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Article

Potential nutritional and antioxidant activity of various solvent extracts from leaves and stem bark of *Anisophyllea laurina* R. Br ex Sabine used in folk medicine

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Anisophyllea laurina is a plant that has been used in folk medicine to treat malaria, dysentery, diabetes, toothache and various skin diseases. Leaves extract had protein content of 9.68% and a high calcium content of 25084.317 mg/100 g while stem bark extract was found to contain greater amounts of calcium (8560.96 mg/100 g), potassium (7649.47 mg/100 g), magnesium (1462.49 mg/100 g) and iron (973.33 mg/100 g). Palmitic acid, linolenic acid, linoleic acid and oleic acid were the most abundant fatty acids in leaves and stem bark extracts. Furthermore, total phenolic (2382.39 mg GAE /100 g) and total flavonoid **(**385.79 mg QE/100 g) contents were abundant in stem bark while leaves extract was rich in total tannin content (3466.63 mg CE/100 g). However, both leaves and stem bark contained great amounts of vitamins and amino acids were a good source of antioxidant activities. For the individual polyphenol, stenophyllanin A (45.87 mg/g), casuarinin (24.55 mg/g) and digalloyl-HHDP-glucopyranose isomer (15.63 mg/g) were found to be the major compounds from the leaves whereas procyanidin tetramer (14.89 mg/g, (-)-Epicatechin (12.18 mg/g) and procyanidin trimer (11.25 mg/g) were the most predominant compounds from the stem bark. Additionally, the results revealed a significant and strong correlation between phenolic compounds and antioxidant activities.

Uniterms: *Anisophyllea laurina/extract/*antioxidant activity. *Anisophyllea laurina/extract/*total phenolic. *Proximate composition. F*lavonoid. Tannin.

INTRODUCTION

Anisophyllea laurina **R. Br. ex Sabine** comprise 25–36 species from the family *Rhizophoraceae (Anisophylleaceae)* are placed in four genera with disjunctive geographic distributions. *A. laurina* is the commonest mangrove and consequently accounts for a considerable area, distributed widely in West Africa (Guinea-Bissau to Sierra Leone), South East Asia, South America and West Malaysia (Sumatra, Peninsular Malaysia and Borneo) (Juncosa, 1988; Zhang, Simmons, Renner, 2007).

Furthermore, Neuwinger (Neuwinger, 2000) reported that *A. laurina* leaves are rich in tannins, a decoction of the leaves is used as a mouth rinse for toothache and the ground leaves are said to have medicinal properties to treat diabetes, emetics and to remove cataracts from the eyes, while the stem bark is used as remedy for dysentery (Lebbie, Raymond, 1995). Both leaves and stem bark were identified and are well-known as traditional medicine for treating malaria in Guinea (Balde *et al.,* 2015). Ethanol and methanol extracts of the leaves and stem bark have shown potential as antibacterial and antifungal agent (Onivogui *et al.,* 2015). In another study conducted by Kargbo, Onivogui and Song (2015) it was reported that the ethanol crude extract from leaves and stem bark of *A. laurina* exerted an inhibitory effect on α-glycosidase and α-amylase.

In recent years, basic research and observational studies have suggested that adequate intake of antioxidant vitamins or minerals may protect against the development of type 2 diabetes via reduction of oxidative stress and its

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associated metabolic abnormalities, including systemic inflammation, endothelial dysfunction, hypertension, and dyslipidemia (Song *et al.,* 2010). The aim of this study was to evaluate the proximate profiles, total phenolic, flavonoids contents and antioxidant activity of leaves and stem bark of *A. laurina.*

MATERIAL AND METHODS

Collection of plant materials

Fresh mature whole leaves and stem bark of *A. laurina* R. Brex Sabine were collected in Coyah of Kindia region in September 2014 and identified by Traore Mohamed Sahar of the Research and Valorization Center on Medicinal Plants, Dubréka, Guinea. A voucher specimen of the plant was deposited with the number 5HK4 at the herbarium of the center.

Proximate analysis of samples

Crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25. Lipid content was analyzed gravimetrically following Soxhlet extraction. Crude fiber was estimated by acid/alkaline hydrolysis of insoluble residues. Crude ash content was estimated by incineration in a muffle furnace at 550 °C. The moisture content was determined gravimetrically. Carbohydrate contents were determined by difference [100-(protein + crude fat + ash + crude fiber). All methodologies followed the recommendations according to the acid hydrolysis method (AOAC, 2000). Triplicate samples were analyzed for each sample.

Mineral analysis

The minerals (Zn, Fe, Cu, Mn, Na, K, Mg, and Ca) were analyzed separately, using an Atomic absorption spectrophotometer (SpectraAA 220, USA Varian). The data reported represent the mean of three determinations. The minerals were expressed as mg/100 g of dry Weight (DW).

Vitamins

Vitamins were analyzed using the method described by Erbaş, Certel and Uslu (2005). Peaks were verified by adding the standard vitamins to samples and individual peak area was calculated according to the peak area of corresponding standard vitamins. Results were calculated on a dry weight basis.

Amino acid analysis

Amino acid contents were analyzed using the method described by Jarrett *et al.* (1986). The amino acid composition was expressed as g of amino acid per 100 g of protein.

Organic acids

Organic acids were determined according to the method described by Usenik, Fabcic and Stampar (2008). The concentrations were expressed as mg per kg dry weight.

Anti-nutritional factors analysis

Total oxalate was determined as described by Day and Underwood (1986). The oxalate content was then calculated by taking 1 mL of 0.05 mol/L of $KMnO_4$ as equivalent to 2.2 mg oxalate (Edwige, 2012). Phytate was determined using the method of Edwige, (Edwige 2012). The following equation gives the percentage of phytic acid:

$$Phytic\ acid = \frac{0.66(10 - V)}{m}$$

where, V is the volume of iron (III) chloride solution in milliliters and m the sample mass in grams.

Fatty acid analysis

Preparation of fatty acid methyl esters (FAME) of both leaves and stem bark was determined according to the method described in AOAC (2000)**.**

Quantification of phenolic compound

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent as described by Gouveia and Castilho (2011). TPC was expressed as mg of gallic acid equivalents per 100 gram of dry weight (mg GAE/ 100 g DW) through a calibration curve of 0-400 µg/mL range. Total flavonoid content (TFC) was also measured as described by Gouveia and Castilho (2011). TFC was expressed as mg of quercetin equivalent per 100 g of dry weight (mg QE/100 g DW), through a calibration curve of quercetin of 0-400 µg/mL. The total tannin content (TTC) was determined using the vanillin-methanol solution as described by Sun, Silva and Spranger (1998). TTC was expressed as mg (+)-catechin equivalents per 100 g of dry weight (mg CE/100 g DW) through a calibration curve of 0-400 µg/mL.

Phenolic profile analysis by UPLC LC-ESI-MS

The mass spectra were recorded using the Waters ACQUITY UPLC® SYNAPTTM High Definition Mass Spectrometer systems (Waters, Milford, USA) equipped with an electrospray ion source and hybrid quadrupole-time-of-flight (Q-TOF) mass spectrometer with the MSE model. The Q-TOF instrument was operated in V mode for MS experiments with the TOF data collected between m/z 100 and 1800. The optimized condition was desolvation gas at 500 L/h at a temperature of 400 ºC, cone gas at 50 L/h and source temperature at 100 ºC, capillary and cone voltages at 3 kV and 30 v, respectively.

UPLC data were produced using the Waters ACQUITY UPLC systems (WATERS MALDI SYNAPT Q-TOF MS, Milford, USA) equipped with a binary pump, an autosampler, a degasser, and a diode-array detector (DAD). The system was controlled with MassLynx V4.1 software. The DAD was monitored in the range 200–600 nm. The chromatographic column UPLC™ BEH AMIDE C18 (2.1 mm×100 mm, 1.7 µm) was used and eluted with a linear gradient of A:100% acetonitrile and B: 0.1 % formic acid at a flow rate of 0.3 mL/min: 0-5% A, 0-17min; 5-60% A, 17-20 min; 60-100 % A, 20-22 min; 100-5% A 22-22.1 min; 5 % A, 22.1-25 min. The temperature was ratting at 45 ºC. The injection volume was 0.5 µL. The accurate mass and composition for the precursor and fragment ions were calculated using the MassLynx 4.1 software. For the quantitative analysis of phenolic compounds, the standard curve of catechin, (-)-epicatechin 3-*O*-gallate and isorhamnetin-3-*O*-glucoside (2-200 µg/mL) were used to quantify the phenolic compounds. The analytical curve was linear (r^2=0.99); the limit of detection was 0.2 µg/mL, and the limit of quantification was 0.5 µg/mL as calculated using the parameters of the analytical curves (standard deviation and the slope).The results were expressed in mg per g of sample. Compounds concentrations were calculated in triplicate and the mean value.

Determination of antioxidant activities

DPPH radical scavenging activity assay followed a reported method by Gouveia and Castilho (2011). The DPPH radical scavenging effect of the sample was expressed based on the Trolox calibration curve, as µmol Trolox equivalent per 100 g of dried fruit weight (µmol eq. Trolox/100 g). Ferric reducing antioxidant power (FRAP) assay was conducted according to Lu *et al.* (2011). A standard curve was made with Trolox, and the results were expressed as µmol Trolox equivalents (TE) per one gram dry weight (DW) of the fruit powders. The ABTS radical scavenging activity assay was performed according to the procedures of Gouveia and Castilho (2011). Results were expressed as µmol Trolox equivalent (TE)/100 g of extract.

RESULTS AND DISCUSSION

The proximate composition and mineral content of leaves and stem bark extracts of *A. laurina* is shown in table I for comparison. The crude protein content of leaves (9.68±0.9%) was high when compared with stem bark, while stem bark extract was found to contain substantially greater amounts of ash (11.93%). The carbohydrate and Food energy (g/calories) amounts were found to be similar in both leaves and stem bark extracts (Table I). In this work, it was observed that leaves and stem bark contained appreciable quantities of several essential mineral macronutrients necessary for human health maintenance. The stem bark appeared to be an important source of mineral elements, such as calcium (8560.96 mg/100 g), potassium (7649.47 mg/100 g), magnesium (1462.49 mg/100 g), iron (973.33 mg/100 g), sodium (242.38 mg/100 g) and zinc (15.91 mg/100 g), while the leaves extract was especially high in calcium (25084.32 mg/100 g) followed by potassium(3583.47 mg/100 g), magnesium (495.36 mg/100 g) and iron (180.86 mg/100 g). The high levels of Ca, Mg and K are collectively known to reduce hypertension and blood pressure as well as used in the prevention and treatment of high blood pressure. The leaves and stem bark of *A. laurina* had high amounts of macro and micro-nutrients especially Ca, K, Mg and Fe when compared with other green leafy vegetables (Abbas *et al.,* 2015; Mendez *et al.,* 2014).

Regarding fatty acids (FA) composition of leaves and stem bark as recorded in Table II, the major fatty acids found in stem bark extracts samples were palmitic acid followed by linoleic acid and oleic acid. For leaves extract linolenic acid, was the most abundant fatty acid, and was followed by palmitic acid, linoleic acid and palmitoleic acid. Furthermore, total polyunsaturated fatty acids (TPUFA) in leaves at 58.40% predominated over total saturated fatty acids (TSFA) and monounsaturated fatty acids (MUFA) at 41.60%. TPUFA content (50.39%) in stem bark was similar to that of TSFA at 49.60 %. Linolenic acid has excellent anti-inflammatory effects since it is transformed by 15-lipoxygenase into an effective metabolite with same pathway as linoleic acid. It also has excellent inhibitory effects against inflammations from burns and erythema (Anitha, 2012). The high content of linolenic acid, oleic acid and linolenic acid in leaves may be beneficial to human health. However, many scientists have reported that saturated fatty acids may impact blood

TABLE I - Proximate composition and mineral contents of leaves and stem bark

Constituents	Stem bark	Leaves
Moisture (%)	87.91±0.10	88.22±0.07
Crude Protein (N x 6.25%=	3.84±0.04	9.6792±0.05
Crude Fiber (%)	3.56±0.05	5.23±0.03
Fats/oil (%)	2.26±0.01	1.6±0.8
Ash (%)	11.93±0.02	7.96±0.03
Carbohydrates (%)	78.41±0.08	75.5308±0.07
Food energy (g/calories)	349.34±0.12	355.24±0.19
Mineral content (mg/100 g)		
Zinc (Zn)	15.914±1.56	44.899±0.67
Iron (Fe)	973.329±1.90	180.86±0.37
Manganese (Mn)	61.225±0.24	41.13±0.65
Copper (Cu)	5.920±0.58	1.096±0.45
Potassium (K)	7649.47±5.3	3583.473±2.05
Sodium (Na)	242.38±1.76	29.81±0.78
Magnesium (Mg)	1462.485±0.7	495.36±0.9
Calcium (Ca)	8560.96±3.05	25084.317±4.10

Values are means± standard deviation of three determinations (n=3).

TABLE II - Fatty acid content of leaves and stem bark of *A. laurina*

Retention time	Fatty acids compound	Stem%	Leaves %
9.393	Lauric acid methyl ester (C12:0)	2.02±0.01	0.55±0.02
12.003	Myristic acid Methyl ester (C14:0)	3.52±0.02	1.43±0.05
14.905	Palmitic acid (C16:0)	36.49±0.58	34.69±0.8
23.886	Arachidic acid (C20:0)	0.56±0.06	0.33±0.07
18.859	Stearic acid (C18:0)	5.76±0.07	4.37±0.01
29.689	Behenic acid (C22:0)	1.26±0.03	0.23±0.01
TPSFA		49.60±0.77	41.60±0.96
15.327	Palmitoleic acid (C16:1)	1.25±0.01	7.58±0.06
19.3	Oleic acid (C18:1)	16.52±0.15	4.77±0.12
20.384	Linoleic acid (C18:2)	27.76±0.58	8.6±0.28
21.999	Linolenic acid (C18:3)	4.86±0.10	37.45±0.26
TPUFA		50.39±1.17	58.4±1.30

Values are means± standard deviation of three determinations (n=3).

cholesterol levels by slowing down the mechanisms that removes circulating LDL from the blood. Palmitic, myristic and lauric acids increase blood cholesterol whereas oleic acid, stearic, linoleic acid and linolenic acid decrease total blood cholesterol content (Fattore *et al.,* 2014).

The vitamin contents and anti-nutrients composition of the leaves and stem bark are presented in Table III. Vitamin C (316.79 mg/100 g) was the most abundant in the leaves extract followed by nicotinamin (208.33 mg/100 g), thiamin (141.67mg/100 g) and pyridoxine (108.33 mg/100 g). In stem bark extract, pyridoxine (108.33 mg/100 g) and nicotinamin (208.33 mg/100 g) were the main vitamins detected whilst vitamin C was not detected. This study suggests that leaves are a rich source of vitamin C and calcium that may be developed as a functional ingredient

in nutraceuticals, and health-promoting phytochemicals. Multivitamins and calcium are the most commonly used dietary supplements in the U.S and are advocated for as an attractive option for preventing chronic diseases, such as cancer, cardiovascular disease and type 2 diabetes (Song *et al.,* 2010)

The results for the anti-nutrients composition showed that leaves and stem bark have similar values of oxalates and phytic acid. The phytic acid (907.53 mg/100 g) and oxalate (27.57 mg/100 g) contents of the leaves did not show any significant difference compared with those of the stem bark. For organic acids the leaves extracts were mainly high in malic acid (6105.66 mg/100 g), followed by citric (64.56 mg/100 g) and tartaric acids (13.06 mg/100 g) (Table III). The high amount of malic acid found in leaves extracts probably contributed to its antimicrobial activity as reported by Onivogui *et al.* (2015). On the other hand, stem bark extracts showed moderate amounts of organic acids such as citric acid (60.82 mg/100 g), acetic acid (34.29 mg/100 g) and malic acid (19.82 mg/100 g). The organic acids contribute towards increased shelf life, stability and microbiological safety of the food (Chen *et al.,* 2014).

Regarding the amino acid profiles in Table IV, the isoleucine, lysine, threonine and alanine contents of leaves and stem bark were not significantly different (P value >0.05), whereas they were significantly different for other remaining amino acids. However, leaves extracts were a good source of principal amino acid as valine, leucine, phenylalanine, arginine and glutamic acid. On the other hand, the non-essential amino acids as aspartic acid, histidine, cysteine, serine, glycine, tyrosine and proline in stem bark were higher than in leaves. The essential amino acids observed in both leaves and stem bark were higher than the suggested amino acids requirements. Therefore leaves and stem bark of *A. laurina* are considered nutritious because their essential amino acid profiles are higher than the reference levels required for children as their bodies are unable to synthesize them (WHO, 2007).

Antioxidant compounds

Results for the antioxidant compounds of leaves and stem bark of *A. laurina* from different extracting solvents are presented in Table V. The total phenolic contents showed great variations in leaves and stem bark extracts, and the range were from 1245.47 to 2294.43 mg GA/100 g and 1506.41 to 2382.39 mg GAE/100 g, respectively. The results showed that the maximum amounts of TPC in the stem bark (2382.39 mg GAE/100 g) was slightly higher than that from leaves (2194.43 mg GAE/100 g). The values for the total phenolic contents obtained in this study were found to be lower than those from leaves and stem bark extracts of *T. Indica* as reported by Razali *et al.* (2012). Regarding total flavonoids content, the results also showed great variation in different extracts, and the ranges were from 104.41 to 346.14 mg QE/100 g in leaves and 292.98 to 385.79 mg

TABLE III - Vitamins, organic acid and anti-nutritional content of leaves and stem bark

Constituents	Stem mg/100 g	Leaves mg/100 g
Ascorbic acid (C)	0.9±0.05	316.79±0.8
Thiamin (B1)	50±0.01	141.667±0.15
Riboflamin(B2)	58.33±0.02	26.667±0.03
Pyridoxine(B6)	300±0.06	108.33±0.33
Nicotinamin (PP)	133.33±0.21	208.33±0.15
Cyanocobalamin	1.75±0.08	3.5±0.01
Malic acid	19.82±0.02	6105.66±0.15
Citric acid	60.82±0.34	64.56±0.21
Tartaric acid	5.8628±2.5	13.06±1.5
Fumaric acid	0.216±0.05	0.31±0.08
Lactic acid	4.70±0.15	5.6±0.02
Acetic acid	34.29±0.03	6.5±0.07
Phytate	895.576±0.45	907.53±0.58
Oxalate	543.54±0.03	275.656±0.01

Values are means± standard deviation of three determinations (n=3).

TABLE IV - Amino acid composition from leaves and stem bark extracts of *A. laurina* as compared to the FAO/WHO/UNU reference pattern (mg/g protein)

Constituents	Stem bark mg/g	Leaves mg/g	FAO/WHO reference protein
Leucine	82.39±0.45	97.49±0.52	20
Isoleucine	60.45±0.52	60.68±0.33	25
Lysine	76.14±0.33	71.00±0.58	30
Phenylalanine	60.20±0.52	75.50±0.45	25
Threonine	43.16±0.23	43.56±0.33	15
Methionine	4.13±0.07	7.46±0.03	15
Valine	7.48±0.06	70.70±0.82	26
Non-essential			
Arginine	50.79±0.78	60.47±0.15	
Histidine	64.14±0.34	30.34±0.45	
Cysteine	11.54±0.54	1.39±0.01	
Aspartic acid	106.63±0.76	93.79±0.23	
Glutamic acid	109.79±0.52	124.70±0.58	
Serine	41.86±0.15	36.98±0.21	
Glycine	76.86±0.07	60.77±0.33	
Alanine	64.26±0.21	64.12±0.15	
Tyrosine	53.17±0.08	41.52±0.03	39
Proline	87.00±0.45	59.54±0.05	

Values (Mean ± SD) in the same column with different letters are significantly at ($P<0.05$), n=3.

QE/100 g in stem bark. TFC of leaves of *A. laurina* was lower compared to that from *T. montanum* leaves 58.48 mg RU/g (Stankovic *et al.,* 2011). Furthermore, the TMAC ranged widely from 8.56to 30.75 mg C3G/100 g for leaves and 2.35 to 60.65 mg C3G/100 g for stem bark. On the other hand, total tannins content of stem bark and leaves extracts in the various solvents ranged from 123.73 to 2391.94 mg CE/100 g and 1322.41 to 3466.63 mg CE/100 g, respectively. The total tannin contents (TTC) of the leaves extracts were higher than those from stem bark and this could probably be due to high diversity of hydrolysable tannins found in leaves by UPLC–Q-TOF-MS analysis. The total tannin contents (TTC) of leaves and stem bark in various extracting solvents was significantly higher than those from leaves of *Aegle marmelos* (12.377 mg/g) (Parvathi, Ramya, Rekha, 2013). With respect to the extraction solvent used in this study, the results showed that antioxidant composition in the leaves and stem bark were significantly different ($p < 0.05$). It was further observed that in all the plant parts used, the highest levels of antioxidant composition were found in the ethanol extract followed by methanol, ethyl acetate and water and this might be attributed to their polarity and good solubility for TPC (Korekar *et al.,* 2011).

Antioxidant activities

Results of the different antioxidant activities for different plant parts of *A. laurina* as determined by ABTS, DPPH and FRAP assay using the different extraction solvents are shown in Figure 1. Results showed that ethanol extracts of *A. laurina* have the highest levels of DPPH. As shown in Figure 1(a), the highest DPPH value was obtained from leaves extract as 76333.5 µmol TE/100 g, followed by stem bark extract with 65262.3 µmol TE/100 g. These results suggest that ethanol was found to be the most efficient solvent to extract DPPH from different parts of *A. laurina*. In this study, the results of FRAP showed good variation and strong antioxidant activity in different extracts, and the ranges were from 19146.6 to 32375.2 µmol TE/100 g in leaves and 10146.6 to 36060.9 µmol TE/100 g in stem bark (Figure 1(b)). The highest levels of activities evaluated by the FRAP assay were observed in ethanol extract of stem bark (36060.9 µmol TE/100 g). These results are in agreement with that of a previous report by Korekar *et al.* (2011) who found the antioxidant activity of *Seabuckthorn* (*Hippophae rhamnoides L.*) in the order of stem bark >leaves. The antioxidant activities of *A. laurina* leaves and stem bark extracts determined as ABTS ranged from 3203.45 to

TABLE V - Total phenolic, flavonoids, tannin contents and antioxidant activity of various solvent extracts from *Anisophyllea laurina* Leaves and stem bark

	solvent	yield mg/100 g	TPC GAE mg/100 g	TFC QE mg/100 g	CTC CE mg/100 g
Leaves extracts	Ethanol	39.34^{a} ±0.01	2294.43^{d}±0.04	346.14^{b}±0.03	3466.63^{d}±0.03
	Methanol	48.18^{c} ±0.02	2155.91^{c}±0.01	305.45^{b}±0.03	2482.76^{b}±0.02
	Ethyl acetate	33.96^{d} ±0.04	2098.06^{a}±0.04	294.83^{a}±0.01	1307.43^{a}±0.03
	Water	26.36^{b} ±0.15	1245.47^{b}±0.01	104.41^{a}±0.02	1322.41^{c}±0.03
Stem bark	Ethanol	22.40^{a} ±0.02	2382.39^{d}±0.05	385.79^{c}±0.07	2391.94^{d}±0.03
	Methanol	34.30^{b} ±0.10	2222.42^{b}±0.02	338.37^{b}±0.01	2055.13^{a}±0.02
	Ethyl acetate	27.84^{d} ±0.70	2191.08^{a}±0.01	315.34^{ab}±0.03	212.57^{c}±0.05
	Water	24.56^{c} ±0.05	1506.41^{c}±0.06	292.98^{a}±0.03	123.73^{b}±0.06

Mean±SD of three replication; for each column, values followed by the different letters are significantly different at P<0.05.

21717.32 µmol TE/100 g and 2263.12 to 26746.6 µmol TE/100 g dry weight (DW), respectively. ABTS values of leaves and stem bark in various extracting solvents were in the following order: ethanol > methanol > ethyl acetate > water. In comparison with other plant parts, *A. laurina* pulp extracts were found to be a good source of antioxidant by ABTS than the leaves and stem bark methanol extract of *Tamarindus indica* L as reported by Razali *et al.* (2012). The correlation tests in leaves showed a strong positive correlation between TPC, TFC, TTC and FRAP (r=0.952, p<0.01), TPC, TFC, TTC and ABTS (r=0.998, p<0.01), TPC, TFC, TTC and DPPH(r=0.982, p<0.01) in both leaves and stem bark. These values are in line with the results reported by Hwang *et al.* (2014) in which high correlation coefficients were found between the *in vitro* antioxidant activities and antioxidant compounds of extracts from cultivated black chokeberry and blueberry.

Phenolic profile by UPLC-ESI-MS

A total of nineteen phenolic compounds were identified from methanol extracts of the leaves and stem bark of *A. laurina* as shown in Table VI and Figure 2. Identification of the MS chromatogram compounds of phenolic compounds by detached molecular weight was done based on the search for $[M-H]^-$ ions, using extracted ion mass chromatograms together with the interpretation of their ESI/IT/MS fragments in comparison with those found in reported literature. These compounds were the most prominent compounds detected at 220-360 nm from the leaves and at λ=223-280 nm from stem bark.

On the basis of the compounds area of each signal in mass chromatograms, Stenophyllanin A, Casuarinin (galloyl-bis-HHDP-glucose) were the most abundant

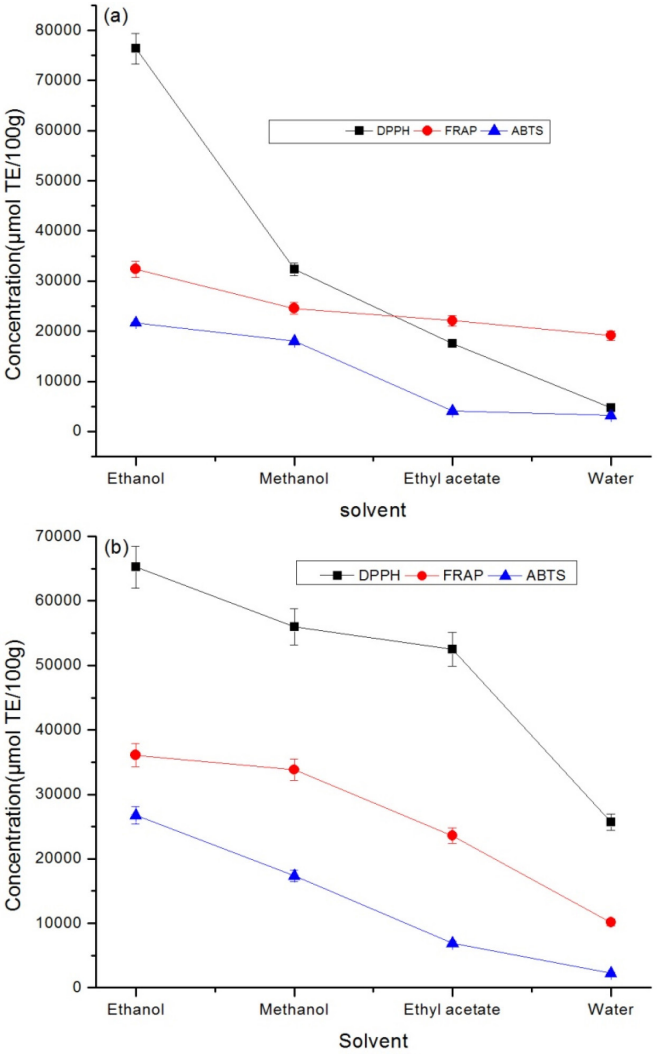

FIGURE 1 - DPPH, FRAP and ABTS from various solvent extracts of leaves (a) and stem bark (b) from *Anisophyllea laurina R. Br. ex Sabine.*

compounds identified in leaves extract (Figure 2 A). On the other hand, Procyanidin tetramer, Procyanidin dimer B1 isomer were the major compounds identified in stem bark (Figure 2 B). The hydrolysable tannins as gallotannin and ellagitannin type were the most abundant compounds identified in leaves extract. Compound 1 was identified as digalloyl-HHDP-glucopyranose isomer with [M-H] at m/z 784.1. Its fragmentation pattern involved the loss of galloyl and HHDP moieties (m/z 633 and 481) respectively. The fragmentation patterns of the majority of this compound have been identified (Barros *et al.,* 2013a). Compound 2 was identified as Pterocarinin A isomer $[M-H]^-$ at m/z 1067.1, with the fragment at m/z 933, 917, 783, 301, 275 by comparing the obtained MS^2 fragmentation patterns with published data. Further, compound 3 was identified as stenophyllanin A/B isomer (galloyl-HHDP-glucose) (Figure 3(a)) with [M-H] at m/z 1207.1. The fragment ions at m/z 917 ($[M–H-289]^-$, loss of Catechin), m/z 602 (deprotonated two ellagic acids), m/z 289 (deprotonated catechin), m/z 275 after loss of CH_2[M-H-14]$^-$, gallic acid itself $[M-H]^-$ at m/z 169 produced a fragment of m/z 125) after the loss of CO_2 whereas compound 4 was identified as casuarinin isomer (galloyl-*bis*-HHDP-glucose) $[M-H]^-$ m/z 935.01 (Figure 3 (b)). The fragment ions at m/z 633, 467, 301,275,169 and 125 were due to the loss of hexahydroxydiphenoyl moieties, galloyl-glucose moieties from the [M−H]− and $[M−H−169]^-$ ionsand m/z125 processed loss of CO_2 group from the carboxylic acid moiety. These compounds have been identified in some plants by comparing the obtained MS^2 profiles with those published (Barros *et al.,* 2013b). On the other hand, compounds 5 and 8 were tentatively identified as procyanidin hexamer with $[2M–H]^{2-}$ ion at m/z 865.3, corresponding to a monoisotopic mass of 1730.5 Daand EuprostinA isomer showing $[M–2H]^{2-}$ ion at m/z 618.98 corresponding to a monoisotopic mass of 1236.97 Da described in *Cistus ladanifer* by Barros *et al.* (2013a). The compound 7 with [M-H]- at m/z 439.1 with its fragment at m/z 409 ($[M–H-30]^-$, loss of two hydrogen and CO), m/z 301 ($[M–H-138]^-$, loss of hexose), m/z 289 ($[M–H-138]^-$, loss of hexose) was assigned to (-)-epicatechin 3-*O*-gallate (Figure 3(d)) whereas compound 8 with $[M-H]^-$ ion at m/z 557.02 and MS^2 fragmentation on negative mode gave $[M-H-80]^-$ion at m/z 477.05 produced a fragment of m/z 315 after the loss of hexose, ([M-H-308]$^-$, the loss of coumaroylhexose) ion at m/z 301 and 169 was tentatively identified as isorhamnetin-3-*O*-glucoside isomer. These two compounds were identified in both leaves and stem bark extracts of *A. laurina.*Some studies have focused on the ability of isorhamnetin to attenuate diabetes complications, such as diabetic cataract, lipid peroxidation and high blood glucose levels (Yokozawa *et al.,* 2002).

For stem bark extract, the procyanidin trimer and hexamer were the most abundant phenolic compounds as shown in Table VI. The compound 1 at t_R=8.182 mn was identified as (Epi)afzelchine(epi)catechin isomer at m/z 561 with fragments ions m/z 407 ($[M-H-154]^-$, loss of gallyol hexose), m/z 289 (deprotonated Catechin), itself $[M-H]^-$ at m/z 289 produced a fragment of m/z 271) after the loss of H_2O which was identified in *Quinchamalium chilensis* by S Simirgiotis *et al.* (2012). On the basis of the mass spectra, three compounds 2, 3 and 5 were identified as procyanidin trimer and tetramer with $[2M–H]^-$ ion at m/z 577.18 corresponding to a monoisotopic mass of 1155.34 Da. MS^2 fragmentation ions m/z 451 $[M-H-126]^-$, 425 $[M-H-152]^-$ after the loss of galloyl group, 407 $[M-H-170]^-$ after the loss of gallic acid and m/z 289 (deprotonated catechin), whereas two procyanidins 4 and 7 were identified as procyanidin hexamer with $[2M–H]^{2-}$ ion at m/z 865.3, corresponding to a monoisotopic mass of 1730.5 Da. The same type of compounds have been found and described in several plants (Dias *et al.,* 2015). The compound 8 was identified as (-)-Epicatechin $[M-H]^-$ at m/z 289.06 (Figure.3(c)), with fragments ions m/z 271 ($[M-H-18]^-$, loss of H_2O), m/z 245 ($[M-H-44]^-$, loss of CO_2), m/z 203 ($[M–H-86]^-$, loss of $C_4H_6O_2$) and 164 ($[M-H-125]^-$ which has been previously described (Khallouki *et al.* 2007). Nevertheless, the compounds 6 and 9 were assigned as Procyanidin A dime $[M- H]^-$ at m/z $[2M–H]^-$ 647.2 with monoisotopic mass of 1295.5 Da and Procyanidin tetramer $[M- H]^-$ at m/z 849.3 respectively, as reported in the root bark of *Anisophyllea dichostyla* R. Br by Khallouki *et al.* (2007).

According the HPLC analysis of individual phenolic compounds, the standard curve of catechin, (-)-epicatechin 3-*O*-gallate and isorhamnetin-3-*O*-glucoside (2-200 µg/mL) were used to quantify the phenolic compounds. The analytical curve was linear (r^2=0.99); the limit of detection was 0.2 µg/mL, and the limit of quantification was 0.5 µg/mL as calculated using the parameters of the analytical curves (standard deviation and the slope). A more detailed knowledge of the phenolic composition of the in leaves and stem bark was obtained with HPLC analysis, as we can see in Table 6. As previously observed in leaves, hydrolysable tannins including stenophyllanin A (45.87 mg/g), casuarinin (24.55 mg/g) and digalloyl-HHDP-glucopyranose isomer (15.63 mg/g) were found to be the major phenolic compounds in the leaves fractions. On the other hand, proanthocyanidins were the major phenolic compounds present in stem bark extracts. Among these compounds, procyanidin tetramer (14.89 mg/g, (-)-epicatechin (12.18 mg/g) and procyanidin trimer

TABLE VI - Detected Compounds in methanol extract of *A. laurina* leaves and stem

No peak	t_R	Compounds	MW	Fragment	mg/g
Leaves extracts					
1	6.39	HHDP di-galloyl hexoside	784.1	301, 275, 169, 163	15.63
2	8.2	Pterocarinin A isomer	1067.1	933, 886, 351, 301, 275, 249, 207	10.71
3	8.79	Stenophyllanin A/B isomer	1207	917, 573, 447, 343, 315, 301, 275, 169	45.87
4	9.25	Casuarinin/casuarictin isomer	935	633, 467, 301, 275, 169, 125	24.55
5	9.59	Procyanidin dimer	577.2	451, 407, 301, 289, 275,169	4.07
6	10.6	Euprostin A isomer	1238	935, 917, 785, 633, 301, 275, 169	0.54
7	14	(-)-Epicatechin 3-*O*-gallate	439	425, 241, 161	1.49
8	14.61	isorhamnetin-3-*O*-glucoside isomer	557	477, 315, 300, 241, 181, 153, 139	3.97
Stem bark extract					
1	8.18	(Epi)afzelchine(epi)catechin isomer	1447.5	723.2, 561, 543, 407, 381, 329, 289	6.22
2	9.33	procyanidin trimer and tetramer	1155.3	865.2, 577.18, 451, 407, 289.1	1.11
3	9.58	procyanidin trimer and tetramer	1155.3	865.2, 577.18, 451, 407, 289.1	11.25
4	9.85	Procyanidin hexamer	1730.5	865.3[2M-H]- 575.2, 407, 289, 287.1, 245	2.09
5	10.22	procyanidin trimer and tetramer	1155.3	865.2, 577.18[2M-H]- , 451, 407, 289.1	4.87
6	10.67	Procyanidin dimer B1 isomer	1295.5	647.2[2M-H]- 467, 289.1, 245, 161	7.74
7	10.84	Procyanidin hexamer	1730.5	1137.4, 919, 865.3 [2M-H]-, 577.2, 407, 289	6.92
8	11.03	(-)-Epicatechin	289.1	271, 245, 203, 137, 125	12.18
9	11.48	Procyanidin tetramer	849.3	723, 577.2, 407, 289, 287, 245, 161, 125	14.89
10	14.02	(-)-Epicatechin 3-*O*-gallate	439.1	425, 241, 161	2.75
11	14.64	isorhamnetin-3-*O*-glucoside isomer	557	477, 315 , 300, 241, 153	1.65

t_R=retention time, MW= molecular weight

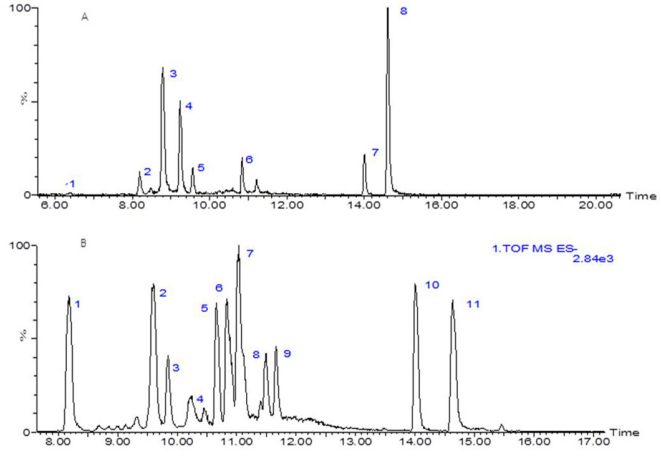

FIGURE 2 - UPLC-TOF-ESI-MS peak chromatograms of leaves (Figure 2A.) and stem bark (Figure 2B), MS in negative ion mode, UV at 280 nm, for the methanol extract from *Anisophyllea laurina.*

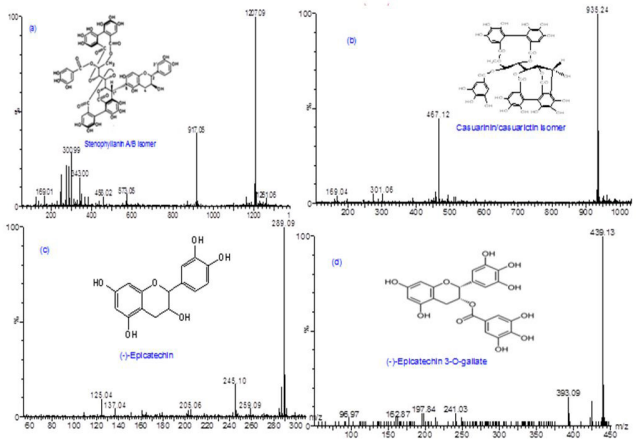

FIGURE 3 - MS2 spectra in negative ion mode of compounds from leaves and stem bark extracts : stenophyllanin A (a), casuarinin (b), (-)-epicatechin (c) and (-)-epicatechin-3-*O*-glucoside (d).

(11.25 mg/g) were the most predominant compound in stem bark.

CONCLUSIONS

The present study reveals that the extracts from leaves and stem bark of *Anisophyllea laurina* were better achieved using ethanol and methanol as extracting solvents of TPC, TFC, TMAC and TTC compared to ethyl acetate and water. However, the total tannin content in seed and leaves extracts were higher than pulp and stem bark extracts. These results have also shown that total

phenolic, flavonoids, anthocyanins, tannin contents and antioxidant compounds from leaves and stem bark were significantly affected by the type of extraction solvent used. The findings in this study have demonstrated that the leaves and stem bark of *A. laurina* could primarily be a good source of mineral contents. Palmitic acid (C16:0), linolenic acid (C18:3n3), linoleic acid (C18:2n6) and oleic acid (C18:1n9) were the most abundant fatty acids in the leaves and stem bark extracts. Among the phenolic compounds identified from samples, Casuarinin isomer and Stenophyllanin A were abundant in leaves whereas stem bark was rich in several procyanidins. This work has quantified for the first time the phenolic compounds of a large number of leaves and stem bark of *Anisophyllea laurina.* It can therefore be concluded that the plant parts of *A. laurina* extracts maybe developed as a functional ingredient in nutraceuticals, and health-promoting phytochemicals.

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