

*Full Length Research Paper*

# Antioxidant activities and GC-MS profiling of fractions of methanol extract of *Andrographis paniculata*

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This study was intended to investigate the antioxidant activities of different solvent fractions (hexane (HEX), chloroform (CHL), ethylacetate (ETHYL) and methanol (MET) fractions) of methanol extract of *Andrographis paniculata* and GC-MS profiling of the most active fraction (MET). The crude methanol extract was fractionated using vacuum liquid chromatography method. Antioxidant activity was evaluated using total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), ferric reducing antioxidant potential (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate (ABTS), and Nitric oxide (NO) radical scavenging abilities. GC-MS was conducted to determine phytochemical present in methanol fraction. The results indicated that MET possessed significantly ( $p < 0.05$ ) higher TPC ( $33.98 \pm 1.63$  mg GAE/g), TFC ( $15.81 \pm 0.9$  5mg QUE/g), TAC ( $21.44 \pm 0.29$  mg AAE/g) and FRAP ( $57.87 \pm 0.88$  mg Fe<sup>2+</sup>/g) than the other fractions. Also, MET exhibited the highest scavenging (ABTS, DPPH and NO) abilities of all the fractions. The GC-MS profiling of methanol fraction showed abundance presence of 2, 5- octadecadienoic-methyl ester and hexadecanoic acid-methyl ester. In conclusion, various fractions of *A. paniculata* have antioxidant abilities and could be used in diseases associated with free radicals.

**Key words:** *Andrographis paniculata*, antioxidant, solvent-fractions, GC-MS.

## INTRODUCTION

Free radicals are highly unstable reactive molecules with at least single unpaired electrons. At physiological level, they play roles in detoxification pathways, phagocytosis, cell signaling and maintaining homeostasis of apoptosis and cell proliferation via oxidative coordination of DNA-transcribing proteins and cascade enzymes (Droge, 2002; Valko et al., 2007; Neupane and Lamichhane, 2020). However, a condition termed as oxidative stress occurs when the free radicals exceed the antioxidant potential of the human system. Antioxidants are molecular species that scavenge or retard the detrimental

role of free radicals in the body (Soetan et al., 2018). Oxidative stress has been linked to the etiology of many diseases like malaria, diabetes mellitus consequent upon generation of excessive free radicals and this necessitated using antioxidant in the management and treatment of these diseases.

*Andrographis paniculata* is a yearly shrub that grows abundantly in many parts of the world. It is a member of Acanthaceae family and popularly known as “king of bitters” due to it having bitter taste and flavour (Subramanian et al., 2012). It is aboriginal to South Asian

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countries and well used in Africa and Asia for the treatment of malaria, diabetes mellitus, typhoid fever, diarrhea, cough and dysentery (Subramanian et al., 2012). The leaves from this plant have been shown to have many phytochemicals with antioxidant and antimicrobial activities (Khushboo et al., 2023). In Ayurvedic system, this plant formed at least 26 formulations and has been added to the World Health Organization (WHO) monographs on chosen herbal plants, and reported to be effective at disrupting the advancement of influenza epidemic of 1919 in India (Polash et al., 2017). In reference to WHO Monograph, *A. paniculata* should not be taken by lactating or pregnant women and those who are allergic to plant of the family of Acanthaceae. Adverse reactions of the plant include urticaria, vomiting and gastric discomfort (Worakunphanich et al., 2021). This medicinal herb has been shown to have antidiabetic, antioxidant, antimicrobial and antimalarial capacities (Hossain et al., 2014; Ahmad et al., 2020; Khushboo et al., 2023).

Gas chromatography mass spectrophotometry (GC-MS) is 2-in-1 analytical procedure comprising gas chromatography coupled with mass spectrophotometry. Gas chromatography (GC) segregates the compounds in a sample and mass spectroscopy (MS) identifies individual compound. It is widely used to determine various phytochemical components in plant extracts and essential oils (Olivia et al., 2021; Saravanakumar et al., 2021).

Several reported medicinal properties of this plant have necessitated the search for a lead component which may eventually assist in the development of drugs from this plant. Therefore, the present study evaluated the polyphenol contents and antioxidant potential of different fractions of methanol extract of *A. paniculata* and profiling of the most active fraction by GC-MS.

## MATERIALS AND METHODS

Leaves of *A. paniculata* used in this work were obtained from farm garden at Ado-Ekiti and identified at Afe Babalola University Ado-Ekiti. They were dried with air at ambient temperature and ground into powdery form.

### Chemicals and reagent

Chloroform, ethyl acetate, n-hexane, methane, sodium carbonate, sodium nitrite, aluminium chloride, sodium acetate, ferric chloride, ferrous sulfate and sodium nitroprusside were bought from LOBA chemie, India. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) were purchased from AK Scientific, USA. Ascorbic acid, quercetin and gallic acid, were gotten from Sigma-Aldrich, USA.

### Extraction of sample

Extraction of the sample was done with the protocol of Karigidi et al.

(2019). 200 g of powdered *A. paniculata* were soaked in 1000 ml of methanol. The resulting solution was shaken at internal for 48 h and later filtered with filter paper. The concentration was done with rotary evaporator at 40°C, and the extract was subjected to fractionation.

### Fractionation of samples

Fractionation was carried out as reported by Karigidi and Olaiya (2020). 40 g of methanol extract was adsorbed using silica gel, packed and successively eluted with hexane, chloroform, ethylacetate and methanol under pressure. The fractions were dried using rotary evaporator to give hexane (HEX), chloroform (CHL), ethylacetate (ETHYL) and methanol (MET) fractions. These fractions were used for further analyses.

### Total phenolic content (TPC)

TPC of the fractions was evaluated with the procedure of Kim et al. (2003). Shortly, 1.0 ml (mg/ml) of the fractions was added to 1.0 ml (10%) of Folin-Ciocalteu phenol reagent. 5 min later, 5.0 ml of 7% Na<sub>2</sub>CO<sub>3</sub> and 5.0 ml of distilled water were added and mixed together. The mixture was permitted for 90 min in the dark at ambient temperature. Spectrophotometric reading was measured at 750 nm and TPC was determined from gallic acid calibration curve as mg GAE/100 g.

### Total flavonoid content (TFC)

TFC of the fractions was assayed with Park et al. (2008). Fraction, 0.3 ml (mg/ml) was added to 3.4 ml (30%) of methanol, 0.15 ml (0.5 M) of NaNO<sub>2</sub> and 0.15 ml (0.3 M) of AlCl<sub>3</sub>·6H<sub>2</sub>O. Five minutes later, 1 ml of 1 M NaOH was introduced. The spectrophotometric reading was measured at 506 nm and TFC was quantified from Quercetin calibration graph as mg QUE/100 g.

### Total antioxidant capacity (TAC)

TAC of the fractions was evaluated with phosphomolybdate method as described by Prieto et al. (1999). Fraction, 0.4 ml (mg/ml) and 4.0 ml of phosphomolybdate reagent (0.6 M sulfuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate) were added together. The mixture was put in water bath for 90 min at 95°C. The mixture was allowed to cool to ambient temperature and spectrophotometric reading was conducted at 695 nm. The TAC was determined from ascorbic acid standard graph as mg AAE/100 g.

### Ferric reducing antioxidant potential (FRAP)

FRAP of the fractions was determined with the procedure of Benzie and Strain (1996). Freshly prepared FRAP reagent activated at 37°C was used. Fraction, 0.2 ml (mg/ml) was mixed with 2.80 ml of the FRAP reagent and incubated in the dark for 30 min. Spectrophotometric reading was taken at 593 nm and FRAP activity was quantified from FeSO<sub>4</sub> calibration graph as mg Fe<sup>2+</sup>E/100 g.

### 2, 2-diphenyl-1-picrylhydrazyl scavenging activity (DPPH)

The DPPH of the fractions assayed with the Gyamfi et al. (1999) method. After reconstitution of sample, 1.0 ml (0.1 - 0.4 mg/ml) was added to 4 ml of freshly prepared DPPH solution (30 mg/l) in

**Table 1.** Phenolics, Flavonoids, TAC and FRAP of Hexane, Chloroform, Ethylacetate and Methanol fractions of Methanol extract of *Andrographis paniculata*.

Fraction	Phenolics (mg GAE/g)	Flavonoids (mg QUE/g)	TAC (mg AAE/g)	FRAP (mg Fe <sup>2+</sup> E/g)
Hexane	9.74±0.93 <sup>d</sup>	6.58±0.95 <sup>c</sup>	11.39±1.65 <sup>b</sup>	11.95±0.53 <sup>c</sup>
Chloroform	11.35±1.18 <sup>c</sup>	10.37±0.16 <sup>b</sup>	12.36±0.77 <sup>b</sup>	6.48±0.58 <sup>d</sup>
Ethylacetate	16.22±2.06 <sup>b</sup>	14.51±0.65 <sup>a</sup>	23.09±3.35 <sup>a</sup>	19.96±0.28 <sup>b</sup>
Methanol	39.88±1.63 <sup>a</sup>	15.81±0.95 <sup>a</sup>	21.44±0.29 <sup>a</sup>	57.87±0.88 <sup>a</sup>

Data were expressed as Mean ± SD. Numbers with different superscript across the same column are different (p < 0.05) significantly.

Source: Authors

methanol. The mixture was shaken and allowed to stay in unlighted area for 1800 s. Spectrophotometric reading was taken at 520 nm. The percentage inhibition was determined as:

Percentage inhibition of DPPH =  $\{(Ab\ control - Ab\ Sample) / (Ab\ Control)\} \times 100$

DPPH reagent was used as control.

## 2, 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability (ABTS)

The ABTS of the fractions was assayed with the procedure of Re et al. (1999). ABTS reagent was prepared with 7 mM ABTS and 2.45 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> in unlit arena for 16 h and spectrophotometric reading at 734 nm was adjusted to 0.700 with ethanol. Fraction, 0.2 ml (mg/ml) was introduced to 2.0 ml ABTS reagent and permitted to incubate for 15 min. Spectrophotometric reading at 734 nm and inhibition percentage was evaluated as:

Inhibition percentage of ABTS =  $\{(Ab\ control - Ab\ Sample) / (Ab\ Control)\} \times 100$

ABTS reagent was used as control.

## Nitric oxide radical scavenging ability (NO)

The NO was assayed using the protocol of Mondal et al. (2006). Sodium nitroprusside (10 mM) prepared in 10 mM, pH 7.4 phosphate buffer was added to 1.0 ml (0.1 - 0.4 mg/ml) of the fraction and incubated for 150 min at 37°C. After that, 1.0 ml of fresh Griess reagent (1% sulfanilic acid and 0.1% naphthylethylene diamine dihydrochloride in 2% phosphoric acid) was added. Spectrophotometric reading was taken at 546 nm and percentage inhibition was determined.

Percentage inhibition of NO =  $\{(Ab\ control - Ab\ Sample) / (Ab\ Control)\} \times 100$

Reaction mixture without extract was used as control.

## GC-MS chromatography

The GC-MS was assayed as described by Karigidi and Olaiya (2020). An aliquot (1 ml) of methanol was pipetted into the column with 350°C as the temperature of injector. The temperature of the oven was initiated at 60°C and held for 120 s till it got to 260°C. Holding was permitted for 9 min at a program rate 5°C min<sup>-1</sup> till

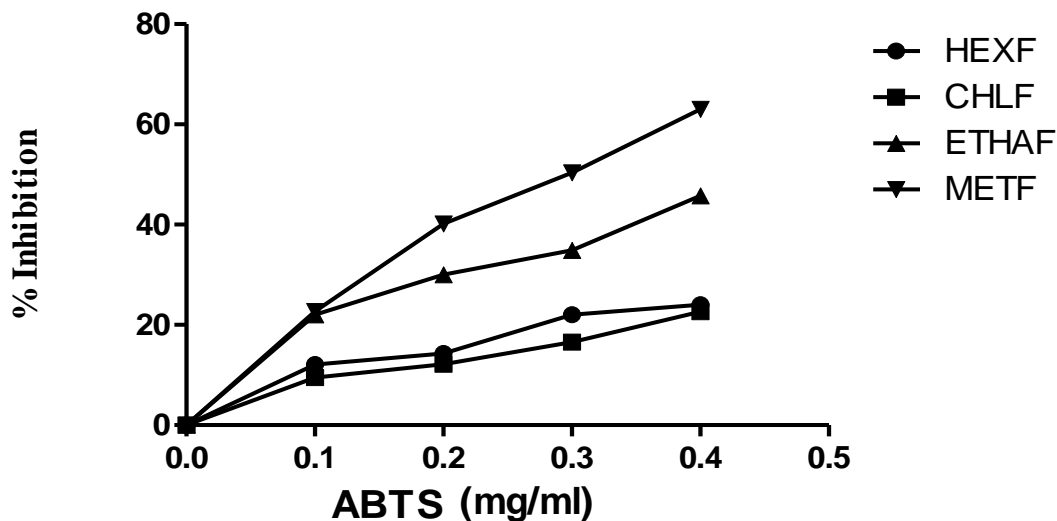
280°C. The temperatures of detector and injector were put at 280 and 250°C, respectively. Temperature of ion source was maintained at 200°C. The mass spectrum of components in the fraction was derived by electron ionization at 70 eV and the detector was operated in scan mode from 45 to 450 atomic mass units (amu). A scan interval of 0.5 s and fragments from 45 to 450 Da was maintained. The accumulated processing period was 54 min. Elucidation of GC-MS mass spectrum was done with the database of National Institute Standard and Technology (NIST) showing more than 62,000 patterns. The spectrum of the component in the fraction was compared with the known components stored in the NIST library.

## Statistical analyses

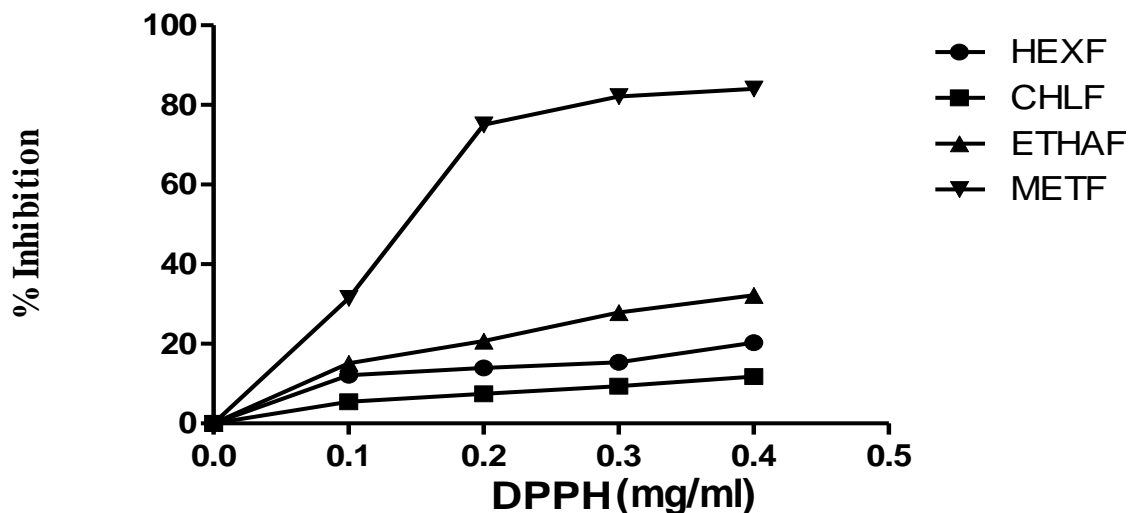
Results were presented as the mean ± SD of three measurements, analyzed using ANOVA, and the means were separated by least significant difference (p < 0.05). Pearson correlation test established correlations between polyphenols and antioxidant abilities.

## RESULTS

The results of polyphenols (phenolics and flavonoids) and some antioxidant abilities (TAC and FRAP) are shown in Table 1. The phenolics content scoped from 9.74 to 39.88 mg GAE/g. The hexane fraction has the lowest phenolics content while the methanol has the highest content. The differences are significant (p < 0.05) when compared with each other. Also, the flavonoids content of the various fractions ranged from 6.58 to 15.81 mg QUE/g. The trend is the same as phenolics but no significant (p < 0.05) difference was noted between ethyl acetate and methanol fractions. The results of TAC and FRAP were presented in Table 1. The TAC ranged between 11.39 and 23.09 mg AAE/g, ethyl acetate fraction has the highest activity while the hexane fraction has the least activity; no significant difference was noted between ethyl acetate and methanol fraction. The results of antioxidant ability (ABTS, DPPH and NO) of different fractions of *A. paniculata* are presented in Figures 1, 2 and 3, respectively. The fractions inhibited the ABTS radical in concentration dependent manner (Figure 1) and their IC<sub>50</sub> calculated (Table 2). In this study, all the fractions inhibited DPPH in a concentration-dependent order and the IC<sub>50</sub> was calculated (Table 2). The highest



**Figure 1.** ABTS scavenging activities of fractions of methanol extract of *A. paniculata*.  
Source: Authors



**Figure 2.** DPPH scavenging activities of fractions of methanol extract of *A. paniculata*.  
Source: Authors

inhibition was found in methanol fraction while the lowest inhibition was found in the hexane fraction. The potential of the fractions to retard the nitric oxide radical generation is presented in Figure 3. The methanol fraction has the lowest  $IC_{50}$  (0.22 mg/ml) while hexane fraction has the highest  $IC_{50}$  (0.55 mg/ml). Pearson correlation was done to establish the association between antioxidant abilities and polyphenol (Table 3). There was positive and significant ( $p < 0.05$ ) relationship between total phenolics and all the antioxidant assays. The same trend was observed for total flavonoids except that the relationship was not significant for DPPH. The GC-MS profiling of the methanol fraction of *A. paniculata* indicated abundant

presence of 2-Monolinolenin, 2TMS derivative, p-Cresyl glycidyl ether and allyl acetate (Table 4 and Figure 4).

## DISCUSSION

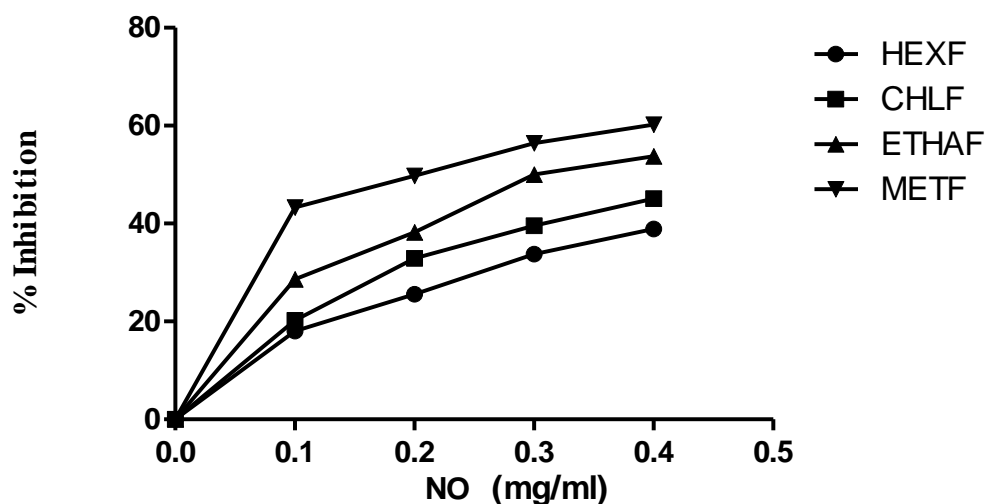
Phenolics compound are one of the secondary metabolites of plant; they are derived from phenylalanine and tyrosine. They exhibit various biological functions through their antioxidant capacity. They are very effective as chain breaking agent and this antioxidant-like activity is consequent upon the presence of their reactive phenol ring. The value of phenolics obtained in this study is

**Table 2.** IC<sub>50</sub> (mg/ml) of the Hexane, Chloroform, Ethylacetate and Methanol fractions against ABTS, DPPH and NO scavenging activities.

Fraction	ABTS	DPPH	NO
Hexane	0.89 ± 0.09 <sup>c</sup>	1.25 ± 0.17 <sup>d</sup>	0.55 ± 0.05 <sup>c</sup>
Chloroform	0.94 ± 0.05 <sup>c</sup>	1.60 ± 0.10 <sup>c</sup>	0.46 ± 0.04 <sup>c</sup>
Ethylacetate	0.51 ± 0.02 <sup>b</sup>	0.60 ± 0.05 <sup>b</sup>	0.30 ± 0.02 <sup>b</sup>
Methanol	0.30 ± 0.01 <sup>a</sup>	0.15 ± 0.01 <sup>a</sup>	0.22 ± 0.02 <sup>a</sup>

Data were expressed as Mean ± SD. Numbers with different superscript across the same column are different ( $p < 0.05$ ) significantly.

Source: Authors

**Figure 3.** NO scavenging activities of fractions of methanol extract of *A. paniculata*.

Source: Authors

**Table 3.** Correlations among TP, TF, TAC, FRAP, ABTS, DPPH and NO.

Correlation	TP	TF	TAC	FRAP	ABTS	DPPH	NO
TP	1						
TF	0.63*	1					
TAC	0.60*	0.74*	1				
FRAP	0.98*	0.55*	0.63*	1			
ABTS	0.70*	0.54*	0.62*	0.71*	1		
DPPH	0.85*	0.43	0.52*	0.89*	0.88*	1	
NO	0.74*	0.60*	0.60*	0.72*	0.93*	0.78*	1

Correlation is significant at the 0.05 level.

Source: Authors

lower than the ones reported by Ahmad et al. (2020) for methanol and acetone extracts of the plant.

Flavonoids are polyphenolic component of plant widely found in human diets; they have prominent antioxidant capacities with many physiological benefits (Adetuyi et al., 2018). The presence of hydroxyl group at position C-3 and C-4 of the B ring of this phytochemicals is

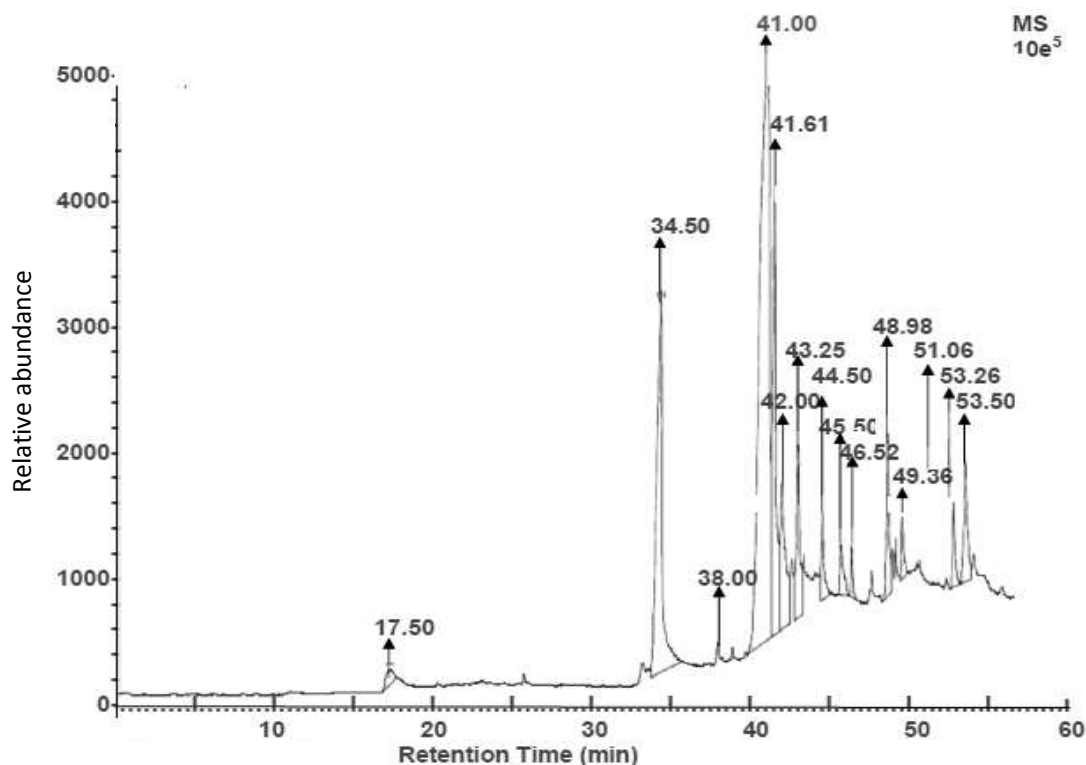
responsible for its antioxidant potentials. The flavonoid contents of the present study are lower when compared with what was reported by Borgohain and Kakoti (2019) for the methanol extract of *A. paniculata*.

Antioxidants are molecular entities that inhibit or slow down the actions of reactive nitrogen species (RNS) and reactive oxygen species (ROS) that lead to chronic and

**Table 4.** Chemical profiling of Methanol fraction of *A. paniculata*.

RT	Compound detected	MF	MW	PA
17.50	Acetic acid, methyl ester	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	74	1.51
34.50	Allyl acetate	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	100	11.48
38.00	2-(2-Hydroxyethoxy) ethyl acetate	C <sub>6</sub> H <sub>12</sub> O <sub>4</sub>	148	2.12
41.00	2-Monolinolenin, 2TMS derivative	C <sub>27</sub> H <sub>52</sub> O <sub>4</sub> Si <sub>2</sub>	496	17.66
41.61	p-Cresyl glycidyl ether	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164	14.24
42.00	2,6- Dimethoxy-4-(2-propenyl)- phenol	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>	194	6.41
43.25	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	7.91
44.50	Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	5.78
45.50	Phytol	C <sub>20</sub> H <sub>40</sub> O	296	3.49
46.52	4-Ethylbenzoic acid, 4-hexadecyl ester	C <sub>25</sub> H <sub>42</sub> O <sub>2</sub>	374	6.23
48.98	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	7.07
49.36	9-Octadecenoic acid (Z)-, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	4.54
53.26	2H-Pyran,2-(7- heptadecyny-loxy) tetrahydro-	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	336	4.59
53.50	2,5- Octadecadiynoic acid, methyl ester	C <sub>19</sub> H <sub>30</sub> O <sub>2</sub>	290	6.76

MF= Molecular formular; MW= molecular weight; PA= percentage abundance.  
Source: Authors

**Figure 4.** GC-MS chromatogram of methanol fraction of methanol extract of *A. paniculata*.

Source: Authors

degenerative diseases (Soetan et al., 2018). Antioxidant assays are usually of two types; hydrogen atom transfer (HAT) and single electron transfer (SET) assays (Adetuyi et al., 2018). The HAT assays are kinetics based while

SET assays measure the antioxidant capacity as a function of colour change when oxidant is reduced.

In this study, SET (ABTS, DPPH and NO) assays are used because of their popularity and accuracy in *invitro*

studies. The TAC relies on the reduction of Molybdate (VI) to Molybdate (V) by an antioxidant species with the development of green phosphomolybdate (V) chromogen. The result obtained in this study had lower values compared to the ones reported by Adetuyi et al. (2018) for aqueous and methanol extract of *Ageratum conyzoides*.

The FRAP is based on the capacity of an antioxidant compound to attenuate iron from the ferric ( $\text{Fe}^{3+}$ ) state to the ferrous ( $\text{Fe}^{2+}$ ) state. The methanol fraction has significantly higher activities than other fractions. The FRAP activity of methanol extract was higher than the one reported for *Rhododendron arboreum* flowers (Kashyap et al., 2017).

The ABTS antioxidant assay measures the loss of the blue-green chromophore of generated ABTS when antioxidant is added to it. The loss of colour is proportional to the antioxidant potential of the extract (Alam et al., 2013). The  $\text{IC}_{50}$  values of both ethyl acetate and methanol fractions were lower than what was reported for aqueous extract of *A. paniculata* by Ismail et al. (2017).

The DPPH scavenging assay evaluates the potential of a reductant to donate hydrogen atom leading to the subsequent loss of its violet colour (Alam et al., 2013). The  $\text{IC}_{50}$  of the ethyl acetate and methanol fractions were lower while hexane and chloroform fractions were higher than the ones reported by Abdul Rahman et al. (2017) for *A. paniculata* extract.

Nitric oxide radical has been one of the striking founts of oxidative stress in cardiac diseases. It is a reactive radical that charade as oxidative signaling molecule in several biological processes, which include blood pressure regulation and neurotransmission, (Valko et al., 2007). In this study,  $\text{NO}\cdot$  is developed at physiological pH from sodium nitroprusside. The  $\text{IC}_{50}$  obtained in this study is greater than the ones reported for different extracts of *A. paniculata* (Borgohain and Kakoti, 2019). This is an indication that the fractions of *A. paniculata* might prevent generation of nitric oxide in the body.

Pearson correlation enabled the determination of the association between polyphenol and antioxidant abilities (Table 3). There was positive and significant ( $p < 0.05$ ) relationship between the total phenolics and all the antioxidant assays. The same trend was observed for total flavonoids except for non-significant relationship observed for DPPH.

The presence of 2, 5- octadecadienoic-methyl ester and hexadecanoic acid-methyl ester might be responsible for the activity of the fraction as Ukwubile et al. (2019) as linked it their presence to anticancer potential.

## Conclusion

Fractions of *A. paniculata* possess polyphenolic compounds with antioxidant properties. Moreover, methanol fraction has the highest polyphenols and

exhibited the highest level of antioxidant ability among the considered fractions. The profiling of the methanol fraction done with GC-MS revealed abundant presence of 2, 5- octadecadienoic-methyl ester and hexadecanoic acid-methyl ester.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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