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Research Article
Antioxidant Parameters and GC-MS Phytochemical Analysis of *Hymenocardia acida* Stem Bark Ethanolic Extract

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Abstract

Background and Objective: Plant-derived medicines have been used in the treatment and management of human diseases. This study was aimed to investigate the antioxidant properties of ethanolic extract of *Hymenocardia acida* stem bark as well as its phytochemical composition using GC-MS. **Materials and Methods:** The stem bark of *H. acida* was collected within the premises of Federal University Wukari, Taraba state, washed, air dried at room temperature for one week and analyzed for antioxidant properties and phytochemical composition. **Results:** The following ranges for the ascertained antioxidant parameters were observed: Total antioxidant capacity (34-72 mg mL^{-1}), Total phenolic content (115-242 mg mL^{-1}) and total flavonoid content (70-122 mg mL^{-1}). The following fatty acids, fatty acids methyl esters and volatile organic compounds were revealed by Gas Chromatography-Mass Spectrometry analysis of *H. acida*: 15-hydroxypentadecanoic acid, oleic acid, palmitoleic acid, 9-octadecenal (Z)-, Behenic alcohol, 1(3H)-iso benzofuranone, 6, 7-dimethoxy-3-[2-(2-methoxyphenyl)-2-oxoethyl, 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene, Heptasiloxane 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13-tetradecamethyl. **Conclusion:** This study showed that *Hymenocardia acida* stem bark has a broad spectrum of antioxidant activity and contains appreciable levels of bioactive compounds such as; Behenic alcohol and Oleic acid which have therapeutic applications.

Key words: Antioxidant, ethanolic, *Hymenocardia acida*, plant-derived medicines, behenic alcohol

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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

Over the years, medicinal plants have been used in the treatment and management of human diseases and hence have been the fundamentals of human medicine[1]. Plant-derived medicines have been of great importance to millions of people in many African countries, particularly the poor living in rural and peri-urban areas where these medicinal plants are readily available and easily affordable[2]. Medicinal plants contain myriads of organic compounds that are capable of causing specific physiological action on the human body[3]. A good number of these organic compounds such as; alkaloids, tannins, flavonoids, terpenoids, saponins and phenolics, generally known as antioxidants have been isolated from plants and found useful in the production of novel drugs to hinder the growth of fungal pathogens, bacterial and to annul or inhibit Reactive Oxygen Species (ROS) via a number of mechanisms to the host cell[4]. These reactive oxygen species, which are majorly chemically reactive molecules containing oxygen are formed as natural products of cellular metabolism. They play vital roles in homeostasis and cell signalling. However, they tend to increase dramatically during environmental stress resulting in significant damage to cell structure[5]. Reactive oxygen species are involved in the aetiology of many chronic diseases due to oxidative damage to nucleic acids, proteins and lipids[6].

Antioxidants exist both in natural and synthetic forms, but due to the toxic and carcinogenic effects of synthetic antioxidants, they have been replaced with natural antioxidants[7]. Natural antioxidants present in plant origin constitute a system that exist in human body which plays a vital role in eradicating excessive free radicals, thus preserving good health[8]. Flavonoids possess a high antioxidant power which can protect cells against adverse effects of ROS[9]. Phenolic compounds are the major antioxidants of human diet and also, they are known to have other biological effects including antimicrobial, antiallergic, anti-inflammatory and anti-platelet actions[10].

Hymenocardia acida is a small browse savannah tree or shrub about 9 m high with palatable foliage, widely distributed within the savannah region of Nigeria. The branches form a fairly heavy, somewhat rounded crown. The branchlets become rusty brown on the bark peels. The leaves are thin, leathery, elliptic-oblong up to 8.75 cm long and 3.75 cm broad, apex being obtuse to rounded, base is obtuse; petiole slender, up to 1.8 cm long. The leaves are usually pubescent when young with a dense mat of fine hairs and with golden glands beneath[11]. It is called "Enache" by Idoma people of North Central Nigeria, "Janyaro" among the hausas in Nigeria, "Uchuo Onyomila" by the Igedes of North Central Nigeria, "Aboopa Orupa" by the Yorubas of Western Nigeria and "Ukwuata" by the Igbos of Nsukka, Enugu state. Experimental studies have shown that crude extracts of the plant have been reported to possess anti-tumor, anti-HIV, anti-inflammatory[11], anti-sickling[12] and anti-ulcer[13]. Among the Idoma and Igede people of North Central Nigeria, the root decoction and stem bark is used in the treatment of diabetes[14]. Also, there have been reports of *in vitro* antitrypanosomal efficacy of leaf[15] and root bark[16] as well as antiplasmodial activities of *H. acida*[17]. Since traditional medicines (plant-derived) are commonly used in Nigeria, it is necessary to study the constituents of *H. acida* stem bark and to assess the potential use of this plant as a medicinal herb. Therefore; the main objective of this study was to examine the antioxidant properties as well as phytochemical composition of ethanolic extract of *Hymenocardia acida* stem bark using GC-MS.

MATERIALS AND METHODS

Plant collection: This research work was carried out in Wukari for about 5 months between May and September, 2018. The stem bark of *H. acida* was collected within the premises of Federal University Wukari, Taraba state. They were identified and authenticated in the Department of Biological Sciences, Federal University Wukari, Taraba state.

Preparation of crude extract: The stem bark was washed, air dried at room temperature for 1 week, pulverized and stored in air-tight container until required. About 100 g of powdered material was soaked in 500 mL of 70% ethanol and stirred intermittently for 48 h at room temperature. The material was filtered using sterile cotton wool and Whatman (No. 1) filter paper, the residue was suspended in the same amount of solvent and the filtered three more times. The pooled filtrates obtained were dried at room temperature under the electric fan. The extracts were stored in air-tight containers at 4°C until needed.

Total polyphenol content: Folin Ciocalteu's reagent was used to analyze for the total polyphenol content of the ethanolic extract of *H. acida* stem bark according to the protocol designed by Singleton and Rossi[18]. About 1 mL of the sample of varying concentrations was incubated in 5 mL of Folin Ciocalteu's reagent and 4 mL of 1 mol L^{-1} Na_2CO_3. After 15 min of incubation, absorbance was measured at 765 nm by spectrophotometer (Shimadzu UV-1700). Gallic acid dissolved in 50% ethanol was used as

standard. The total polyphenol content was reported in terms of mg mL^{-1} of gallic acid equivalents 1 g of extracts (GAEs).

Total antioxidant capacity (Phosphomolybdenum method): Total antioxidant capacity was determined by phosphomolybdenum method[19]. About 0.1 mL of the sample was combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 Mm ammonium molybdate). The mixture was incubated at 95°C for 90 min and then cooled at room temperature. Absorbance was measured at 695 nm. Antioxidant capacity of each sample was expressed as ascorbic acid equivalent.

Total flavonoid content: Total flavonoids were estimated using the method of Ordonez *et al.*[20]. Here, 0.5 mL of 2% $AlCl_3$ ethanol solution was added to 0.5 mL of extract and allowed to stand for 60 min at room temperature before the absorbance was measured at 420 nm. The extract was evaluated at a final concentration of 1 mg mL^{-1}. Total flavonoids content was calculated as quercetin equivalent (mg g^{-1}) using the equation based on the calibration curve: $y = 0.025x$, $R^2 = 0.9812$; where, x is the absorbance and y is the Quercetin Equivalent (QE).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: GC-MS plays a key role in the analysis of unknown components of plant origin. About 2 µL of the ethanolic extract of *H. acida* was employed for GC-MS for analysis of different compounds. Instruments and chromatographic conditions GC-MS analysis were carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm×ID×1 µm of capillary column, composed of 100% Dimethylpolysiloxane) operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL min^{-1} and an injection volume of 0.5 El was employed (split ratio of 10:1) inject or temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10-200°C min^{-1}, then 5-280°C min^{-1}, ending with 9 min isothermal at 280°C. Mass spectra was taken at 70 eV; a scan interval of 0.5 sec and fragments from 45-450 Da. The eluted component is detected in the mass detector. The spectrum of the unknown component is compared with the spectrum of the known components stored of the NIST library and determines the name, molecular weight, fatty acids, volatile compounds and some of the useful components analyzed in the GC-MS study[21].

Statistical analysis: Data were expressed as means±standard deviations (SD) of three replicate determinations and then analyzed by SPSSV-16 (statistical program for social sciences). Statistical analysis was done by one way analysis of variance (one-way ANOVA). The $p \leq 0.05$ was considered to be statistically significant.

RESULTS

The result for Total Antioxidant Capacity (TAC) showed that fraction 6 had the highest antioxidant capacity (72 mg mL^{-1}) with fraction 7 having the lowest (34 mg mL^{-1}). For Total Flavonoid Content (TFC), fraction 6 had the highest flavonoid content (122 mg mL^{-1}) and fraction 5 had the lowest (070 mg mL^{-1}). The result for Total Phenolics Content (TPC) revealed that fraction 6 had the highest phenolics content (242 mg mL^{-1}) with fraction 2 having the lowest (115 mg mL^{-1}) as shown above in Table 1.

GC-MS phytochemical analysis of *Hymenocardia acida* stem bark ethanolic extract revealed the following compounds: 15-hydroxypentadecanoic acid, Palmitoleic acid, Behenic alcohol, Oleic acid, 9-octadecenal (Z)-, 1(3H)-isobenzofuranone, 6,7-dimethoxy-3-[2-(2-methoxyphenyl)-2-oxoethyl, 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene, Heptasiloxane 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13-tetradecamethyl (Table 2).

Table 1: Total antioxidant capacity (TAC), total flavonoid content (TFC) and total polyphenols content (TPC) of *Hymenocardia acida* stem bark ethanol extract

Fraction	Solvent combination	TAC (mg mL^{-1})	TFC (mg mL^{-1})	TPC (mg mL^{-1})
1	Chlo: (100:00)	48±0.00	102±2.00	195±1.73
2	Chlo: Eth Ac (50:50)	40±1.16	100±1.53	115±3.00
3	Eth Ac: Meth (100:00)	53±2.00	102±1.73	195±3.00
4	Eth Ac: Meth (50:50)	58±1.00	102±3.46	240±2.08
5	Eth Ac: Meth (00:100)	46±1.53	070±2.08	192±1.00
6	Meth: Water (50:50)	72±2.00	122±2.52	242±1.16
7	Meth: Water (00:100)	34±1.53	082±1.00	210±1.73

Each data represents the mean of 3 concentrations ±SD, Chlo: Chloroform, Eth Ac: Ethyl acetate, Meth: Methanol

Table 2: Different compounds obtained from Gas Chromatography-Mass Spectrometry analysis of ethanolic extract of *Hymenocardia acida* stem bark

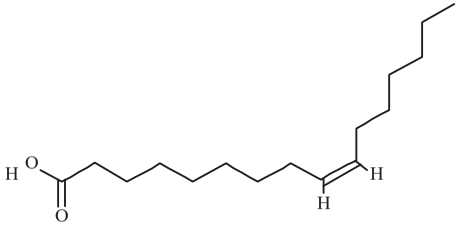
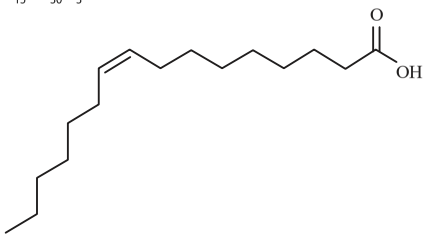
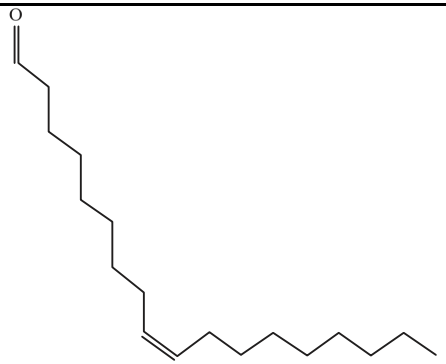
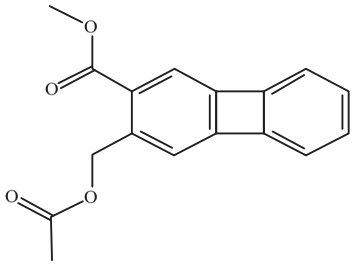
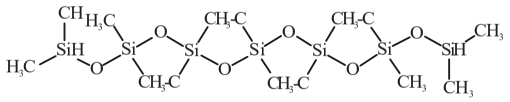
Peak	Retention time	IUPAC name	Chemical structure	Nature and uses
1	36.9203	15-hydroxypentadecanoic acid	 $C_{15}Ho_{30}O_3$	Fatty acid: It is used as an internal standard in the quantification of formation of 11-hydroxylauric acid by Gas-Chromatography. It is also used as a suitable reagent in the synthesis of 16DCA (DCA-dicarboxylic acid) by one-carbon elongation procedure at C-15
2	37.06	Palmitoleic acid	 $C_{16}H_{30}O_2$	Fatty acid: It is used to produced soaps, cosmetics and industrial mold release agents. Palmitic acid undergoes hydrogenation to yield cetyl alcohol, which is used to produce detergents and cosmetics
3	40.6885	Behenic alcohol	 $C_{22}H_{46}O$	Fatty acid: Behenic alcohol is used as a pharmaceutical antiviral agent for reducing the duration of cold sores caused by the herpes simplex virus in the OTC medication. It can also be used traditionally as an emollient, emulsifier and thickener in cosmetics and nutritional supplement (as an individual entity and also a constituent of policosanol
4	42.9831	Oleic acid	 $C_{18}H_{34}O_2$	Fatty acid: It is a component of many foods, in the form of triglycerides. It is used commercially in the preparation of oleates and lotions and as pharmaceutical solvent. It can also be used in manufacturing surfactants, soaps and plasticizers. Small amounts of oleic acid are used as an excipient in pharmaceuticals and it is used an emulsifying or solubilizing agent in aerosol products

Table 2: Continue

Peak	Retention time	IUPAC name	Chemical structure	Nature and uses
5	43.7673	9-octadecenal (Z)-	 $C_{18}H_{34}O$	Volatile organic compound: It is a component of flavouring agents in food
6	49.9809	1(3H)-isobenzofuranone, 6, 7-dimethoxy-3-[2-(2-methoxyphenyl)-2-oxoethyl		
7	50.0425	2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	 $C_{17}H_{14}O_4$	Volatile organic compound: It provides a novel self-crosslinking antireflective coating polymers which can be used to reduce outgassing
8	50.0425	Heptasiloxane 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13-tetradecamethyl	 $C_{14}H_{44}O_6Si_7$	Volatile organic compound: It is used as materials for pharmaceutical and coatings

DISCUSSION

In this study, investigation of antioxidant properties of *Hymenocardia acida* stem bark revealed that among the substances investigated the presence of phenols and flavonoids were detected. Total Flavonoid Content (TFC) and Total Phenolics Content (TPC) of ethanolic extract of *H. acida* stem bark varied from 070-122 mg mL^{-1} and 115-242 mg mL^{-1}. The result of the phytochemical screening of the ethanolic extract of *Hymenocardia acida* stem bark showed the presence of flavonoids, phenolics and absence of anthraquinones, this observation is similar to the previous report[22]. The presence of these metabolites suggests that the plant might be of medicinal importance.

Most plants reported to possess antioxidant properties usually have flavonoids and phenolic compounds as major constituents[23]. Phenolic compounds are good eradicators of free radicals in the body system[24]. They exhibited antioxidant activity by rendering lipid free radicals inactive. They also prevented the decomposition of hydrogen peroxides into free radicals[25]. Flavonoids exhibited their antioxidant activity by scavenging harmful free radicals and reactive oxygen species[26]. A known contributory mechanism to their antioxidant activities is their ability to stabilize membranes by reducing the fluidity of the membranes as well as partitioning flavonoids into the hydrophobic core of the membrane[27]. It has been captured by many reports that antioxidants play a vital role in the prevention and treatment of free radical-related disorders[26,27]. Therefore, the phenolics and flavonoid contents of *H. acida* stem bark suggested its potential ability in neutralizing free radicals thus, preventing free-radical related disorders.

In this study, GC-MS phytochemical screening of ethanolic extract of *H. acida* confirmed diverse class of organic compounds present in the ethanolic extract, ranging from saturated and unsaturated fatty acids such as (palmitoleic acid, oleic acid, 15-hydroxypentadecanoic acid), alcohols such as (Behenic alcohol) and volatile organic substances such as (9-octadecenal (Z)-, 1(3H)-isobenzofuranone, 6, 7-dimethoxy-3-[2-(2-methoxyphenyl)-2-oxoethyl, 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene, Heptasiloxane 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13-tetradecamethyl). Similar results of biologically functional compounds were obtained by a previous study which reported that the 15-hydroxydecanoic acid is a fatty acid with the molecular formula $C_{15}H_{30}O_3$ is used as internal standard (reagent) for the normalization of intensities in the mass spectra of plant cut in polymer. It could also be used as an internal standard in the quantification of formation of 11-hydroxylauric acid by gas chromatography[28]. Palmitoleic acid is a fatty acid which has a molecular formula of $C_{16}H_{30}O_2$. It is used to produce soaps, cosmetics and industrial mold release agents. It undergoes hydrogenation to yield acetyl alcohol which is used to produce detergents[29]. Oleic acid is a fatty acid with the molecular formula $C_{18}H_{34}O_2$. The principal use of oleic acid is as a component in many foods in the form of its triglycerides. It is used commercially in the preparation of oleates and lotions and as pharmaceutical solvent. It is also used as an emollient. Oleic acid has been shown to possess anti-diabetic properties[30]. Behenic alcohol has $C_{22}H_{46}O$ as its molecular formula. It is used as a pharmaceutical antiviral agent for reducing the duration of cold sores caused by herpes simplex virus in OTC medication. It also used traditionally as an emollient, emulsifier and thickener in cosmetics and nutritional supplement (as an individual entity and also as a constituent of policosanol)[21]. The 9-octadecenal (Z)- is a volatile organic compound whose molecular is $C_{18}H_{34}O$. It is a component of flavouring agents in food[31]. The 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene provided a novel self-crosslinking antireflective coating polymers which can be used to reduce outgassing[21]. Its molecular formula is $C_{17}H_{14}O_4$. Heptasiloxane 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13-tetradecamethyl has its molecular formula is $C_{14}H_{44}O_6Si_7$. It is volatile organic compound. It can be used as a material for pharmaceuticals and coatings. These biologically functional compounds have been reported to have multiple effects in regulating the general physiological and biochemical parameters[21], rightly justifying why *H. acida* has been a famous home-made remedy for a number of diseases since ancient times, to this day in many tribal and traditionally bound societies.

CONCLUSION

From this study, it was concluded that the stem bark of *Hymenocardia acida* contains myriads of bioactive compounds, making the plant to have a broad spectrum of antioxidant activity as well as applications in pharmaceutical industry thus supporting the traditional use of this plant as medicine.

SIGNIFICANCE STATEMENT

This study discovered certain phytochemicals and bioactive compounds present in ethanolic extract of *Hymenocardia acida* stem bark that has application in pharmaceutical industry thus supporting the traditional use

of this plant as medicine and can be beneficial in neutralizing free radicals thus, preventing free-radical related disorders, respectively. This study will help the researchers to uncover the critical areas of phytomedicine that many researchers were not able to explore. Thus a new theory on drug production may be arrived at.

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