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Original Research

Antioxidant and phytochemical profiling of ethanol leaf extract of *Ficus pachyneura* and derived fractions

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ABSTRACT

The Ficus genus consists of about 850 species, many of which are used traditionally to treat several oxidative stress-related health conditions. This study was designed to evaluate the *in vitro* antioxidant activity and the Gas chromatography-mass spectra analysis of ethanol leaf extract of *Ficus pachyneura* (ELEFP) and its chloroform and ethyl acetate derived fractions. The antioxidant potential of various extractives were evaluated using different antioxidant assays such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) assay, nitric oxide (NO) and 2,2-azinobis-ethylbenzothiozoline-6-sulphonic acid (ABTS) using UV spectrophotometer. The result showed that ELEFP and its fractions exhibited significant antioxidant activities by reducing ferric ion in ferric- tripyridyl traiazine complex to ferrious ion, donating an electron to NO to form less toxic formazon, converting DPPH unstable state to an unstable non-toxic form and free radical scavenging capacity against 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals as the concentrations were increased. . The GC-MS analyses of ELEFP and its fractions revealed amongst others, the abundance of 13-octadecadienoic acid, hexadecanoic acid, tetradecanoic acid, 2-4-Di-tert-butylphenol, and heptafluorobutyric acid, which are known for their potent hepatoprotective, antihistaminic, anti-inflammatory and antioxidant activities. These activities could be attributed to the relatively high concentration of polyphenol and the important compounds, revealed in the GC-MS of ELEFP and the derived chloroform and ethyl acetate fractions. This finding suggested that these medicinal plants possess a significant antioxidant potential and are important source of natural antioxidants and can be effectively used in treating oxidative stress disorders.

Keywords: Antioxidant activity, Ficus pachyneura, derived fractions, oxidative stress

INTRODUCTION

Maintaining the redox equilibrium is important in preserving the correct functionality of cellular vital functions (Valko *et al.,* 2007). A disturbance of the balance between formation of ROS and the rate at which they are scavenged by either enzymatic (superoxide dismutase, catalase, Malondialdehyde and glutathione peroxidase) and non-enzymatic (ferric reducing antioxidant power (FRAP), nitric oxide (NO), 2,2-diphenyl picryl hydrazyl (DPPH) and 2,2-azino-bis- 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) antioxidants, will lead to increase production of reactive species, otherwise referred to as oxidative stress (Zaka-Ur-Rab *et al.*, 2016). Oxidation is a reaction process that produces these free radicals, leading to chain reaction that damage the cells organisms, whereas, compounds that inhibits these oxidation processes are referred to as antioxidants which scavenge free radicals and reduce the oxidative stress in the living and non-living systems (Grison-Pigé *et al.,* 2002; Kumari *et al.* 2012; Nawaz *et al.,* 2019).

Antioxidants are known to protect cells against the damaging effects of reactive oxygen species otherwise called free radicals (Cheng, 2006). The antioxidants possess electron donating ability and inhibit the free radical-mediated oxidative reactions by various mechanisms, such as, hydrogen donation, metal chelation, metal and lipid reduction, inhibition of lipid peroxidation and free radical inhibition (Singh and Goel, 2009; Ahmed and Urooj, 2010). Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative disorders, rheumatic disorders, DNA damage and ageing (Uddin *et al.,* 2008). Routinely, antioxidant potentials of plants are assessed by their ability to increase ferric reducing antioxidant power (FRAP) activity and scavenge 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) (Ogunmoyole *et al.,*

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2012).Gas Chromatography-Mass Spectrometry (GC-MS) remains one of the best techniques used for the identification and quantification of compounds (Aneesh *et al*., 2013; Senthil *et al*., 2016).

The Ficus genus consists of about 850 species, with about 200 different varieties occurring as woody trees, shrubs and vines in the forests of tropical and subtropical regions of the African and Asian continents (Gregory *et al.,* 2009; Awad *et al.,* 2011; Nawaz *et al.,* 2019). Evidence-based biological activities of Ficus species are documented in literature. They include: anticancer (Khan & Sulana, 2005), anthelmintic (Aswar *et al.* 2008), antioxidant (Kirana *et al.* 2009; Joseph & Raj 2010)), antimicrobial (Salem *et al.* 2013), antidiabetic (Deepa *et al.* 2018) and hepatoprotective (Wilson & Wilson, 2013) effects. This study was aimed at profiling the *in vitro* antioxidant and phytochemical (GC-MS) analysis of *Ficus pachyneura* ethanol leaf extract and its chloroform and ethyl acetate derived fractions.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Analytical grades of the ethanol, chloroform and ethyl acetate solvents (BDH Chemicals Ltd, Poole, England) and other reagents such as 1,1-diphenyl-2- picril hydrazyl (DPPH), trichloro acetic acid (TCA), sodium phosphate, sodium nitrate, ammonium molybdate, sodium hydroxide, potassium ferricyanide, catechin (CA), ferrous ammonium sulphate, butylated hydroxytoluene (BHT), gallic acid (GA), ascorbic acid (AA), $AlCl_3$, $FeCl_3$, potassium chloride, potassium acetate, phosphate buffer, 2-deoxy-D-ribose, thiobarbituric acid (TBA), HCl, H_2SO_4, H_2O_2, folin-ciocalteus's phenol reagent and sodium carbonate were obtained from Merck (Sigma-Aldrich, Germany), were used for the study.

PLANT COLLECTION/ IDENTIFICATION

Fresh leaves of *Ficus pachyneura* were obtained from Ameke, Afarata Ibeku, in Umuahia North Local Government Area of Abia State. It was identified and authenticated in the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State. A voucher specimen was kept in the departmental herbarium.

EXTRACT PREPARATION

The leaves of *Ficus pachyneura* were washed under running water immediately after collection and air- dried to constant weight, then pulverized into coarse powder using a contact mill machine. The quantity of the coarse powder was weighed with an electronic digital balance and was macerated in 1:5 w/v of ethanol for 72 hours with vigorous agitation every 2 hours. The mixture was filtered through Whatman filter paper into an already measured beaker at room temperature. The filtrate obtained was concentrated to dryness under reduced pressure using a rotary evaporator (Cole-Parmer type N-1110, China).

SOLVENT-SOLVENT PARTITIONING

This was done using the protocol designed by Kupchan & Tsou (1973), with slight modification Wagenen *et al.* (1993). A known weight of the crude extract (ELEFP) was dissolved in ethanol in a 1:9 concentrations. The resulting solution was then partitioned successively in equal volume of solvents in increasing polarity *viz* chloroform and ethyl acetate. The two derived fractions were obtained after evaporation to dryness, using a rotary evaporator. The percentage yield (w/w) of the crude extract and the fractions were calculated as described by Sofawora (1993).

$$\% \text{ Yield} = \frac{W_1}{W_2} \times \frac{100}{1}$$

Where;

W_1 = Weight of dried extract/fraction,

W_2 = Weight of the pulverized plant material/crude extract.

The final products (crude extract and derived fractions) were refrigerated at 4°C until time of use. Thereafter, they were evaluated for their *in vitro* antioxidant potential and GC-MS analysis

ANTIOXIDANT ACTIVITY OF ETHANOL LEAF EXTRACT OF *Ficus pachyneura* AND ITS DERIVED FRACTIONS

2, 2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) PHOTOMETRIC ASSAY

The free radical scavenging activity of ELEFP and its chloroform and ethyl acetate derived fractions were investigated by the DPPH assay (Mensor *et al.*, 2001), at varying concentrations (25, 50, 100, 150, 200, 250, 300 µg/mL) mixed with 1 mL of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark, at room temperature. The experiment was done in triplicates. The percentage antioxidant activity was calculated as follows.

% antioxidant activity (AA) = 100-[{(ABS sample—ABS blank) ×100}/ABS control]

One mL of methanol + 2.0 mL of the extract/fraction was used as the blank while 1.0 mL of the 0.5 mM DPPH solution + 2.0 mL of methanol was used as the negative control. Ascorbic acid (vitamin C) was used as reference standard (Iwalewa *et al.,* 2008).

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The ferric reducing antioxidant power was carried out as described by Benzie and Strain, (1999).

Reagents used include; Acetate buffer (300 mM), pH 3.6 (3.1g sodium actate.$3H_2O$ and 16 mL glacial acetic acid in 1000 mL buffer solution); 2, 4, 6-triphridyl-s-triazine

(TPTZ) (10 mM) in 40 mM HCL and $FeCl_3$ $6H_2O$ (20 mM) in distilled water. The FRAP working solution was freshly prepared by mixing the 3 reagents above in the ratio of 10:1:1, respectively. The FRAP reagent (3 mL) and 100 µl of the extract/fraction solution at concentrations of 25, 50, 100, 150, 200, 250 and 300 µg/mL was mixed and allowed to stand for 4 minutes. The absorbance was recorded at 593 nm, at 37°C. The ascorbic acid was tested in a parallel process. The absorbance of each test tube was taken at 0 and 4 minutes after addition of sample.

FRAP value = $abs\ 4\ \text{minutes} - abs\ 0\ minute$.

NITRIC OXIDE SCAVENGING ASSAY

The nitric oxide scavenging activity was estimated according to the method described by Tejero *et al.* (2014). Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different concentrations of ELEFP/fractions and incubated at 25°C for 150 min. The samples were then mixed with Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with 1-naphthyl ethylene diamine dihydrochloride was read at 546 nm using a UV–VIS spectrophotometer. The inhibition of nitric oxide formation was determined with respect to standard potassium nitrite in the same way with Griess reagent. The results were expressed as potassium nitrite equivalent used as a standard.

2,2-AZINO-BIS (3-ETHYLBENZOTHIAZOLINE-6-SULFONIC ACID) (ABTS) RADICAL SCAVENGING ASSAY

2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity of ELEFP and its chloroform and ethyl acetate derived fractions were determined as described by Pellegrini *et al.* (1999). Summarily, 37.5 mg of potassium persulfate was dissolved in 1 ml of distilled water. A total of 44 µl of this solution was added to 9.7 mg of ABTS dissolved in 2.5 ml of distilled water so as to prepare ABTS solution. The ABTS solution was allowed to stand in the dark for about 15 h at room temperature. The working solution was freshly prepared by mixing 1 ml of ABTS solution with 88 ml of 50% ethanol. A total of 25 µl of different concentrations of ELEFP/fraction were mixed with 250 µl of ABTS working solution and allowed to stand for 4 minutes. The absorbance was read at 734 nm in a UV–VIS spectrophotometer. The result was expressed as ascorbic acid equivalent which was used as a standard. All the analyses were conducted in triplicates. Appropriate blanks (methanol) and standard (BHT) were carried out simultaneously.

GAS CHROMATOGRAPHY-MASS SPECTRA (GC-MS) ANALYSIS

The GC-MS analysis was performed on an Agilent Technologies interfaced [Model: 7890A (GC)] with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion source temperature at 250 ^oC. Helium gas of about 99.9% purity was used as carrier gas, while HP-5ms (30 mm × 0.25 mm × 0.320 µm) was used as the stationary phase. The initial temperature of the oven was 140^oC, held for 5 minutes at 4^oC per minute to the final temperature of 240 ^oC, which was sustained for 15minutes, then 1µ/l of extract/fraction was auto injected.

IDENTIFICATION OF PHYTOCHEMICAL COMPOUNDS

Identification of phytochemical compounds and interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components using computer searches on a NIST Ver.2.1 MS data library. The name, molecular weight and structure of the components of the test materials were established.

STATISTICAL ANALYSIS

Data obtained from this study were descriptively analyzed and expressed in terms of means ± standard errors. One-way analysis of variance (ANOVA) coupled with appropriate post-hoc statistics were conducted to separate means of the graded concentrations of the extract, while statistical confidence was set at 95 % ($p < 0.05$). Each test was conducted in triplicate.

RESULTS

YIELD OF THE SOLVENT-SOLVENT PARTITIONING EXTRACTION

Yield (g) of the Ethanol crude extract of *Ficus pachyneura* leaf, the ethyl acetate and chloroform fractions were 156.08, 44.40 and 26.12 grams respectively.

The result presented in Figure 1 shows the percentage scavenging activities of the crude ethanol extract of *F. pachyneura* and its chloroform and ethyl acetate derived fractions. At the highest concentration (300 µg/mL), the crude extract of *F. pachyneura* and its derived ethyl acetate and chloroform fractions evoked 97.73, 95.77 and 94.62% antioxidant activity respectively, comparable with the standard ascorbic acid (93.65% antioxidant activity). The concentration of the inhibitor (IC_{50}) values of the crude extract, ethyl acetate and chloroform derived fractions of *F. pachyneura* (Figure 1) were found to be 13.09, 10.27 and 13.26 µg/mL respectively, compared with the 11.48 µg/mL of the standard (ascorbic acid).

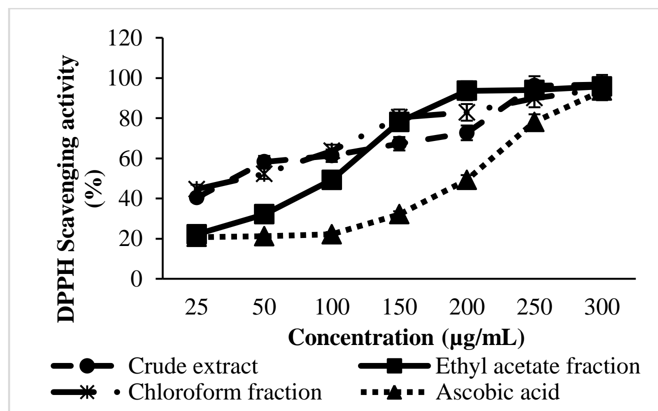

Figure I: DPPH scavenging activity of *F. pachyneura* leaf extractives

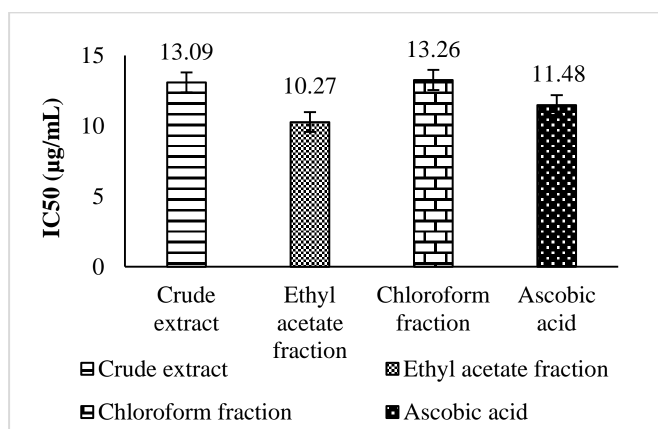

Figure II: DPPH IC_{50} of different extract fractions

NITRIC OXIDE SCAVENGING ACTIVITY

The result of nitric oxide scavenging activity showed a concentration-dependent capability with the highest nitric oxide scavenging activity; 91.03, 84.39 and 90.99 % by the crude extract, ethyl acetate and chloroform fractions of Ficus pachyneura respectively, occurring at the highest concentration, whereas, the standard Ascorbic acid had 91.45% Nitric oxide scavenging activity at the same concentration 400 µg/ml (Figure III).

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ACTIVITY

The FRAP activity of the crude extract and derived ethyl acetate and chloroform fractions of *Ficus pachyneura* showed a concentration-dependent scavenging activity, with 79.09, 85.89 and 86.14% activity observed at 400 µg/ml concentration respectively, while the reference drug had more FRAP activity (92.29 %) at the same concentration. The crude extract, derived ethyl acetate and chloroform fractions of *Ficus pachyneura* recorded 15.23, 14.57 and 14.08 of IC_{50} values, respectively, compared with the 13.04 of the reference drug (Figure III).

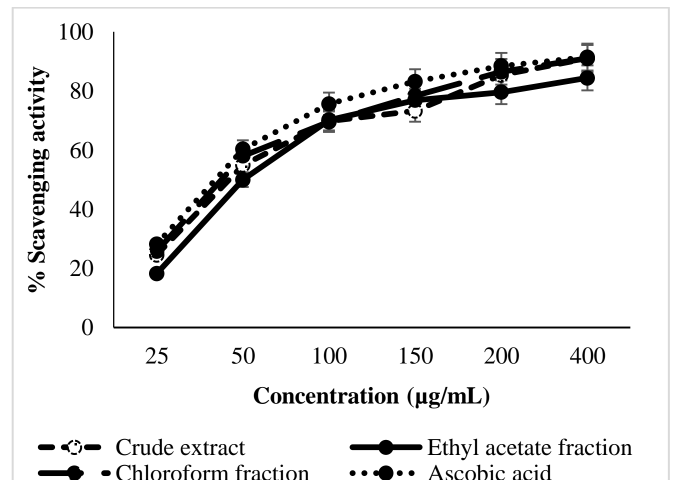

Figure III: Nitric oxide scavenging activity of *F. pachyneura* leaf extractives.

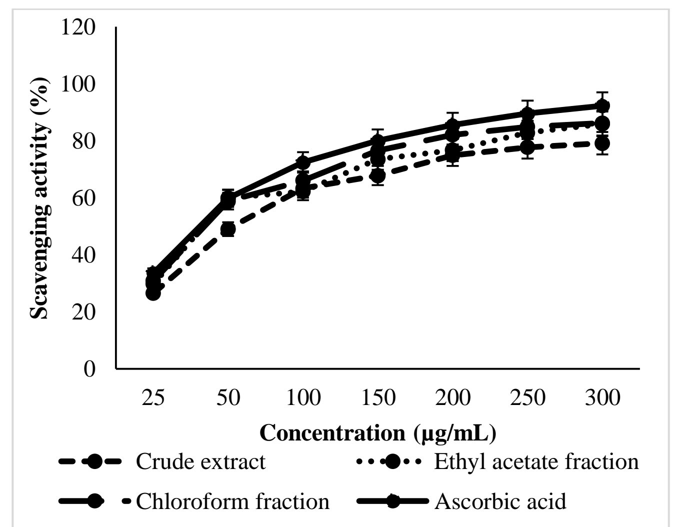

Figure IV: FRAP activity of *F. pachyneura* leaf extractives.

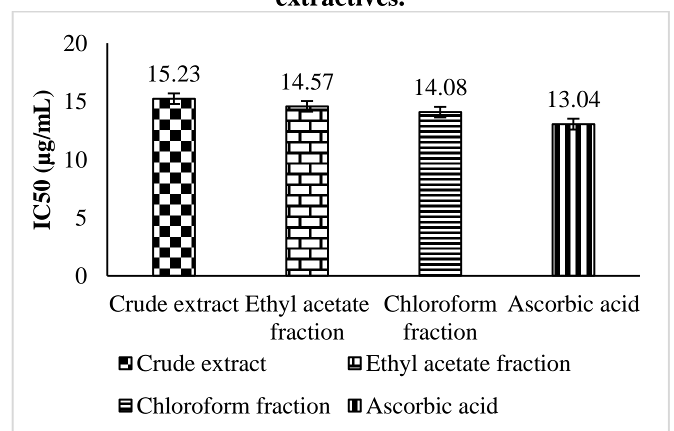

Figure V: IC_{50} of *F. pachyneura* leaf extractives

2,2-AZINO-BIS 3-ETHYLBENZOTHIAZOLINE-6-SULFONIC ACID (ABTS)

The ethanol extract of *Ficus pachyneura* and its chloroform and ethyl acetate derived fractions exhibited concentration-dependent antioxidant activity in the scavenging of the ABTS free radicals. The maximum activity of the crude extract, ethyl acetate and chloroform fractions were 83.71, 69.67 and 82.37 %, respectively, at 400 μg/ml compared with 85.69 % ABTS scavenging activity of the standard Ascorbic acid at the same concentration (Figure). The ethyl acetate fraction showed the least ABTS inhibitory activity at the highest concentration used in this study.

The GC-MS analysis of the crude extract of *Ficus pachyneura* leaf revealed the presence of over 80% volatile compounds, long and branched chains hydrocarbon, equal percent distribution of alcohols, fatty acids and ester compounds were identified in the chloroform fraction, whereas, more than 60% of the compounds identified in the ethyl acetate fraction were esters. Thus indicating that hydrocarbons of alcohols, fatty acids and esters are the major constituents determined from the GC-MS analysis. These included; unsaturated (essential fatty acid; 13-octadecadienoic acid, methyl ester; 13-octadecadienoic acid, methyl ester-; etc), saturated fatty acids (hexadecanoic acid; tetradecanoic acid, heptyl ester; methyl stearate), ethylester alkaloids (ethyl oleate), amide (hydroxylamine-o-decyl) and ketones (2-4-Di-tert-butylphenol).

DISCUSSION

Oxidation is a reaction process that produces free radicals, leading to chain reactions that damage the cells. Compounds that inhibits these oxidation processes are referred to as antioxidants which scavenge free radicals and reduce the oxidative stress in the living and non-living systems (Grison-Pigé *et al.,* 2002; Kumari *et al.* 2012; Nawaz *et al.,* 2019). The antioxidants possess electron donating ability and inhibit the free radical-mediated oxidative reactions by various mechanisms, such as, hydrogen donation, metal chelation, metal and lipid reduction, inhibition of lipid peroxidation and free radical inhibition (Singh & Goel, 2009; Ahmed &Urooj, 2010).

Different concentrations of ELEFP and its fractions showed significant antioxidant activity in terms of ferric reducing antioxidant power (FRAP), nitric oxide (NO) scavenging ability, free radical scavenging capacity against 2,2-diphenyl picryl hydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals. The reducing power is generally associated with the presence of reductants, which exert antioxidant action through breaking the free radical chains by donating hydrogen atom (Mohamed *et al.,* 2010; El-Fishawy *et al.,* 2011; Rajiv & Sivaraj, 2012). In this study, the presence of reductants in ELEFP and its chloroform and ethyl acetate fractions reduced Fe3+/ferricyanide complex to the Fe2+/ferrous form.

GC-MS remain one of the best technique used for the identification and quantification of compounds (Aneesh *et al*., 2013; Senthil *et al*., 2016). The results from the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of ELEFP and its fractions confirmed the predominant compound to be 2,4-Di-tert-butylphenol in the crude and the derived fractions, 13-Octadecenoic acid methyl ester and Tetradecanoic acid- 2-hydroxy- methyl ester in the fractions, which are known to exhibit physiologic and pharmacological activities such as hypo-cholesterol, antispasmodic, anticancer, antiviral, antihistaminic, hypocholesterolemia, antieczemic effect and of great importance as anti-inflammatory and antioxidant. 13-Octadecenoic acid has also demonstrated higher activity in increasing NO release in macrophage cells, playing an important role in host defense mechanisms as an antioxidant (Santos *et al.,* 2004). The 2,4-Di-tert-butylphenol or 2,4-bis (1,1-dimethylethyl)-phenol (2,4-DTBP) is a common natural product that exhibits potent toxicity against almost all testing organisms (Chuah, 2015), and a toxic lipophilic phenol which act as an antioxidant by donating a hydrogen atom that could quench active free radicals and stop the propagation of lipid peroxidation (Ali *et al*., 2013; Zhang, 2018). It has been found to decrease the expression of tumor necrosis factor alpha (TNF-α), interleukin Il-6 and IL-1b genes, with strong activity on the expression of cyclooxygenase-2 (Cox-2) (Nair *et al.,* 2018).

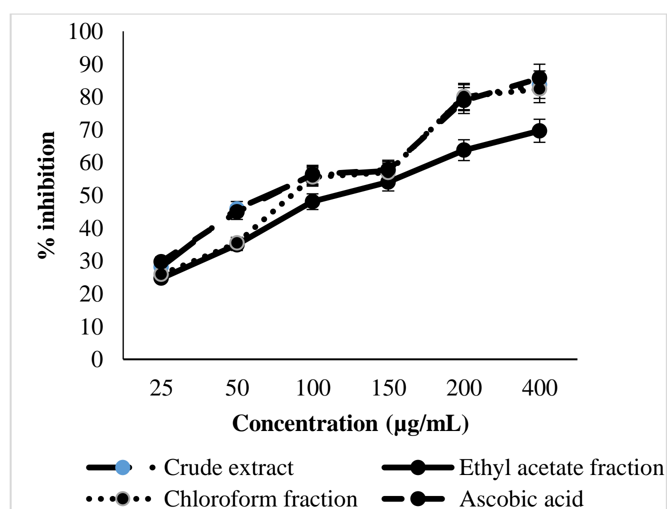

Figure VI: ABTS scavenging activity of *F. pachyneura* leaf extractives.

Table 1: GC-MS Compounds identified in the Ethanol Leaf Extract of *F. pachyneura*

S/no.	Reaction time (min)	Peak area (%)	Compounds name	Molecular formula	Molecular weight (g/mol)	
1	5.310	0.20	Methylene chloride	CH_2Cl_2	84.933	
2	6.366	0.37	Benzene, 1,2,3-trimethyl-	C_9H_{12}	120.1916	
3	6.846	1.57	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	147.002	
5	7.828	0.18	Acetic acid, chloro-, 1-methylbutyl ester	$C_7H_{13}ClO_2$	164.630	
6	8.259	0.82	Octane, 1,1'-oxybis-	$C_{16}H_{34}O$	242.4406	
7	8.644	0.82	10-Methylnonadecane	$C_{20}H_{42}$	282.5475	
8	8.787	8.26	Decane, 2,4,6-trimethyl-	$C_{13}H_{28}$	184.3614	
9	8.958	7.20	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.4146	
10	9.119	4.37	Pentane, 2,2,3,3-tetramethyl-	C_9H_{20}	128.2551	
11	9.486	2.76	Octane, 6-ethyl-2-methyl-	$C_{11}H_{24}$	156.3083	
12	9.806	6.61	Nonane, 3-methyl-	$C_{10}H_{22}$	142.2817	
13	9.915	0.79	Heptane, 2,4-dimethyl-	C_9H_{20}	128.2551	
14	10.025	8.01	2,6-Dimethyldecane	$C_{12}H_{26}$	170.3348	
15	10.845	0.22	Hydroxylamine, O-decyl-	$C_{10}H_{23}NO$	173.2957	
16	11.778	0.37	Naphthalene	$C_{10}H_8$	128.1705	
17	12.028	0.41	4-Dodecene, (E)-	$C_{12}H_{24}$	168.3190	
18	14.947	0.47	Benzocycloheptatriene	$C_{11}H_{10}$	142.1971	
19	17.625	1.25	Cetene	$C_{16}H_{32}$	224.4253	
20	18.347	0.14	Naphthalene, 1,6-dimethyl-	$C_{12}H_{12}$	156.2237	
21	20.977	6.67	2,4-Di-tert-butylphenol		$C_{14}H_{22}O$	206.3239

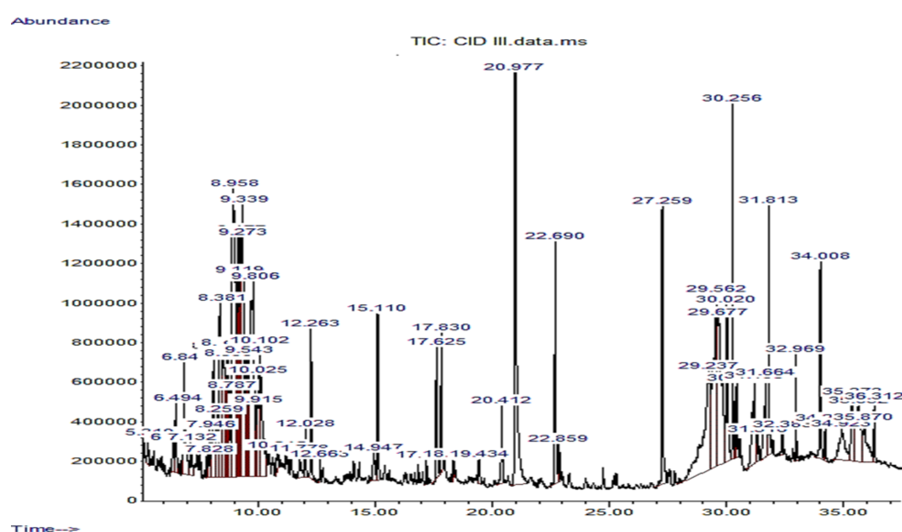

Figure VII: Chromatogram peaks of compounds in crude ethanol leaf extract of *F. pachyneura* (ELEFP).

Table II: GC-MS compounds identified in Ethyl acetate fraction of Ethanol Leaf Extract of *F. pachyneura*

S/no.	**Reaction time (min)**	**Peak area (%)**	**Compounds name**	**Molecular formula**	**Molecular weight (g/mol)**
1	6.301	7.51	beta.-Pinene	$C_{10}H_{16}$	136.2340
2	6.494	1.38	Octane, 4-ethyl-	$C_{10}H_{22}$	142.2817
3	6.846	1.22	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	147.002
4	7.203	4.58	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	134.2182
5	9.486	1.05	Carbonic acid, prop-1-en-2-yl tetr adecyl ester	$C_{18}H_{34}O_3$	298.4608
6	9.637	1.28	Carbonic acid, nonyl vinyl ester	$C_{12}H_{22}O_3$	214.3013
7	15.278	0.46	Thymol	$C_{10}H_{14}O$	150.2176
8	17.625	1.43	7-Tetradecene, (Z)-	$C_{14}H_{28}$	196.3721
9	18.346	1.39	Aromandendrene	$C_{15}H_{24}$	204.3511
10	20.976	7.66	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.3239
11	29.559	1.80	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507
12	30.018	1.61	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3435
13	30.256	2.67	Cycloeicosane	$C_{20}H_{40}$	280.5316
15	31.151	0.85	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721
16	31.195	7.13	13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879
17	31.380	1.66	Methyl stearate	$C_{19}H_{38}O_2$	298.5038
18	31.633	0.39	Ethyl Oleate	$C_{20}H_{38}O_2$	310.5145
19	34.006	1.88	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561
20	36.312	0.53	Squalene	$C_{30}H_{50}$	410.7180

Table III: GC-MS result showing compounds identified in the chloroform fraction of ELEFP

S/no.	Reaction time (min)	Peak Area (%)	Compounds name	Molecular formula	Molecular weight (g/mol)
1	6.301	1.13	beta.-Myrcene	$C_{10}H_{16}$	136.23
2	6.365	0.56	Benzene, 1,2,4-trimethyl-	C_9H_{12}	120.1916
3	6.496	1.22	Oxirane, (chloromethyl)-	C_3H_5ClO	92.524
4	6.849	1.52	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	147.002
5	7.201	1.65	p-Cymene	$C_{10}H_{14}$	134.2182
6	7.949	0.44	Hexane, 2,2,5-trimethyl-	C_9H_{20}	128.2551
8	8.382	3.58	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.4146
9	8.958	13.51	Heptadecane, 2,6,10,14-tetramethyl	$C_{21}H_{44}$	296.5741
10	9.543	1.30	Carbonic acid, nonyl vinyl ester	$C_{12}H_{22}O_3$	214.3013
11	20.974	9.27	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.3239
12	30.022	1.18	1,2-Benzenedicarboxylic acid, buty l 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334.4498
14	31.666	0.50	Ethyl Oleate	$C_{20}H_{38}O_2$	310.5145
15	34.008	1.50	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561
16	34.216	1.44	Tetradecanoic acid, 2-hydroxy-, methyl ester	$C_{15}H_{30}O_3$	258.3969
17	35.582	2.22	9,19 -Cyclolanost-24-en-3-ol, (3.beta.)-	$C_{30}H_{50}O$	426.7174

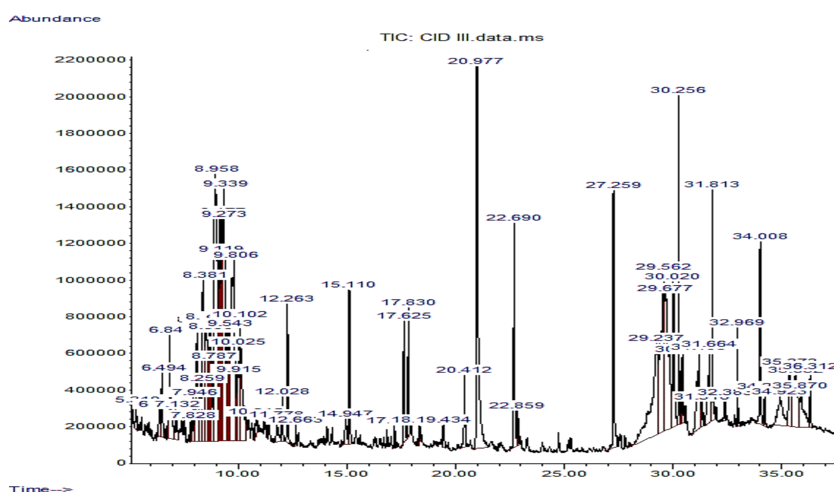

Figure VIII: Chromatogram peaks of compounds in ethyl acetate fraction of ethanol leaf extract of F. pachyneura

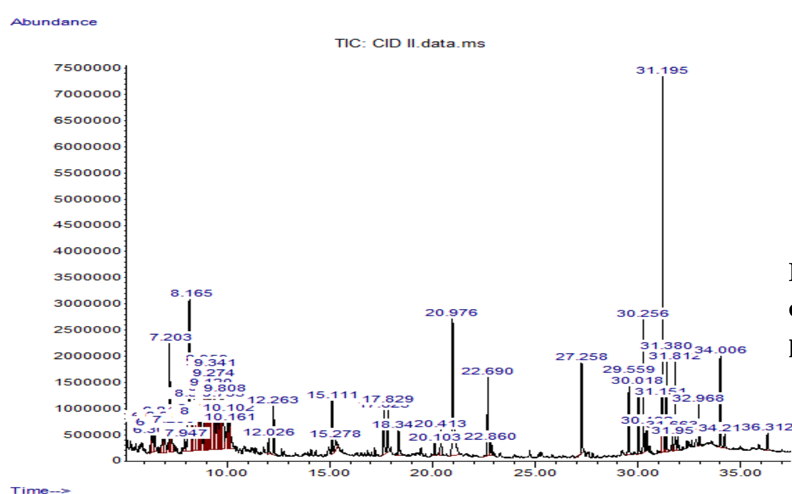

Figure IX: Chromatogram peaks of compounds in chloroform fraction of F. pachyneura

CONCLUSION

The phytochemical and the GC-MS findings revealed the presence of important phytoconstituents, noted for their various biological ingredients with wide varieties of pharmaco-active effects. The ELEFP and its fractions exhibited very high degree of antioxidation and free radical scavenging activities in a concentration dependent manner, and which, by extension, could confer protection against oxidative stress.

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