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Anti-Tumor Activities/Alternative Therapy of Some Selected Nigerian Medicinal Plants

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ABSTRACT

Cancer is a high mortality disease of public concern. Effectiveness of chemotherapy is often limited by toxicity to untargeted tissues among other serious side's effects. Alternative therapy such as herbal remedies for cancer, which may have the potential to offer more efficacy and less side effects have, however, not been rigorously studied or tested. The Aim of this present study therefore was to validate the anti-cancer properties (using tumor cells) of some selected Nigerian medicinal herbal remedies used by the population in Watsilla, Michika LGA. Phytochemical analysis, Antimicrobial and antioxidant activities of the selected medicinal plants were carried out as preliminary studies. The selected herbal extracts revealed the presence of alkaloid, essential oil, phenol, glycosides, flavonoid, tanin, terpenoid, proteins and saponins. The medicinal plants demonstrated good antioxidant activity with IC_{50} ranging from 0.42-0.54 which is lower than IC_{50} of Ascorbic acid (IC_{50} 0.60 and 0.66) in both DPPH and H_2O_2 free radical scavenging activity model used in this study.The brine shrimp toxicity results, a preliminary anti-cancer studies, indicated that 100% of the plant extracts tested had LC_{50} values lower than 1000 µg/mL; *Heamatostaphisbarteri* (LC_{50} 23.31µg/mL),*Cyanoglossum officinal*'(LC_{50} 50.32µg/mL), *Ximenia americana* (LC_{50}86.57µg/mL) and *Anchus officinalis*(LC_{50} 54.37µg/mL), are excellently none toxic and surely have no obvious danger of outright toxicity during acute exposure. Thus, from the analysis it was concluded that, thesemedicinal plants demonstrated good phytochemical constituents, antioxidant activities and notoxicity on brine shrimp, with a potential to reducing the risk of tumor causing diseases, thereby confirming folkloric information of the usage of the plant as a remedy for common maladies like tumors that lead to various cancers.From the results it was found that the antimicrobial, antioxidant and antitumor activities showed better results than the standard. Bergenin has been found to have some antimicrobial, antioxidants and anti-tumor activities.

Key words: Medicinal plants, pyhtochemistry, anti-tumor, antioxidant activity, chemotherapy.

INTRODUCTION

Nature has provided mankind with a complete store house of remedies to cure most/ all of their ailments [1]. The use of plants in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been found that many plants have medicinal values [2]. The high cost of conventional medicines and their limited availability especially to rural communities in Africa and other developing regions have driven the continued dependence on traditional therapeutics [3]. About 75-90 % of the world population still relies on plants and plant extracts as a source of primary health care [4]. Plants use in traditional médicine, also called phytomedicine are plant-derived medicines that contain chemicals, more usually, mixtures of

chemical compounds that act individually or in combination on the human body to prevent disorders and to restore or maintain health [5]. About two (2) decades ago, 3.4 billion people in the developing world were reported to depend on plant based traditional medicines. Owing to poverty, unawareness and unavailability of contemporary health facilities, most people, especially rural dwellers are still compelled to practice traditional medications for the treatment of their day to day illnesses [6]. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses [7]. Traditional medicine has been used for thousands of years with great contributions made by practitioners to human health, particularly as primary health care providers at the community level [7]. With these descriptions, various forms of medicines and therapies such as herbal medicine, massage, homeopathy, mud bath, music therapy, wax bath, reflexology, heat therapy, therapeutic fasting and dieting, spinal manipulation, psychotherapy, placebo effect etc. are elements of traditional medicine. It does show that, a large country of the size of Nigeria, with diverse cultures and traditions, should be rich in traditional medicine and should have eminent and respected traditional healers to take care of the teeming population [8]. Most Nigerians, especially those living in rural areas, do not use orthodox medicine and it is estimated that about 80% of the populace still prefer to solve their health problems consulting traditional healers [9]. Where access to orthodox medicine exists, the rising cost of imported medications and other commodities used for medicine has posed big problems. Beside, many rural communities have great faith in traditional medicine, particularly in the inexplicable aspect as they believe that, it is the wisdom of their fore-fathers which also recognize their socio-cultural and religious background which orthodox medicine seems to neglect [10]. Traditional medicine is the oldest, most tried and tested form of medicine and is as old as man himself [11]. Traditional medicine covers a wide variety of therapies and practices which vary from country to country and region to region. In some countries, it is referred to as "alternative" or "complementary" medicine (CAM) [11]. Plants, especially used in traditional medicine can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity [12]. The small fractions of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structures from about 90 species of plants. Some of the useful plant drugs include: vinblastine, vincristine, taxol, artemesinin, digitoxigenin and camptothecin. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification / purification of the active principles and elucidation of the mechanism of drug action are of paramount importance. Hence, work in both mixture of traditional medicine and single active compounds are very important [13]. With the continuous use of antibiotics, microorganisms have become resistant. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppression and allergic reactions [14]. This has created immense clinical problems in the treatment of infectious diseases [15]. The use of medicinal plants in traditional medicine has been recognized and widely practiced. According to the [16], 80% of the world`s populations rely on traditional medicines to meet their health regiments because, plant therapies treat a wide range of substances that can be used to treat chronic and acute infectious diseases.

Despite the claimed ethno medicinal uses of the plants in the treatment of tuberculoses, swelling, diarrhea and to stop bleeding during pregnancy, there is

little or no scientific evidence to support the traditional claims and there is no data to standardize the drug for quality control. As a result of this, it becomes extremely important to make an effort towards standardization of the plant as crude drugs and also to establish scientific evidences of their traditional uses as anti-tumor agents. Cancer is one among the dangerous diseases leading to so many deaths worldwide which is without cure, it accounted for an estimated 9.6 million deaths in 2018 [17]. Medicinal plants have been used traditionally for treatment of cancer, but with unspecified dosages. For instance, the selected plantshave been used traditionally for the treatment of cancer in Lala district Gombi L.G.A Adamawa State but there is no scientific evidence to backup the claim of these traditional users. Therefore these plants were selected for this research to find out their anti-tumor activity in order to provide scientific bases for the use of these plants for their cancer treatment. Thus, the aim of this work was to phytochemically screen, evaluate their antitumor and visualize their antioxidant activities.

MATERIALS AND METHODS

Sample Collection and Authentication; Matured plants were collected from Watsilla, Michika, Local Government Area, Adamawa State in the month of 0ctober,2023 and the plants were identified by Forestry Research Institute, Ibadan where their voucher specimens (FHI) were deposited.

Sample Preparation; The roots and leaves of *Heamatostaphis barteri hook, Avena sativa, Ximenia americana Linn, Solonumdul camara*were thoroughly washed with tap water to remove dusts and other unwanted materials accumulated on the roots and leaves from their natural environment. The dust free roots and leaves were allowed to dry under shade. The dried samples were pulverized by using mortar and pestle. Finally, fine powder was obtained from the powdered samples by sieving through the kitchen strainer and used for extraction.

Ethanol and aqueous Extraction; The powdered samples (100 g) were weighed and soaked in 350 mL of ethanol and water in a conical flask. The flask containing the samples were shaken, corked and left to stand for 48 h at room temperature. After 48 h, the mixtures were filtered and the extract were collected and concentrated by evaporation to dryness in evaporating dish and screened for phytochemicals [18], [19].

Antioxidant Potential of the Plants Extract; The antioxidant activity of ethanol and aqueous extracts of plant samples were determined by *in vitro* methods. DPPH (2, 2-diphenyl-2-picryl hydrazyl) free radical scavenging assay and Nitric oxide (NO) assay methods were employed to assess the antioxidant potential. All the assays were carried out in triplicates.

Determination of 2, 2-diphenyl-2-picryl hydrazl (DPPH) Radical Scavenging Capacity

DPPH radical scavenging capacity of the extracts of the plant materials was determined according to the method of [20] and [21]. DPPH (2,2-diphenyl-2-picryl hydrazyl), a stable free radical, when acted upon by an antioxidant, is converted into diphenyl-picryl hydrazine with a colour change from deep violet to light yellow colour. This can be quantified spectrophotometrically at 518 nm to indicate the extent of DPPH scavenging activity by the plant extracts. The effect of the Ethanol and aqueous extracts of the plants on DPPH radical was estimated adopting the method of [20]. The plant parts (roots and leaves) extracts of the different solvents were treated with different concentrations ranging from100 µg/mL to 1000 µg/mL in 95% (v/v) ethanol. 1mL of freshly prepared DPPH solution was added in each of these test tubes and was shaken and incubated for 25 min at room temperature. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard. Control sample was prepared without any extract or Ascorbic acid. 95% ethanol was

used as blank. Percent scavenging of the DPPH free radical was measured using the following equation [22].

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Where:

Absorbance of control = Absorbance of DPPH radical + ethanol
Absorbance of sample = Absorbance of DPPH radical + sample extract /standard.

Scavenging of hydrogen peroxide; The ability of the extracts to scavenge hydrogen peroxide was determined according to the method by [23] method. A solution of hydrogen peroxide (2M) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen was determined by absorption at 285nm using a UV/Visible spectrophotometer. The samples at '1 mg/mL, 0.5 mg/mL, 0.25 mg/mL 0.125 mg/mL, and 0.0625 mg/mL' were added to H_2O_2. The decrease in absorbance of H_2O_2 at 285nm was measured spectrophotometrically after ten minutes (10 min) against a blank solution containing the test samples in phosphate buffer solution (PBS) without H_2O_2 and blank solution containing phosphate buffer without hydrogen peroxide (control). All the tests were performed in triplicates. The percentages of hydrogen peroxide scavenged by the extracts were calculated as follows:

$$\% \text{ scavenged } H_2O_2 = [(A_c - A_s) / A_c] \times 100$$

Where A_c, is the absorbance of the control, A_s, the absorbance in the presence of the sample of extract and standard, [22]. The values of % inhibition were obtained from the above equation. The 50% inhibitory concentration (IC_{50}) of the extracts were obtained from the graphs of the concentration of the samples (Aqueous and Ethanol extracts) versus % inhibition (% H_2O_2 reduction)

Brine Shrimp Lethality Assay; For this experiment, brine shrimp eggs without shells "*Artemia* Revolution" 120g were obtained from NT labs (Fry care) laboratories LTD UK, Serial No. 7//3380900038///3.Made in England. Eggs were stored in a refrigerator at 5^{o}C, till needed for use

Preparation of Artificial Sea water; Artificial sea water was prepared by dissolving 35g of sea salt in1litre of distilled water for hatching the brine shrimp eggs [24].

Hatching of Brine Shrimp; Artificial seawater was Prepared at full strength. To obtain an optimum result, a solution of specific gravity of 1.022 at 24^{o}C was prepared by dissolving 35g sea salt sodium chloride NaClper- liter of water. The seawater was added to the brine shrimp Hatcher in a heated aquarium aerate from bottom of the unit so that all eggs are kept in suspension and moving. The brine shrimp bottle was shaken before dispensing into the aquarium (each drop gives from 1500 to 2000 nauplii, three drops (4500 - 6000 nauplii, average of 5,000) and are hatched in approximately 250 mL sea water. The hatcher is illuminated very well for a minimum of three hours preferably for 12hours. The hatching time depends on temperature at 24^{o}C (which is average tropical aquarium temperature) hatching takes place between 24-48 hours (maximum hatch 44-48hours). The Nauplii are then used directly for the cytotoxicity test [24].

Preparation of Test Sample; Samples were prepared by dissolving 20mg of the plant extracts in 10mL of suitable solvent (stock solution # 1). Solution of varying concentrations (1000, 500, 250,125,100 µg /mL) were obtained by the serial dilution technique.

Cytotoxicity Test (Bioassay); Brine shrimp lethality bioassay was carried out using brine shrimp larvae (*Artemiasalina*)

to determine the cytotoxicity of the plant extracts. To each sample vial corresponding to 1000,500,250,125, and 100 μg/mL. 4.0 mL of artificial seawater was added and 10 brine shrimps were introduced into the tubes using pipette, and the final volume in each vial was adjusted with artificial seawater to make a total volume of 5mL. The test tubes were left uncovered in the light, the nauplii were counted against a lighted background using magnifying hand lens and the number of the surviving shrimps were counted and recorded after 6, 12 and 24hours. Control test was also carried out using artificial seawater only. Nauplii were considered dead if they were lying immobile at the bottom of the vial.

Lethal Concentration (LC_{50}) Determination; The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC_{50}) was determined from the 24 h counts and the dose-response data were transformed into a straight line by means of a trendline fit linear regression analysis ; the LC_{50} was derived from the best-fit line obtained. LC_{50} values were obtained from the best-fit line, plotted on concentration against Percentage mortality, [25].

Thin Layer Chromatography (TLC)

TLC was carried out on all the fractions using a solvent system of 9:1 v/v chloroform in methanol.The fractions that will be obtained were combined and allowed to dry. The dried sample was then be dissolved in ethyl acetate. This was carefully washed. The coloured component was purified, spotted on the TLC plate and then labeled.

Nuclear Magnetic Resonance (NMR) Analysis

Fraction(s) of the VLC, and fraction (s) of the sephadex column was sent for NMR analyses. The NMR spectra were run at SIPBS, University of Strathclyde, Glasgow, UK. On a JEOL-LA-400 MHz FT-NMR spectrophotometer. The procedures for proton NMR are for five minutes using electronics programme solfware respectively

RESULTS AND DISCUSSION

Table 1: Qualitative phytochemical analysis of the ethanol extracts of four Nigerian medicinal plants

Plant extract	Alk	EO	Ph	Gly	Sap	Flav	Tan	Terp	proteins
Heamatostaphisbarteri	+	+	+	+	_	+	+	+	+
Cyanoglossum officinal'	+	+	+	_	_	+	_	+	+
Ximenia Americana	+	+	+	+	+	+	+	+	_
Anchus officinalis	-	+	_	_	_	+	+	+	_

Keys:+=present- =below detection levels

Table 2: Brine Shrimp Study of ethanol extract of three plants

Extracts	conc. ppm	No. nauphi	6hr	12hr	24hr	mean	% dead	
Heamatostaphis barteri	10	18	2	1	3	2	67	
	100	19	3	4	6	4	21	
	200	20	5	6	7	6	30	23.31
	500	20	3	8	9	7	35	
	1000	20	2	7	8	6	30	
Cyanoglossum officinal'	10	18	0	1	3	2	11	
	100	18	0	3	4	2	11	
	200	20	2	5	7	5	25	50.32.
	500	19	3	7	8	6	32	
	1000	20	5	9	10	8	40	
Ximenia Americana	10	20	3	4	5	4	20	
	100	19	7	10	15	11	58	
	200	20	10	13	14	12	60	86.57
	500	16	9	12	15	12	75	
	1000	19	12	16	17	15	79	
Anchus officinalis	10	19	0	2	3	2	11	
	100	16	1	3	5	3	19	
	200	18	2	4	6	4	22	54.37.
	500	20	5	7	8	7	35	

Table 3: DPPH Radical Scavenging Activity of three Medicinal Plant Extract

Conc (µl)	*Heamatostaphis barteri* (%)	*Cyanoglossum officinal*'(%)	*Ximenia americana* (%)	*Anchus officinalis*(%)	Ascorbic Acid (%)
5	91.47 ± 1.51[ab]	82.32 ± 3.33 [a]	76.31 ± 14.44[a]	29.31 ± 0.77	80.43 ± 5.06
10	57.96 ± 3.51[a]	67.56 ± 1.68[a]	84.49 ± 5.77 [ab]	21.13 ± 0.58	48.09 ± 5.74
15	74.19 ± 6.29[ab]	43.68 ± 1.55[a]	64.89 ± 4.66[a]	19.68 ± 0.16	18.22 ± 10.22
20	72.04 ± 2.05[a]	31.15 ± 20.72[a]	70.30 ± 4.48[a]	11.31 ± 0.56	13.26 ± 1.79
25	46.46 ± 11.18[a]	23.12 ± 5.48[a]	63.06 ± 0.82[a]	9.51 ± 0.89	6.25 ± 1.22
IC_{50}	**0.44**	0.50	**0.45**	1.42	0.60

[a]Significantly ($p < 0.05$) higher compared to Ascorbic acid at the same concentration
[b]Significantly ($p < 0.05$) higher compared to other extracts at the same concentration

Table 4: Hydrogen Peroxide Radical Scavenging Activity of three Medicinal Plant Extract

Conc (µl)	*Heamatostaphis barteri* (%)	*Cyanoglossum officinal*'(%)	*Ximenia Americana* (%)	*Anchus officinalis*(%)	Ascorbic Acid (%)
5	90.07 ± 8.44[a]	93.07 ± 4.10[ab]	87.00 ± 7.63[a]	37.02 ± 10.70	80.43 ± 5.06
10	80.95 ± 2.27[a]	81.52 ± 8.18[a]	88.89 ± 4.51[ab]	20.65 ± 2.68	48.09 ± 5.74
15	66.90 ± 2.15[a]	46.06 ± 1.72[a]	68.34 ± 4.63[a]	19.60 ± 0.75	18.22 ± 10.22
20	56.38 ± 1.40[a]	45.08 ± 14.90[a]	71.49 ± 0.55[a]	12.02 ± 3.26	13.26 ± 1.79
25	39.71 ± 7.38[a]	24.25 ± 0.89[a]	66.89 ± 1.07[ab]	11.20 ± 5.46[a]	6.25 ± 1.22
IC_{50}	**0.42**	0.44	**0.41**	1.22	0.60

[a]Significantly ($p < 0.05$) higher compared to Ascorbic acid at the same concentration
[b]Significantly ($p < 0.05$) higher compared to other extracts at the same concentration

The ^{1}H and ^{13}C chemical shifts data (**Tables 4**) of the white solid obtained from root of *Heamatostaphis barteri* were in agreement with literature data reported by [26], and [27]. Similarly, melting point (237-240 ^{o}C) of the isolated compound was in agreement with that (238-240 ^{o}C) reported by [6]. Based on these experimental analyses and comparison with literature reports, the white solid was identified as bergenin. Bergenin is a dihydroisocoumarin derivative of glucopyranosyl gallic acid [5]. Bergenin has been isolated from several plant species including; *Ardisia crenata, Rodgersiasam bucifolia*, *Bergenia ligulata*, *Ficus glomerata*, *Peltophorum africanum* and *Peltophorum pterocarpum* etc. It has been reported that bergenin has several pharmacological properties such as antiplasmodial, antimicrobial, antiviral and antifungal; as well as exhibiting antioxidant activity [9].

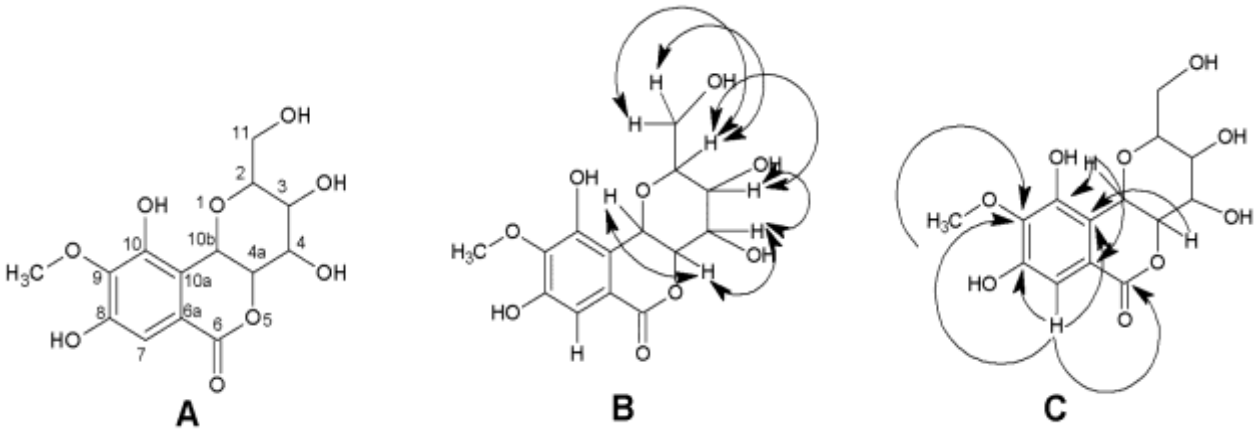

Fig. 1: A;IUPAC Numbering of Bergenin [28]; B;Important COSY and C; HMBC-Correlations of Bergenin 2016. Results of the phytochemical screening showed the presence of saponins, tannins, steroids/sterols, tannins, terpenoids, flavonoids, reducing Sugars, cardiac glycosides and proteins,[28].

Table 3; showed the results of DPPH free radical scavenging activities of each plant extract (% inhibition) and the IC_{50} µg/mL. It showed that *Heamatostaphis barteri*exhibited significant ($p>0.05$) % inhibition (91.41 ± 1.51^{ab} as compared with the standard drug ascorbic acid with the $IC_{50}$of 0.44, µg/mL.*Anchus officinalis*showed that there have lower $IC_{50,}$ 0.50 µg/mL as compared with standard drug ascorbic acid, *Ximenia americana* 0.45µg/mL with the % inhibition (84.49 ± 5.77^{ab}) are significantly different compared to the standard drug ascorbic acid with the IC_{50} of 0.66 µg/mL and *Cyanoglossum officinal* was non-active in the antioxidant analysed compared to the standard drug.

Table 4; showed the hydrogen perioxide free radical scavenging activity. The results showed that*Heamatostaphisbarteri* had lower IC_{50} of 0.42µg/mL compared to the standard drug. *Anchus officinalis*showed lower IC_{50} 0.44µg/mL with % inhibition of (93.07 ± 4.10ab) compared to the standard drug. *Ximenia americana* showed lower IC_{50} of 0.44µg/mL with % inhibition (88.89 ± 4.51^{ab} and 66.89 ± 1.07^{ab}) than the control, ascorbic acid and *Cyanoglossum officinal*1.22µg/mL showed non-significance ($p>0.05$) difference with the IC_{50} as compared with the standard drug ascorbic acid.

Table 2; Showed lethality of a test sample in zoological organisms such as the brine shrimp (*Artemiasalina*). The results showed that *Cyanoglossum officinal*(LC_{50}54.37), *Anchus officinalis*(LC_{50} 50.32), ***Ximenia Americana*** (LC_{50} 86.57),

Heamatostaphis barteri (LC_{50} 23.318) all have lethality values < 1000, showing how non toxic the plants / alternative medicinal therapies are.

DISCUSSION

Phytochemical screenings of the plants vary from one place to another, which may be due to geographical variation, climate conditions and soil composition of the area. Thus, it is possible to have different chemical composition of the same plant under research in other areas. The results of phytochemical screening indicated that the plants extracts contain alkaloid, flavonoids phenols, terpenoids alkaloids and other phytochemicals tested. The antimicrobial activity of the extracts might be attributed to the presence of the foresaid secondary metabolites in the extracts. The good zone of inhibition shown by some ethanol extract in **Table 3,** of the plants might be due to an important part of natural products from plants, biomolecules and secondary metabolites usually exhibits some kind of biological activities. They are widely used in the human therapy, veterinary, agriculture, scientific research and in countless other areas [7]. The usefulness of plant materials medicinally is due to the presence of bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds [4]. Alkaloids play some metabolic role and control development in living system. They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids. Tannins are known to inhibit pathogenic fungi. Flavonoids are known to inhibit the initiation, promotion and progression of tumors [4]. Flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenicetc, effects, [29]. Anthraquinones are considered to be one of the most active agents in metastatic breast cancer. The antimicrobial activity of the extracts could be explained by the presence of tannins. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting cell protein synthesis [7]. Plant phytochemicals such as phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers [28]. The Phytochemical profiles of the plants investigated, showed the presence of alkaloids, saponins, flavanoids, terpenoids, phenolics and reducing sugar for all the plants (**1**). The presence of phenolics and flavonoids demonstrates the plants as potential sources of antioxidants [30]. Antioxidant activities of ethanol extracted from *Heamatostaphis barteri and Ximenia americana* using various established ***in vitro*** systems. Data generated suggest that among the four plant samples (extraction with ethanol) showed significant inhibitory effects on superoxide radical and DPPH with IC_{50} values of **0.42,0.44** and **0.45, 0.41**µg/mL, its reducing power was also the strongest among the four samples. These ***in vitro*** results, establish the clear possibility that plants extracts have very active components and could be used as effective ingredient in health or functional food to reduce **oxidative stress**. The brine shrimp test results indicated that all the plant extracts tested had LC_{50} values lower than 1000 µg/mL which suggests that they are practically nontoxic. As traditional medicines, most of the extracts are prepared as decoctions, which, in a way are mirrored on the ethanol extracts, the results of which suggest that the way they are used poses no threat of acute toxicity as opined by this study and the work of Meyer *et al.,* 1982. All of the extracts, including *Heamatostaphisbarteri* (LC_{50} 23.31µg/mL), and *Ximenia americana* (LC_{50}86.57µg/mL) are mildly toxic and probably have no obvious danger of outright toxicity during acute exposure. However extracts from these plants used as traditional medicines are unlikely to have any ill effects on patients as they are not on the highly toxic category. Some brine shrimp results that are already available [31] provide a circumstantial evidence that plant extracts with LC_{50} values below 1000 µg/ml have a

likelihood of yielding anticancer compounds. This corroboration is demonstrated by *Brideliacathartica* [32], *Croton macrostachys* [32]. In a 2004 study using brine shrimps, *Phyllanthusengleri*gave an LC50 of 0.47 µg/ml [32], and recently the plant yielded englerin A, a selective anticancer compound against kidney cancer cells [9], which provided further corroborative evidence on the potential of brine shrimp test to predict the presence of anticancer compounds in plant extracts. A white amorphous solid was obtained from the VLC of ethyl acetate crude extract of *Heamatostaphis barteri,* TLC of the solid gave a single dark spot (R_f = 23 i.e. 0.23 x100) when the plate was sprayed with 10 % sulphuric acid in methanol and heated at 120 °C. This is suitable for detection of most polar compounds. The solid melted at a temperature of 237–240 °C. A blue black colouration obtained from $FeCl_3$ test suggested that the white solid could be galloyl tannin [33]. Proton NMR spectrum (Table 4) showed two phenolic- OH downfield signals at δ_H (9.75, 8.47) as singlets; three hydroxyl signals at δ_H(5.65, 5.45, 3.86), all singlets, which suggested presence of an aromatic and aliphatic groups in the compound. Seven oxymethine signals (at δ_H3.85, 3.65, 3.44, 3.21, 4.99, 3.57, 4.02) (d, dd and ddd) were also observed from the spectrum which corresponded to signals due to H-11, H-4, H-11, H-3, H-10b, H-2 and H-4a, respectively. These signals are similar to those reported by [27] for bergenin. The positions of these protons were confirmed by analysis of ^{1}H-^{1}H COSY and HSQC spectra. In addition, two singlet signals at δ_H3.78 and δ_H6.99 were assigned to a methoxy group (-OCH_3) and an aromatic proton (H-7), respectively, in accordance to [34]. ^{13}C-NMR spectrum (Table 4.11) showed a methoxy and a lactone carbon signals at δ_C60.3 and δ_C164.3 (C-6), respectively. The remaining twelve signals were characteristic of carbons from a glucosyl unit and an aromatic nucleus [34]. These data were comparable to those published for bergenin by [27] and [26]. HSQC spectrum showed the following correlations between C and H atoms: δ_{CH}; 3.57, 82.3 (CH aliphatic, C-2); 3.21, 71.2 (CH aliphatic, C-3); 3.65, 74.2 (CH, aliphatic, C-4); 4.02, 80.3 (CH aliphatic, C-4a); 6.99, 110.2 (CH aromatic, C-7); 4.98, 72.6 (CH aliphatic, C-10b); 3.85 and 3.44, 61.7 (CH_2 aliphatic, C-11) and 3.78, 60.3 (-OCH_3). The HSQC correlations confirmed that only one proton (δ_H 6.99, H-7) was attached to the aromatic carbon (δ_C110.2, C-7). HSQC also revealed correlations typical of an aliphatic nucleus. A correlation typical of anomeric proton (δ_H4.98, H-10b) and carbon (δ_C72.6, C-10b) was also observed. This further suggested the presence of an aromatic and sugar nucleus [35]. It was evident from the HMBC spectrum that the aromatic proton signal at δ_H6.99 (H-7) correlated to a carbonyl carbon at δ_C 164.3 (C-6) and two oxygenic aromatic carbons at δ_C; 141.3 (C-9) and 151.9 (C-8). The aromatic proton (H-7) also correlated to a tertiary aromatic carbon at δ_C116.6 (C-10a). Therefore, a carbonyl group could be attached at C-6a that is ortho to the carbon bearing the only aromatic proton. Furthermore, the methoxy proton signal at δ_H 3.78 correlated to an aromatic carbon at δ_C141.3 (C-9), thus implying that, the methoxy group could be attached on the aromatic ring at C-9 which was meta to C-7 but para to C-6a- bearing the benzoyl carbon (C-6, δ_C 118.6). It was also noted from HMBC spectrum that H-10b, δ_H4.99 (anomeric proton) correlated to one oxygenic aromatic carbon at δ_C148.6 (C-10) and two other aromatic carbons at δ_C116.6 (C-10a) and δ_C118.6 (C-6a) [34]. The later (C-6a) was observed to correlate to H-4a (δ_C4.02) which suggested that the sugar unit was C-C joined at C-10a of the aromatic ring via anomeric carbon (δ_C 72.6, C-10b) [34]. The downfield signal of H-4a (δ_H4.02) appeared to be higher than those of other sugar protons except the anomeric proton (δ_H 4.99). This suggested that C-4a (δ_C 80.3) could be linked to C-6 (carbonyl carbon- electron withdrawing) through ethereal oxygen in a cyclic ester (lactone) formation.In conclusion all of the extracts of the four plants tested are none toxic. Ethanol extracts testedhave LC_{50} values that suggest a remote

possibility that they may yield cancer cell line active compounds. Thus, most of the extracts of the plants tested are likely to be innocuous on short term use.

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