

Chemical composition and antioxidant activity of essential oil of leaves and flowers of *Alternanthera sessilis* red from Sabah

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ABSTRACT

In the present study, chemical composition and antioxidant activity of essential oil from the aerial parts of *Alternanthera sessilis* red has been investigated. The chemical composition of essential oil of *A. sessilis* red was determined by GC-MS analysis. Determination of antioxidant nature of *A. sessilis* red was carried out by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method using butylated hydroxytoluene (BHT) as the positive control. The major components of essential oil of leaves, as analyzed by the GC-MS were found to be 1,1,1,5,5,5-hexamethyl-3,3-bis(trimethylsilyloxy)trisiloxane (15.43%), S,S-dioxide trans-2-methyl-4-N-pentylthiane (11.27%), didodecylphthalate (10.62%) and tetrahydro-2,5-dimethoxy furan (10.01%). However, the major components of essential oil of flower were 1,1,1,5,5,5-hexamethyl-3,3-bis(trimethylsilyloxy)trisiloxane (17.76%), trans-4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-1,3-dioxolane (11.12%) and tetrahydro-2,5-dimethoxy furan (9.10%).

INTRODUCTION

Alternanthera sessilis red or red carpet weed belongs to the family *Amaranthaceae* which known as a medicinal plant in Asia (Vinodh and Kumar, 2014). In Malaysia it is commonly known as keremak, pudohrumputaoh and serapat depending upon the local areas. Young shoots and leaves of this weed are harvested as vegetables. It also serves as good ground cover and fodder. According to CRC world dictionary of Medicinal and poisonous plants *A. sessilis* has reddish-purple stem at base, papery leaves with white bracts and pink whitish perianth and inflorescence bracts (Quattrocchi, 2012). *A. sessilis* grows under variety of soil and climatic conditions. Wet soil condition is found to be the best for its growth, but it is habitant for marshy areas and wetlands (Vennila and Nivetha, 2015). It is a weed of rice in tropical areas and a pest of sugarcane and bananas (Das and Borua, 2014). Several therapeutic benefits of *Alternanthera*

sessilis has been investigated which includes anti diabetic, anti-inflammatory, cytotoxic effect toward pancreatic cancer lines and free radical scavenging activity (Tan and Kim, 2013). Previous biological analysis of *A. sessilis* revealed the presence of different phytochemical compounds from its various organic extracts. Triterpenoids, phytosterols and glycosides were found to be the main phytoconstituents for anti-diabetic activity of this weed (Das *et al.*, 2015). Nayak *et al.* (2010) has reported its antipyretic activities. The aqueous alcoholic extract of the entire plant was found to have the ability to modulate the histamine system and act as a smooth muscles relaxant.

An ethereal extract was found to possess anti-ulcerative property (Das and Borua, 2014). The plant was also found to be rich in antioxidant carotene (Patel *et al.*, 2015). The above therapeutic studies have been reported in the different organic extracts. Therefore, the aim of this study was to determine the chemical composition of the essential oil of its aerial parts and investigate their antioxidant activity. At the best of our knowledge there are no published studies that have reported these results for the essential oil of *A. sessilis*.

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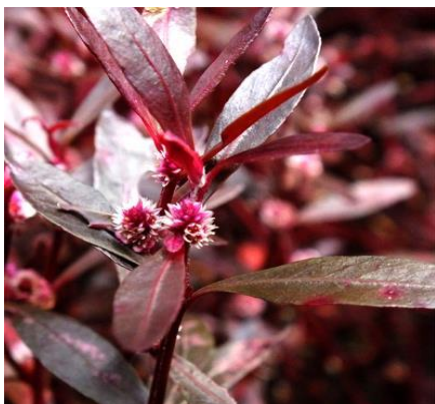
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Most of the medicinal plants which are mean to cure daily ailments have been found to possess antioxidant properties (Hossain and Shah, 2015). Natural antioxidant from medicinal plants sources are always popular in the food industries. They can be added to variety of foods for the prevention of oxidation via inhibiting the initiation of the radical chain reaction and also propagation steps resulting in the termination of the oxidation reaction processes (Nimse and Pal, 2015). The popularity of natural antioxidants is growing based on the fact that few synthetic antioxidants such as butylated hydroxyanisole (BHA) and BHT are now suspected to be harmful to human health as they confer some degree of carcinogenicity.

MATERIALS AND METHODS

Plant material

Alternanthera sessilis red was collected at flowering stage from Orchard De Villa which is located at Luyang Kota Kinabalu, Sabah, Malaysia (Fig. 1). The botanical identification and confirmation was achieved by Borneo Herbarium, Universiti Malaysia Sabah where a voucher specimen 00001-LPK/LYY was deposited in the herbarium.



(a)



(b)

Fig.1: *Alternanthera sessilis*: (a) Reddish-purple plant (b) Flower

Sample collection

The fresh plant which was in good condition was thoroughly washed with water. The leaves were collected and

chopped in small pieces and dried in shades on laboratory benches at ambient temperature for one week to ensure it is completely dried. About 1Kg leaves were pulverized in a grinder for 3 min and stored in dark bags to protect them from humidity and light prior to analysis.

Isolation of essential oil

The extraction of essential oil of the dried aerial parts was conducted by hydrodistillation process in a Clevenger-type apparatus for 3h. The essential oil obtained was then dried over anhydrous sodium sulphate (Na_2SO_4), filtered and stored in sealed glass vial at 4 °C in dark, for further analysis.

GC-MS analysis of essential oil

The samples of *A. sessilis* red leaves were prepared in methanol and analyzed by injecting into GC-MS branded Perkin Elmer (Model Perkin Elmer Clarus 500, USA) with a split injector at 300 °C. The VF-5 MS fused silica capillary column (30 m x 0.25 mm x 0.25 μm) was employed. The temperature was adjusted to 50 °C, held for 10 min, increased at 3 °C/min to 250 °C and finally hold for 10 min. Inert helium gas was employed as a carrier gas at constant flow rate of 1.0 ml/min. The compounds were identified by comparison of their retention indices (RI), with those provided in the National Institute of Standards and Technology (NIST) library. Identification was assumed when a good match of RI was achieved.

DPPH radical scavenging activity

The antioxidant activity of the extracts was quantitatively assessed on the basis of free radical scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, according to the method reported by Brand-Williams with slight modifications (Brand-Williams, 1995). 1ml of plant extracts of various concentrations, ranging between 50 to 200 $\mu\text{g/ml}$ were mixed with 1 ml of 0.5mM DPPH solution in methanol. Incubation, was done for 30 min in dark at 37 °C. BHT was used as a positive control and negative control was without any inhibitor or plant extract. Absorbance was measured calorimetrically, at 517nm. The experiments were carried out in triplicate. The percentage inhibition was calculated using the following formula:

$$\text{DPPH Scavenging Activity (\%)} = \left[\frac{(\text{A}_o - \text{A}_s)}{\text{A}_o} \right] \times 100$$

Here, A_o is absorbance of the control (no sample, DPPH solution only) and A_s is absorbance in presence of the sample.

RESULTS

Hydro-distilled essential oil from fresh flowers and leaves of *A. sessilis* red were analyzed by GC-MS. The oil was pale yellow with strong aromatic odor. The chemical constituents, retention time and their relative percentage are shown in Table 1 and Table 2. GC-MS analysis of the EO oil of flowers and leaves identified 22 and 18 compounds representing 94.6% and 98.4% of the total oil respectively.

Table 1: Chemical composition of essential oil from leaves of *Alternanthera sessilis*.

Entry	Compound Name	Retention Time	% Peak area
1	Methyl propionate	2.03	1.85
2	2,4-Dimethyl-3-pentanol	2.16	1.10
3	2-(1-Methylpropyl)cyclopentanone	2.24	1.01
4	2,3,4-Trimethylpentanoic acid	2.78	0.99
5	Methyl-2- <i>O</i> -methyl- α - <i>D</i> -xylofuranoside	3.25	3.13
6	2,6-Dimethyl-7-octen-3-ol	4.37	0.99
7	Tetrahydro-2,5-dimethoxy furan	4.94	10.01
8	9-Heptadecanone	8.68	2.06
9	1-Fluorododecane	10.09	6.60
10	1,1'-Oxybis heptane	12.61	2.41
11	1-Chlorooctadecane	17.61	3.99
12	Octyl ester 2-propenoic acid	23.80	9.32
13	2,4,4,6,6,8,8,-Heptamethyl-1-nonene	25.88	1.64
14	(<i>Z</i>)-7-tetradecene	30.79	3.12
15	1,1,1,5,5,5-Hexamethyl-3,3-bis(trimethylsilyloxy)trisiloxane	30.84	15.43
16	Cis-4-pentyl-5-propyl-2,2-bis(trifluoro methyl)-1,3-dioxolane	36.05	2.29
17	3-Methyl-2-(2-oxopropyl)furan	36.83	1.12
18	S,S-Dioxide-trans-2-methyl-4- <i>N</i> -pentyl-thiane	39.03	11.27
19	Didodecyl phthalate	42.35	10.62
20	(<i>S</i>)-Methylester-12-methyl-tetradecanoic acid	43.03	1.13
21	Tetracontane-1,40-diol	45.04	1.53
22	Trimethyl(4-terbutylphenoxy)silane	46.10	2.98

Table 2: Chemical composition of essential oil from flowers of *Alternanthera sessilis*.

Entry	Compound Name	Retention Time	% Peak Area
1	2-Methyl-1-undecene	2.02	3.50
2	Meso-2,5-dimethyl-3,4-hexanediol	2.15	3.43
3	2-(1-Methylpropyl)cyclopentanone	2.24	4.54
4	2,3,4-Trimethylpentanoic acid	2.77	4.17
5	Methyl-2- <i>O</i> -methyl- α - <i>D</i> -xylofuranoside	3.24	2.98
6	Tetrahydro-2,5-dimethoxy furan	4.92	9.10
7	2-Ethylhexyl ester-2,2-dimethyl propanoic acid	6.43	5.62
8	1-Fluorododecane	10.08	7.96
9	<i>N</i> -Dodecylpyridinium chloride	14.89	2.35
10	Bis(trimethylsilyl) 4-methyl-4-cyclohex-ene-1,2-dicarboxylic acid	23.81	7.71
11	1,1,1,5,5,5-Hexamethyl-3,3-bis(trimethylsilyloxy) trisiloxane	30.84	17.76
12	S,S-Dioxide-2- <i>N</i> -propylthiolane	33.73	1.16
13	1R, 2C, 3T, 4T-Tetramethyl cyclohexane	39.04	3.63
14	(-)-Cis-3,4-dimethyl-2-phenyltetrahydro-1,4-thiazine	39.08	4.52
15	2-Pentyl-1-heptene	42.87	5.26
16	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)- phenoxy]silane	43.15	3.54
17	3-Methyl-2-(2-oxopropyl)furan	44.36	0.09
18	Trans-4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-1,3-dioxolane	45.78	11.12

Table 3: Antioxidant activity of Essential oil from *Alternanthera sessilis* red.

	EO of Leaves	EO of Flowers	BHT
DPPH IC ₅₀ (μ g/ml)	179 \pm 0.06	170 \pm 0.02	88 \pm 0.04

The major components of EO of flower based on their peak area were 1,1,1,5,5,5-hexamethyl-3,3-bis(trimethylsilyloxy)trisiloxane (17.76%) which is an oxygenated diterpenes followed by trans-4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-1,3-dioxolane (11.12%) and tetrahydro-2,5-dimethoxy furan (9.10%). The other characteristics compounds determined were 1-fluorododecane (7.96%), 2-ethylhexyl ester-2,2-dimethylpropanoic acid (5.62%), bis(trimethylsilyl)-4-methyl-4-cyclohexene-1,2-dicarboxylic acid (7.71%), 2-pentyl-1-heptene (5.26%), 2-(1-methylpropyl)cyclopentanone (4.54%), (-)-cis-3,4-dimethyl-2-phenyltetrahydro-1,4-thiazine (4.52%), 1R,2C,3T,4T-tetramethylcyclohexane (3.63%) and 2-methyl-1-undecene (3.50%). However, the major components of EO of leaves, based on their peak area, were 1,1,1,5,5,5-hexamethyl-3,3-bis(trimethylsilyloxy)trisiloxane (15.43%) which is an

oxygenated diterpenes followed by S,S-dioxide trans-2-methyl-4-*N*-pentylthiane (11.27%), didodecylphthalate (10.62%) and tetrahydro-2,5-dimethoxy furan (10.01%). The other characteristics compounds determined were, octyl ester-2-propenoic acid (9.32%), 1-fluorododecane (6.60%), 1-chlorooctadecane (3.99%), methyl-2-*O*-methyl- α -*D*-xylofuranoside (3.13%), (*Z*)-7-tetradecene (3.12%), trimethyl(4-terbutylphenoxy)silane (2.98%), 1,1'-oxybis-heptane (2.41%), cis-4-pentyl-5-propyl-2,2-bis(trifluoromethyl)-1,3-dioxane (2.29%) and 9-heptadecanone (2.06%).

From the comparative analysis of scavenging activity of *A. sessilis* essential oil of flowers (170 \pm 0.02 μ g/ml), leaves (179 \pm 0.06 μ g/ml) and BHT (88 \pm 0.04 μ g/ml), it has been concluded that the essential oil showed moderate activity than that of the synthetic BHT (Table 3). However, analysis showed that the

radical scavenging activity of essential oil increases with the increase in concentration (Fig. 2).

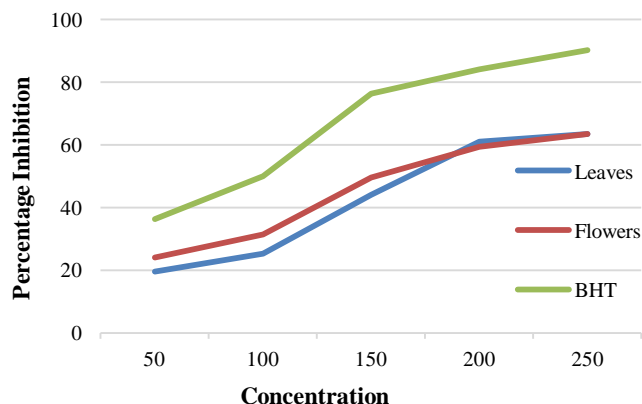


Fig 2: DPPH scavenging activity of essential oil of flowers and leaves of *Alternanthera sessilis*.

DISCUSSION

Among the detected compounds most of the compounds were found to be quite good therapeutic agents. For instance, the didodecylphthalate (10.62%) possesses antioxidant and antibacterial activities (Kalaiarasan, 2012; Sujatha *et al.*, 2015). The analysis of essential oil showed the presence of oxygenated sesquiterpenes, diterpenes, ketones, fatty acid and esters. These different phytochemicals have been reported to have antioxidant, anti-inflammatory and immune modulating activities (Tacouri *et al.*, 2013; Silveria *et al.*, 2013; Prakasia and Nair, 2015). Terpenes have promising biological activities which protect human cell from oxidative stress due to free radicals (Zengin and Baysal, 2014). It has been proved that the accumulated toxins from liver and kidney can be reduced by the intake of proper dose of terpene which contains antioxidants (Soares *et al.*, 2013; Karau *et al.*, 2015). In addition, sesquiterpenes are also used in pharmaceuticals which are highly effective against inflammatory and allergic infections (Meyers *et al.*, 2013). The reported antioxidant activity of the essential oil from flower and leaves is expected due to the presence of different terpenes and fatty acids. Methyl esters were proven to show antioxidant activities. S,S-dioxide-2-N-propylthiolane compounds with different alkyl chains have been reported to show antioxidant activities. 1-Fluorododecane also reported to show antibacterial activity (Siddiqui, 2014). Compounds having suffixes 'siloxanes' belongs to the class of organo-silicon compounds. The chemical constituents of siloxanes comprises of organic as well as inorganic compounds which includes silicon, oxygen, carbon and hydrogen. Cosmetic industries predominantly use siloxanes, to produce sunblocks, deodorant, hairspray and skincare (Omoruyi *et al.*, 2014). There were seven compounds which have been found to be present in the essential oil of leaves and flowers, indicating that these are evenly distributed throughout the plant. These compounds are, 2-(1-methylpropyl)-cyclopentanone, 2,3,4-trimethylpentanoic acid, methyl 2-O-methyl- α -D-xylofuranoside,

tetrahydro-2,5-dimethoxy furan, 1-fluorododecane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]trisiloxane, and 3-methyl-2-(2-oxopropyl)furan.

The antioxidant effect on DPPH radical scavenging capacities was conceived to their hydrogen donating ability (Abed *et al.*, 2014). The principle is based on availability of electron or hydrogen radicals to neutralize the DPPH free radical to become a stable diamagnetic molecule. The degree of decolorization indicates the scavenging potential of compounds in short time. The free radical scavenging activity of essential oil on DPPH may be due their hydrogen donating ability which reduces the stable DPPH free radical violet colour to pale yellow DPPH-H. The results of antiradical activities of essential oil from flower and leaves were usually represented by IC₅₀ value. IC₅₀ value is the concentration of effective sample needed to scavenge 50% of DPPH present in the sample solution.

CONCLUSION

The analysis of essential oil showed the presence of oxygenated sesquiterpenes, diterpenes, ketones, fatty acid and esters. These result suggested that the essential oil of *A. sessilis* red exhibits moderate antioxidant property. The scavenging activity of *A. sessilis* essential oil of flowers and leaves were found to be 170 ± 0.02 µg/ml and 179 ± 0.06 µg/ml respectively as compared to BHT (88 ± 0.04 µg/ml).

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