World Journal of Agricultural Research, 2013, Vol. 1, No. 6, 101-103
Available online at http://pubs.sciepub.com/wjar/1/6/1

DOI:10.12691/wjar-1-6-1

Standardization of *Gyrinops Walla* Gaertn. (Thymalaeaceae): Newly Discovered, Fragrant Industrial Potential, Endemic Plant from Sri Lanka

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Received August 07, 2013; Revised October 12, 2013; Accepted October 14, 2013

Abstract *Gyrinops walla* Gaertner. (Thymelaeaceae), is an endemic resinous plant used in traditional medicine, perfume production, incenses, aroma therapy and cosmetic industry. Due to the high economic value, large-scale smuggling of *G. walla* from Sri Lanka has been practiced for a long time. Therefore, present study was undertaken to study the phytochemical and volatile oil components of *G. walla*. Phytochemical analysis of leaf, bark and stems were performed according to the methods described in WHO guidelines. Aerial parts were hydro-distilled in a Clevenger-type apparatus for 8 h. Oil was analyzed using GC-MS and identification of components of volatile oils was carried out based on retention indices and fragmentation patterns of the mass spectra. Phytochemical screening of leaf, stem and bark crude extracts of *G. walla* revealed the presence of tannins, saponins, steroid glycosides, flavonoids and alkaloids in all three parts tested. Thin layer chromatographic profiles observed under UV light at 366 nm exhibited higher number of spots in CH_2Cl_2 extracts for all three parts followed by hexane and methanol fractions, respectively. Sky blue spot present in all three fractions [hexane (R_f = 0.83), dichloromethane (R_f = 0.66) and methanol (R_f = 0.77)] of stem extracts was characteristic to the stem extract. The yield of the essential oils obtained from stem parts of *G. walla* was 0.20 ± 0.01%. Major constituents, which exist more than 1% in heartwood of *G. walla* oil, were gamma-selinene (72.49%), 3-Phenyl-2-butanone (2.04%), 3-pentanone (2.02%), beta-patchoulene (1.37%) respectively. Present study reports the presence of preliminary phytochemicals, TLC finger prints and GC-MS analysis of essential oil of *G.walla* for the first time in Sri Lanka. Presence of high content of γ-selinene and β-patchoulene (73.86%) indicates potential for commercial production of world class perfume and other scented products.

Keywords: *Gyrinops walla Gaertner, Thymelaeaceae, essential oil, Thin Layer Chromatography, GC-MS, phytochemicals*

Cite This Article: R.M. Dharmadasa, Asitha Siriwardana, Kosala Samarasinghe, and Adhihetty, P., "Standardization of *Gyrinops Walla* Gaertn. (Thymalaeaceae): Newly Discovered, Fragrant Industrial Potential, Endemic Plant from Sri Lanka." *World Journal of Agricultural Research* 1, no. 6 (2013): 101-103. doi: 10.12691/wjar-1-6-1.

1. Introduction

Gyrinops walla Gaertner. (Thymelaeaceae), commonly known as Walla Patta in Sinhala and Agarwood in English, is an endemic fragrance producing resinous plant grown in wet and intermediate zones in Sri Lanka [1]. Resin extracted from *Gyrinops* species has its unique fragrance and therefore, it is widely used as an ingredient in traditional medicine, world class perfume [2], incenses [3], aroma therapy [4], cosmetic and preservatives of accessories [5,6]. Moreover, agar wood is used as cultural and religious purposes across the Asia [7]. Due to the high economic value in perfumery and other industries excessive exploitation of *Gyrinops* and *Aquilaria* species from natural habitats have been reported from many countries [8]. Therefore, they are classified as endangered species in the international trade [8]. *Gyrinpos walla* is the only *Gyrinpos* species available in Sri Lanka. Since this species possess pleasant fragrance which has lucrative in Middle Eastern market where it is used as a component to manufacturing of expensive perfumes, large scale smuggling of *G. walla* from Sri Lanka has been practiced for a long time. In the context of conservation purpose, *G. walla* has been protected under the Flora and Fauna Protection Ordinance (FFPO) in Sri Lanka since 2004 and Convention on International Trade in Endangered Species (CITES) since 2005. Therefore, exploration of this valuable plant and scientific identification of plant material and volatile oil components which are responsible for fragrance, are timely important in order to ensure the sustainable utilization and conservation of this valuable plant.

2. Materials and Methods

2.1. Plant Materials

Leaf, stem and bark samples were obtained from fully matured *G. walla* plants grown in a home garden at

Awissawella which belongs to low country wet zone in Sri Lanka. Herbarium specimen was prepared and deposited in institutional herbarium (HTSGW-21). Collected samples (1kg) were cut into pieces and air-dried for 3 days at room temperature (28 ± 2°C). Then materials were ground to coarse powder using grinder (Janke and Kundel, Germany). All solvents used for the extraction of phyto-chemicals were from Sigma Chemical Company (USA).

2.2. Sample Preparation for Chemical Analysis

Coarsely powdered plant material (10 g) of leaf, bark and stem were sequentially extracted, separately with 50 mL of hexane, dichloromethane and methanol by using Soxhlet apparatus. The extract was concentrated at 45 °C using rotovapour (Buchi Rotavapour, Type-R-114A29 B-480, Switzerland).

2.3. Phytochemical Screening and Thin Layer Chromatography (TLC)

Methanol extracts of leaf, bark and stem of *G. walla* were screened for preliminary phytochemicals such as alkaloids, flavonoids, saponins, steroidal glycosides and tannins according to the method described by Farnsworth [9]. Thin layer chromatographic analysis of plant extracts were performed according to the method described by Stahl [11] with slight modifications. Pre-coated Silica gel 60 GF_{254} plates were used. Approximately 8 µL of each sample was spotted on the TLC plate, air-dried and placed in the chromatographic chamber previously saturated with the solvent system (Hexane 2: ethyl acetate 1: methanol 1) developed TLC plates were observed under UV 366 nm and after spraying with vanillin-sulfuric acid followed by heating at 105°C for 3-5 minutes.

2.4. Distillation of Essential Oil

Coarsely powdered heart wood sample (1 kg) of *G. walla* were hydro distilled in a Clevenger-type apparatus for 8h. The volatile oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4°C until analysis. The yield of the oils was calculated based on dry weight of plant materials.

2.5. GC-MS Analysis of Essential Oil

GC-MS analysis was performed on a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The oven temperature was programmed from 60 -280°C at 4 °C/min. Helium was used as carrier gas at a flow rate of 2 mL/min. The gas chromatograph was coupled to a Hewlett-Packard 6890 mass selective detector. The MS operating parameters were; ionization voltage, 70 eV; and ion source temperature, 200°C.

3. Results and Discussions

3.1. Phytochemical Screening

Phytochemical screening of leaf, stem and bark crude extracts of *G. walla* revealed the presence of tannins, saponins, steroidal glycosides, flavonoids and alkaloids in all three parts tested (Table 1).

Table1. Phytochemical analysis of heartwood of *Gyrinops walla*

Phytochemicals	Part of the plant		
	Leaf	Stem	Bark
Tannins	+	+	+
Saponins	+	+	+
Steroidal glycosides	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+

(+) – Present; (-) –Absent

3.2. Thin Layer Chromatography

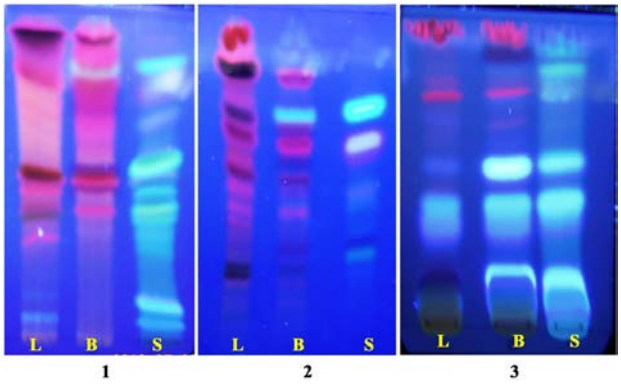

Figure 1. Thin layer chromatogrammes of different parts of *Gyrinops walla* observed under UV 366 nm (1- Hexane fraction; 2- Dichloromethane fraction; 3- Methanol fraction; L- leaf; B- bark; S- stem solvent system Dichloromethane : Cyclohexane : Methanol 1 : 1 : 0.1)

Thin layer chromatographic profiles observed under UV 366 nm exhibited comparatively higher numbers of spots in dichloromethane extracts for all three parts (leaf, bark and stem) followed by hexane and methanol fractions, respectively Stem extracts exhibited a prominent sky blue spot for hexane (R_f = 0.83), dichloromethane (R_f = 0.66) and methanol (R_f = 0.77). In addition, the same spot was present in bark extracts of hexane and dichloromethane extract but did not present in methanol extract (Figure 1). Further, light pink colour spot at R_f = 0.30 was characteristic for leaf extract of hexane fraction. Finger print analysis using thin layer chromatography has become the most potent tool for standardization and quality control of plant materials and plant base herbal medicines due to its simplicity, cost effectiveness and reliability. Use of thin layer chromatographic profiles for quality control of *Bacopa monnieri* and its products [12], determination of active phytoconstituents present in hexane and aqueous extracts of *Rumex vesicarius* L. [13] and determination of quercetin in *Calendula Officinalis* [14] have been reported.

Although there were reports on resin composition of *G walla* found in Sri Lanka, [15], information on standardization and composition of essential oil, in the present study, essential oil was distilled by using hydro distillation, which is classically practiced method for oil extraction of agar wood [16]. Essential oil of *G. walla* was either reddish or yellowish brown in colour with intrinsic pleasant aroma. Oil percentage was 0.2% w/v. Results obtained in this study are in agreement with the values reported by previous work [17]. The oil content was varied from 0.15-0.85% w/v composition according to the

method of inoculation. Further, variation of oil colour in different parts varied from greenish brown to dark reddish brown.. Results of GC-MS analysis of essential oil of *G. walla* are presented in Table 2.

Table 2. Essential oil composition of *Gyrinops walla* Gaertner

Compound	Area %	Retention time (min.)
3-Phenyl-2-butanone	2.04±0.05	11.78
11-dimethyltetracyclo-undec-9-en-8-ol	0.24±0.02	12.57
11-isopropylidentricyclo-undec-3-en-10-one	0.55±0.03	13.45
β-patchoulene	1.37±0.07	13.73
gamma-selinene	72.49±2.0	15.49
3-pentanone	2.02±0.30	18.23

Total six chemical constituents were identified from the *G. walla* oil. As shown in Table 2, major constituents, which exist more than 1% in heartwood of *G. walla* oil, were γ-selinene (72.49%), 3-Phenyl-2-butanone (2.04%), 3-pentanone (2.02%) and beta-patchoulene (1.37%), respectively. All other constituents present were minor amounts (< 1%). Present results exhibited high content of γ-selinene (72.49%). Presence of special aroma containing compound such as γ-selinene and β-patchoulene (73.86%) might be responsible for the intrinsic pleasant aroma of oil fraction of *G. walla*. However, literature showed that the variation of chemical compositions and amount of individual components' depend on the location [18], who compared chemical profiles of selected agar wood oils from peninsular Malaysia reported that 3-phenyl-2-butanone content was varied from 0.79 to 7.80%. In addition, he pointed out that chemical composition also exhibited the variation according to the location. Moreover, presence of 3-phenyl-2-butanone and γ-selinene in essential oil of agar wood has been reported [18,19].

4. Conclusions

We have reported presence of preliminary phytochemicals, TLC fingerprints and GC-MS analysis of essential oil of *G.walla* for the first time in Sri Lanka. Present study revealed the presence of tannins, saponins, steroidal glycosides, flavonoids and alkaloids in all three parts tested. Moreover, TLC fingerprints could be successfully used for the identification of stem and leaf samples even in powder form. Presence of special aroma containing compound such as γ-selinene and β-patchoulene (73.86%) indicates that this has very good potential for commercial production of excellent perfume and other scented products. However, further optimization of distillation method could be suggested.

Conflicts of Interests

Authors declare that there is no conflict of interest.

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