S., Rahim, A.A., Taha, K.K. and Abdallah, K.B., Characterization of Methanolic Extracts of Agarwood Leaves. *Journal of Applied and Industrial Sciences*, 2013, 1 (3): pp 78-88.

[9] Chen H., Yang Y., Xue J., Wei J., Zhang Z. and Chen H., 2011, "Comparison of compositions and antimicrobial activities of essential oils from chemically stimulated agarwood, wild agarwood and healthy *Aquilaria sinensis* (Lour.) Gilg trees", *Molecules*, 16: pp 4884-4896.

[10] Regula Naef, "The volatile and semi-volatile constituents of agarwood, the infected heartwood of *Aquilaria* species: a review", 2011, Vol. 26(2), pp 73–87.

[11] Wetwitayaklung, P., Thavanapong, N. and Charoenteeraboon, J., 2009, "Chemical constituents and antimicrobial activity of essential oil and extracts of heartwood of *Aquilaria crassna* obtained from water distillation nd supercritical fluid carbon dioxide extraction", *Silpakorn University Science and Technology Journal* 3, pp 25–33.