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GC-MS Analysis and Antioxidant Activity of EtOAc Extract Fraction of *Acassia sieberiana (Fabaceae) for* the Presence of Flavonoids

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research is aimed at analyzing the flavonoid content of ethyl acetate (EtOAc) extract fraction of *Acassia sieberiana* through Gas chromatography mass spectrometry (GC-MS) and testing its radical scavenging activity on DPPH. The stem bark of the plant was collected from Pankshin local

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government area of Plateau state. It was made into fine powder and macerated in methanol. The concentrated extract was fractionated in EtOAc and water. The EtOAc fraction was concentrated using rotary evaporator. The concentrated EtOAc extract fraction was subjected into phytochemical screening which revealed the presence of cardiac glycosides, tannins, saponins, flavonoids, phenolic compounds and terpenes. The components of the EtOAc fraction were separated by TLC using solvents mixture of n-hex:EtOAc in the ratio 3:1 and n-hex:EtOAc:MeOH in the ratio 5:1:1. Flavonols, aurones, flavones, flavonones and catechines were indicated. The GC-MS showed the presence of Ergosta–5,22-dien-3-01-acetate(93β,22E), 4H-Benzopyran-4-one,5,6,7-trimethoxy-2-(4-methoxyphenyl), Ethyliso-allocholate, 3,9- epoxypreg-16-en-14-ol-20-one-11,18-diacetoxy-3-methoxy and 10-Octadecnoicacidmethyl ester at retention times 30.038, 31.685, 32.057, 34.148 and 38.780min respectively. The antioxidant activity of the EtOAc extract fraction was appreciable in the sense that it has IC_{50} of 57.18ug/ml though less than that of ascorbic acid which was found to be 52.04ug/ml.

Keywords: EtOAc; antioxidant activity; GC-MS; flavonoid.

1. INTRODUCTION

Acacia sieberiana is a member of the Family Fabaceae. It is a tree that grows up to 15 m high with light-colored bark and often with a flat crown. The leaves are ten to fifteen centimeter long with straight white thorns at their base, the branches and often the leaves are covered with yellow hairs. The seeds are contained in straight pods, Eight to twelve centimeters long and two to three centimeter width. The flower heads are spherical shape and cream colored. *Acacia sieberiana* grows in various regions in the world and Africa [1]. Medicinal plants have served as an alternative means for treating and curing various disorders in different communities and cultures. Herbal medicines are widely used for the treatment and prevention of various diseases in Africa and other developing countries of the world. These herbs are generally accessible, affordable and acceptable by most of the consumers [2]. In recent years, there has been interest in the use of herbal medicine in the treatment of a number of diseases among which are cancer, diarrhea, high fever and hypertension.

Flavonoids are group of polyphenols present in plants and responsible for their color, growth, development and immunity. They are compounds with $C_6C_3C_6$ framework. Epidmiological studies suggest that the regular consumption of flavonoids protects humans against diseases linked with oxidative stress [3, 4]. Many flavonoids found in plants have biological and pharmacological activities, such as antimicrobial, antiinflammatory, and antiallergic actions [5,6]. Flavonoids are also a kind of natural antioxidant substances capable of scavenging free superoxide radicals, thus displaying anti-aging properties and reducing the risk of cancer. A commonly used method of separating a mixture of organic compounds is known as liquid-liquid extraction and other chromatographic techniques. For instance, new imidazole was synthesized and characterized through tthree components reaction [7]. All compounds prepared are usually identified using different chemical techniques, such as (^{1}H.NMR spectra, ^{13}C.NMR-spectra [8].

GC-MS has been regarded as forensic substance identification because it is used to perform a 100% specific test which positively identifies the presence of a particular substance [9,10]. After identification of chemical structures and pharmacological activities of various flavanoids, their chemical structures can be modified by using computer-aided drug design programs, use them in nanoformulations, change their dosage and forms, modify or synthesize specific flavonoids with a stronger targeting strategy, lower toxic side effects and make them more adaptable to the complex physiological conditions of the human body [11].

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The stem barks of *Acacia sieberiana* were collected from Pankshin local government area of Plateau State, Nigeria. They were dried at room temperature in the chemistry laboratory of University of Jos Nigeria. The stem bark was transformed into fine powder with the aid of pestle and mortar and stored for further use.

2.2 Extraction and Fractionation

Five hundred gram (500g) of *A. sieberiana* stem bark powder was macerated in 1500ml methanol

and stirred constantly for 15 minutes. The mixture was allowed to stand for 24 hours. It was then decanted and filtered using Whatman No 1 filter paper. The extract was concentrated using rotary evaporator at 65ºC. Ten gram (10g) of the methanol extract was dissolved in 100ml distilled water and successively partitioned in 50ml ethyl acetate (EtOAc). The lower aqueous fraction layer was collected in a beaker by opening carefully the tap of the separating funnel. The upper EtOAc fraction was concentrated further using rotary evaporator.

2.3 Phytochemical Screening

Preliminary phytochemical screening of the EtOAc extract fraction was carried out using standard methods reported by Sofowora (1993) and Evans (1983).

2.4 Thin Layer Chromatography (TLC)

The TLC analyses of the extract of the EtOAc fraction was carried out using precoated aluminium plates. The plates were carefully cut to slides of 3cm length and 10cm height. The solvent system of n-hexane:EtOAc mixture in the ratio 3:1 and n-hexane:EtOAc:MeOH mixture in the ratio 5:1:1 were prepared. The extract of the EtOAc fraction was spotted on each aluminium plate 1cm each (drawn with a pencil) above from the bottom. The plates were then placed in the saturated developing tanks containing the solvent mixture ratios each mentioned above. The solvent mixtures were allowed to move up the plate until were about 1cm from the top. They were removed from the tank and the solvent front immediately marked. The plates were allowed to dry and were viewed in UV lamp (254nm) and ammonia (NH_3) vapour. The retention factor (R_f) values of the spots were calculated using the relationship.

$$Rf = \frac{distance\ between\ the\ starting\ point\ and\ spot\ line}{distance\ between\ the\ starting\ point\ and\ Solvent\ front}$$

2.5 Antioxidant Activity

The preparation of 0.04% DPPH was achieved by dissolving 0.01g DPPH in 250ml distilled water in a volumetric flask. The mass 0.05g each of standard ascorbic acid and the EtOAc extract fraction of *A. sieberiana* were dissolved separately in 50ml ethanol to obtain 500ug/ml each. Other concentrations of 400, 300, 200, 150, 100, 75, 10, 5 and 1ug/ml were prepared through serial dilutions. All experiments were performed in triplicates. To each concentration of freshly prepared standard ascorbic and EtOAc extract fraction of *A. sieberiana*, 3ml of 0.04% DPPH reagent was added. It was allowed to develop for 30 minutes in the dark and the absorbance was measured using the UV-spectrometer at 517nm. The percentage scavenging activities for each of the EtOAc extract fraction of *A. sieberiana* and standard ascorbic acid on DPPH were calculated using the formula reported by Kaur et al. [12].

$$\%\ scavenging\ activity = \frac{Ao - As \times 100\%}{Ao}$$

Where A_o is the absorbance of negative control and A_s the absorbance of the sample. The concentrations of both standard ascorbic acid and the EtOAc extract fraction of *A. sieberiana* extract that can scavenge 50% of the DPPH free radicals (IC_{50}) were also calculated.

2.6 Gas Chromatography Mass Spectroscopy (GC-MS)

The EtOAc extract fraction was subjected to GC-MS analysis in order to identify the type of compound present. It was carried out using a QP2010 PLUS Gas Chromatography coupled with Mass Spectroscopy (Shimadzu, Japan). The ionization voltage was 70eV. The gas chromatography was conducted in a temperature programming mode with a Restek column (0.250mm, 60m; XT1-5). The initial column temperature was 80^{o}C for 1 minute then increased linearly at 7^{o}C min^{-1} to 220^{o}C hold for 3 minutes followed by linear increased temperature 10^{o}C min^{-1} up to 290^{o}C hold for 10 minutes. The temperature of the injection port was 290^{o}C and the GC-MS interface was maintained at 290^{o}C. The sample was introduced through an all-glass injector working in the split mode with a Helium carrier gas flow rate of 1.2 ml/min. The identification of components was accomplished by comparison of retention time and fragmentation pattern as well as with mass spectra in the NIST spectral library stored in the computer software (Version 1.10 beta, Shimadzu) of the GC-MS.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening of EtOAc extract fraction of *A. sieberiana* indicated the presence of various secondary metabolites like tannins,

flavonoids, saponins, steroids/terpenes, phenolic compounds and glycosides while alkaloids were not detected (Table 1). These components have phytochemical properties for curing various ailments and possess antimicrobial, antioxidant and anti-diarrheal potentials. Earlier reports showed that various extracts of *A. sieberiana* possess terpenoids, lactones, proteins and carbohydrates [13,14]. The compounds presented in Table 1 are natural chemicals that have either antioxidant or hormone-like actions and are promoted for the prevention and treatment of many health conditions, perhaps because they are readily available and could be sourced from various plants/parts such as fruits, vegetables, beans, grains etc, as well as the fact that scientists have identified thousands of them in materials selected for studies because of local uses in traditional medicines. These phytochemical compounds are the key candidates in the medicinal value of the plant.

Table 1. Phytochemical constituents of EtOAc extract fraction of *A. sieberiana*

S/No	Phytochemical	Observation
1	Cardiac glycosides	+
2	Steroids/terpenes	+
3	Saponins	+
4	Alkaloids	-
5	Tannins	+
6	Flavonoids	+
7	Phenols	+

3.2 Thin Layer Chromatography (TLC)

The chromatograms developed in the TLC analysis of EtOAc extract fraction using the solvent system of n-hexane:EtOAc (3:1) and n-hexane:EtOAc: MeOH (5:1:1) were viewed under UV light. Both solvent systems gave six coloured components each. The yellow components with R_f values 0.87 and 0.96 indicate the presence of flavonols and aurones. The pale green colours with R_f values 0.61 and 0.74 and a black colour with R_f of 0.50 indicate the presence of flavones. The blue and the pale blue colours with R_f values of 0.80, 0.85 and 0.11 indicate the presence of could be flavonones. The colours became more intense after the chromatogram was passed over ammonia vapour. The red and pale red colours with R_f values 0.51, 0.39, 0.71 and 0.60 indicate the presence of anthocyadins/chalcones.

3.3 Antioxidant Activity

The DPPH assay is one of the most common and relatively quick methods used for testing radical scavenging activity of various plant extracts [15]. The quantitative assay on Table 3 showed antioxidant activity of the EtOAc extract fraction of *A. sieberiana.* The standard ascorbic acid showed higher antioxidant activity than the EtOAc extract fraction. The percentage inhibition of the EtOAc extract fraction on DPPH increases with increase in concentration but became nearly constant at 300, 400 and 500ug/ml (Table 3). The EtOAc fraction depicted better antioxidant activity of 17.72% at the lowest concentration of 1ug/ml than the ascorbic acid with only 2.3%. In general, the EtOAc extract fraction showcased a direct linear relationship on DPPH as seen in Fig. 2 in such a way that as the concentration increases, the scavenging effect also increases. The ability of the EtOAc extract fraction to depict antioxidant activity could be ascribed to the appreciable number of compounds (phenolic) seen from the number of spots produced on the TLC plates (Table 2). The IC_{50} of the EtOAc extract fraction was calculated from the calibration curve (Fig. 2) to be 52.04ug/ml while that of the standard ascorbic acid was found to be 57.18ug/ml. This has proven that the ascorbic acid indicated more radical scavenging activity than the EtOAc fraction because lower IC_{50} signifies more scavenging ability [16]. The presence of phenolic compounds and flavonoids in this plant may be responsible for the antioxidant activity observed in this study. Flavonols are considered to act as UV protectants and free radical scavengers [17]. For this reason, the antioxidant properties observed in this plant may support the local usage of the stem bark for the treatment of radical related diseases such as high fever, cancer and hypertension.

3.4 Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS analysis of compounds was carried out on the EtOAc extract fraction and the major peaks were identified at retention times 10.011, 31.685, 34.057, 35.860 and 38.780min as shown in Table 4. The compounds identified include Ergosta–5,22-dien-3-01-acetate(93β,22E), 4H-Benzopyran-4-one-5,6,7-trimethoxy-2-(4-methoxyphenyl), Ethyl iso-allocholate which has been reported by Malathi et al. [18] to have antimicrobial activity. 3,9-Epoxypreg-16-en-14-ol-20-one-11,18-diacetoxy-3-methoxy which has been reported to possess antioxidant, anticancer and antiinflamatory activities. 10-Octadecnoic acid methyl ester has been reported to possess

antibacterial, antifungal and hepatoprotective properties [19]. It also exhibit analgesic, antiinflammatory, antibacterial and anticancer properties [20].

Table 2. TLC analysis of EtOAc extract fraction of *A. sieberiana*

Solvent system	No of components	UV light	NH_3/UV light	R_f values	Remark
n-hex:EtOAc (3:1)	6	Yellow	Intense yellow	0.87	Flavonoid
		Blue	Intense blue	0.80	Flavonoid
		Pale green	Green	0.61	Flavonoid
		Red	Intense red	0.51	Flavonoid
		Pale red	Red	0.39	Flavonoid
		Pale blue	Blue	0.11	Flavonoid
		Yellow	Intense yellow	0.96	Flavonoid
n-hex:EtOAc:MeOH (5:1:1)	6	Blue	Intense blue	0.85	Flavonoid
		Pale green	Green	0.74	Flavonoid
		Red	Intense red	0.71	Flavonoid
		Pale red	Red	0.60	Flavonoid
		Black	Black	0.50	Flavonoid

Table 3. Free radical scavenging activity of EtOAc extract fraction of *A. sieberiana* and standard ascorbic acid on DPPH

Concentration (µg/ml)	Scavenging activity on DPPH (%)	
	EtOAc extract fraction of *A. sieberiana*	Ascorbic acid
1	17.72±1.21b	2.30±0.32a
5	20.00±0.47c	47.34±0.30c
10	24.31±1.33d	59.17±0.38e
50	31.59±0.64d	86.77±0.52b
100	34.77±0.05d	94.49±0.22c
200	70.90±0,56e	93.99±0.74b
300	71.81±0.08b	94.37±0.01a
400	71.81±0.08c	95.84±0.04d
500	71.81±0.15e	94.44±0.85c

Values presented as mean±SEM (n = 3), Values in the same column sharing same letters are not significantly different (p ≤ 0.05)

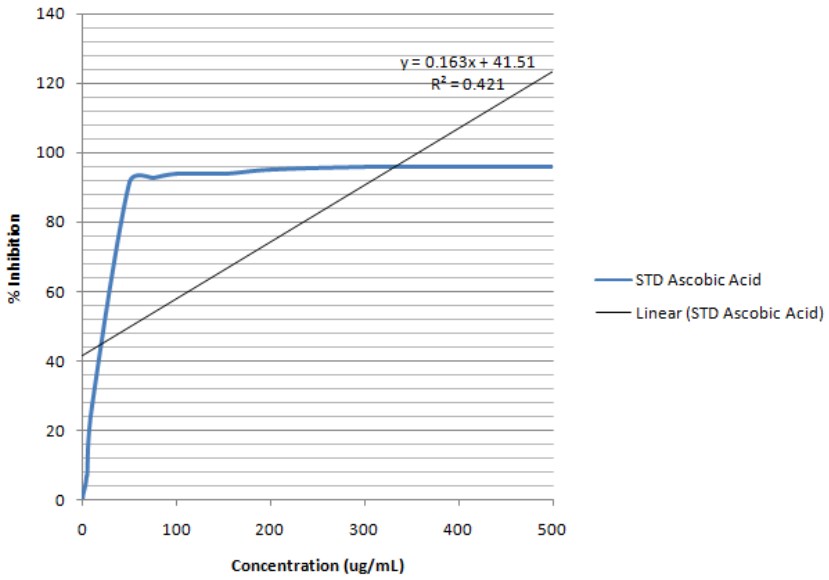


Fig. 1. Percentage inhibition against concentration (0-200µg/mL) of standard ascorbic acid

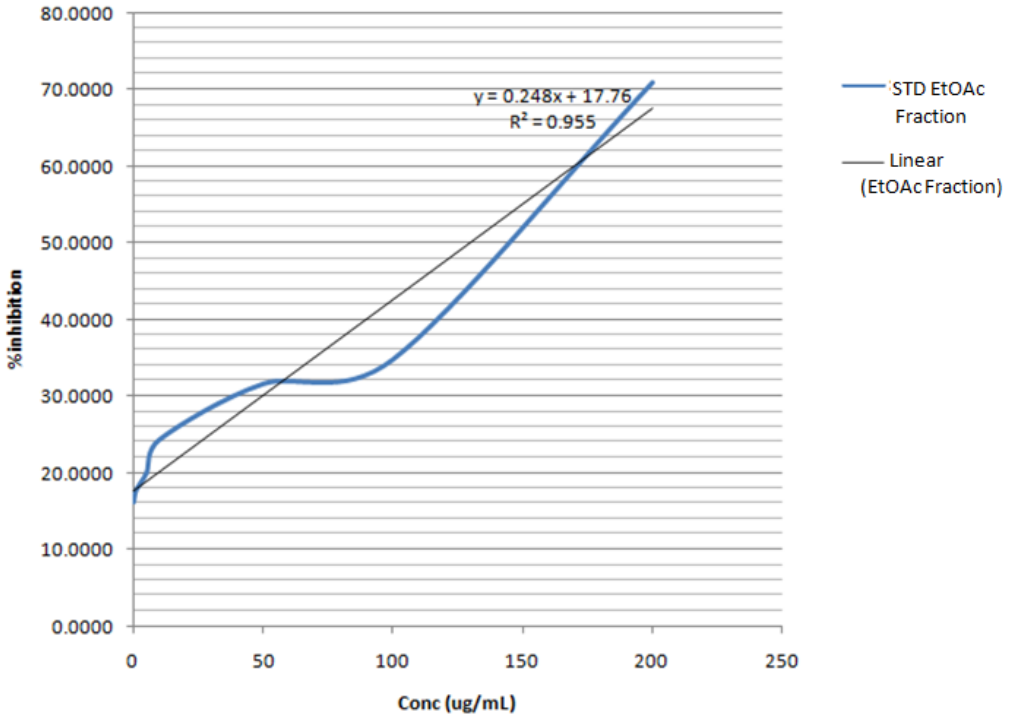


Fig. 2. Percentage Inhibition against concentration (0-200µg/mL) of EtOAc extract fraction of *A. sieberiana*

Table 4. Major compounds identified in EtOAc extract fraction of *A. sieberiana*

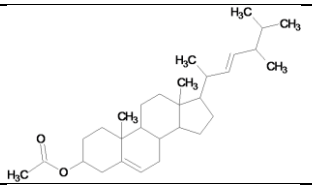
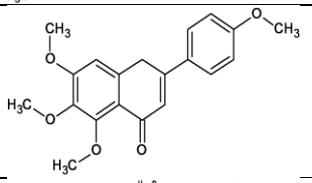
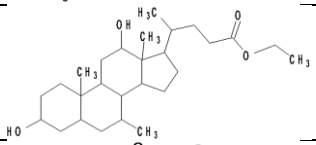
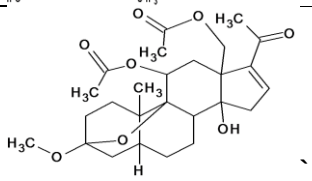
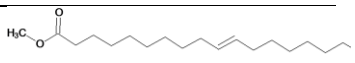
RT (min)	Molecular formula	Molecular mass	IUPAC name	Chemical structure
10.011	$C_{30}H_{48}O_2$	440	Ergosta – 5, 22- dien- 3-01-acetate (93β,22E)	
31.685	$C_{19}H_{18}O_6$	342	4H-Benzopyran-4-one,5,6,7,-trimethoxy-2-(4-methoxyphenyl)	
34.057	$C_{26}H_{44}O_5$	436	Ethyl iso-allocholate	
35.860	$C_{26}H_{36}O_8$	476	3,9- Epoxypreg-16-en-14-ol-20-one, 11,18-diacetoxy-3-methoxy	
38.780	$C_{19}H_{36}O_2$	296	10-Octadecnoic acid methyl ester	

Table 5. Biological activity of some of the identified compounds

Compound	RT	Activity
Ethyl iso-allocholate	34.057	Anticancer, Antiseptic
10-octadecanoic acid methyl ester	38.780	Antibacterial, Hepatoprotective
3,9- Epoxypreg-16-en- 14-ol-20-one, 11,18-diacetoxy-3-methoxy	35.860	Anticancer, Antioxidant, Antiinflammatory

Dr Duke's www.ars-gov/cgi-bin/duke/. [Online database] (2013)

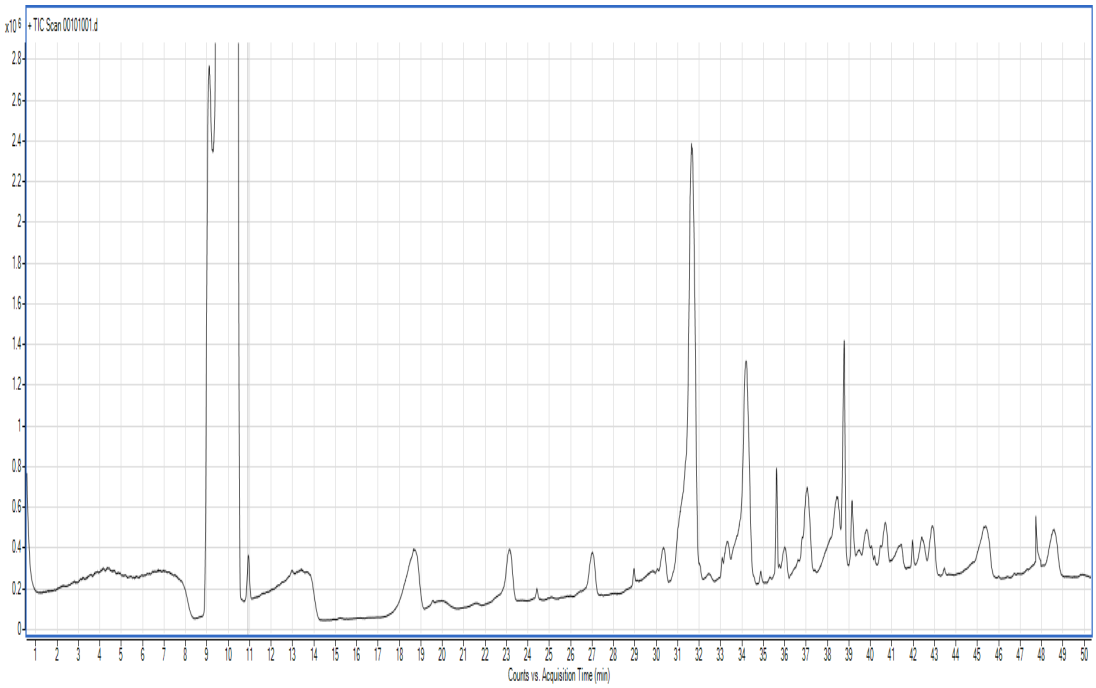


Fig. 3. GC-MS Chromatogram of EtOAc Fraction of *A. sieberiana*

4. CONCLUSION

A. sieberiana is an essential medicinal plant with vast medicinal applications. This study has examined the bioactive compounds present in the plant extract based on the solvent used. It has also reported the antioxidant activity of the extract fraction. The result revealed that the extract fraction (EtOAc) contains flavonoids and terpenes and other phenolic compounds which possess therapeutic activities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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