Asian Journal of Applied Chemistry Research

6(3): 14-26, 2020; Article no.AJACR.59399
ISSN: 2582-0273

Phytochemical Screening, GC-MS Analysis and Antioxidant Activity of Three Medicinal Plants From Nigeria

Ezekwe Ahamefula S.[1*], Ordu Kenneth S.[2] and Oruamabo Ralphael S.[3]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Rivers State University, Nkpolu Oroworokwo, Port Harcourt, Nigeria.
[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, Rivers State University, Nkpolu Oroworokwo, Port Harcourt, Nigeria.
[3]Department of Paediatrics, College of Medical Sciences, Rivers State University, Nkpolu Oroworokwo, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors EAS and OKS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EAS and ORS managed the analyses of the study. Authors EAS, OKS and ORS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2020/v6i330161
Editor(s):
(1) Dr. Angélica Machi Lazarin, State University of Maringá, Brazil.
Reviewers:
(1) Gayatri Sanjeevkumar Kamble, ABMSP's Anantrao Pawar College of Engineering and Research, Savitribai Phule Pune University, India.
(2) Bhagwan Singh Chandravanshi, Addis Ababa University, Ethiopia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59399

Original Research Article

Received 20 May 2020
Accepted 27 July 2020
Published 07 August 2020

ABSTRACT

Phytochemical screening, GC-MS analysis and antioxidant activity of some medicinal plants were investigated using standard methods. Leaves of *Gongronema latifolium*, *Petrocarpus mildbraedii,* and *Piper guineense* plants were prepared properly and used in the entire study. Results of phytochemical screening for the plants revealed the presence of tannins, saponins, alkaloids, phenolic compounds, reducing sugars amongst others at different concentrations. Results of GC-MS analysis for *G.latifolium* showed a total of thirty-three compounds of which Cyclohexano, 2-l(methlaminomethyl)-trans had the highest retention time, squalene had the highest molecular weight and phytol had the highest peak area. GC-MS result for *P.mildbraedii* revealed a total of twenty-three of which Acatamide,2,2,2-trichloro- had the highest retention time, Erucic acid had the

**Corresponding author: Email: ezekweahamefularsu@gmail.com;*

highest molecular weight whereas n-Hexadecanoic acid the highest peak area. Only ten compound were observed in *P.guineense* of which Ethyl (3-hydroxyphenyl) carbamate had the highest retention time, Benzaminde, N-(4,5-dichloro-1,3-benzothiazol-2yl)-3,5-dimethoxy- had the highest molecular weight and Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- has the highest peak area in *P. guineense*. The antioxidant activity of the plant extracts as assessed, followed the order *P.guineense* > *G.latifolium*> *P. mildbraedii* in comparative with ascorbic acid used as the control. The observed compounds become very important when the usefulness of these plants as medicinal plants are considered. They could as well be behind the antioxidant property of the plants in this study. This study has revealed the phytochemical screening, GC-MS analysis and antioxidant activity of some medicinal plants.

Keywords: Antioxidants activity; GC-MS analysis; phytochemicals; medicinal plants.

1. INTRODUCTION

Plants have contributed greatly to the benefit of mankind on this planet Earth [1-12]. The numerous species of plants are easily identified when either their flowers, leaves or fruits are available [13]. It has been reported that plants such as trees and shrubs abound in Nigeria [14-17]. Generally, plants are versatile materials having a wide range of applications [2,5,9,12,13, 18-22]. A wide range of them have been implicated against fungi as antifungal, bacteria as antibacterial, and inflammation as anti-inflammatory agents [5,10,23-25]. Studies have shown that parts of some plants such as leaves, roots, barks, and seeds could serve as analgesic, active antipyretic laxative, amongst others [7,19,22,24-30]. Apart from serving as food materials [8,20,22,26,31-36], it has been noted that different parts of plants are appreciated for their texture, flavor, chemical and nutritional properties [18-20]. The chemical and nutritional properties of plants have been noted by different authors as being behind their therapeutic applications [2-7,9-11,14-19,23-30]. Okwu [37] noted that the chemical constituents in plants are known as phytochemicals. Duru et al. [34] and Duru et al. [38] reported that both phytochemicals and nutrients such as vitamins, minerals, proteins and amino acids; and carbohydrates found in plants are biological active in nature and are effective against disease causing pathogens. According to Morebise and Fafuns [23]; Sofowora [25]; Okigbo and Mmeka [27]; Duru et al. [38], Duru et al. [39]; and Amadi et al. [40] plants with biologically active constituents are termed medicinal plants.

Gongronema latifolium Benth, *Petrocarpus mildbraedii* Harms and *Piper guineense* Schum. & Thonn are amongst the plants termed as medicinal plants with wide range of applications. *Gongronema latifolium*, known commonly as amaranth globe, is a glaborous plant that grows in tropical Africa. It is a climber with characteristics greenish yellow flowers [41]. It can inhabit secondary forest, deciduous forest, rainforest or mangrove forest. It can be propagated by seed, stem cutting or softwood [16,41-42]. The flower grows around July and August. Aside Nigeria, the plants can also be found in found in Senegal, Chad, and DR Congo [16,42-44]. In Nigeria, the Igbos, Efik/Ibibios call *G.latifolium* “utasi”, the Yorubas call it “arokeke” or “madumaro”. According to Edim et al. [43], the Akan-asantes in Ghana, the Serers in Senegal and the Kissis in Sierra leone call it “kurutu nsurogya”, “gasub” and “ndondo-polole” respectively. *G.latifolium* have sweetly bitter in taste. It is used as in soups, salads and sauces as spice [45]. It is also used in the local brewing of beer. The stem is sometimes used as chewing stick [41-43]. The plant has wide application in folkric medicine against abnormal blood glucose levels, diarrhoea, tussive, etc [16,41-42]. It has bioactive constituents that are effective against disease causing microorganisms and certain diseases [46-48]. *P. mildbraedii* grows upto 15–25 m tall. It has fast growth tendency and cappices well. *P. mildbraedii* has superficial root system and most of them are in the top 30 cm of the soil. It has an intermittent pattern of leaf flushes. Flushes appear in the dry season when other leafy vegetables are scarce. It is a lowland green or semi-deciduous forest. It belongs to Papilionaceae family. It is known as “uruhe”, “mádoóbìyaá” and “ọ̀ha” by Edo, Hausa and Igbo tribes of Nigeria respectively [17,49]. *P. mildbraedii* has green stiffy leaves, which are used for soup preparation in Nigeria. Extracts from *P.mildbraedii* are used in the treatment of headaches, pains, fever, convulsions, respiratory disorders, and as antimicrobial agents [17,50-52]. Its wood is also applied in wood construction [50,53]. *Piper guineense* respectively known as “uziza” and “iyere” by the Igbo and Yorubas

tribes of Nigeria, is an evergreen climbing shrub that produces woody stem of about 4-20 meters tall. The plant bears the capacity to support itself on other plants with the help of its adventitious roots, which are produced with stems [17,52-53]. The plant and its pepperish. fruit product are common spices in Africa [52-54]. It could be harvested as wild or semi-cultivated. It is also cultivated for use both as a spice and also as a medicine [52,54]. The fruit of *P. guineense* both fresh and dried are used as spice in flavouring soups, rice and other foods [52,54]. The fruits and leaves are used fresh and dried as components of medicinal preparation [52,54]. The roots are chewed and the juice swallowed as an aphrodisiac. The root is also used as chewing stick for cleaning of teeth [17,52-54].

The production of reactive oxygen species (ROS) has been associated with normal cellular metabolism [55], and their excessive production result in progressive damage as well as ultimately cell death. Various stresses leading to excessive production of reactive oxygen species have been recognized. According to Sharma et al. [55], a cell is said to be in state of oxidative stress when the level of ROS exceeds the defense mechanisms. The defense mechanisms include group of complex antioxidative system comprising of nonenzymatic such as the major cellular redox buffers ascorbate (AsA) and glutathione (γ-glutamyl-cysteinyl-glycine, GSH) as well as tocopherol, carotenoids, and phenolic compounds; and enzymatic components such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH) cycle ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [56-57]. They scavenge and as well combat the activities of ROS and prevent them from being excessive as to pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells [55]. The active oxygen species (AOS) such as singlet oxygen, super oxide radical anion, hydrogen peroxide associated with oxidative stress has been linked to play cardinal role as free and non-free radicals in the etiology of several diseases like arthritis, cancer, atherosclerosis, diabetes, cardio- and cerebrovascular diseases, tumors, epilepsy, mutagenesis, carcinogenesis, arteriosclerosis, Alzheimer's disease, tissue injury [58-60].

Due to the linkage of certain disease conditions with oxidative stress and coupled with the facts that natural compounds found in plants or their synthetic forms are the basis of modern pharmacopeia [25,61-62], there is a renewed interest on medicinal plants with antioxidants activity. The present study investigated the phytochemical constituents of *G.latifolium*, P. guineense and *P.mildbraedii* and *P.guineense* with advanced method of GC-MS and their antioxidant activity.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The samples of *G. latifolium*, *P.mildbraedii*, and *P.guineense* used were collected from Imo State University school farm and were identified in the Department of Plant Science and Biotechnology of the same institution by Botanist in the Department. The leaves of interest were collected, properly cleaned, shade dried and coarsely powdered for further usage.

2.2 Extracts Preparation

Ten grams (10 g) of each sample was extracted by maceration in 50 ml of water for 3 days with frequent agitation at a speed of 280 rpm at 28°C in dark. Between extractions, the samples were centrifuged for 10 min with 2000 rpm. The combined supernatants were collected, filtered through Whatman No. 1 filter paper and concentrated in vacuum. They were kept in a vacuum desiccator for complete removal of solvent. The yield extracts were thus used for some of phytochemical screening, GC-MS analysis and assessment of antioxidant activity.

2.3 Qualitative Phytochemical Determinations

2.3.1 Test for tannins

To 1 mL each of the plant extract, equal volume of bromine water was added. The formation of a greenish to red precipitate was taken as the presence of tannins,

2.3.2 Test for saponins

1 mL of each extract was boiled with 5 mL of distilled water for 5 min. and decanted while hot.

4 mL of distilled water was added to 1 mL each of the filtrate before they were shaken vigorously for observation of stable froth on standing.

2.3.3 Test for flavonoids

0.5 g of each selected plant extract were added in a test tube and 10 ml of distill water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml concentrated H_2S0_4. Indication of yellow color shows the presence of flavonoid in each extract.

2.3.4 Test for alkaloids

1 mL each of the plant extract was shaken with 5 mL of 2% HCl on a steam bath and then filtered. To I mL each of the filtrate, Wagner`s reagent (iodine in potassium –iodide solution) was added and reddish brown precipitates was observed for positive result.

2.3.5 Test for steroids

The amount of 0.5 g each of the extract was dissolved in 10 mL anhydrous chloroform and filtered. The filtrate was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

2.3.6 Test for terpernoids

1 g of selected plant samples was taken in a test tube, then poured 10 mL of methanol in it, shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform were mixed in extract of selected plant samples and 3 mL of sulphuric acid were added in selected sample extracts. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

2.3.7 Cardiac glycosides

1 mL each of the plant extract was dissolved in in 2 mL of chloroform in a test tube. 1 mL conc.H_2SO_4 was carefully added to each of the test tubes through their sides and they were observed for a red or reddish brown colouration at the interphase, which indicates positive result.

2.3.8 Test for phlobatannins

One percent aqueous hydrochloric acid was added was added to each of the plant extract in a test tube (about 2 mL), and then boiled with the help of Hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

2.3.9 Test for phenolic compounds

To 2 mL of each plant extract, 1% $FeCl_3$ was added and observation was made for blue, violet, purple, green or red-brown colour.

2.3.10 Test for proteins

Five drops of 1% hydrated copper sulphate was added to 2 mL each of the sample extract in a test tubes. Two mL of 40% NaOH was also added, and the test tubes were shaken vigorously to mix the contents and presence of purple colouration indicated the presence of proteins.

2.3.11 Test for reducing sugars

One mL of ethanol was mixed with 2 mL each of the plant extract, after which 1 mL each of Fehling solution A and B were added to the test tubes. The test tubes were heated to boiling while observation was made for presence of reddish brown colouration which indicates positive results.

2.3.12 Test for anthroquinones

One gram of the ground plant was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate was added with an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration, which for the presence of anthraquinones. This was repeated with all the plant samples.

2.4 GC-MS Analysis of the Extracts

GC-MS analysis of the aqueous extracts was carried out using AOC-20i auto sampler and gas chromatograph interface to a mass spectrometer (GC-MS) instrument. Employing the following conditions; column Elite-1 fused silica capillary column (30 mm×0.25 mm ID×1µM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/ min, and an injection volume of

0.5µl, Split ratio of 10:1), with injector temperature 250°C; and ion-source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 mins isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time was 36 mins. The plant extract was dissolved in aqueous and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test were ascertained. The process was repeated fro each extract.

2.5 Determination of Antioxidant Activity

2.5.1 DPPH (1, 1-Dipheny l-2-picrylhydrazyl) radical scavenging assay

The free radical scavenging activity was measured by DPPH assay method. Four mg of DPPH (0.1 mM) was dissolved in 100 mL of distill water to obtain working solution. One mL of each extract was mixed separately with 2.0 mL of 0.1 mM DPPH followed by 30 min incubation in dark. The reduction of the DPPH free radical was measured by taking the absorbance at 517 nm [63]. Colour of DPPH was reduced from purple to yellow. The antioxidant activity of each extracts was evaluated by calculating the inhibition % of free radical formation using the formula:

% inhibition = $[(A-A_1)/A] \times 100$; A= absorbance of the blank (DPPH); A1= absorbance of the extract (DPPH+ extract).

3. RESULTS AND DISCUSSION

The phytochemical screening of *G. latifolium*, *P. mildbraedii* and *P.guineense* represented in Table 1 shows the presence of tannins, saponins, flavonoids, alkaloids, steroids, terpernoids, cardiac glycosides, phlobactanins, phenolic componds, proteins, reducing sugars and anthroquinones at different concentrations in the plants. Saxena et al. [64] noted that phytochemicals are chemicals that protect cells in plants against pathogenic attack and environmental hazards. However, it was in recent times that it was clearly observed that phytochemicals play important roles in human health, when they are appropriately taken in significant concentrations [65-66]. Phytochemicals become important when their roles or functions are considered in humans [40]. The concentration of tannins was high in *G. latifolium* while *P. guineense* and *P.mildraediii* had moderate concentrations of tannins. According to Saxena et al. [64] and Serrano et al. [67], many health benefits have been recognised for the intake of tannins. Epidemiological associations have linked tannins to decreased frequency of chronic diseases [64]. Tannin containing plant extracts are used as astringents, as diuretics, against diarrhoea, stomach and duodenal tumours. Tannins are also potent antiseptic, antioxidant, anti-inflammatory and haemostatic pharmaceuticals [68-69]. Saponins are group of secondary metabolites known for their stable foam or foaming property in aqueous solutions [70]. Extensive research studies have revealed the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins. Saponins have also been found to be effective against protozoans, molluscs, fungi and viruses [64]. Saponin concentration was high in *G. latifolium* while *P. guineense* in the present study but was moderate in *P.mildraediii.* Flavonoids are ubiquitous in nature and are known to have numerous derivatives [64]. They protect carbohydrates, proteins, lipids and DNA macromolecules from oxidative processes and protect the biological systems from harmful effects of such oxidative processes [64]. Flavonoids were moderately present in *G. latifolium* and *P.guineense*, but absent in *P. mildbraedii* in this study. Alkaloids offer significant protection and ensure survival of plants. They exhibit antihypertensive, antiarrhythmic, antimalarial, and anticancer effect [64]. Some alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug [71]. Alkaloid was high in *P.guineense*, moderate in *G. latifolium*, but absent in *P. mildbraedii.* Steroids were only observed in *P. mildbraedii.* The roles of steroids in the formation if steroid hormones have long been noted. Terpenoids have anticarcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial or diuretic activity. It is on record that drugs have been manufactured from some terpenoid derivatives [72-73]. Cardiac glycosides at low concentrations are highly beneficial to the heart [70]. Terpernoids and cardiac glycosides were moderately observed in

P.guineense and *P. mildbraedii* in the present study. The anti-inflammatory, analgesic and wound healing properties of phlobatannins have been reported [70,74]. Phlobatannins were moderately concentrated in in *G. latifolium* and *P. mildbraedii.* Phenolic compounds have been implicated against increased bile secretion, reduction of lipid levels and blood cholesterol. They also exhibit antimicrobial activity against some strains of bacteria. *G. latifolium* had high concentration of phenolic compounds while *P. mildbraedii* and *P.guineense* had moderate concentrations of the compounds. Proteins are reported sources of amino acids, which invariably repairs and build worn-out tissues. Proteins occurred moderately in *G.latifolium* but were highly concentrated in *P. mildbraedii* and *P.guineense.* Reducing sugars and anthroquinones occurred moderately in *P. mildbraedii* and *P.guineense* while *G. latifolium* had high concentration of *anthroquinones*.

Result of GC-MS analysis of *G.latifolium* showing retention time, molecular formula, molecular weight and peak area as presented on Table 2 revealed the presence of 33 constituents showing their retention time in min., molecular formular, molecular weight and peak areas in percentage. Among the compounds observed in *G.latifolium* were 4-Aminobutanoic acid, Ethylene oxide, Methyltetradecanoate, M'N-Diacetylenediamine, Metheyl 8-methyldecanoate, Urea, butyl, Teichloroacetic, undec-10-enylester, 8-none-2-one, 3,8-Dioxatricylo (5.1.0.0(2,4) octane, 4-ethyl, Undecanoic acid, 10-methyl, methylester, 7-Hexadecanoic acid, methyl ester (Z), Hexadecanoic, methyl ester, 1-Tridecanamine, n-Hexadecanoic acid, Cyclopentanemethanamine 2-amino, Acetaldehyde, 9,12-Octadecanoic acid, methyl ester, 15-Octadecanoic acid, methyl ester, Phytol, Methyl sterate, Cis-vaccenic acid, Di-Phenylephrine, 9-Octodecanoic acid (Z), methyl ester, 9-Hexadecanoic acid, 9-Oxabicyclo[6.1.0]nonane, cis, 13-Docosenic acid, methyl ester, Erucic acid, 6-Nitroundec-5-ene, Cis-9-Hexadecanoic acid, Squalene, 6-Nitronudec-5-ene, 2-Methylaminomethyl-1,3-dioxolane,and Cyclohexano, 2-l(methlaminomethyl)-trans. Cyclohexano, 2-l(methlaminomethyl)-trans had the highest retention time, squalene had the highest molecular weight while phytol had the highest peak area in *G.latifolium* as observed in the present study.

Result of GC-MS analysis of *P. mildbraedii* showing retention time, molecular formula, molecular weight and peak area as presented on Table 3 revealed the presence of 23 constituents showing their retention time in min., molecular formular, molecular weight and peak areas in percentage. Among the compounds observed in *P. mildbraedii* were Ethylene, 2,4 (1H, 3H)-Pyrimidinedione, dihydro-5-hydroxy, N-(3-Methylbutyl) acetamide, Bicyclo[3,1,1]heptane-2,6,6-trimethyl-[1R-(1α, 2α, 5α]-,1-Heptadecanamine, 2-Butene ozonide, Cyclopentanol, 2-(aminomethyl)-,trans-, Hexadecanoic acid, methyl ester, 1H-Azonine,octahydro, n-Hexadecanoic acid, n-Hexadecanoic acid, Heptane, 1-Heptadecanamine,N,N`-Bis (3-aminopropyl)ethylenediamine, Cis-Vaccenic acid, Octadecanoic acid, Acetamide 2,2,2-trichloro-, Cis-11-Eicosenoic acid, Di(pent-4-4nyl)amine, Cyanoacetylurea, Erucic acid, 3-fluoroamphetamine, Benzeneethanamine,4-fluoro, and Acatamide,2,2,2-trichloro-. Acatamide,2,2,2-trichloro- had the highest retention time, Erucic acid had the highest molecular weight whereas n-Hexadecanoic acid the highest peak area in *P.mildbraedii*.

Table 1. Phytochemical screening of *G. latifolium*, *P. mildbraedii,* and *P. guineense*

Phytochemical	*G. latifolium,*	*P. mildbraedii*	*P. guineense*
Tannins	++	+	+
Saponins	++	+	++
Flavonoids	+	-	+
Alkaloids	+	-	++
Steriods	-	+	-
Terpernoids	-	+	+
Cardiac glycosides	-	+	+
Phlobatannins	+	+	-
Phenolic compounds	++	+	+
Proteins	+	++	++
Reducing sugars	-	+	+
Anthroquinones	++	+	+

++: present in high concentration; +: moderate concentration; -: absent

Table 2. Result of GC-MS analysis of *G.latifolium* showing retention time, molecular formula, molecular weight and peak area

S/N	RT	Name of compound	Molecular formular	Molecular weight	Peak area %
1	2.493	4-Aminobutanoic acid	$C_6H_9NO_2$	103	1.37
2	4.312	Ethylene oxide	$C_6H_{12}N_2O$	44	0.74
3	8.227	Methyltetradecanoate	$C_6H_{30}O_2$	242	2.79
4	8.548	M'N-Diacetylenediamine	$C_{12}H_{24}N_2O_2$	144	1.51
5	8.741	Metheyl 8-methyldecanoate	$C_{12}H_{24}O_2$	200	1.00
6	8.944	Urea, butyl	$C_5H_{12}N_2O$	116	0.62
7	9.019	Teichloroacetic, undec-10-enylester	$C_{13}H_{21}Cl_3O_2$	314	1.83
8	9.062	8-none-2-one	$C_9H_{10}O$	140	1.93
9	9.313	3,8-Dioxatricylo (5.1.0.0(2,4) octane, 4-ethyl	$C_8H_{10}O$	138	0.77
10	9.308	Undecanoic acid, 10-methyl, methylester	$C_{13}H_{26}O_{26}$	214	0.66
11	9.489	7-Hexadecanoic acid, methyl ester (Z)	$C_{17}H_{32}O_2$	268	2.24
12	9.663	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$	268	2.24
13	9.800	1-Tridecanamine	$C_{13}H_{29}N$	199	1.20
14	9.944	n-Hexadecanoic acid	$C_{15}H_{32}O_2$	256	6.96
15	10.105	Cyclopentanemethanamine 2-amino	$C_6H_{14}N_2$	114	1.51
16	10.286	Acetaldehyde	C_2H_4O	44	0.52
17	10.720	9,12-Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294	3.94
18	10.762	15-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296	3.98
19	10.832	Phytol	$C_2H_{40}O$	296	17.44
20.	10.913	Methyl sterate	$C_{19}H_{38}O_2$	298	2.62
21	11.051	Cis-vaccenic acid	$C_{18}H_{34}O$	282	5.07
22	11.051	Di-Phenylephrine	$C_9H_{13}NO_2$	167	1.02
23	11.950	9-Octodecanoic acid (Z), methyl ester	$C_{19}H_{36}O_2$	296	5.09
24	12.217	9-Hexadecanoic acid	$C_{16}H_{30}O$	254	1.72
25	12.913	9-Oxabicyclo[6.1.0]nonane, cis	$C_8H_{24}O$	126	1.18
26	13.036	13-Docosenic acid, methyl ester	$C_{23}H_{44}O_2$	352	4.38
27	13.276	Erucic acid	$C_{22}H_{42}O_2$	338	1.34
28	13.966	6-Nitroundec-5-ene	$C_{11}H_{21}NO_2$	199	1.43
29	14.624	Cis-9-Hexadecanoic acid	$C_{16}H_{30}O_2$	254	0.65
30	14.624	Squalene	$C_{30}H_{50}$	410	0.61
31	15.057	6-Nitronudec-5-ene	$C_{11}H_{21}NO_2$	199	1.43
32	18.047	2-Methylaminomethyl-1,3-dioxolane	$C_5H_{11}NO_2$	117	0.59
33	19.144	Cyclohexano, 2-l(methlaminomethyl)-trans	$C_8H_{17}NO$	143	1.56

RT= Retention time

Table 3. Result of GC-MS analysis of *P. mildbraedii* showing retention time, molecular formula, molecular weight and peak area

S/N	RT	Name of compound	Molecular formular	Molecular weight	Peak area %
1	2.374	Ethylene	C_2H_4O	44	0.79
2	4.681	2,4 (1H, 3H)-Pyrimidinedione, dihydro-5-hydroxy	$C_4H_6N_2O_3$	130	1.17
3	8.537	N-(3-Methylbutyl) acetamide	$C_7H_{15}NO$	129	2.96
4	9.013	Bicyclo[3,1,1]heptane-2,6,6-trimethyl-[1R-(1α, 2α, 5α]-	$C_{10}H_{12}$	138	4.67
5	9.051	1-Heptadecanamine	$C_{17}H_{37}$	255	1.05
6	9.211	2-Butene ozonide	$C_4H_8O_3$	104	1.71
7	9.307	Cyclopentanol, 2-(aminomethyl)-,trans-	$C_6H_{23}NO$	115	2.14
8	9.628	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.87
9	9.783	1H-Azonine,octahydro	$C_8H_{17}N$	127	3.65
10	9.933	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	24.08
11	10.752	Heptane	$C_7H_{24}O$	114	1.44
12	10.816	1-Heptadecanamine	$C_{17}H_{37}N$	255	1.58
13	10.912	N,N`-Bis (3-aminopropyl)ethylenediamine	$C_7H_{19}N_3$	145	0.85
14	11.040	Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	18.25
15	11.174	Octadecanoic acid	$C_{18}H_{36}O_2$	284	10.33
16	11.944	Acetamide 2,2,2-trichloro-	$C_2H_2Cl_3NO$	161	0.87
17	12.201	Cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	7.59
18	12.324	Di(pent-4-4nyl)amine	$C_{10}H_{19}N$	153	2.41
19	13.030	Cyanoacetylurea	$C_4H_5N_3O_2$	127	1.38
20	13.271	Erucic acid	$C_{22}H_{42}O_2$	338	7.20
21	15.148	3-fluoroamphetamine	$C_9H_{12}FN$	153	1.06
22	19.117	Benzeneethanamine,4-fluoro	$C_9H_{12}FNO_2$	185	0.97
23	21.080	Acatamide,2,2,2-trichloro-	$C_2H_2Cl_3NO$	161	0.98

RT= Retention time

Table 4. Result of GC-MS analysis of *P. guineense* showing retention time, molecular formula, molecular weight and peak area

S/N	RT	Name of compound	Molecular formular	Molecular weight	Peak area %
1	5.62	4,9-decadienoic acid, 2-nitro-,ethyl ester	$C_{12}H_{19}O_4$	241	2.64
2	5.90	(+)-2-carene, 4-alpha,-isopropenyl	$C_{13}H_{20}$	176	3.21
3.	6.93	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	208	56.27
4	9.63	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	3.19
5	9.96	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.50
6	10.68	Cyclotetradecane	$C_{14}H_{28}$	196	6.87
7	10.83	Phytol	$C_{20}H_{40}O$	296	11.27
8	11.05	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282	4.92
9	14.50	Benzaminde, N-(4,5-dichloro-1,3-benzothiazol-2yl)-3,5 dimethoxy-	$C_{16}H_{12}Cl_2N_2O_{3S}$	382	2.16
10	14.65	Ethyl (3-hydroxyphenyl) carbamate	$C_9H_{11}NO_3$	181	1.97

RT= Retention time

Result of GC-MS analysis of *P. guineense* showing retention time, molecular formula, molecular weight and peak area as presented on Table 4 revealed the presence of 10 constituents showing their retention time in min., molecular formular, molecular weight and peak areas in percentage. Among the compounds observed in P. guineensewere 4,9-decadienoic acid, 2-nitro-,ethyl ester, (+)-2-carene, 4-alpha,-isopropenyl, Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-, Hexadecanoic acid, methyl ester, Hexadecanoic acid, Cyclotetradecane, Phytol, Cis-vaccenic acid, Benzaminde, N-(4,5-dichloro-1,3-benzothiazol-2yl)-3,5- dimethoxy- and Ethyl (3-hydroxyphenyl) carbamate. Ethyl (3-hydroxyphenyl) carbamate had the highest retention time, Benzaminde, N-(4,5-dichloro-1,3-benzothiazol-2yl)-3,5-dimethoxy- had the highest molecular weight and Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- has the highest peak area in *P. guineense*.

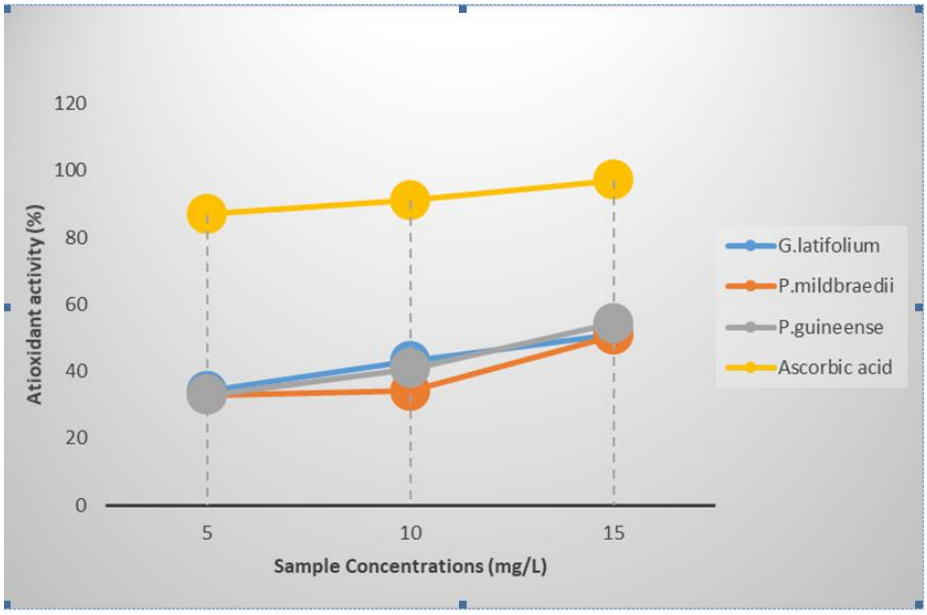

Fig. 1. Antioxidant activity of *G. latifolium, P. mildbraedii* and *P. guineense*

The observed compounds revealed by GC-MS in the plants and those of phytochemical screening become important when their functions and contributions in nature are considered.

The antioxidant activity of the *G.latifolium, P.mildbraedii* and *P.guineense*. as presented in Fig. 1 revealed increased % inhibition of 34%, 43% and 51% respectively for 5 mg/L, 10 mg/L and 15 mg/L concentrations of *G.latifolium*. % inhibition for *P. mildbraedii* were observed to be 32.89%, 34.13% and 50.78% respectively for concentrations of 5 mg/L, 10 mg/L and 15 mg/L of *P. mildbraedii***.** The % inhibition activity of 32.8%, 40.8% and 54.27% respectively with concentrations of 5 mg/L, 10 mg/L and 15 mg/L were observed for *P.guineense*. Generally, the antioxidant activity of the plants increased with increase in concentration of the extracts, and they followed the order *P.guineense* > *G.latifolium*> *P. mildbraedii* in comparative with ascorbic acid used as the control.

4. CONCLUSION

G.latifolium is enriched with enormous compound, followed by P.mildbraedii and then *P.guineense*. Their antioxidant capacity followed the order *P.guineense* > *G.latifolium*> *P. mildbraedii.* These componds as revealed by GC-MS analysis could no doubt be behind the observed antioxidant activity of the plants and as well as behind their efficacies as medicinal plants.This study has revealed the phytochemical screening, GC-MS analysis and antioxidant activity of some medicinal plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abulude FO. Phytochemical screening and mineral contents of leaves of some Nigeria woody plants. Research Journal of Phytochemistry. 2007;1(1):33-39.
2. Monago-Ighorodje C, Duru M, Adindu E, Nwauche K, Ezekwe A, Nosiri I, Odika P, Onyeabo C, Ogar I, Berezi EP, Ugoh AI, Eboagwu I, Otta E. Effect of ethanolic leaf extract of *Vinca major* L. on biochemical parameters and glucose level of alloxan induced diabetic rats. African Journal of Biotechnology. 2019;18(32):1054-1068.
3. Duru MKC, Arukwe U, Amadi, BA. Bioactive constituents and macronutrients composition of anti-malarial concoction used in Umunchi village in Isiala Mbano L.G.A of Imo State, Nigeria. International Science Research Journal. 2011;3:61–64.
4. Ibegbulem CO, Eyong EU, Essien EU. Biochemical effects of drinking Terminalia catappa Linn. decoction in Wistar rats.

African Journal of Biochemistry Research. 2011;5(8):237-243.

5. Nwachukwu MI, Duru MKC, Nwachukwu IO, Obasi CC, Uzoechi AU, Ezenwa CM, Anumodu CK. *In-vitro* phytochemical characterization and antibacterial activity of *Newbouldia laevis* (boundary tree) on *Escherichia coli* and *Staphylococcus aureus*. Asian Journal of Microbiology and Biotechnology. 2017;2(1):30-36.
6. Duru M, Amadi B, Ugbogu A, Eze A. Effect of "udu" an antimalarial herbal preparation on visceral organ weight and blood lipid profiles in wistar rats. Journal of Pharmacy and Clinical Sciences. 2014;8:1-7.
7. Okwu DE. Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. J. Sustain. Agric Environ. 2004;6:30-34.
8. Amadi B, Onuoha N, Amadi C, Ugbogu A, Duru M. Elemental, amino acid and phytochemical constituents of fruits of three different species of eggplants. International Journal of Medicinal and Aromatic Plants. 2013;3(2):200-202.
9. Nwachukwu MI, Duru MKC, Amadi BA, Nwachukwu IO. Comparative evaluation of phytoconstituents, antibacterial activities and proximate contents of fresh, oven-dried uncooked and cooked samples *of Buchholzia coriacea* s*eed* and their effects on hepatocellular integrity International. Journal of Pharmaceutical Science Invention. 2014;3(6):41-4.
10. Nwachukwu MI, Duru MKC, Nwachukwu IO. Antifungal properties and effect of fresh, oven dried uncooked and cooked seeds *of Buchholzia coriacea* on haematology and kidney. Elixir Food Science. 2013;64:19350-19356.
11. Duru MKC, Amadi BA, Eze AE, Ugbogu AE, Onuoha N. *In vivo* studies of *Solanum aethiopicum* fruit on some biochemical parameters using rats. Journal of Chemical and Pharmaceutical Research. 2013;5(2): 1-4.
12. Amadi BA, Agomuo EN, Duru, MKC. Toxicological studies of *Asmina triloba* leaves on haematology, liver, kidney using rat model. International Science Research Journal. 2013;4(2):11-17.
13. Aju PC, Popoola, L. Trees in the traditional farming system in southeastern Nigeria. A case study of Imo State. J. Environ. Ext. 2005;5:25:31.
14. Ugbogu AE, Okezie E, Uche-Ikonne C, Duru M, Atasie OC. Toxicity Evaluation of the aqueous stem extracts of *Senna alata* in wistar rats. American Journal of Biomedical Research. 2016;4(4):80-86.
15. Okoroh PN, Duru MKC, Onuoha, SC, Amadi BA. Proximate composition, phytochemical and mineral analysis of the fruit of *Ficus capensis*. International Journal of Innovative Research and Development. 2019;8(8):84-88.
16. Neuwinger HD. African traditional medicine, a dictionary of plants application, Medpharm GmbH Publishers, Stuttgart, German. 2000;589.
17. Burkill HM. The useful plants of west tropical Africa, Royal Botanic Gardens, Kew (K). 1985;3.
    Avliable:https://plants.jstor.org/stable/10.5555/al.ap.upwta.3_699
    (Date Accessed; 11/09/2018).
18. Abulude FO, Onibon VO, Oluwatoba F. Nutritional and antinutritional compositions of some tree bark. Nig. J. Basic Applied Sci. 2004;13:43-49.
19. Agoha RC. Medicinal plants of Nigeria. Vol. 3. Offset Drik Keriji Faculteitder Wiskunde. 1976;102-103.
20. Amadi B, Duru M, Agomuo E, Amadi P, Onedibe O. Nutritional, phytochemical and sensory evaluation of Mberiagworagwo traditional food of Uruagunnewi people in Anambra State, Nigeria. Journal of Advances in Biology & Biotechnology. 2017;14(1):1-8.
21. Duru M, Amadi C, Ugbogu A, Eze A, Amadi B. Phytochemical, vitamin and proximate composition of *Dacryodes edulis* fruit at different stages of maturation. Asian Journal of Plant Science and Research. 2012;2(4):437-441.
22. Duru M, Amadi B, Eze A, Ugbogu A. Evaluation of mgbam traditional food on haematological profile and some selected biochemical parameters following consumption. Elixir Food Science. 2013; 64:19345-19349.
23. Morebise O, Fafunso MA. Antimcrobial and phytotoxic activities of saponin extracts from two Nigeria edible medicinal plants. Biokemistri. 1998;82:69-77.
24. Morebise O, Fafunso MA, Makinde JM, Olajide OA, Awe EO. Anti-inflammatory property of *Gongronema latifolium*. Phytother. Res. 2002;16(l):S75-S77.
    DOI: 10,1002/ptr.784
25. Sofowora EA. Medicinal Plants and traditional remedies in Africa. University of

Ile-Ife press, Nigeria. Spectrum books Ltd. Ibadan, Nigeria.1993;1-23.

26. Duru M, Ugbogu A, Amadi B. Effect of *Solanum macrocarpon* fruit on haematology, hepatic and renal function. Advances in Biochemistry. 2013;1(2):28-32.
27. Okigbo RN, Mmeka EC. An appraisal of phytomedicine in Africa. KMITL Sci. Tech. J. 2006;6(2):83-94.
28. Nwanjo HU, Okafor MC, Oze GO. Anti-lipid peroxidative activity of *Gongronema latifolium* in strptozotocin-induced diabetic rats. Nig. J. Physiol. Sci. 2006;21(1-2):61-65.
29. Nwinyi OC, Chinedu NS, Ajani OO. Evaluation of antibacterial activity of *Pisidium guajava* and *Gongronema latifolium.* J. Med. Plants Res. 2008;2(8): 189-192.
30. Duru MKC, Agomuo EN, Amadi BA. Biochemical studies on 'Udu' an antimalarial concoction used in Umunchi village, Isiala Mbano L.G.A of Imo State, Nigeria, Continental Journal of Pharmacology and Toxicology Research. 2012;5(2):28–34.
31. Duru MKC, Agomuo EA, Amadi BA. Nutrient composition of Nduduagworagwo, a traditional food of Akokwa people in Ideato North L.G.A of Imo State, Nigeria. Continental J. Food Science and Technology. 2012;6(3):27–32.
32. Amadi BA, Arukwe U, Duru MKC, Amadi CT, Adindu EA, Egejuru L, Odika PC. Phytonutrients and antinutrients screening of *D.edulis* fruits at different maturation stages. J. Nat. Prod. Plant Resour. 2012; 2(4):530-533.
33. Amadi BA, Arukwe U, Duru MKC, Adindu EA, Ufornwa EC, Odika PC. The effect of fermentation on anti-nutrients, carbohydrates and vitamin contents of *Pentaclethra macrophylla* Seed. International Science Research Journal. 2011;3:74-77.
34. Duru M, Nwadike C, Ezekwe A, Nwaogwugwu C, Eboagwu I, Odika P, Njoku S, Chukwudoruo C. Evaluation of nutritional, anti-nutritional and some biochemical studies on *Pleurotus squarrosulus* (Mont.) singer using rats. African Journal of Biochemistry Research. 2018;12(2):7-27.
35. Duru M, Eboagwu I, Kalu W, Odika P. Nutritional, anti-nutritional and biochemical studies on the oyster mushroom, *Pleurotus ostreatus*. EC Nutrition. 2019:14(1):36- 59.
36. Amadi B, Agomuo E, Duru M, Anyanwu E, Onyeabo C, Odika P. Amino acid profiles, anutinutrients, concentrations, of minerals and antinutrient-mineral molar ratios of akidiagworagwo and nwagbaraoti traditional foods. Polish Journal of Natural Sciences. 2020;35(1):57-74.
37. Okwu D.E. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. Int. J. Mol. Adv. Sci. 2005;1(4):375-381.
38. Duru M, Amadi B, Agomuo E, Eze A. Chemical profile of an anti-malarial concoction Udu used in Umunchi autonomous community in Isiala Mbano L.G.A of Imo State, Nigeria. Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS). 2012;3(3): 444-447.
39. Duru M, Ugbogu A, Amadi B, Odika P, Chima-Ezika R, Anudike J, Osuocha K. Chemical constituents *of Buchholzia coriacea* se*ed.* Proceedings of the 35th Annual International Conference, Workshop & Exhibition of Chemical Society of Nigeria. 2012;*2:*39-45.
40. Amadi BA, Duru MKC, Agomuo EN. The chemical profiles of leaf, stem, and flower *of Ageratum conyzoides*. Asian Journal of Plant Sciences and Research. 2012*;*2(4): 428-43.
41. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Ibadan; 2006.
42. Gill LS. Ethnomedical uses of plants in Nigeria. University of Benin Press, Nigeria. 1992;215.
43. Edim, EH, Egomi, UG, Uwem EF, Archibong OE. Review on *Gongronema latifolium (*Utasi): A novel antibiotic against Staphylococcus *aureus* related infections. International Journal of Biochemistry and Biotechnology. 2012;1(8):204-208.
44. Ugochukwu NH, Babady NE. Antioxidant effects of *Gongronema latifolium* in hepatocytes insulin dependent diabetes mellitus Filoterapia. 2002;73:(7-8)612-618.
45. Anaso HU, Onochie CC. A comparative study of nutrients in *Gongronema latifolium, Piper Guidneense* and *Piper nigrum*, to testify high acceptability in local dishes. J. Sci. Eng. Technol. 1999;6(2): 1321–1832.
46. Eleyinmi AF. Chemical composition and antibacterial activity of *Gongronema*

latifolium. Journal of Zhejiang University Science B. 2007;8(5):352-358.

47. Ross, I.A. Medicinal plants of the world, Vol. 2: Chemical Constituents, Traditional and Modern Uses. Humane Press, Totowa NJ 07512. 2001;487.
48. Schneider C, Rotscheidt K., Breitmaier E. 4 new pregnane glycosides from *Gongronema latifolium* (Asckepiadaceae). Liebigs Annalen Der Chemie, 1993;10: 1057-1062.
49. Ajibesin KK, Bala DN, Ekpo BAJ, Adesanya, SA. Toxicity of some plants implicated as poisons in Nigerian ethnomedicine to rats. Nigerian Journal of Natural Products and Medecines. 2002; 6:8–10.
50. Akinnifesi FK, Kang BT, Ladipo DO. Structural root form and fine root distribution of some woody species evaluated for agroforestry systems. Agroforestry Systems.1998;42:121–138.
51. Akpanyung EO, Udoh AP, Akpan EJ. Chemical composition of the edible leaves of *Pterocarpus mildbraedii*. Plant Foods and Human Nutrition. 1995;48(3):209–215.
52. Burkill, HM. The useful plants of West Tropical Africa. 2nd Edition. Volume 3, Families J–L. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 1995; 857.
53. Iwu MM. Handbook of African medicinal plants, 1st edition, CRC press Inc., Florida. 1993;239-239.
54. Ken F. Tropical plants database.
    Available:http\\tropical.theferns.info.tropical.theferns.info/viewtropical.php?id=Piper+guineense
    Accessed on 7/07/ 2020).
55. Sharma P, Jha AB, Dubey RS, Pessarakli M. Active oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany. 2012;2012:26, Article ID 217037.
    Available:http://dx.doi.org/10.1155/2012/217037
56. Duru MKC, Akubugwo EI, Chinyere GC, Alisa CO, Nwaogwugwu JC. Effect of seasonal water fluctuation of a water body on antioxidant activity of selected plants of lower phylum (A case study of Nche stream). Academic Journal of Chemistry. 2017;2(8):2519-7045.
57. Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Biology. 1998;49:249–279.
58. Sulekha M, Satish Y, Sunita Y, Rajesh N. Antioxidants: a review. Journal of Chemical and Pharmaceutical Research. 2009;1(1): 102-104.
59. Halliwell B. How to characterize an antioxidant: an update, Biochem. Soc. Symp. 1995;61:73-101.
60. Halliwell B, Gutteridge JMC.Free Radical in Biology and Medicine", 2nd Ed., Clarendon Press, Oxford University Press, Oxford; 1998.
61. Agomuo E, Duru M, Amadi B, Amadi P, Ugwokaegbe P. Effect of caffeine on some selected biochemical parameters using rat model. Advances in Biology. 2017;2017: 8 pages, Article ID 9303276.
    Available:https://doi.org/10.1155/2017/9303276
62. Duru MKC, Amadi BA, Amadi CT, Lele KC, Anudike JC, Chima-Ezika OR, Osuocha K. Toxic effect of carica papaya bark on body weight, haematology, and some biochemical parameters. Biokemistri. 2012; 24(2):67-71.
63. Brand-Williams W, Cuvelier ME, Barset C. Use of free radical method to evaluate antioxidant acitivty. LWT-Food Science and Technology. 1995;28(1):25-30.
64. Saxena, M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2013;1(6):161-182.
65. Hasler CM, Blumberg JB. Symposium on phytochemicals: Biochemistry and physiology. Journal of Nutrition. 1999;129: 756S-757S.
66. Gibson EL, Wardel J, Watts CJ. Fruit and vegetable consumption, Nutritional Knowledge and beliefs in mothers and children. Appetite. 1998;31:205-228.
67. Serrano J, Puupponen-Pimia R, Dauer A, Aura A, Saura-Calixto F. Tannins: current knowledge of food sources, intake, bioavailability and biological effects. Molecular Nutrition Food Research. 2009; 53:S310–29.
68. De Bruyne T, Pieters L, Deelstra H, Vlietinck A. Condensed vegetables tannins: biodiversity in structure and biological activities. Biochemical System Ecology. 1999;27:445–59.
69. Dolara P, Luceri C, De Filippo C, Femia AP, Giovannelli L, Carderni G, Cecchini C,

Silvi S, Orpianesi C, Cresci A. Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutation Research. 2005; 591:237–46

70. Agomuo EN, Duru MKC, Amadi BA. Some bioactive constituents of *Asmina triloba* (paw paw variety). International Science Research Journal. 2013;4(2):18-22.
71. Rao RVK, Ali N, Reddy MN. Occurrence of both sapogenins and alkaloid lycorine in Curculigo orchioides. Indian Journal Pharma Science. 1978;40:104-105.
72. Dudareva N, Pichersky E, Gershenzon J. Biochemistry of plant volatiles. Plant Physiology. 2004;135:1893–1902.
73. Langenheim JH. Higher plant terpenoids: A phytocentric overview of their ecological roles. Journal of Chemical Ecology. 1994; 20:1223-1280.
74. Sofowora EA. Medicinal plants and transitional medicine in Africa, John Wiley and Sons Ltd, New York. 1982;191-234.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59399