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Evaluation of Phytochemical Screening and Anti-Tumor Activities of Some Selected Medicinal Plants in Nigeria

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ABSTRACT

Cancer is a high mortality disease of public concern. Effectiveness of chemotherapy is often limited by toxicity to untargeted tissue 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 of ten selected medicinal herbal remedies used by the population in Lala District, Gombi LGA, phytochemical analysis, Antimicrobial and antioxidant activities of the ten medicinal plants were carried out as preliminary studies. The selected herbals' extract revealed the presence of alkaloid, essential oil, phenol, glycosides, flavonoid, tanin, terpenoid, proteins and saponins. The medicinal plants demonstrated good antioxidant activity with IC50 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 scarving activity model used in this study.The brine shrimp toxicity results indicated that 50% of the plant extracts tested had LC50 values lower than 1000 μg/ml; Heamatostaphisbarteri (LC50 23.31μg/ml)is mildly toxic and probably have no obvious danger of outright toxicity during acute exposure. It can be concluded that any two of the for medicinal plants showed can be selected for further studies as they can be demonstrated to have good phytochemical consitituent, antioxidant activities and mild toxicity on brine shrimp.

Keywords: pyhtochemistry, anti-trmor, medicinal plants, chemotherapy

INTRODUCTION

Nature has provided mankind with a complete store house of remedies to cure all ailments of mankind [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 practise 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 [8]. 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 [9]. Most Nigerians, especially those living in rural orthodox medicine and it is estimated that about 80% of the populace still prefer to solve their health problems consulting traditional healers [10]. 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 [11]. Traditional medicine is the oldest, most tried and tested form of medicine and is as old as man himself [12]. 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) [13]. 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 [14]. The small fractions of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure 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 of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, work in both mixture of traditional medicine and single active compounds are very important [15]. 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 [9]. This has created immense clinical problem in the treatment of infectious diseases [11].

The use of medicinal plants in traditional medicine has been recognized and widely practiced. According to the World Health Organization (2010) [13], 80% of the w populations rely on traditional medicines to meet their health regiments. Plant used for traditional medicine contains a wide range of substances that can be used to treat chronic and acute infectious disease. Despite the claimed ethno medicinal uses of the plants in the treatment of tuberculoises, 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 drug and also to establish scientific evidence of its traditional use as anti-tumor agent. Cancer is one among the dangerious diseases leading to so many death in the worldwide which is without cure accounting for an estimated 9.6 million deaths in 2018 (www.who.Int/news-room/fact retrieved 22nd september, 2020). Medicinal plants have beem used traditionally for treatment of cancer, but with unspecified dosage. This could cause another death threat of the plants which have been used traditionally for 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 the treatment of cancer. The aim is to phytochemically screen, evaluate it antitomur and characterization of the active principles.With the following objectives to carry out phytochemical evaluation of crude ethanol extracts of the medicinal plant, to carry out antibacterial activities, antioxidant of the medicinal plant and antitumor activitiesand to determine the IC_{50} and LC_{50} of the ethanolic crude extracts.

MATERIALS AND METHODS

Sample Collection and Authentication

Matured plants were collected from Lala District in Gombi Local Government Area, Adamawa State in the month of July 2018.

Sample Preparation

The roots **of both *Haematostaphisbarteri hook* and *XimeniaAmnericana Linn***were thoroughly washed with tap water to remove dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were allowed to dry under shade. The dried leaves were pulverized by using mortar and pestle. Finally, fine powder was obtained from the powdered leaves by sieving through the kitchen strainer and used for extraction.

Ethanolic Extraction

The powdered leaves (100 g) was weighed and soaked in 350 mL of methanol in a conical flask. The flask containing the leaves were shaken, corked and left to stand for 48 h at room temperature. After 48 h, the mixture was filtered and the extract were collected and concentrated by evaporation to dryness in evaporating dish.

Antioxidant Potential of the Plants Extract

The antioxidant activity of Ethanol and aqeuous 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 will be employed to assess the antioxidant potential. All the assays will be carried out in triplicate.

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 methods of [12]. 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 methods of [12]. The plant parts (Roots and Stem bark) extracts of the different solvents was treated with different concentrations ranging from 100 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 [6].

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

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 [8] the method was modified. A solution of hydrogen peroxide (2mM) 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' was 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 sample 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 triplicate. The percentage of hydrogen peroxide scavenged by the extracts was 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 and A_s the absorbance in the presence of the sample of extract and standard [8,9]. The values of % inhibition were obtained from the above equation. The 50% inhibitory concentration (IC_{50}) of the extracts was obtained from the graphs of the concentration of the samples (Aqueous and Ethanol extracts) versus % inhibition (% H_2O_2 reduction)

Brine Shrimp Lethality Assay

Brine shrimp eggs were commercially available. For this experiment, brine shrimp egg 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 5^0C.

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 (www.idpublications.org retrieved 2nd may, 2021).

Hatching of Brine Shrimp

An artificial seawater was Prepared at full strength. To obtain an optimum result a solution of specific gravity of 1.022 at 24^0C was prepared by dissolving 35g sea salt sodium chloride NaCl pre- 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 (5000 nauplii) and are hatched in approximately 250ml

sea water. The hatcher is illuminated very well for a minimum of three hours preferably for 12hours. The hatching time depend on temperature at 240C (which is average tropical aquarium temperature) hatching take place between 24-48 hours (maximum hatch 44-48hours). The Nauplii is then used directly for the cytotoxicity test (www.idpublications.orgretrieved 2nd may, 2021).

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 100μg/ml, 4ml 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 of concentration against Percentage mortality.

Table 1: Qualitative analysis of three medicinal plants of ethanolic extracts

Plant extract	Alk	EO	Ph	Gly	Sap	Flav	Tan	Terp	Proteins
Heamatostaphisbarteri	+	+	+	+	_	+	+	+	+
Avena sativa	+	+	+	_	_	+	_	+	+
Solonumdulcamara	-	+	_	_	_	+	+	+	_

Keys:
+=present
-=absent

Table 2: BrimeShrim Study of ethanolic extract of three plants

Extracts	conc. ppm	No. nauphi	6hr	12hr	24hr	Mean	% dead	
Heamatostaphisbarteri	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	
Avena sativa	10	18	0	1	3	2	11	
	100	18	0	3	4	2	11	
	200	20	2	5	7	5	25	5032.69
	500	19	3	7	8	6	32	
	1000	20	5	9	10	8	40	
Solonumdulcamara	10	19	0	2	3	2	11	
	100	16	1	3	5	3	19	
	200	18	2	4	6	4	22	5437.51
	500	20	5	7	8	7	35	
	1000	19	6	5	9	7	37	

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

Conc (µl)	*Heamatostaphisbarteri* (%)	*Avena sativa* (%)	*Solonumdulcamara* (%)	Ascorbic Acid (%)
5	91.47 ± 1.51[ab]	82.32 ± 3.33 [a]	29.31 ± 0.77	80.43 ± 5.06
10	57.96 ± 3.51[a]	67.56 ± 1.68[a]	21.13 ± 0.58	48.09 ± 5.74
15	74.19 ± 6.29[ab]	43.68 ± 1.55[a]	19.68 ± 0.16	18.22 ± 10.22
20	72.04 ± 2.05[a]	31.15 ± 20.72[a]	11.31 ± 0.56	13.26 ± 1.79
25	46.46 ± 11.18[a]	23.12 ± 5.48[a]	9.51 ± 0.89	6.25 ± 1.22
IC_{50}	**0.44**	0.50	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 1 showed that alkaloids, essential oils, phenols, glycosides, flavonoids, tanins, terpenoids and proteins were present but saponin absent. *Solonum dulcamara* also showed that essential oils, flavonoids, tannins, terpenoids, were present while alkaloids, phenols, glycosides, saponins and proteins, were absent. **Table 2** 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.*Avena sativa* showed that there have lower IC_{50}0.50 µg/mL as compared with standard drug ascorbic acid, and *Solonum dulcamara solanaceae* shows non-active in the antioxidant analysed compared to the standard drug.

Table 3 showed the hydrogen perioxide free radical scavenging activity. The results showed that*Heamatostaphisbarteri* showed lower IC_{50} of 0.42µg/mL compared to the standard drug. *Avena sativa* showed lower IC_{50} 0.44µg/mL with % inhibition of (93.07±4.10ab) compared to the standard drug. And *Solonum dulcamara* 1.22µg/mL showed non-significance (p>0.05) difference with the IC_{50} as compared with the standard drug ascorbic acid.

DISCUSSION

Phytochemical screening of plants varies 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 indicate that the plant extracts contains alkaloid and flavonoids. 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 ethanolic extract in **Table 3.** Of the plants might be due to an important part of natural products from plants, biomolecules and secondary metabolites usually exhibit some kind of biological activities. They are widely used in the

human therapy, veterinary, agriculture, scientific research and in countless other areas [8]. The usefulness of plant materials medicinally is due to the presence of bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds [7]. 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 tumours [8]. The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenicetc [6]. 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 [8]. Plant phytochemicals such as phenolics are a major group of compounds acting as primary antioxidants or **free radical** scavengers [4]. The Phytochemical profiles of the plants investigated, showed the presence of alkaloids, saponins, flavanoids, terpenoids, phenolics and reducing sugar for all the plants [5]. The presence of phenolics and flavonoids demonstrates the plants as potential sources of antioxidants [9]. Antioxidant activities of ethanolic extracted from *Heamatostaphisbarteri and Ximenia Americana* using various established ***in vitro*** systems. Data generated suggest that among the ten 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 three samples. These ***in vitro*** results, establish the clear possibility that plants extracted from *ten different plant in which two (Heamatostaphisbarteri and Ximenia Americana) are most active* could be used as effective ingredient in health or functional food to reduce **oxidative stress**. The brine shrimp test results indicate that 50% of the plant extracts tested had LC_{50} values lower than 1000 µg/ml which suggests that they are practically non-toxic. 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 this study agreed with [7]. Some of the extracts, including *Heamatostaphisbarteri* (LC_{50} 23.31µg/ml), mildly toxic and probably have no obvious danger of outright toxicity during acute exposure. However extracts from these two 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 [8] 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* [6], *Croton macrostachys* [4]. In the 2004 study using brine shrimps, *Phyllanthusengleri*gave an LC50 of 0.47 µg/ml [11], and recently the plant yielded englerin A, a selective anti-cancer compound against kidney cancer cells [13], which provides further corroborative evidence on the potential ofthe brine shrimp test to predict the presence of anti-cancer compounds in plant extracts. It istherefore possible that in this study, dichloromethane extracts of *Heamatostaphisbarteri* (LC_{50} 23.31µg/ml) and *Ximenia Americana* (LC_{50}86.57µg/ml) may have potential to yield compounds active against cancer cell lines and probably not too farfetched to speculate of their possibility to yield cancer cell line active compounds. In conclusion most of the extracts of the plants tested seem to be innocuous on short term use. Dichloromethane extracts of

Heamatostaphisbarteri among the tested extracts have LC_{50} values that suggest a remote possibility that they may yield cancer cell line active compounds. In conclusion most of the extracts of the plants tested seem to be innocuous on short term use. Ethanolic extracts of *Heamatostaphisbarteri* among the tested extracts have LC_{50} values that suggest a remote possibility that they may yield cancer cell line active compounds.

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