

GC-MS BASED CHEMICAL PROFILING AND EVALUATION OF ANTIOXIDANT POTENTIAL OF LEAVES AND STEMS OF *ALTERNANTHERA SESSILIS* RED FROM SABAH, MALAYSIA

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ABSTRACT

Objective: The aim of the current study was to determine the chemical composition of the leaves and stem of *Alternanthera sessilis* via GC-MS analysis and evaluating their total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA).

Methods: Determination of AA was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Chemical profiling of leaves and stem was conducted via GC-MS analysis of methanol and hexane extracts, which resulted in the identification of different types of low and high molecular weight compounds consisting of carbohydrates, fatty acid and vitamins. TPC and TFC were determined by the spectroscopic method and aluminium chloride calorimetric method.

Results: Among all the extracts, methanolic leaf extract exhibited highest DPPH radical scavenging activity with IC₅₀ value of 0.194±0.003 mg/ml, followed by methanolic stem extract (0.470±0.006 mg/ml), hexanolic leaf extract (0.786±0.002 mg/ml) and hexanolic stem extract (2.026±0.003 mg/ml), respectively. Butylated hydroxytoluene (BHT) used as the positive control showed IC₅₀ value of 0.037±0.002 mg/ml. The results suggested that the crude extracts of *Alternanthera sessilis* red leaves and stems exhibited good AA. The TPC and TFC of leaves were determined by using methanol and dichloromethane (DCM) as extracting solvents and was estimated higher for methanolic extract (TFC=142.7±0.0003 mg QE/g; TPC=279.8±0.0002 mg GAE/g). The AA was also reported to be higher for methanolic leaf extract which was 0.194 mg/ml.

Conclusion: TPC was found to be more than TFC. Methanolic extract (highly polar) was reported to display higher TPC and AA when compared to DCM. Among the methanolic and hexanolic extracts of leaves and stem, the AA was reported highest for the methanolic leaf extract.

Keywords: *Alternanthera sessilis*, Total phenolics, Total flavonoids, DPPH radical scavenging activity, BHT, GC-MS analysis

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INTRODUCTION

Tropical rainforests are among the biggest attractions of Malaysia. It is the land of some of the dense rainforests of the world. These rainforests are home to many creatures and pharmacologically important plants that are rich in various medicinal properties [1]. Around 2,000 plant species found here have therapeutic characteristics and can be used in traditional treatment [2]. Most of the medicinal plants which are meant to cure daily ailments are reported to have antioxidant properties which are beneficial to health [3]. Different parts of the plants such as leaves, flowers, seed and roots were reported to have important biological and pharmacological activities, such as antioxidant, anti-allergic, antibiotic and anti-carcinogenic properties. According to research, almost 80% of people globally used herbal medication as health care due to the potency of herbal medication [4]. Natural antioxidants from medicinal plants sources are always popular in the food industries. They can be added to a 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 [5]. 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 oxidation reaction processes. The popularity of natural antioxidants is growing based on the fact that few synthetic antioxidants such as BHA and BHT are now suspected to be harmful to human health as they confer some degree of carcinogenicity. Therefore, the surge for natural antioxidants has ever since increased. Phenolic compound derivatives became an exciting topic within this era because of their highly antioxidative effect. The polyphenols prevent various diseases and anti-aging [6]. Vegetables, fruits and tea are believed to account mainly for antioxidant activity of many plants by distributing the phenolic compounds with antioxidant activity in it [7]. Moreover, due to the

higher antioxidant activity, investigation of phenolic compounds in medicinal herbs has become more imperative in the past few years [8]. Flavonoids have been of particular interest because of their multiple roles in plants and their benefits in human health [9]. The main dietary sources of flavonoids along with wine and tea are fruits and vegetables [10]. Experiments have proven that flavonoids affect the heart and circulatory system and also strengthen the capillaries. They are often referred to as 'biological stress modifiers' since they serve as protection against environmental stress [11]. They are also known to have synergistic effects with ascorbic acid. Their protective actions are mainly due to membrane stabilizing and antioxidant effects.

Alternanthera sessilis red belongs to the family *Amaranthaceae*, which grows under a variety of soil and climatic conditions [12]. In Malaysia it is commonly known under various names such as keremak, pudoh rumput aoh and serapat, depending upon the local areas. Wet soil condition is found to be the best for its growth, but it is a habitant for marshy areas and wetlands [13]. Several therapeutic benefits of *A. sessilis* have been investigated which include antidiabetic, anti-inflammatory, cytotoxic effect toward pancreatic cancer lines and free radical scavenging activity [14]. The main aim of the present study was to determine the total phenolic content, total flavonoid concentration and antioxidant activity of various solvent extracts obtained from the plant *A. sessilis*. All these organic solvent extracts were also subjected to phytochemical screening.

MATERIALS AND METHODS

Chemicals, reagents and instruments

Follin-Ciocalteu phenol reagent, BHT, DPPH, aluminium chloride, quercetin, ascorbic acid, gallic acid and sodium carbonate (Na₂CO₃) were obtained from Sigma-Aldrich. Hexane, DCM and methanol were purchased from Merck. All chemicals were of analytical grade

and used without any further purification. The GC-MS analysis was conducted on a Perkin Elmer Clarus 500, GC-MS spectrometer equipped with VF-5 MS fused silica capillary column of 30 m x 0.25 i.d. and 0.25 μ m film thickness. Perkin Elmer Lambda 20 UV-Vis spectrophotometer was used to measure the absorbance for the determination of AA, TPC and TFC of all the crude extracts.

Collection and preparation of plant material

The medicinal plant *Alternanthera sessilis* red was bought from Orchard De Villa which is located at Luyang Kota Kinabalu, Sabah, Malaysia. The botanical identification and confirmation were achieved by Borneo Herbarium, Universiti Malaysia Sabah where a voucher specimen 00001-LPK was deposited in the herbarium. The fresh plant, which was in good condition, was thoroughly washed with water. The leaves were collected and dissected in small pieces and dried under shades inside the laboratory benches at ambient temperature for one week to ensure it is dried completely. About 1 kg leaves were pulverized in a grinder for 3 min and stored in dark bags to protect from humidity and light, prior to analysis.

Preparation of crude extract

The dried leaves powder (200 g) was extracted with methanol using Soxhlet extraction method at room temperature. The methanolic extract was recovered by evaporating the solvent by vacuum rotator evaporator. This crude extract was further diluted with water and subsequently extracted with hexane and dichloromethane to get their respective residual fractions. These fractions were filtered using Whatman's filter paper and then evaporated under reduced pressure *via* rotary evaporator in order to obtain the crude extracts. The extraction processes were repeated in triplicate. After solvent evaporation, all the crude extracts were weighed and kept for further usage in capped vials at 4 °C.

GC-MS analysis of crude extract

The samples of *Alternanthera sessilis* red leaves and stems were prepared in methanol and hexane and analyzed by injecting into GC-

MS with a split injector at 300 °C. The VF-5 MS fused silica capillary column (30 m x 0.25 mm x 0.25 μ m) was employed. The temperature programme was 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 a constant flow rate of 1.0 ml/min. The compounds were identified by comparison of their retention indices (RI) with those provided in National Institute of Standards and Technology (NIST) library. Identification was assumed when a good match of RI was achieved.

Determination of TPC

The TPC was determined using the spectroscopic method as described by Ainsworth and Gillespie, 2007 [15]. The reaction mixture was prepared by mixing 1 ml plant extracts (1 mg/ml), 1 ml of 10 % Folin-Ciocalteu's reagent dissolved in 13 ml of deionized water followed by the addition of 5 ml of 7 % Na₂CO₃ solution. The mixture was mixed thoroughly and kept in the dark at room temperature for 2 h. The blank solution was also prepared. The absorbance was recorded using UV-Vis spectrometer at 760 nm. All the analysis was repeated three times and the mean value of absorbance was obtained. TPC was determined by extrapolating calibration line which was constructed by the gallic acid solution. The TPC was expressed as gallic acid equivalent (mg GAE) per gram of the dried sample.

Determination of TFC

The TFC of the *Alternanthera sessilis* Red was determined by using aluminium chloride calorimetric method based on the methodology reported by Afify *et al.* [16]. 0.5 ml of sample (1 mg/ml) was mixed with 1 ml of 10 % aluminium chloride, 1 ml of potassium acetate (1 M) and 2.5 ml of distilled water. Quercetin was used as the internal standard. The absorbance of the mixtures was measured at 415 nm by using UV-spectrophotometer. The TFC was expressed in terms of quercetin equivalent (mg QE/g of sample). All the analyses were repeated three times and the mean value of absorbance was obtained.



Fig. 1: Soaked and dissected plant samples in methanol and hexane

In order to study GC-MS based chemical profiling, the leaves and stem of *Alternanthera sessilis* red were extracted with hexane and methanol (fig. 1). The initial weight of the sample used was 300 g for each *viz.* leaves and stem. The highest percentage yield of solid residue was obtained for methanolic stem extract (table 1).

DPPH radical scavenging activity

The AA of all the four extracts were quantitatively assessed on the basis of free radical scavenging activity of stable DPPH radical according to the reported method of Brand-Williams *et al.* 1 ml of plant extract solution of various concentrations, ranging from 0.05-0.20 mg/ml was mixed with 1 ml of 0.5 mmol DPPH solution in methanol. Incubation of the resulting solution was carried out for 30 min in dark room at 37 °C. BHT was used as the positive control

under the same assay condition. The absorbance was measured calorimetrically at 517 nm. The experiments were carried out in triplicate. The percentage inhibition was calculated using the following formula.

$$\% \text{ of radical scavenging activity} = 100 \times \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}$$

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

RESULTS AND DISCUSSION

Table 1: The percentage yield of extracts obtained from the solid residue of plant material

Extracts	Weight of crude extract (g)	Yield (%)
Methanol leaves	9.48	3.16
Methanol stem	19.77	6.59
Hexane leaves	13.18	4.39
Hexane stem	10.26	3.42

The methanolic and hexanolic crude extracts were analyzed by using GC-MS which led to the identification of different organic compounds of polar and nonpolar in nature, respectively. The identified chemical compounds are listed in table 2, table 3, table 4 and table 5 according to their elution order on a VF-5 capillary column. The major chemical compounds found in the hexanolic leaf extract were, (Z,Z)-9,12-Octadecadienoic acid (25.33 %), Vitamin A aldehyde (11.94 %) and 12-bromododecanoic acid (11.37 %), respectively. The major chemical compounds found in hexanolic leaf extract were hexamethyl cyclotrisiloxane (14.37 %), 10,13-dimethyl tetradecanoic acid, methyl

ester (13.91 %), (Z,Z,Z)-9,12,15-octadecatrienoic acid methyl ester, (10.94 %), trimethyl-[4-(1,1,3,3-tetramethylbutyl)phenoxy]silane (10.39 %) and hexamethylcyclotrisiloxane-1-monolinoleoyl glycerol trimethylsilyl ether (9.18 %). The methanolic stem extract led to the identification of methyl-2-O-methyl- α -D-xylofuranoside (30.47 %), followed by 2-(diethyl boryl oxy)-ethanethiol, (20.21 %) and tetrahydro-2,5-dimethoxy-furan (12.11 %) as major compounds whereas the major compounds in methanolic leaf extract were 2,2-dimethyl-1,3-propanediol (41.14 %), 1,3-dimethoxy-3-methyl-2-pentanone (14.22 %) and 2-(1-methylpropyl)cyclopentanone (11.49 %).

Table 2: Chemical composition of hexanolic crude extract of stem of *Alternanthera sessilis* red

No	Ret. time	Name of compounds	Molecular formula	M. W	Peak area %
1	2.02	2-Methyl-2-decanol	C ₁₁ H ₂₄ O	172	0.07
2	2.77	2,3,4 Trimethylpentanoic acid	C ₈ H ₁₆ O ₂	144	0.80
3	3.24	1,3-Dimethoxy-3-methyl-2-pentanone	C ₈ H ₁₆ O ₃	160	3.69
4	4.93	3-(2-Methoxyethoxymethoxy)-2-methylpentan-1-ol	C ₁₀ H ₂₂ O ₄	206	1.81
5	5.11	1,1-Diethoxydecane	C ₁₄ H ₃₀ O ₂	230	1.27
6	10.10	1-Fluorododecane	C ₁₂ H ₂₅ F	188	1.33
7	26.56	Cyclohexane-1,3-dione,2-allylaminomethylene-5, 5-dimethyl-	C ₁₂ H ₁₇ NO ₂	207	0.19
8	30.84	1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane	C ₁₂ H ₃₆ O ₄ Si ₅	384	2.00
9	31.55	1-O-(10-Undecenyl)- β -D-Mannofuranoside	C ₁₇ H ₃₂ O ₆	332	0.48
10	37.47	Octadecanoic acid,phenylmethyl ester	C ₂₅ H ₄₂ O ₂	374	0.84
11	41.84	3-Methyl-2-(2-oxopropyl)furan	C ₈ H ₁₀ O ₂	138	1.32
12	42.06	Phthalic acid, bis(7-methyloctyl) ester	C ₂₆ H ₄₂ O ₄	418	0.86
13	42.74	Phthalic acid, isobutyl 2-methylallyl ester	C ₁₆ H ₂₀ O ₄	276	0.64
14	43.03	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270	3.16
15	43.47	12-Bromododecanoic acid	C ₁₂ H ₂₃ BrO ₂	278	11.37
16	43.87	10-Bromododecanoic acid, ethyl ester	C ₁₂ H ₂₃ BrO ₂	278	3.79
17	44.34	1-Fluoro-Dodecane	C ₁₂ H ₂₅ F	188	2.08
18	44.96	6,11-Eicosadienoic acid, methyl ester	C ₂₁ H ₃₈ O ₂	322	4.54
19	45.03	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester	C ₁₉ H ₃₂ O ₂	292	2.73
20	45.15	Phytol	C ₂₀ H ₄₀ O	296	5.50
21	45.36	7-Pentyl-Bicyclo[4.1.0]heptane	C ₁₂ H ₂₂	166	4.55
22	45.43	(Z,Z)-9,12,-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	25.33
23	45.71	Z,Z-6,28-Heptatriactontadien-2-one	C ₃₇ H ₇₀ O	530	8.26
24	45.82	Vitamin A aldehyde	C ₂₀ H ₂₈ O	284	11.94

The result of total TPC was estimated by using Folin-Ciocalteu reagent. The phenolic content in plant extracts of *Alternanthera sessilis* red is expressed as milligram of Gallic acid equivalent (GAE) per gram of dry weight of extract, which is solvent dependent. The higher the polarity of solvent the more will be the TPC and TFC in the extract (table 7). Therefore, two polar solvents were chosen in order to obtain the TPC and TFC i.e. methanol and DCM. The

flavonoid concentration expressed as quercetin equivalent was determined by using aluminium chloride method. Higher concentration of flavonoids was found in the methanol extract (142±0.0003) followed by the dichloromethane (79.8±0.0001). The percentage yield obtained for both the extraction solvents are given in table 6, which significantly depends on the polarity of the solvent. The more is the polarity, the higher is the yield.

Table 3: Chemical composition of hexanolic crude extract of leaves of *Alternanthera sessilis* red

No	Ret. time	Name of compounds	Molecular formula	M. W	Peak area %
1	2.13	1-Hexen-4-ol, 1-chloro-3,5-dimethyl-	C ₈ H ₁₅ OCl	162	1.62
2	2.29	Phytol	C ₂₀ H ₄₀ O	296	0.38
3	2.50	5-Hydroxy-4-methyl-6-hepten-3-one	C ₈ H ₁₄ O ₂	142	0.72
4	2.75	2,3,4-Trimethylpentanoic acid	C ₈ H ₁₆ O ₂	144	2.25
5	3.21	Methyl 2-O-methyl- α -D-xylofuranoside	C ₇ H ₁₄ O ₅	178	8.99
6	3.49	Methyl 2,4-dimethylhexanoate	C ₉ H ₁₈ O ₂	158	0.76
7	4.89	Hexamethyl-Cyclotrisiloxane	C ₆ H ₁₈ O ₃ Si ₃	222	14.37
8	5.05	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane	C ₁₇ H ₃₀ OSi	278	10.39
9	30.83	1,1,1,3,5,5,7,7-Nonamethyl-3-(trimethylsiloxy) tetrasiloxane	C ₁₂ H ₃₆ O ₄ Si ₅	384	3.45
10	34.98	Isophytol	C ₂₀ H ₄₀ O	296	1.86
11	42.94	1,10-Hexadecanediol	C ₁₆ H ₃₄ O ₂	258	2.47
12	43.01	10,13-Dimethyl-Tetradecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	13.91
13	43.78	Z,Z-6,28-Heptatriactontadien-2-one	C ₃₇ H ₇₀ O	530	1.64
14	43.91	Methyl 4-(2-methyl-2-propen-1-yl)-3-cyclohexene-1-carboxylate	C ₁₂ H ₁₈ O ₂	194	1.46
15	44.95	(Z,Z)-9,12-Octadecadien-1-ol	C ₁₈ H ₃₄ O	266	5.96
16	45.02	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester	C ₁₉ H ₃₂ O ₂	292	10.94
17	45.14	1-Pentatriacontanol	C ₃₅ H ₇₂ O	508	5.06
18	45.30	Heptacosanoic acid, 25-methyl-, methyl ester	C ₂₉ H ₅₈ O ₂	438	1.55
19	45.78	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	498	9.18
20	46.09	Trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-Silane	C ₁₃ H ₂₂ OSi	222	3.05

Table 4: Chemical composition of a methanolic crude extract of stem of *Alternanthera sessilis* red

No.	Ret. time	Name of compounds	Molecular formula	M. W	Peak area %
1	2.77	2,3,4 Trimethyl pentanoic acid	C ₈ H ₁₆ O ₂	144	5.77
2	3.24	Methyl-2-O-methyl- α -D-xylofuranoside	C ₇ H ₁₄ O ₅	178	30.47
3	4.92	Furan, tetrahydro-2,5-dimethoxy-	C ₆ H ₁₂ O ₃	132	12.11
4	6.42	Tritetracontane	C ₄₃ H ₈₈	604	3.69
5	8.91	Ethanethiol, 2-(diethylboryloxy)-	C ₆ H ₁₅ BOS	146	20.21
6	10.07	1-fluorododecane	C ₁₂ H ₂₅ F	188	6.13
7	27.46	Methyl 3-[(1E)-5-hydroxy-1-pentenyl]benzoate	C ₁₃ H ₁₆ O ₃	220	4.96
8	37.88	Z,Z-6,28-Heptatriactontadien-2-one	C ₃₇ H ₇₀ O	530	1.02
9	41.12	7-Methyl-4-octanol	C ₉ H ₂₀ O	144	2.39
10	43.43	3-Methyl-2-(2-oxopropyl)furan	C ₈ H ₁₀ O ₂	138	3.78

Table 5: Chemical composition of a methanolic crude extract of leaves of *Alternanthera sessilis* red

No.	Ret. time	Name of compounds	Molecular formula	M. W	Peak area %
1	2.15	Scyllo-inositol	C ₆ H ₁₂ O ₆	180	3.33
2	2.24	2-(1-Methylpropyl)cyclopentanone	C ₉ H ₁₆ O	140	11.49
3	2.77	2,3,4 Trimethylpentanoic acid	C ₈ H ₁₆ O ₂	144	2.76
4	3.24	1,3-Dimethoxy-3-methyl-2-pentanone	C ₈ H ₁₆ O ₃	160	14.22
5	4.21	2,2-Dimethyl-1,3-propanediol	C ₅ H ₁₂ O ₂	104	41.14
7	4.93	4,4-Dimethoxycyclohexanone	C ₈ H ₁₄ O ₃	158	4.60

Table 6: The percentage yield of extracts obtained from solid leaf residue of plant material

Plant extracts	Percentage yield (%)
Methanol	2.49
Dichloromethane	2.45

Table 7: Total phenolic and total flavonoid contents in methanol and DCM extracts of *Alternanthera sessilis* red leaves

Plant extracts	Total phenolic contents (mg GAE/g)	Total flavonoid contents (mg QE/g)
Methanol	279.8±0.0002	142.7±0.0003
Dichloromethane	84.5±0.0003	79.8±0.0001

^aData are expressed as mean±SD, ^bResults are the mean of triplicate values (n=3)

DPPH radical is widely used as a model to determine the scavenging potential of several natural compounds such as phenolics or crude extract of plants [17]. The basic principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant. The degree of discoloration indicates the scavenging potential of antioxidant compounds of the extracts. The absorption strength decreases in the presence of a free radical scavenging

antioxidant. The leaves and stem of *A. sessilis* red in different organic solvents were tested for their free radical scavenging activity using DPPH assay. The results obtained showed an increase in the percentage inhibition as the concentration increases. The highest percentage inhibition exhibited by BHT was 88.84 %. Meanwhile, for the methanolic leaf extract, methanolic stem extract, hexanolic leaf extract and hexanolic stem extract the highest percentage inhibition were 70.81 %, 53.74 %, 7

48.66 % and 40.10 % respectively. The higher is the percentage inhibition, the higher is the AA (fig.2). Further, the IC_{50} value was calculated. IC_{50} value is the concentration of sample at which the inhibition percentage reaches 50%. In the case of methanolic extract, the IC_{50} was reported to be 0.194 ± 0.003 mg/ml and 0.470 ± 0.006 mg/ml for leaves and stem extracts, respectively. Whereas, for hexanolic extracts the IC_{50} was reported to be 0.786 ± 0.002 mg/ml for leaf and 2.026 ± 0.003

mg/ml for stem, respectively. BHT was used as standard had IC_{50} value of 0.037 ± 0.002 mg/ml (fig. 3). The difference in their IC_{50} value is due to the presence of different phytochemicals in different crude extracts of *Alternanthera sessilis* red leaves and stem. The lower the IC_{50} value, the higher the free radical scavenging activity. Therefore, methanolic leaf extract displayed the strongest free radical scavenging properties over the rest three when compared to BHT.

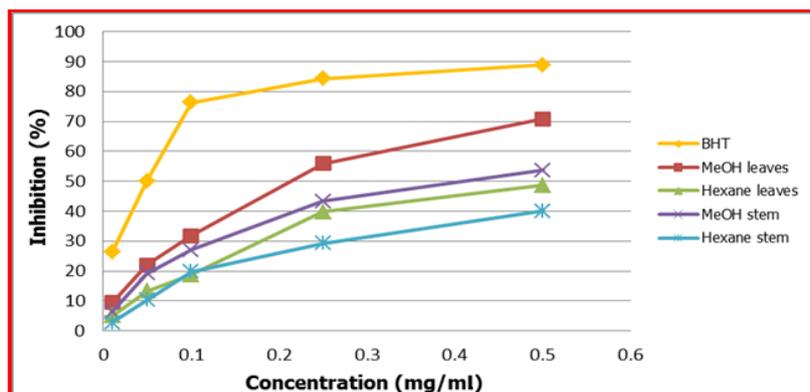


Fig. 2: Percentage inhibition of BHT and various *A. sessilis* red extracts against different concentration, ^aResults are the mean of triplicate values (n=3)

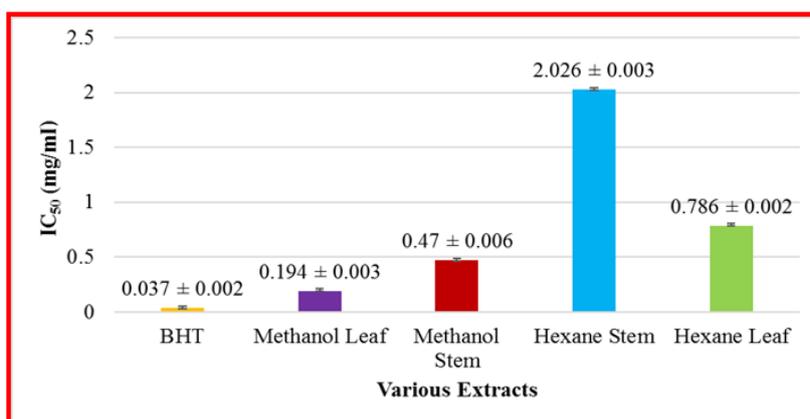


Fig. 3: IC_{50} value of BHT and various *A. sessilis* red extracts against different concentration, ^aExperiments were run in triplicate (n=3), ^bResults are reported in mean \pm SD

CONCLUSION

In conclusion, this study shows a higher ratio of total flavonoid content (142.7 ± 0.0003 mg QE/g) and total phenolic content (279.8 ± 0.0002 mg GAE/g) in methanol extract followed by DCM extract. The highest AA was reported to be 0.194 ± 0.003 mg/ml, for strongly polar methanol leaf extract followed by methanol stem extract (0.470 ± 0.006 mg/ml), which proves that polar solvent are good extracting solvents for the identification of TPC, TFC as well as free radical scavenging properties.

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AUTHORS CONTRIBUTIONS

MSK designed the study, the main conceptual ideas and proof outline. LYY, WZ and MSK performed the experiments. SKY worked

out almost all of the technical details, performed the numerical calculations for the suggested experiment and took the lead in writing the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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