ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2022.146.155

Research Article
GC-MS Characterization and Antioxidant Properties of Partially Purified Ethanol Extract of *Nauclea latifolia* (African Peach) Stem Bark

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Abstract

Background and Objective: Many synthetic antioxidants are very effective but they possess some side effects and toxic properties in human health, thus warranting the increasing interest in natural antioxidants, e.g., polyphenols, present in medicinal and dietary plants. This study aimed at fractionation, determination of antioxidant properties and phytochemicals of *Nauclea latifolia* stem bark. **Materials and Methods:** Extraction was carried out using absolute ethanol in the ratio 1:5 w/v for exactly 48 hrs and elution was done with solvent combinations in order of increasing polarity, beginning from chloroform, ethyl acetate, methanol and finally water. While GC-MS analysis was carried out using a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk CTO6859). The *in vitro* antioxidant activity, total flavonoid and total phenolic contents of seven fractions of the ethanol extract of *Nauclea latifolia* stem bark was determined. **Results:** Antioxidant activity in mg mL^{-1} was within the range of 50 ± 2.52 to 95 ± 1.16 mg mL^{-1}. The total flavonoid concentrations varied from 100 ± 2.00 to 190 ± 2.65 mg mL^{-1} of quercetin equivalent (QE). The total phenolic content ranged from 154 ± 2.65 to 330 ± 3.61 mg mL^{-1} of gallic acid equivalents (GAE). *Nauclea latifolia* water fraction showed the highest antioxidant activity and total flavonoid content while the chloroform fraction has the highest total polyphenolic content. The extract of *N. latifolia* stem bark exhibited a strong positive correlation between the total antioxidant capacity, total flavonoids and total phenolic content (especially the methanol:water fraction). The GC-MS analysis revealed the presence of lipids, their esters and phenolic compounds. **Conclusion:** Hence, the plant has potential for both pharmaceutical and industrial applications. The methanol:water fraction was found to be of outstanding potential, hence, can be processed further for drug development.

Key words: Chromatographic, antioxidant, phenolics, *Nauclea latifolia*, pharmaceutical and industrial applications, Perkin Elmer Turbo Mass spectrophotometer

Citation: Yakubu, O.E., M.R.C. Schetinger, K.A. Arowora and C.O. Shaibu, 2022. GC-MS characterization and antioxidant properties of partially purified ethanol extract of *Nauclea latifolia* (African peach) stem bark. Biotechnology, 21: 146-155.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade[1]. In developed countries such as the United States, it is estimated that plant drugs constitute as much as 25% of the total drugs, whereas, in developing countries including China and India, the contribution[2] is as much as 80%. This underscores the increased research interest in medicinal plants and traditional medicine all over the world[3]. Plant-derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins, amino acids[4] and vitamin C[5] have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity[6].

Oxidation reactions produce free radicals that can start multiple chain reactions that eventually cause damage or death to the cell[7]. Antioxidants remove these free-radical intermediates by being oxidized themselves and inhibit other oxidation reactions, thus stopping the harmful chain reactions. Such oxidative processes are dangerous for all living cells[8]. Human beings are exposed daily to certain toxic chemicals and pathogens, which cause certain serious health problems, certain chemicals and reagents that were thought to be health-friendly, have been proved to have serious adverse effects on health[8,9].

Continuous research by scientists has shown that some plant extracts have antioxidant properties that can help remove free radicals produced by oxidation. An example of such a plant is *Nauclea latifolia* which have been reported to have certain medicinal properties in traditional settings. This plant has been used locally in the treatment of ailments such as diabetes, malaria and hypertension.

Nauclea latifolia (family: Rubiaceae) is commonly known as African peach. It is a straggling shrub or small tree commonly found in tropical Africa and Asia[10,11]. The plant has some synergistic and protective effects against certain hepatocellular injury[12]. Lagnika *et al.*[13] reported that the ethanolic extract of *N. latifolia* leaves possesses antioxidant activity. Leaves of *N. latifolia* have a protective role against diabetes and hypertension[14]. In this study, GC-MS characterization and antioxidant properties of partially purified ethanol extract of *Nauclea latifolia* (African peach) stem bark was carried out to investigate its potential as a potent source of herbal medicine and to corroborate its use in traditional African medicine for the treatment/management of oxidative stress-related diseases and infections.

MATERIALS AND METHODS

Study area: This research project was conducted from November, 2016 to August, 2017 at the Federal University Wukari, Nigeria.

Sample collection and preparation: The plant stem bark was collected from the Faculty of Agriculture garden of Federal University Wukari campus, Wukari LGA of Taraba State, Nigeria. The stem barks were examined to be free from diseases. Only healthy plant parts were used. The stem bark was cut into pieces using a kitchen knife and dried under shade for 14 days to reduce moisture content and prevent enzyme action. The dry stem bark was pulverized using a laboratory blender.

Sample extraction: Exactly100 g of powdered sample was soaked in absolute ethanol in the ratio of 1:5 w/v (100 g:500 mL) for exactly 48 hrs. The extract was filtered out first using a clean white sieving mesh and then using the Whatman No. 1 filter paper. The filtrate was concentrated using a thermostatic water cabinet at 40°C for 7 days. The concentrated extract was then transferred to air-tight containers, corked and preserved in a refrigerator at 4°C before analysis.

Partial purification: The ethanol extract was subjected to column chromatography to separate the extract into its component fractions. Silica gel was used in packing the column while different solvent combinations based on increasing polarity were used as the mobile phase as described by Yakubu *et al.*[14].

Elution: The ethanol extract (5 g) was dissolved in 5 mL absolute ethanol and the solution was applied to a chromatographic column. Elution of the extract was done with a solvent system of gradually increasing polarity, beginning from chloroform, ethyl acetate, methanol and finally water. The following ratio of the solvent combination was sequentially used in the elution protocol:

- **Chloroform:** Ethyl acetate 100:0, 50:50 and 0:100
- **Ethyl acetate:** Methanol 50:50 and 0:100
- **Methanol:** Water 50:50 and 0:100

A measured volume (400 mL) of each solvent combination was poured into the column each time using a separating funnel. The eluted fractions were collected in aliquots of 400 mL in fraction collection tubes.

Determination of total antioxidant capacity (TAC): The scavenging action of the plant extracts and the resulting fractions from ethanol extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined colourimetrically at 517 nm using Trolox as standard according to the method described by Saranraj and Sivasakthivelan[15]. The absorbance was measured at 517 nm in triplicate for each fraction. Total antioxidant capacity (TAC) was calculated as mg mL^{-1} of trolox equivalent (TE) using the regression equation from the calibration curve.

Determination of total flavonoid content (TFC): Flavonoids were determined using the aluminium chloride colorimetric method of Chang *et al.*[16]. Quercetin was used for derivation of the calibration curve and total flavonoids content was expressed as mg mL^{-1} quercetin equivalent (QE). The concentration of flavonoids in the sample was estimated using the calibration curve.

Estimation of total polyphenol content (TPC): Total polyphenol component was estimated colourimetrically at 765 nm as described by Lachman *et al.*[17], using Folin-Ciocalteu reagent and expressed as gallic acid equivalent (GAE). The reactions were conducted in triplicates and the absorbance of the sample was measured against the reagent blank at 765 nm.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: This was carried out as described by Thomas *et al.*[18] on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859 and USA) which includes a Perkin Elmer Autosampler XLGC. Analysis was carried out using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL Software.

Data analysis: The results were analyzed by One-way ANOVA, using SPSS Statistical Package Version 21. All data were expressed as Mean±SD and the difference between groups was considered significant at $p<0.05$.

RESULTS

Table 1 shows the results of the total antioxidant capacity (TAC), total flavonoid content (TFC) and total polyphenols content (TPC) for *Nauclea latifolia* stem bark ethanol extract.

The total antioxidant capacity (TAC), total flavonoid content (TFC) and total polyphenols content (TPC) revealed that the methanol:water fraction has the highest total antioxidant activity (95±1.16) and then continues in the following order ethyl acetate:methanol (00:50) fraction>chloroform (100:00)>methanol:water>chloroform: ethyl acetate (50:50)>ethyl acetate:methanol (50:50)>ethyl acetate:methanol (100:0). The flavonoid content is in the order methanol:water methanol (50:50) (190±2.65)>ethyl acetate: methanol (00:50)>chloroform (100:00)>chloroform: (50:50)> ethyl acetate:methanol (50:50)>ethyl acetate:methanol (100:00)>methanol:water (00:100). The polyphenolic content is in the order chloroform (100:00) (330±3.61)>ethyl acetate:methanol (00:50)>methanol:water (50:50)> chloroform:ethyl acetate (50:50)>methanol:water (00:100)> ethyl acetate:methanol (100:00)>ethyl acetate:methanol (50:50).

Table 2 shows GC-MS results for *N. latifolia* stem bark ethanolic extract with details of compounds identified and their functions.

Linear correlation: Figure 1 shows a strong positive correlation ($R^2 = 0.8566$) between total flavonoid content and total antioxidant capacity. Figure 2 shows a moderate positive correlation ($R^2 = 0.4638$) between total polyphenolic content and total antioxidant capacity of *Nauclea latifolia* stem bark ethanol extract. While Fig. 3 shows a moderate positive correlation ($R^2 = 0.4244$) between polyphenolic content and total flavonoid content of *Nauclea latifolia* stem bark ethanol extract in Fig. 3.

Table 1: Total antioxidant capacity (TAC), total flavonoid content (TFC) and total polyphenols of fractions obtained from *Nauclea latifolia* stem bark ethanol extract

Fractions	Solvent combinations	TAC (mg mL^{-1})	TFC (mg mL^{-1})	TPC (mg mL^{-1})
1	Chlo (100:00)	84 ± 2.45^{d}	168 ± 1.73^{c}	330 ± 3.61^{e}
2	Chlo:Eth Ac (50:50)	65 ± 1.00^{b}	122 ± 1.16^{b}	186 ± 5.29^{b}
3	Eth Ac:Meth (100:0)	50 ± 2.52^{a}	102 ± 3.61^{a}	156 ± 0.58^{a}
4	Eth Ac:Meth (50:50)	52 ± 1.73^{a}	102 ± 2.00^{a}	154 ± 2.65^{a}
5	Eth Ac:Meth (00:50)	90 ± 1.00^{e}	180 ± 0.00^{d}	252 ± 3.61^{d}
6	Meth:Water (50:50)	95 ± 1.16^{f}	190 ± 2.65^{e}	208 ± 2.00^{b}
7	Meth:Water (0:100)	70 ± 3.00^{c}	100 ± 2.00^{a}	184 ± 0.00^{b}

Each data represents the mean of 3 concentrations±SD, Chlo: Chloroform, Eth Ac: Ethyl acetate, Meth: Methanol and values with different superscript along the column are statistically significant

Table 2: GC-MS results for *N. latifolia* stem bark ethanolic extract

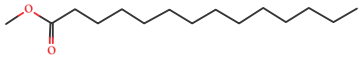
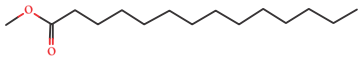
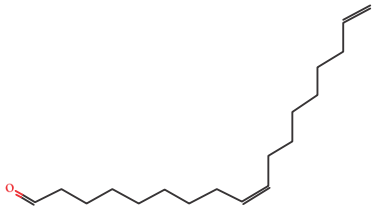
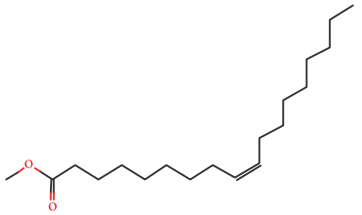
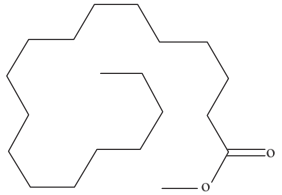
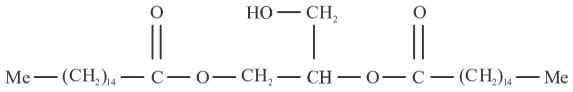
Peak	Retention time	IUPAC name	Chemical structure	Nature and uses
1	29.1445	Methyl tetradecanoate	$C_{15}H_{30}O_2$ 	Fatty acid, as flavouring agent in food, as emollient and skin conditioning in cosmetics
2	33.538	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ 	Fatty acid, to induce significant aortic dilation, inhibit prostaglandin and phagocytic activity
3	36.9417	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$ 	Volatile organic compound, in film developing and paint stripping product
4	37.0614	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$ 	Fatty acid, as Intermediate for detergents, emulsifiers and wetting agents
5	37.5302	Methyl stearate	$C_{19}H_{38}O_2$ 	Fatty acid, as lubricants for plastics, paint and ink additives, surfactants and base materials for perfumery
6	40.6748	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$ 	Fatty acid, is used as an emulsifier for cream, milky lotion and hair conditioner

Table 2: Continue

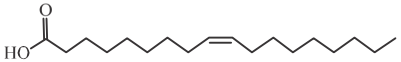
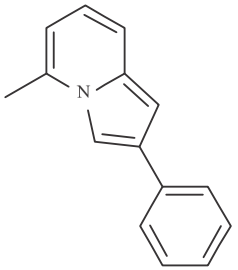
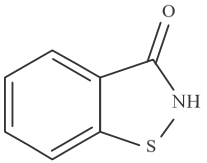
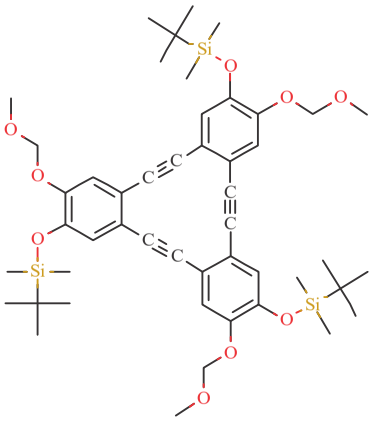
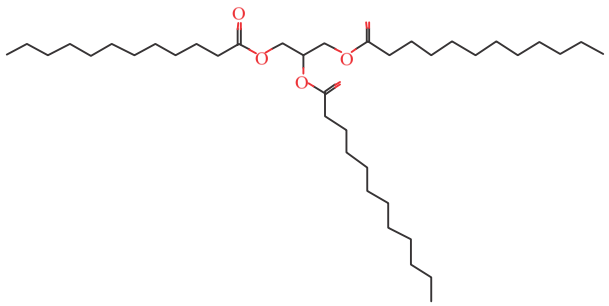
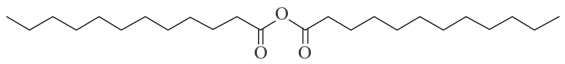
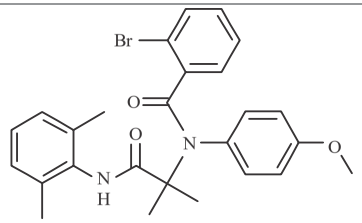
Peak	Retention time	IUPAC name	Chemical structure	Nature and uses
7	43.7601	Oleic Acid	$C_{18}H_{34}O_2$ 	Fatty acid, as an emulsifying agent, as an emollient
8	48.618	5-Methyl-2-phenylindolizine	$C_{15}H_{13}N$ 	Volatile organic compound, as a flavouring agent in food and beverages
9	48.6636	1,2-Benzisothiazol-3-amine, TBDMS derivative	C_7H_4ONS 	Organic compound, as a preservative in emulsion paints and adhesives
10	48.7405	Tris(tert-butyldimethylsilyloxy) arsane	$C_{48}H_{66}O_9Si_3$ 	Volatile organic compound, used in the beverage industry

Table 2: Continue

Peak	Retention time	IUPAC name	Chemical structure	Nature and uses
11	48.7886	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$ 	Volatile organic compound, component of insecticides and herbicides, in the cosmetic product as a thickening agent
12	48.8358	Lauric anhydride	$C_{24}H_{46}O_3$ 	Fatty acid, treatment of wool in presence of cresol
13	49.0331	Benzamide, 2-bromo-[2-(3-fluorophenyl)-5-benzoxazolyl]-		Volatile organic compound, component of insecticides and herbicides

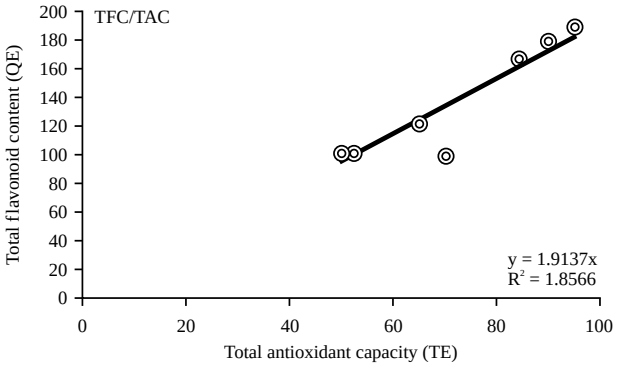


Fig. 1: Linear correlation between total flavonoids content and total antioxidant capacity of fractions

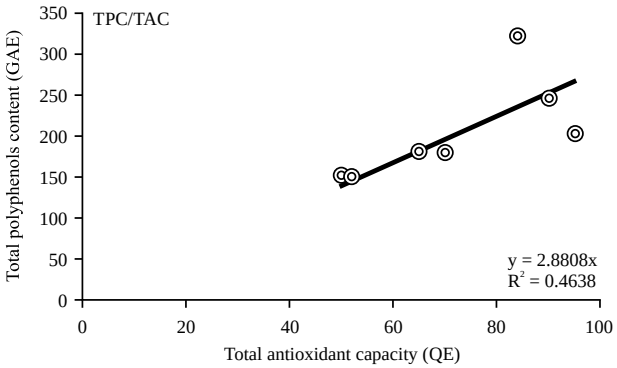


Fig. 2: Linear correlation between total polyphenolic content and total antioxidant capacity of fractions

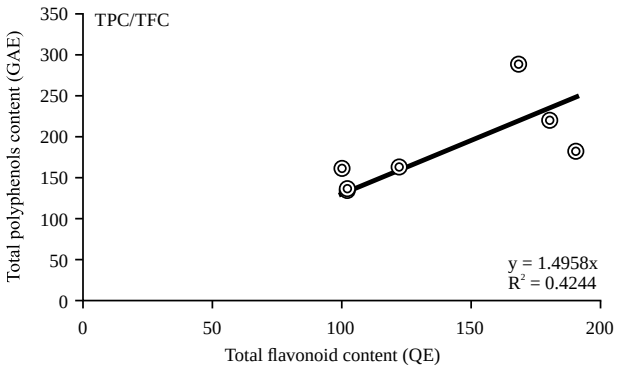


Fig. 3: Linear correlation between total polyphenolic content and total flavonoid content of fractions

Table 2 shows the GC-MS profile of compounds identified for *N. latifolia* stem bark ethanolic extract ranging from 33.54 (Methyl tetradecanoate) to 49.03 (Benzamide, 2-Bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]-). The peaks in the chromatogram were integrated and compared to the peaks of known compounds in the GC-MS library. These peaks are shown in Fig. 4.

DISCUSSION

Antioxidant properties of many plant extracts have been exploited in the treatment of diverse ailments. The total antioxidant capacity of the ethanol extract of *Nauclea latifolia* stem bark range from 50-95 mg mL^{-1} this may be due to the presence of flavonoids such as resveratrol, catechins, anthocyanins and isoflavone as well as phenolic acids and lignan[19], that play a major role as antioxidants, especially the flavonoids that can play a role in phyto-preventive therapies[20-23]. The flavonoid content was correlated with the antioxidant activity ($R^2 = 0.8566$), as it is been established that flavonoids have the strongest radical-scavenging power among all-natural phenolic compounds[14].

According to Manuswamy *et al.*[23], there is a strong relationship between the total phenolic content and antioxidant activity in selected fruits and vegetables, this may also be true for *Nauclea latifolia* although a moderately positive correlation ($R^2 = 0.4638$) was observed in this study (Fig. 2), which could be based on the quantity of flavonoid content present in the total phenolic content that showed $R^2 = 0.4244$ when correlated (Fig. 3).

Although not consistent, the total antioxidant capacity of ethanol extract of *N. latifolia* stem bark may be partly dependent on the polarity of the eluting solvent which also concurs with the studies of researchers[14,24-26]. The variation in the effects of flavonoids and phenolics from natural products may be influenced by the type of plant material, the chemical nature of the extractable compounds and the effectiveness of extraction solvents to solubilize such compounds[27-29]. Solvents with intermediate polarity are reportedly preferred to be used in the extraction of phenolics and antioxidants as compared to those highly polar such as water or non-polar solvents such as hexane[29]. Similarly, previous research have demonstrated that the aqueous extracts of *C. olitorius* significantly scavenged DPPH free radicals and this property was attributed to their high total phenol, total flavonoid and ascorbic acid contents[30-32].

In the current study, there was a strong relationship ($R^2 = 0.8566$) between antioxidant activity and total flavonoid contents and but a moderate relationship ($R^2 = 0.4638$) between antioxidant activity and total phenolic content of the fractions of *N. latifolia* stem bark. Therefore, it can be said that the antioxidant capacity of the fractions is majorly dependent on their flavonoids content although there is a wide grade of variation between different phenolic compounds in their effectiveness as antioxidant[33] which may be a contributing factor to the moderately positive correlation.

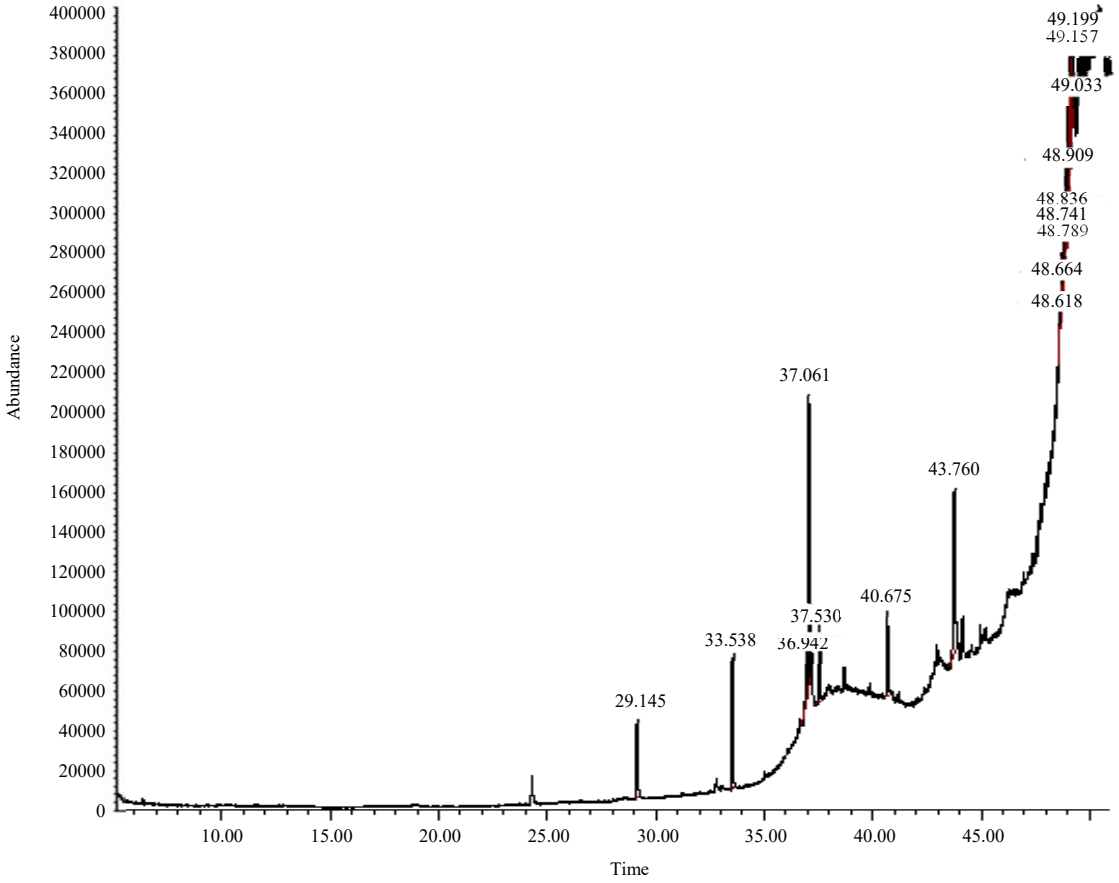


Fig. 4: GC-MS peak scan for *N. latifolia* stem bark ethanolic extract

The GC-MS analysis revealed the presence of Methyl tetradecanoate, Hexadecanoic acid, methyl ester, 9,17-Octadecadienal, (Z)-, 9-Octadecenoic acid (Z)-, methyl ester, Methyl stearate, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, Oleic Acid, 5-Methyl-2-phenylindolizine, 1,2-Benzisothiazol-3-amine, TBDMS derivative, Tris(tert-butyldimethylsilyloxy) arsane, Dodecanoic acid, 1,2,3-propanetriyl ester, Lauric anhydride and Benzamide, 2-Bromo-N-[2-(3-fluorophenyl)-5-benzoxazolyl]-. Details on the functions of these are elucidated in Table 2.

CONCLUSION

The results of this study showed that the highest antioxidant activity and total flavonoid content of *N. latifolia* were exhibited by the water fraction while the chloroform fraction has the highest total polyphenolic content. Hence, the ethanol extract of *N. latifolia* stem bark exhibited the best correlation between the total antioxidant capacity, total flavonoids and total phenolic content (especially the methanol:water fraction) which gives it a better chance of being recommended for the development of drugs used for combating diseases of oxidative stress origin.

ACKNOWLEDGMENT

The authors thank Prof. S.E. Atawodi, Ahmadu Bello University, Zaria, for his kind support and guidance.

SIGNIFICANCE STATEMENT

This study discovered that *N. Latifolia* could best be extracted by ethanol especially the methanol:water fraction. This could be beneficial in the utilization of this plant for medicinal purposes. This study will help researchers to further exploit the potentials of *N. Latifolia* in combating diseases of oxidative stress origin.

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