European Journal of Biology and Medical Science Research
Vol.7, No.2, pp.21-33, June 2019
Published by European Centre for Research Training and Development UK (www.eajournals.org)

FREE RADICAL SCAVENGING ACTIVITIES OF EXTRACTS AND BIOACTIVE CONSTITUENTS FROM THE ROOTS OF *PLUMBAGO ZEYLANICA* (LINN.)

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ABSTRACT: *Bioactive constituents from the methanolic extract (ME) and ethylacetate extract (EA) of Plumbago zeylanica were characterized by gas chromatography-mass spectrometry. The free radical scavenging activities of ME and EA was determined using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Major bioactive compounds found in the ME includes oxalic acid allyltridecyl ester, decane, 2-piperidinone-N-[4-bromo-n-butyl] and tetradecane, compounds obtained from EA were 1-(ethenyloxyl) octadecane and cis-13-octadecenoic acid. Cis-13-octadecenoic acid, though known, was isolated for the first time from the root of P. zeylanica. The highest percentage antioxidant activities of ME and EA were 98.5% and 45.5% respectively at 350 µg/ml concentration of extracts. Results obtained from this study justify the use of P. zeylanica in traditional medicine for treatment of different ailments and it could be a potential source for novel drug compounds.*

KEY WORDS: *Plumbago zeylanica*, gas chromatography-mass spectrometry, DPPH, bioactive compounds

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INTRODUCTION

Generation of free radicals or reactive oxygen species (ROS) during metabolic process and other activities of biological system create oxidative stress. Oxidative stress is implicated in heart diseases, neurodegenerative diseases, malaria, acquired immune deficiency syndrome (AIDS), cancer and ageing (Sian, 2003 and Zima *et al.*, 2001).

Antioxidants are secondary metabolites found in plants (Krishnaiah *et al.*, 2007). Antioxidants reported in plants include phenols, flavonoids, carotenoids, cinnamic acids, tocopherols, benzoic acids, ascorbic acids, folic acids and tocotrienols (Walton and Brown, 1999). Bioactive compounds with antioxidant properties present in plants protect cells from damage caused by the free radicals

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

and protection against cellular oxidation reactions (Seethalaxmi *et al.*, 2012 and Praveen *et al.*, 2007).

Plumbago zeylanica L. is a medicinal plant in the family Plumbaginaceae and it is widely distributed in Asian and West African countries. *P. zeylanica* is popularly called *inabiri* by the Yoruba natives of South western Nigeria and is known by various names in different parts of the world viz: *leadwort/Ceylon leadwort* (in English), *bleiwurz/zahnkraut* (in German), *chitrak/chitramol* (in Indian), *ensain/enkin* (in Arabia), and *sanza* (in Swahili) (Pant *et al*., 2012). In Nigerian traditional medicine the root, stem-bark and leaves are used as medicinal herbs for a variety of treatments. The roots are used to treat rheumatic swelling, scabies and ulcers in Nigeria (Olagunju *et al.*, 2006) while the powdered roots or leaves are used to treat gonorrhea, syphilis, tuberculosis, rheumatic pain, swellings and wounds in Ethiopia (Pant *et al.*, 2012). *P. zeylanica* roots have been reported for the treatment of fever, diseases of the spleen, dysentery, diarrhea, leprosy, and also bacterial, microbial and helmith infections (Kirtikar and Basu, 1984; Thakur *et al.*, 1989).

Plumbagin, a naphthoquinone and a major bioactive constituent in the plant, and several other components are considered to be responsible for its therapeutical and pharmacological activities such as antiplasmodial, anticonvulsant, antioxidant, hepatoprotective, anti-inlammatory, hypolipidaemic, antitumour, anticacinogenic, antifungal, antiviral and antibacterial (Tilak *et al.*, 2004; Neubert *et al.*, 2006; Simonsen *et al.*, 2001 and Vishnukanta and Rana, 2010).

Various analytical methods have been developed to isolate, characterize and identify the bioactive components of *P. zeylanica*. Unnikrishnan *et al.*, (2008) developed and validated a reverse phase HPLC method with UV detection to quantify plumbagin. Kishore *et al.*, (2010) characterized difuranonaphthaquinones, naphthaquinone, lapachol and plumbagin from *P. zeylanica* using spectroscopic data. Ajayi *et al.*, (2011) reported 2,4-bis(1,1-dimethylethyl), an antioxidant compound from *P. zeylanica* using GC-MS technique.

In furtherance of our search for useful antioxidant phytochemicals from *P. zeylanica*, we investigated the ME and EA extracts of *P. zeylanica*. We hereby report in this study, antioxidant potential of extracts and bioactive constituents of *P. zeylanica*. A compound, cis-13-Octadecenoic acid (Figure 4), a fatty acid was isolated for the first time, from the root of the plant. In this paper, we also report the isolation and structure of the compound as revealed by GC-MS.

MATERIALS AND METHODS

Plant collection and authentication

Roots of *P. zeylanica* collected at Babajakan village, Ayedaade Local Government Area, Osun State, Nigeria, in June, 2010. The plant was authentication by Mr. Adeleke of Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria where a voucher specimen of the plant was deposited in the herbarium, with Accession Number QC 488.

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Extraction of plant materials

Air-dried and ground roots of *P. zeylanica* (700 g) was extracted with 70% methanol at room temperature for 48 h by merceration. The total filtrate was concentrated to dryness using rotary evaporator (30°C) and weighed. 30 g of ME was dissolved in distilled water, exhaustively and successively partitioned with EtOAc and n-butanol to yield EtOAc, n-butanol and water extracts, after concentration using rotary evaporator at 30°C and weighed.

Fractionation and isolation of ethylacetate extracts of *P. zeylanica*

The EtOAc extract of P. zeylanica was subjected to column chromatography using silica gel (200-400 mesh size), using gradient elution of hexane : EtOAc (100:0, 95:5 to 5:95, 0:100). 105 fractions (10mL x 5) were collected. Fractions were bulked into 3 subfractions based on TLC profile, using aluminium TLC plates (silica gel 60 F_{254} pre-coated plates), fractions were concentrated using rotary evaporator and weighed (F_1). Further fractionation of F_1 was carried out using gradient elution of hexane : (100:0 to 0:100), pooled together based on TLC profile to yield compound 1, after evaporation to dryness, using rotary evaporator and weighed.

GC-MS analysis of ME, EA and compound 1

The ME, EA and compound 1 was subjected to GC-MS analysis. GC-MS analysis was carried out using Agilent Technologies Network Gas Chromatograph System (Model 7890 A series) equipped with a flame ionization detector and injector, MS transfer line temperature of 250 ^oC. The GC was equipped with a fused silica capillary column- HP-5MS (30 m x 0.320 µm), film thickness 0.25 µm. The oven temperature was held at 80 ^oC for 3 min holding time and raised from 80 – 300 ^oC at a rate of 8 ^oC /min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 3.5414 cm/sec. The extract (1 mg dissolved in 1 ml absolute alcohol) (1.0 micron), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent Technologies Network Mass Spectrometer (Model 5975 C series) coupled to Agilent Technologies Gas Chromatograph (Model 7890 A series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200 ^oC, scanning rate of 1 scan/sec. Mass spectrum of each sample was compared with the known compounds stored in the software database library.

DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity

The free radical scavenging activity of crude methanolic extract of *P. zeylanica*, ethylacetate extract (EA) and ascorbic acid (AA) was determined using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as described by Shimada *et al.*, (1992) and reported by Mazumder *et al.*, (2012). 1.0 mL of ethanolic solution of DPPH (0.1 mM) was added separately to 3.0 mL of ME, EA and AA (standard) at concentration range of 50-350 µg/mL in triplicates. The mixtures were allowed to stand in the dark at room temperature for 30 min and the absorbances were read against the control (without sample or standard) at 517 nm on a UV-Visible Spectrophotometer (Jenway UV-VIS 6305). The mean of the three readings was calculated. The percentage DPPH scavenging effect was calculated using the equation:

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DPPH scavenging effect (%) = Absorbance (control) – Absorbance (sample) x 100

Absorbance (control)

RESULTS

GC-MS analysis

GC-MS chromatogram of the root ME exhibited 19 major peaks (Figure 1). The bioactive compounds corresponding to the peaks are shown in Table 1. The major compounds include oxalic acid,allyl tridecyl ester (peak 1, 11.511%), Decane (peak 2, 11.527%), 2-piperidinone, N-[4-bromo-n-butyl]- (peak 18, 11.208%), tetradecane (peak 5, 10.053%), tridecane (peak 4, 9.998%) and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (peak 16, 7.926%) (Table 1). GC-MS chromatogram of ethylacetate fraction of *P. zeylanica* showed a prominent peak (Figure 2) and the corresponding compound include octadecane, 1-(ethenyloxy)- (peak 10, 82.709%) (Table 2). Figure 3 showed the GC-MS chromatogram of isolated compound from ethylacetate sub-fraction of *P. zeylanica* showing fused peaks and the corresponding compound is cis-13-otadecenoic acid (peaks 3 and 4, 98%) (Table 3) while the structure of the compound is represented in Figure 4.

DPPH free radical scavenging activity of *P. zeylanica*

The result of the free radical scavenging activity showed that the scavenging activity increases as the concentration of the extracts of *P. zeylanica* and ascorbic acid increases (Figure 5). The ethylacetate fraction (EAX) exhibited a lower scavenging activity as compared with the methanolic crude extract (MPZX) and ascorbic acid (AA). However, the scavenging effect of MPZX was quite close to the standard AA.

DISCUSSION

It has been reported in our previous studies that secondary metabolites such as alkaloids, tannins, cardiac glycosides, steroids, flavonoids, saponins, anthraquinones, phlobatinnins and carbohydrates are present in ethanolic root extract of *P. zeylanica* (Ajayi *et al.*, 2011). These secondary metabolites and phytochemical compounds in plants have been reported to have a wide range of biological activities on physiological systems and such activities include antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, hypocholesterolemic, cancer preventive, antiarthritic, antidiabetic anticoronary (Olagunju *et al.*, 2006 and Kumar *et al.*, 2010). The antioxidant effect of 1% aqueous, boiled water, ethanolic and boiled ethanolic extracts of *P. zeylanica* has been shown to be effective in DPPH (Tilak *et al.*, 2004). This antioxidant activity was reported to be due to its richness in flavonoids, phenolics and nonphenolic compounds present in the root extracts (Aquino *et al.*, 2001; Miller *et al.*, 1993 and Benzie and Strain, 1996). The high free radical scavenging activity of MPZX compared to the EAX may be due the synergistic effects of several phytochemical components present in MPZX which may have been removed during fractionation process.

Phytochemical antioxidants neutralize oxidants or reactive oxygen species (ROS) that are produced in the human body during metabolic process of macromolecules leading to diseases such

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as cancer, cardiovascular diseases and so on (Krishnaiah *et al.*, 2007). These compounds are therefore beneficial in the management of various diseases (Aderogba *et al.*, 2008). cis-13-Octadecenoic acid was isolated from the EAX fraction of the root of *P. zeylanica*. The compound was found to be the most abundant in EAX fraction having 98% of total compounds (Table 3).

CONCLUSION

This study has clearly shown through GC-MS analysis that the root extract of *P. zeylanica* was rich in phytochemical compounds and these compounds may be responsible for the free radical scavenging property of the plant. Furthermore, isolation of cis-13-Octadecenoic acid, a fatty acid is a major finding in this research work.

ACKNOWLEDGEMENT

Authors express their gratitude and thankfulness to the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Oyo State, Nigeria for allowing the use of her laboratory facilities to carry out this research work.

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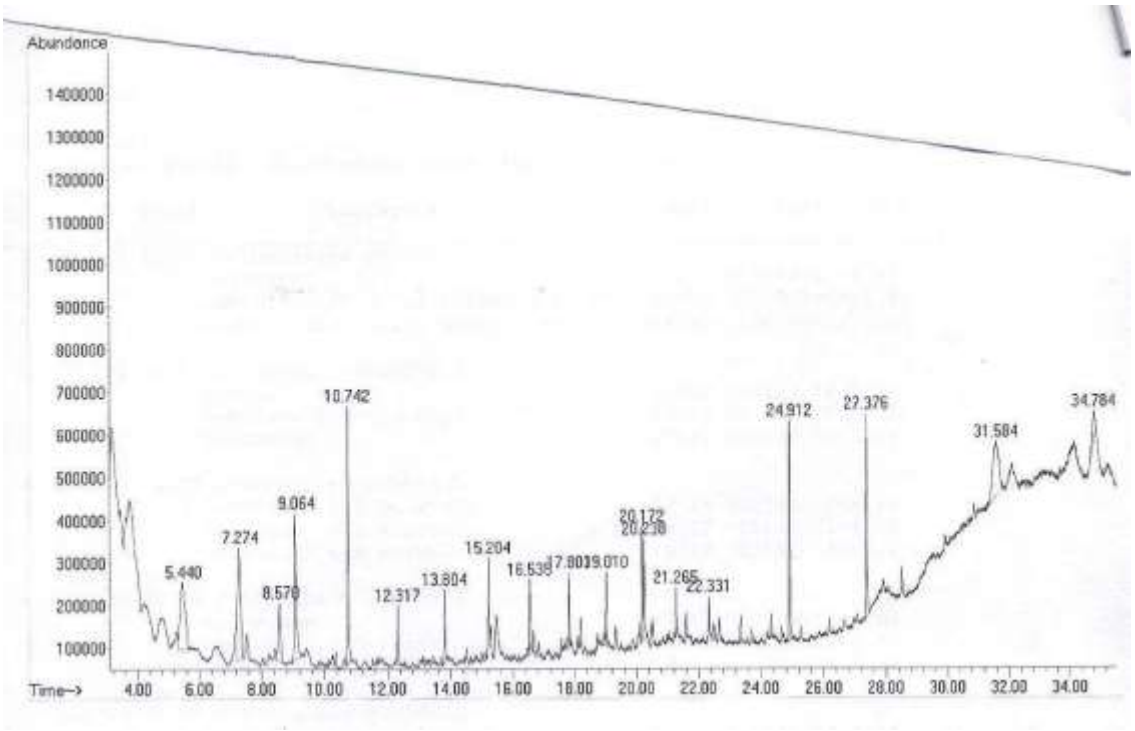

Figure 1: GC-MS chromatogram of ME of the root of *P. zeylanica*.

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Table 1: List of phytochemical components identified in ME of *P. zeylanica*

Pk	RT	Library/ID	% of total	Quality
1	5.439	Oxalic acid, allyl tridecyl ester	11.511	64
2	7.275	Decane	11.527	91
3	8.569	Dodecane, 4,6-dimethyl	4.519	68
4	9.066	Tridecane	9.998	93
5	10.743	Tetradecane	10.053	94
6	12.316	Dodecane, 2,6,10-trimethyl	2.109	90
7	13.804	Hexadecane	2.421	95
8	15.206	Tetradecane	1.949	92
9	16.534	Hexadecane	2.493	91
10	17.804	Nonadecane	2.314	94
11	19.011	Eicosane	2.394	90
12	20.173	9,12-Octadecadienoic acid, methyl ester	3.870	95
13	20.236	Trans-13-Octadecenoic acid, methyl ester	3.784	99
14	21.266	Octacosane	1.260	87
15	22.330	Tricosane	1.380	93
16	24.910	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) Ester	7.926	91
17	27.377	2,6,10,14.18,22-Tetracosahexaene, 2,6,10,15,19, 23-hexamethyl-, (all-E) –	6.622	95
18	31.582	2-Piperidinone, N-[4-bromo-n-butyl] -	11.208	86
19	34.787	2-Piperidinone, N-[4-bromo-n-butyl] -	2.660	95

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Pk = Peak, RT = Retention time, ID = Library identification

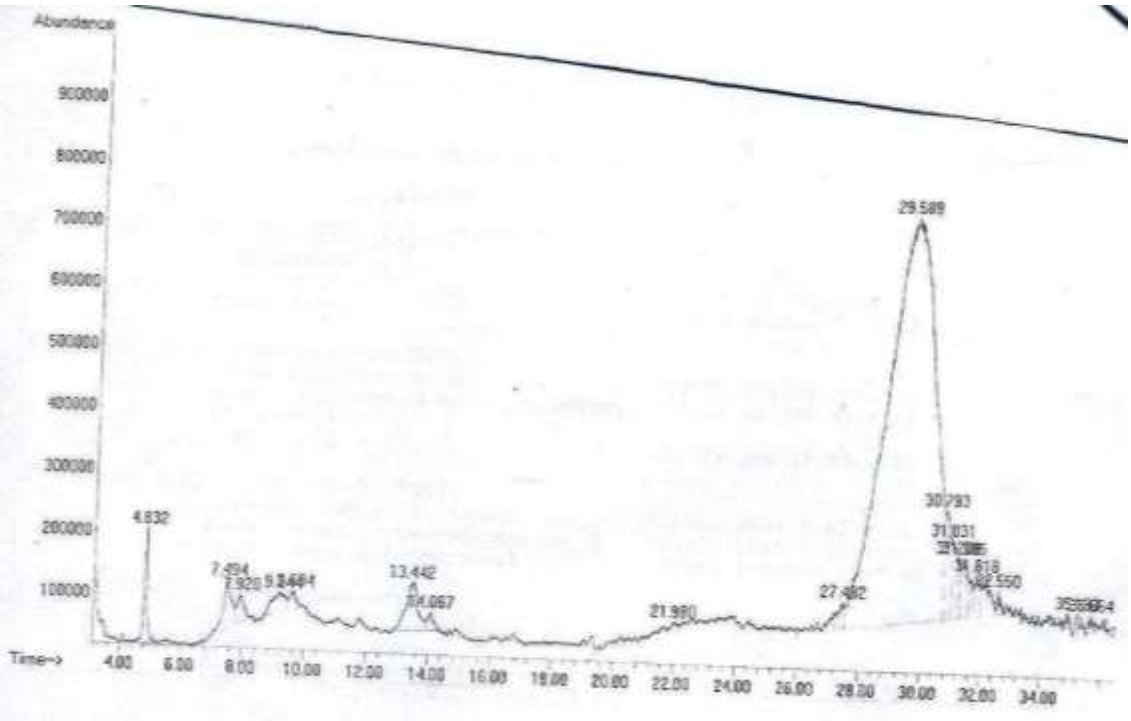

Figure 2: GC-MS chromatogram of EA fraction of the root of *P. zeylanica*.

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Table 2: List of phytochemical components identified in EA fraction of *P. zeylanica*

Pk	RT	Library/ID	% of Total	Quality
1	4.832	Naphthalene	1.489	96
2	7.493	15-Octadecenoic acid, methyl ester	1.620	68
3	7.922	Octadecanoic acid, methyl ester	0.846	55
4	9.244	9-Oxabicyclo [6,10] nonane, cis-Dodecyl Acrylate	1.092	55
5	9.633	Z- (13,14-Epoxy) tetradec-11-en-1-ol Acetate	0.490	43
6	13.444	Tetracosanoic acid, methyl ester	2.522	70
7	14.067	1,2,4,5-tetrathiane	0.444	9
8	21.981	Oxirane, tetradecyl-	0.262	52
9	27.480	Oleic acid	0.677	72
10	29.591	Octadecane, 1- (ethenyloxy) -	82.709	53
11	30.793	cis-vaccenic acid/cis-13-Octadecenoic acid	2.692	83
12	31.033	Oleic acid	1.405	93
13	31.205	cis-vaccenic acid/cis-13-Octadecenoic acid	0.689	70
14	31.393	cis-vaccenic acid/cis-13-Octadecenoic acid	1.515	64
15	31.817	Oleic acid	0.981	70
16	32.549	6-Octadecenoic acid, (Z) -	0.308	42
17	35.193	Butyl 9-tetradecenoate	0.222	42
18	35.662	Butyl 9-tetradecenoate	0.037	55

Pk=Peak, RT=Retention time, ID=Library identification.

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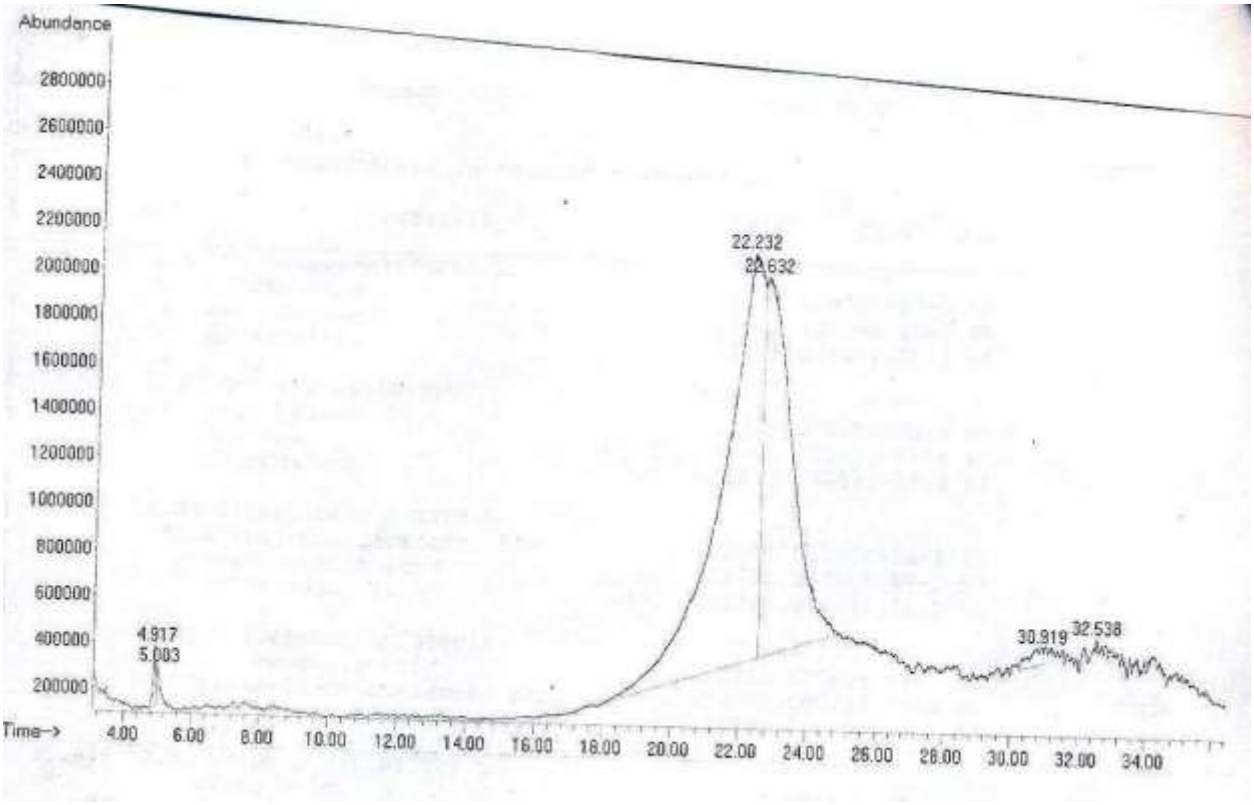

Figure 3: GC-MS chromatogram of compound 1

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Table 3: Phytochemical component of compound 1 isolated from EA fraction of *P. zeylanica*

Pk	RT	Library/ID	% of Total	Quality
1	4.918	Naphthalene	0.480	96
2	5.004	Naphthalene	0.469	96
3	22.233	cis-vaccenic acid/cis-13-Octadecenoic acid	58.036*	97
4	22.633	cis-vaccenic acid/cis-13-Octadecenoic acid	39.933*	97
5	30.918	Oleic acid	0.933	89
6	32.538	6-Octadecenoic acid, (Z) -	0.089	89

Pk=Peak, RT=Retention time, ID=Library identification,
* = cis-vaccenic acid/cis 13-Octadecenoic acid as pure compound with % of Total equals 98%.

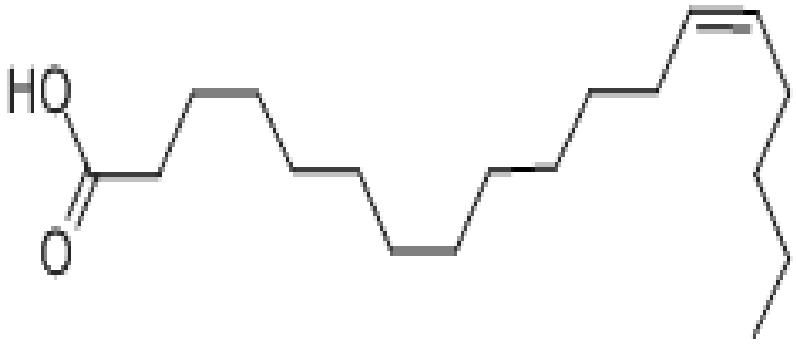

Figure 4: Cis-13-Octadecenoic acid

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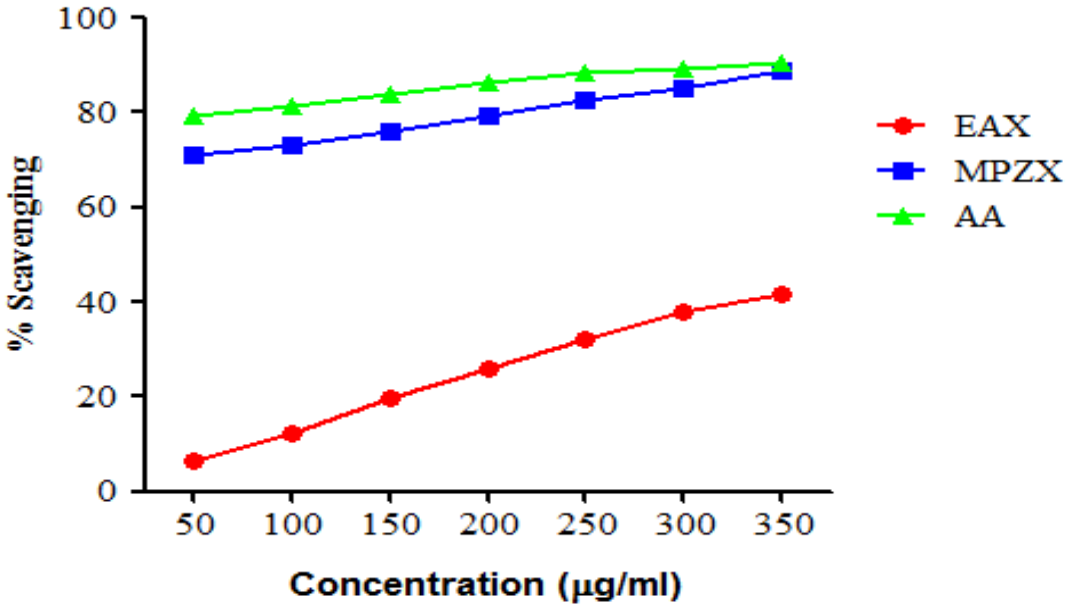

Figure 5: DPPH free radical scavenging activities of methanolic extract of *P. zeylanica* (ME=MPZX), ethylacetate extract (EA=EAX) and ascorbic acid (AA). Values were represented as mean ± SEM of 3 readings.

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