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Compositions of *Thunbergia grandiflora* Leaf and Root Essential Oils

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Authors' contributions

This work was carried out in collaboration between both authors. Author DOM designed the study, wrote the protocol and managed the analyses of the study. Author KAM performed the statistical analysis and wrote the first draft of the manuscript. Authors KAM and DOM managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Fresh samples of leaves and roots of *Thunbergia grandiflora* were collected from forest- reserved area, Ibadan, Nigeria for extraction of their volatile oils. Hydro-distillation method was used in an all-glass Clevenger apparatus designed to British pharmacopeia specifications. It gave yields of 0.09% and 0.10% respectively. The oils were analyzed using gas chromatography [GC], gas chromatography-mass spectrometry [GC-MS] in University of Botswana. A total of thirty-eight compounds were identified in the leaf and root essential oils, twenty-seven compounds were identified in leaf oil, which account for 98.13% of it while seventeen identified compounds are responsible for 73.98% of root oil. Most abundant compounds in leaf oil are α-3-octanol (45.96%), 3,7 dimethyl 1,6-octadien-3-ol (13.22%) and 2- methoxy-3-(2-propenyl) phenol (8.36%). Dominant compounds in root oil are methyl salicylate (44.80%), 2,21,5,51-tetramethyl-1,11 - biphenyl (5.05%) and i-propyl hexadecanoate (2.87%). leaf oil is dominated by following classes of compounds (%); alcohols (68.58), carboxylic acid (4.01), aldehydes (12.90), ketones (5.18), esters (2.62), aromatics (0.66) and also sulphurates (0.45). Classes of compounds in root oil are esters (47.67), alcohols (2.64), carboxylic acids (0.55), aldehydes (7.69), sulphurates (1.61), aromatics (5.05) and alkane

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(5.02). The two oils contain alcohols and esters in good amounts. Our results indicate that alcohols, esters and aldehydes dominate *Thunbergia grandiflora* essential oils. We present important compounds in the leaf and root essential oils of *Thunbergia grandiflora*, which have not been reported earlier in literature.

Keywords: Thunbergia grandiflora; acanthaceae; essential oil; terpenoids; Gas Chromatography (GC) and Gas Chromatography – Mass Spectrometry (GC-MS).

1. INTRODUCTION

Thunbergia grandiflora Roxb.exRottler (*Acanthaceae*) is an evergreen vine. It is a large climbing and twining plant, native to China, India, Nepal, Indochina and Burma and many tropical countries of Africa [1-4]. It is also found throughout the Bangladesh, especially in forests of Gajipur and Chittagong [5]. Generally; it is known as black clock vine and blue trumpet vine. Other common names include Bengal clockvine, Bengal trumpet, blue skyflower, blue thunbergia, clock vine, skyflower and skyvine [3]. In Bangladesh, it is known as “Kauathuti”, “Nallata” and “Nillata” [4]. This Plant grows to about 20 metres in height and has a tuberous root system with a deep tap root [2]. The stalked, opposite leaves, which have rough surface, are quite variable in shape, they may be triangular or ovate and the margins may be toothed, lobed or entire. Length of the leaf is up to 20 cm and width is up to 6 cm broad, broad-based narrowing to a pointed tip, usually with deeply scalloped lobes towards the base. The shape and texture of the leaf as well as being oval and narrowing to pointed tip makes the plant different from other *thunbergia* species. The trumpet-shaped flowers have a short broad tube, blue to mauve on the outside, pale yellow inside, which expands to five rounded, pale lavender-blue petals, one larger than the others. The seed is flat, up to 1 cm long and covered with brown scales, the pods containing the seeds are ejected several metres upon ripening [2]. The plant is cultivated as house plant in temperate regions and has gained the Royal Horticultural Society's Award of Garden Merit [6,7].

Thunbergia grandiflora has very rampant growth habit and is great for the quick covering of arbors, trellises and fences. It is used as container plant, marvelous cascading over wall. The plant climbs and blankets native vegetation, with the weight of the vine often pulling down mature trees. *T. grandiflora* is the most widespread pest species, having been used as a garden ornamental for its attractive large leaves and hanging groups of large, pale lavender flowers [8]. Other species of *Thunbergia* includes *T. laurifolia, T. laevis nees, T. alata, T. erecta, T. coccinea*. Traditionally, some of these species have been used for treating fever, wounds, skin problems, back and joint pains, eye inflammation, piles and rectal cancer. They have also been used as aphrodisiac and antidote for snake bite [9-11]. *Thunbergia laurifolia* was found to possess antioxidant, hepaprotective, neuroprotective and hypoglycaemic properties [12-15]. *Thunbergia laurifolia* have been reported to contain compounds such as grandifloric acid, 8-epigrandifloric acid, 3′-O-β-glucopyranosyl-stilbericoside, benzyl β-glucopyranoside, (E)-2-hexenyl-β-glucopyranoside, hexanol-β-glucopyranoside and 6,8-di-Cglucopyranosylapigenin [16]. *T. laevis nees* was reported to display significant antioxidant and anti-diabetic properties [17,18]. Iridoid glucosides, apigenin glucosides, stilbericoside and thunbergioside were also obtained from *T. laevis nees* [19-21] as well as essential oil constituents, phytochemical analyses, and antimicrobial studies on leaf and stem of *thunbergia laevis* nees [22]. *Thunbergia grandiflora* is used for the treatment of blood dysentery, cataract, conjunctivitis, diabetes, gout, hydrocele, hysteria, malaria, marasmus, ophthalmia, postpartum pre-eclampsia, rheumatism, spermatorrhoea, stomach complaints, elephantiasis and urinary bladder stone [23-25]. They are used as traditional medicines for cardiac diseases and blood purification. Leaves and stem of *T. grandiflora* are used as a poultice in stomach complaints and to treat eye diseases in Chittagong Hill Tracts and it has anthelmintic activity [26]. The methanolic extracts of *T. grandiflora* flowers showed presence of alkaloids and phenols [27,28]. The novel iridoid glycosides; isounedoside and grandifloric acid were isolated from *thunbergia grandiflora* [29]. Research has not been done on the essential oil composition of *T. grandiflora*; we therefore report the leaf and root essential oil compositions of *thunbergia grandiflora*, which is scarce in literature.

2. EXPERIMENTAL DETAILS

2.1 Plant Material

Fresh samples of leaves and roots of *Thunbergia grandiflora* were collected from forest reserved area, Ibadan, Nigeria for extraction of their volatile oils on 8th June, 2015. Plant was authenticated at the Herbarium, Department of Botany, University of Ibadan, where voucher samples have been deposited, with voucher number UIH-22440.

2.2 Isolation of Essential Oils

Sample of *Thunbergia grandiflora* was collected, about 1.5 Kg, which was separated into leaf and root parts giving 235 g of leaf and 865 g of root. They were crushed separately and hydro distilled for 2.5 to 3 hours in an all glass Clevenger-type apparatus designed to British Pharmacopeia specifications. Oils were collected under iced condition with very small quantity of distilled n-hexane, which the analyzing GC corrected. Leaf and root essential oils were procured in 0.09% and 0.1% yields respectively.

2.3 Gas Chromatography

Leaf and root essential oils were analyzed using an Agilent 5975C series Gas Chromatograph - Mass Spectrometer (GC-MS) system. Data analysis was done using Productivity Chemstation Version E.02.01.1177 in conjunction with the Automated Mass Spectral Deconvolution and Identification System (AMDIS). The oven program for the column was as follows: The equilibration time was set at 0.25 min; the initial temperature was set at 70°C, held there for 4 minutes, and then ramped at 6°C/min to 140°C for 6 min. It was further ramped at 15°C/min to 300°C for 4 minutes. The total run time was 33.60 minutes. 1 µL of sample extract was injected in the splitless mode. The injector temperature was set at 280°C and the column head pressure was 56.756 kPa yielding a total flow of 64 mL/min. The transfer line temperature was 280°C. A HP-5MS 5% Phenyl Methyl Silox column with dimensions of 30 m x 250 µm x 0.25 µm and maximum recommended temperature (MRT) of 325°C was used throughout. Helium was used as a carrier gas at a flow rate of 1 mL/min.

2.4 Mass Spectrometry

The acquisition mode was full scan with a solvent delay of 4.00 minutes. Acquisition mass range was set at *m/z* 50– 600 and the electron multiplier detector voltage was auto set to 1082 V. The mass spectrometer source and quadrupole mass analyzer temperatures were set to 230°C and 150°C respectively. The filament emission current was auto set to 34.610 µA.

2.5 Identification of Components

Identification of the essential oil components were based on their retention indices (determined with reference to a homologous series of n-alkanes), and by comparison of their mass spectral fragmentation patterns with the National Institute for Standards and Technology (NIST) library version 8.0. The AMDIS software version 2.71 build 134.27 was used for deconvolution i.e. to compare the NIST library spectra with component spectra. The minimum match factor was for deconvolution on AMDIS was set to 70 and all identified components had match factors between 70 and 100. Some mass spectral fragmentation patterns with characteristic retention were compared with standard inbuilt data of Adams (1995) [30].

3. RESULTS AND DISCUSSION

3.1 Results

Volatile oils from leaf and root parts of *Thunbergia grandiflora* Roxb.exRottler, a medicinal plant, were obtained by hydro-distillation and gave yields of 0.09% and 0.1% respectively [Table 1]. The oils were analyzed using gas chromatography [GC], gas chromatography-mass spectrometry [GC-MS]. The chromatograms are presented in Figs. 1 and 2. Twenty-seven compounds were identified in leaf and seventeen compounds in root. Results of the 38 identified compounds in both are presented in Table 2. Comparison of the amounts of the ten classes of compounds found in the two *T. grandiflora* essential oils studied is presented in Table 3.

3.2 Discussion

Volatile oils from leaf and root of *Thunbergia grandiflora* gave characteristic distinctive notes: Leaf oil obtained in 0.09% yield had a leafy odour while root oil in 0.10% yield, had woody smell (Table 1). Compounds identified in each are listed in Table 2. Thirty-eight volatile compounds were identified in the two essential oils. The leaf oil contain 27 compounds, which make-up

98.13% of it, while root oil have 17 compounds accounting for 73.98% of it; Dominant compounds (%) in leaf essential oil are:α-3-octanol (45.96), 3,7 dimethyl 1,6-octadien-3-ol (13.22) and 2-methoxy-3-(2-propenyl) phenol (8.36). While dominant compounds (%) in root essential oil are: methyl salicylate (44.80), $2,2^1,5,5^1$-tetramethyl-$1,1^1$ - biphenyl (5.05) and i-propyl hexadecanoate (2.87). Leaf oil is dominated by the following classes of compounds (%): alcohols (68.58), carboxylic acid (4.01), aldehydes (12.90), ketones (5.18), esters (2.62), aromatics (0.66) and sulphurate (0.45). Classes of compounds (%) in root oil are esters (47.67), alcohols (2.64), carboxylic acids (0.55), aldehydes (7.69), sulphurate (1.61), aromatics (5.05) and alkane (5.02). Leaf and root oils contain different amounts of ten classes of compounds shown in Table 3. The root essential oil is a good source of esters while the leaf essential oil is a good source of alcohols. Compounds present in both leaf and root essential oils include (%);$2,2^1$, 5, 5^1, tetramethyl $1,1^1$ - biphenyl (0.66 in leaf and 5.05 in root), 6,10,14, - trimethyl – 2- pentadecanone (0.35 in leaf and 0.92 in root), (Z) 9,17 octadecadienal (0.96 in leaf and 2.84 in root), pentadecanal (1.18 in leaf and 2.06 in root) and phytol (2.00 in leaf and 1.15 in root) (Table 2). The presence in significant amount of oxygenated compounds (esters, alcohols, acids, terpenoids) in these oils is an important quality determining factor, they are known to have flavouring and pharmacological properties as well as being important precursors in acetate and shikimate biosynthesis of important metabolites [31]. Phytol present in both oils, is an important compound used in cosmetics, shampoos, toilet soaps, and household cleaners as it shows antimicrobial, anticancer, antidiuretic, antioxidant, anti-inflammatory, antitumor, antimicrobial, and chemopreventive properties and used in vaccine formulations [32,33]. Methyl salicylate in root oil is used in high concentrations as analgesic to treat joint and muscular pain [34]. 3-Octanol in leaf oil is utilized in alcoholic beverages as a flavouring agent. n- hexadecanoic acid in leaf oil is reported to possess anti-inflammatory, antioxidant, hypocholesterolemic and antibacterial activities. Medicated oils rich in n-hexadecanoic acid is used for the treatment of rheumatic symptoms in the traditional medical system [35,36]. The identified 38 compounds play important role in the vast ethno-medicinal uses and biological activities demonstrated by *T. grandiflora* [23,27,29,28]. The ubiquitous terpenoids are not common in these essential oils.

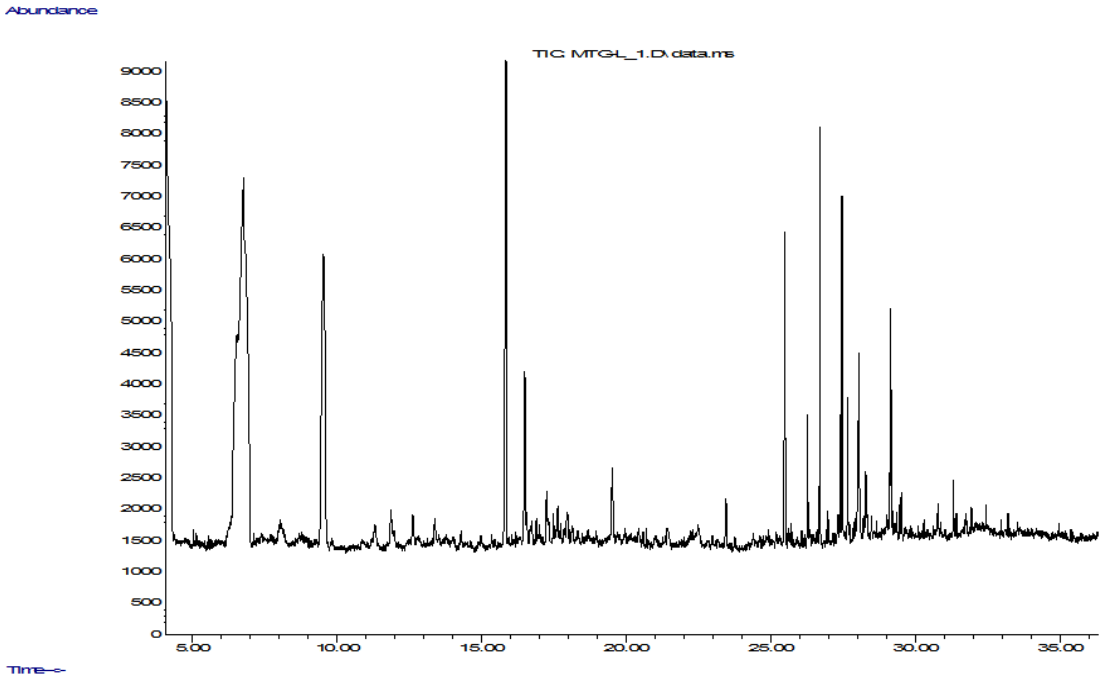

Fig. 1. Gas chromatogram of the leaf essential oil of *Thunbergia grandiflora* [see GC conditions under experimental procedures]

Table 1. Essential oils procured from leaf and root parts of *Thunbergia grandiflora*

Plant part	Weight of sample(g)	Weight of essential oil procured (g)	%Yield of essential oil procured	Odour assessment
Leaf	235	0.2	0.09	Leafy odour
Root	865	0.9	0.10	Woody smell

Table 2. Chemical composition of volatile oils of leaf and root *Thunbergia grandiflora*

S/N	RI	Compound	% composition in	
			Leaf	Root
1	1055	3 - octanol	45.96	-
2	1071	3,7 dimethyl 1,6 – octadien - 3 - ol	13.22	-
3	1762	Methyl salicylate	-	44.80
4	2152	2 – methoxy - 3- (2 – propenyl) phenol	8.36	-
5	2226	1 – (2,6,6 – trimethyl – 1,3,cyclohexa – 2 – buten – 1 - one	2.61	-
6	2315	2,3 dihydro -1,1-dimethyl 1H indene – 4 – carboxylic acid	0.52	-
7	2361	4 – (2,2- dimethyl – 6- methylene cyclo – 3 – buten – 2 -one	0.67	-
8	2403	α phenylethylbutanoate	0.69	-
9	2526	Nonylcyclopropane	-	2.50
10	2596	4 – (2,6,6, - trimethyl – 1- cyclohexen – 3- buten – 2 - one	1.55	-
11	2761	phytol	2.00	1.15
12	3091	2 – formyl methyl – 4,6,6 – bicyclo [3.1.1] hept – 3 - ene	0.86	-
13	3343	$2,2^1$, 5, 5^1 , tetramethyl $1,1^1$ - biphenyl	0.66	5.05
14	3348	tetradecenal	3.82	-
15	3446	n - pentadecanol	1.04	-
16	3454	9, 10 – dihydodiethyl – anthracene – 9,10 – biimine – 11,12 - dicarboxylate	0.66	-
17	3501	hexadecanal	3.58	-
18	3535	6,10,14, - trimethyl – 2- pentadecanone	0.35	0.92
19	3577	1 - hexadecanol	-	2.64
20	3580	1,3,3 – trimethylbicyclo [2.2.1] heptane	0.43	-
21	3589	(Z) 9,17 octadecadienal	0.96	2.84
22	3596	(Z) 7 tetradecenal	-	2.79
23	3597	Cis – 9 - hexadecenal	2.51	-
24	3621	pentadecanal	1.18	2.06
25	3671	n – hexadecanoic acid	3.49	-
26	3697	Ethyl hexadecanoate	0.56	-
27	3702	(Z) – 13- octadecenal	0.85	-
28	3726	i – propyl hexadecanoate	-	2.87
29	3765	2 – (4^1 – methoxyphenyl) – 2- (3^1 – methyl – 4^1methoxyphenyl) propane	-	0.63
30	3780	(E) 3 - Eicosene	-	1.68
31	3849	2 – chloroethyllinoleate	0.31	-
32	3855	Methyl 11,14,17, Eicosatrienoate	0.40	-
33	3953	2,7 dimethyl octane	-	0.82
34	3979	Benzo[b] thiophene – 3 – carboxylic acid	-	0.55
35	4026	3,3 – dimethyl hexane	0.44	-
36	4095	hexadecane	-	1.07
37	4161	Decyl -2- ethyl hexyl sulphurate	-	0.63
38	4224	2 ethyl hexyl isohexylsulphurate	0.45	0.98
% identified			**98.13**	**73.98**

RI means retention index

Table 3. Classes of compounds in *T. grandiflora* leaf and root essential oils

S/N	Class of compound	% in each of *Thunbergia grandiflora* essential oils	
		Leaf	Root
1	alkene	0.86	1.68
2	aldehyde	12.90	7.69
3	ketone	5.18	0.92
4	alkane	0.87	5.02
5	aromatics	0.66	5.05
6	esters	2.62	47.67
7	alcohols	68.58	2.64
8	carboxylic acid	4.01	0.55
9	sulphurate	0.45	1.61
10	terpenoid	2.00	1.15

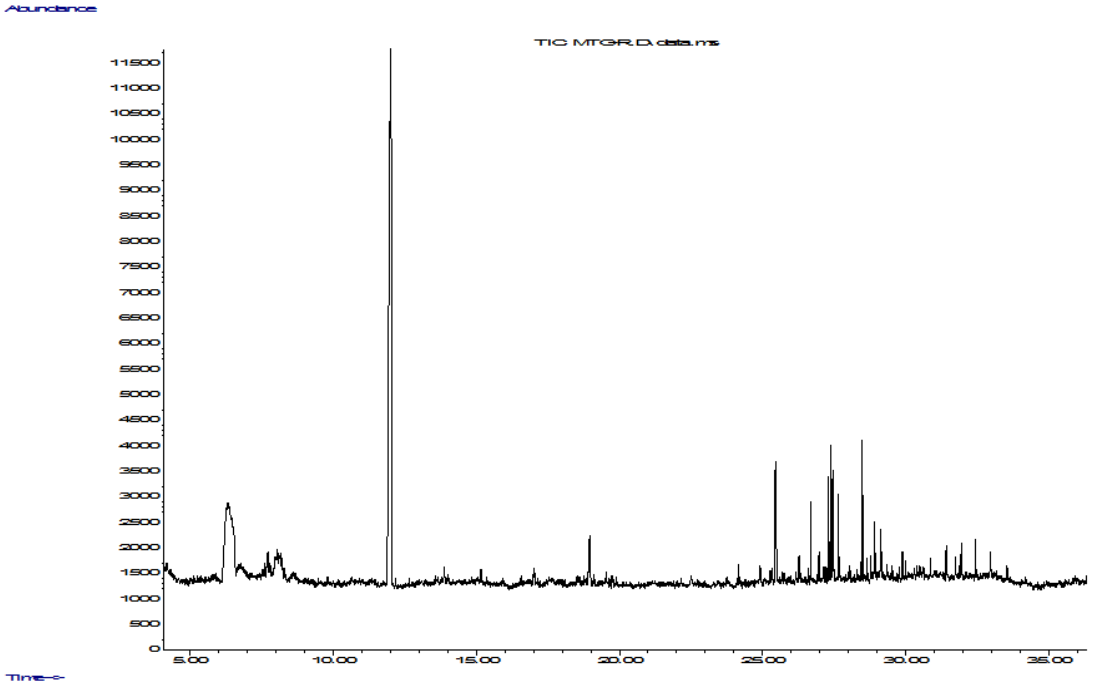

Fig. 2. Gas chromatogram of the root essential oil of *Thunbergia grandiflora* [see GC conditions under experimental procedures]

4. CONCLUSION

Our results indicate that alcohols, esters and aldehydes dominate *Thunbergia grandiflora* essential oils. This accounts for its important uses in ethno-medicinal activities. Our study revealed important compounds in the leaf and root essential oils of *Thunbergia grandiflora*, which have not been reported earlier in literature.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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