Bima Journal of Science and Technology, Vol. 5(2) Dec, 2021 ISSN: 2536-6041

PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF *Xeromphis nilotica* STEM-BARK EXTRACT

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ABSTRACT

Free radicals are reactive molecules involved in several physiological processes and have been associated with many ailments such as cancer, arthritis and liver injury that affect a great number of people around the globe. Therefore, there is a need to explore indigenous plant with free radical scavenging activity or antioxidant potentials. Phytochemical analysis as well as estimation of the total phenolic and flavonoid contents of *Xeromphis nilotica* stem-bark was done using established protocols. Antioxidant and free radical scavenging activity of the extract was assessed by using various *in-vitro* models. 1,1-Diphenyl-2-picrylhydroxyl (DPPH) quenching assay, hydrogen peroxide scavenging test, total antioxidant capacity using phosphomolybdenum assay were employed and ascorbic acid was used as standard. Alkaloids, carbohydrates, flavonoids, phenolic acids, saponin, steroids, terpenoids and tannins were tested positive however, anthraquinone was not detected. A significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The extract was also shown to have high total phenolic and total flavonoid contents of 116.32mg GAE/ml and 56.79mg QE/ml respectively. These results clearly indicate that *Xeromphis nilotica* could be a potential source of natural antioxidant and may possibly be effective against free radical mediated diseases.

Keywords: *Xeromphis Nilotica*., Antioxidant activity, Flavonoids, Free radicals, Phytochemicals, Total Phenolics

INTRODUCTION

There is strong evidence that many dangerous pathophysiological processes, such as cancer, diabetes, cardiovascular and neurodegenerative diseases are associated with the accumulation of free radicals (Islam et al.,2013). A free radical is an atom or molecule that has an unpaired electron and is therefore unstable. This unstable radical has the tendency to become stable through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells, thus causing tissue damage (Gilgun-Sherki et al., 2002). All biological systems have intrinsic antioxidant defence mechanisms that remove damaged molecules, but these mechanisms can be inefficient. Therefore, dietary intake of antioxidants is imperative to protect cells from damage caused by free radicals.

Oxidation is a chemical reaction that involves transfers of electron, the substance that gives away the electron is subsequently oxidized, which results in production of free radicals that start chain reactions with subsequent cellular damage. These species may be either oxygen derived (ROS) or nitrogen derived (RNS). The most common reactive oxygen species include superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH) (Partap et al., 2014). Free radicals are natural

by-products of our own metabolism with very short half-life, high reactivity and damaging activity towards macromolecules like proteins, DNA, lipids as well as cell membrane and mitochondria. The adverse effects of free radicals can cause lipid peroxidation and decrease membrane fluidity, gene mutation leading to cancer, cardiovascular disease, liver damage (Vasudevan., et al., 2016).

Antioxidants are substances that prevent and stabilize the damage caused by free radicals by supplying electrons to the affected macromolecules. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body. Consumption of antioxidant enriched fruits and vegetables is known to lower the risk of several diseases caused by free radicals (Hamid et al., 2019). Such health benefits are mainly due to the presence of phytochemicals such as polyphenols, carotenoids, vitamins (Rahman et al., 2015). Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health (Gurib-Fakim., 2006). For example, phenolic and polyphenolic such as flavonoids, flavones and other phenolics could be responsible for different bioactivity such as anti-inflammatory, anti-carcinogenic, antimicrobial and anti-oxidative activities of plant extracts (Diouf et al., 2009). The use of herbal remedies as alternative medicine plays a significant role in the cultures and beliefs of the indigenous populations of Nigeria (Mohammed et al., 2014). Therefore, it is pertinent to assess the antioxidant activity of plants used in ethno-medicinal practice or either to elucidate the mechanism of their pharmacological activity.

Xeromphis nilotica is a lowland shrub that grows wild in savannah regions of Africa and Asia (Farooqui et al., 2013). The vernacular names for *Xeromphis nilotica* are *gial-gotel*, *kwanarya* in northern Nigeria. *Xeromphis nilotica* is expected to have antioxidant potentials as the stem bark concoction is used by the local communities in the treatment and management of pain related ailment and also is reputed to be of medicinal value in folkloric treatment and management of various disease (Adzu et al.,2014). Ethnobotanical and ethno-pharmacological studies of *Xeromphis nilotica* indicate the potential use of these plants for the treatment of a large variety of diseases. But, there is dearth of scientific information on the efficacy and mechanism of action of these remedies. Hence there is a need for more precise validation of ethno-pharmacological claims, and this study is an attempt to experiment the antioxidant properties of the *X. nilotica* stem-bark.

MATERIALS AND METHODS

Plant material and Extract Preparation

Fresh *Xeromphis Nilotica* stem-bark was collected from Kumo Gombe State Nigeria in the month of January 2017. It was authenticated by taxonomist, specimen voucher (BUM 326) was deposited in herbarium at the Department of Biochemistry, University of Maiduguri. Nigeria.

Xeromphis Nilotica stem-bark was washed and allowed to dry under shade pulverize to fine powder using mortar and pestle. Hundred (100g) of the powdered plant material was macerated in 70% ethanol for 72h with intermittent shaking. The mixture was filtered twice through Whatman filter paper. The resulting filtrate was subjected to evaporation in a Rotary Evaporator for 10 min at 60°C. Dried extract was packed in air tight container and reconstituted when required.

Phytochemical Analysis of Extract

The extract was subjected to various qualitative screenings for the identification of phytochemical constituents. The crude extract was screened for the presence of primary and secondary metabolites such as alkaloids,

steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones using standard procedures previously described by Trease and Evans (1997).

Total phenol content

The total phenol content was determined by Folin-ciocalteau reagent method, 0.5ml of extract (1:5 dilution) and 0.1ml of Folin-Ciocalteau reagent (0.5N) were mixed and incubated at room temperature for 15min. 2.5 ml saturated sodium carbonate was added, incubated for 30min at room temperature and absorbance was measured at 760nm. The total phenol content was expressed in terms of Gallic acid equivalent (mg/g) (Ainworth and Gillespie., 2007).

Total Flavonoid

A solution of each extract was prepared by sonicating 3mg in 10ml methanol for 10min. To 300µl in a test tube, 3.4ml of 30% methanol was added to obtain a clear solution. Then, 150µl of 0.5M sodium nitrite solution was added, followed by 150µl 0.3M aluminium chloride solution. After 5min. 1ml of 1M sodium hydroxide was added, content was mixed thoroughly absorbance was measured at 506nm on a UV visible spectrophotometer against a blank. A calibration curve of Quercetin was obtained. The total flavonoid content of each extract was expressed as µg of Quercetin equivalents per ml (Chandra et al.,2014).

***In vitro* Antioxidant Activity Tests**

DPPH radical method

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1-diphenyl-2- picrylhydrazyl as previously described by (Hossain et al., 2015)

The reaction mixture consists of 1.0 ml of DPPH in methanol (0.3 mM) and 1.0 ml of the extract. After incubation for 10 min in dark, the absorbance was measured at 517 nm. DPPH scavenging activity was expressed in terms ascorbic acid equivalent

% inhibition of DPPH radical =[{Ao – A1/Ao}] ×100.

Where:

Ao is the absorbance before reaction,

A1 is the absorbance after reaction has taken place.

Quercetin equivalents per ml

Hydrogen Peroxide Radical Scavenging Activity Analysis

Hydrogen peroxide rapidly decomposed into oxygen and water with hydroxyl radicals that can initiate lipid peroxidation and subsequent DNA damage. Hydroxyl radical scavenging activity was determined by the method of (Ruch et al., 1989) A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of *X. Nilotica* (20-60 mg/ml) in phosphate buffer were added to a H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing phosphate buffer without H_2O_2 was used

The percentage H_2O_2 scavenging of *X. Nilotica* and Ascorbic acid as standard compound was calculated.

% H_2O_2 scavenged = [{Ao – A1/Ao}] ×100.

Where:

Ao is the absorbance of control

A1 is the absorbance of test.

Total antioxidant Capacity (TAC) Assay using Phosphomolybdenum

The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyze and subsequent formation of a green phosphate Mo (V) complex at acidic pH. An aliquot (0.1) ml of extract in eppendoff tube was incubated with 1 ml of reaction mixture (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in boiling water bath

at 95°C for 90min. After the sample had cooled to room temperature, the absorbance of the aqueous solution is measured at 695nm against blank in UV spectrophotometer. (Kasangana et al.,2009). A typical blank solution contained 1ml of reagent solution and the appropriate volume of the same solvent used for the test sample. Total antioxidant capacity was expressed as Ascorbic acid/100mg dry weight of extract

where is $A_{control}$ is the absorbance of the control reaction and
A_{sample} is the absorbance of the tested extract samples.

Statistical Analysis

All the experiments for determination of total phenolics, total flavonoids, and antioxidant properties using DPPH, hydrogen peroxide, phosphomolybdum and TBA were conducted in triplicates. The values are expressed as the mean ± standard deviation (SD). The statistical analysis of the results was done by using SPSS. Analysis of variance and significance of difference among means were tested by one-way ANOVA and values ($p<0.05$) are considered significant.

RESULTS

The preliminary phytochemical analysis of *Xeromphis nilotica* stem-bark (Table 1) revealed the presence of alkaloids, carbohydrates, flavonoids, phenolic acids, saponin, steroids, terpenoids and tannins, however, anthraquinone was not detected.

Table 1 showing results of preliminary phytochemical assay; Various analytical assays for phytocomponents were performed. Carbohydrate, Alkaloids, Saponins, Terpenoids, Steroids, Phenolic acid, Flavonoids and Tannins were tested positive. However, Anthraquinone glycoside was not detected and Fehlings test for reducing sugars tested negative.

S/no	Phytochemicals	Specific tests	Results
1.	Carbohydrates	Molisch's test	Positive
		Fehling's test for reducing sugar	Negative
2.	Alkaloids	Mayer's reagent	Positive
		Dragendroff's Test	Positive
		Wagner's test	Positive
3.	Saponin	Frothing Test	Positive
		Haemolysis test	Positive
4.	Terpenoids	Salkwoski's test	Positive
5.	Steroids	Salkwoski's test	Positive
6	Anthraquinone	Bontrager's test	Negative
7.	glycosides Phenolic acid	Ferric chloride test	Positive
8.	Flavonoids	Shinoda's test	Positive
		Ferric chloride test	Positive
9.	Tannins	Lead ethanoate test	Positive
		Ferric chloride test	Positive

***Xeromphis nilotica* Stem-Bark Shows High Total Phenolic and Total Flavonoid Contents**

Phenolic compounds are known as powerful chain breaking antioxidant and are very important plant constituents because of their radical scavenging ability, which is due to their hydroxyl groups (Adesegun et al.,2009). Total phenolic content was calculated with a standard calibration curve using a formulae $y=0.0005x + 0.1408$ ($R^2 = 0.986$). Gallic acid was obtained (for concentrations ranging from 75 mg/L to 750 mg/L) and the total phenolic

content of the extract was expressed as µg of Gallic acid equivalents per ml. The ethanol stem-bark extract of *X. nilotica*, was found to contain116.32 ug/g GAE of total phenolic content.

Total Flavonoids Content

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, anti-ulcer, anti-allergic, anti-viral and anti-cancer activities (Umamashewari et al., 2008). They are capable of effectively scavenging the reactive O_2 species because of their phenolic hydroxyl groups and so they are potent antioxidants (Mansuri et al., 2014). The total flavonoid content of the extract was expressed as µg of Quercetin equivalents (QE) per mL, and calculated using the formula, $y = 0.0008x + 0.0225$ ($R^2 = 0.9647$). In this study the ethanolic extracts of *Xeromphis nilotica* stem-bark was found to contain 56.79mg/g of flavonoids in terms of quercetin equivalent

In-vitro Antioxidant Activity of *Xeromphis Nilotica* Stem-Bark Extract

Ethanolic extract of *Xeromphis nilotica* stem-bark showed robust free radical scavenging activity in response to different invitro models employed, DPPH scavenging, H_2O scavenging and total antioxidant capacity by reducing Mo(IV) to Mo(V).

i) DPPH radical scavenging activity

In evaluating the free radical scavenging activity of crude extracts and single compounds, the agent of choice is often the DPPH radical (Ko et al., 2015). Here, the radical scavenging activities of the crude ethanolic extract of *X. nilotica* stem-bark was assessed by measuring the decrease in absorbance of DPPH in the presence or absence of the extract in the assay mixture, ascorbic acid solution was as standard. The percentage scavenging of DPPH radicals were recorded against the selected concentrations (62.5, 125, 250,500, 1000 µg/ml). (Table2) Using standard inhibition curve for DPPH scavenging activity of *X. nilotica* stem-bark extract and Ascorbic acid respectively. The extract showed potent scavenging activity against DPPH radical with IC_{50} values of 150 µg/ml. The percentage scavenging activity of the extract on the DPPH radical was concomitantly increased with increase in extract concentration.

Table 2: showing percentage scavenging activity of the DPPH radicals by *X. nilotica* stem-bark extract and ascorbic acid (standard control) at different concentrations. Data is SEM representation of three independent experiments. The IC_{50} of *X.nilotica* stem-bark extract was 150(µg/ml); and that of Ascorbic acid was 100(µg/ml

Conc (µg)	Percentage Scavenging Activity of DPPH	
	Ascorbic Acid	Plant Extract
62.25	45	40
125	52	51
250	63	60
500	78	70
1000	96	87
IC_{50} (µg/ml)	100	150

ii) Hydrogen peroxide scavenging activity

Hydrogen peroxide radical scavenging activity of *Xeromphis nilotica* stem-bark extract with ascorbic acid solution as standard is shown in (Table 3). The radical scavenging activity of the plant extract seems to be in a dose dependent manner. The inhibition data were recorded against the selected concentrations (62.25, 125,250, 500,1000 µg/ml). The IC_{50} values of hydrogen peroxide radical scavenging activities of *X.nilotica* extract and ascorbic acid were calculated using regression equation. IC_{50} value for standard (Ascorbic acid) and *X. nilotica* stem-bark extract were 250 µg/ml and 280 µg/ml respectively which represent the

antioxidant potential of the standard and extract samples.

Table 3: showing the percentage hydrogen peroxide radical scavenging activities of *X. nilotica* stem-bark extract and ascorbic acid (standard control).

Conc (µg)	Percentage Scavenging Activity of H_2O_2	
	Ascorbic Acid	Plant Extract
62.25	40	23
125	43	30
250	51	41
500	67	56
1000	95	82
IC_{50} (µg/ml)	250	280

The result demonstrates that radical scavenging activity is in dose dependent manner. Data is SEM representation of three independent experiments. IC_{50} value for standard ascorbic acid and *X. nilotica* stem-bark extract were 250 µg/ml and 280 µg/ml respectively.

Total Antioxidant Capacity (TAC) assay

The result of the total antioxidant capacity of the *X.nilotica* stem-bark extract using Phosphomolybdenum method was presented in (Figure 1). Reduction of Mo (VI) to Mo (V) by the plant extract and subsequent formation of a green phosphate Mo (V) complex at acidic pH. The total antioxidant capacity of the plant extract seems to be concentration dependent manner.

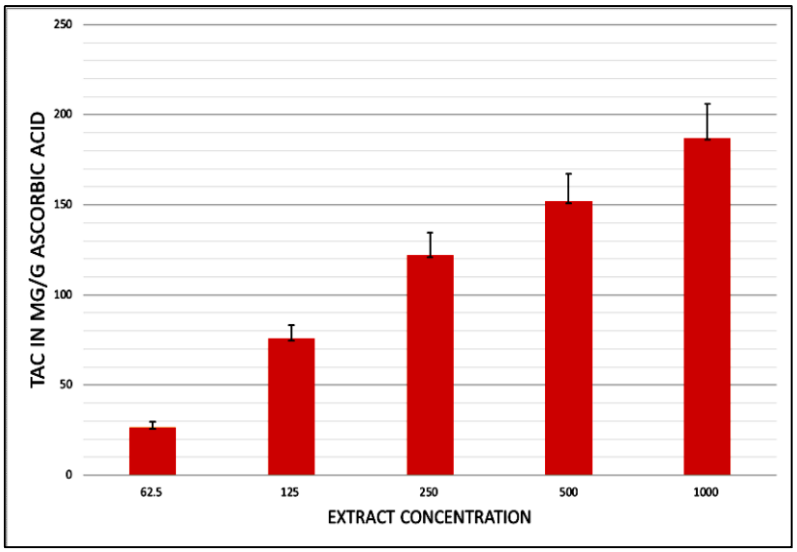

Figure 4: A histogram representing the concentration dependent total antioxidant capacity of *Xeromphis nilotica* stem-bark extract using Phosphomolybdenum method. Reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate Mo (V) complex at acidic pH. The results of the total antioxidant capacity show a concentration dependent activity.

DISCUSSIONS

Pytochemical Screening and Determination

Plants are important source of bioactive compounds for the development of new therapeutic agents. Phenolic compounds are widely distributed in the tissues of plants as well as play a vital role as an effective free radical scavengers and antioxidants (Molan et al., 2012). They are commonly found in both edible and in non-edible herbs, cereals, fruits, vegetables, oils, spices, and other plant materials (Miliauskas et al., 2004). Scientific information on the antioxidant properties of

endemic plants is scare because the availability of endemic plants is limited to certain regions and only known by local populations. Therefore, the assessment of such properties remains an interesting and useful task, particularly to find promising sources of natural antioxidants for functional foods and nutraceuticals.

The antioxidant activity of polyphenols is mainly due to their redox properties as hydrogen donor and singlet oxygen quenchers (Banerjee and Bonde., 2011). Typical phenolic compounds that possess antioxidant activity are known to be phenolic acids and flavonoids. These compounds possess diverse biological activities such as anti-inflammatory, anti-carcinogenic, antimicrobial and these activities might be related to antioxidant activity. This activity is due to their ability to adsorb, neutralize and to quench free radicals (Huyut et al., 2017).

Our preliminary phytochemical analysis for *Xeromphis nilotica* stem-bark (Table 1) tested positive for the presence of alkaloids, carbohydrates, flavonoids phenolic acids, saponin, steroids, tannins and terpenoids. Also *Xeromphis nilotica* extract shown to have high amount of total phenolics and flavonoids contents and these could be attributable to the radical scavenging activity of the extracts. Phenolics and flavonoids constitute a major group of compounds, which act as primary antioxidants (Bursal and Gulan., 2011) and are known to react with hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals (Karagoz et al., 2015). They are also known to protect DNA from oxidative damage, inhibit growth of tumor cells and possess anti-inflammatory and antimicrobial properties.

DPPH Radical Scavenging Activity

Free radical is a molecule with an unpaired electron and is involved in tissue damage, inflammation, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases (Braca et al., 2002). Our results demonstrated that the ethanolic extract of *Xeromphis nilotica* stem-bark possess free radical scavenging activity by using the following *in vitro* models; DPPH scavenging, H_2O_2 scavenging, phosphophomolybdum antioxidant capacity.

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of plant extracts and scavenging of the radical is related to inhibition of lipid peroxidation. (Tarachand et al., 2012). It accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Partap et al., 2014). Radical scavenging activities are very important in inhibiting the deleterious effects of free radicals in different diseases, including cancer. In the DPPH assay, violet colour DPPH solution is reduced to yellow coloured product, diphenylpicryl hydrazine, in response to extract addition. Our results reveal that ethanolic stem-bark extract *Xeromphis nilotica* displayed marked DPPH radical scavenging activities that mimics ascorbic acid a natural antioxidant used as standard (Table 2). It can be hypothesized the odd electron of the nitrogen atom in DPPH is reduced by receiving a hydrogen atom from the antioxidant. This effect could be attributed to the hydrogen donating ability of the phenolic compound present in the *X. nilotica extract* (Ashafa et al., 2010).

H_2O_2 Scavenging Activity

H_2O_2 is a weak oxidising agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, inside the cell, H_2O_2 can probably react with Fe2+, and /or Cu2+ ions to form hydroxyl radical and this might probably be the basis of its toxic effects (Zhang et al.,2020). In this experiment, scavenging of H_2O_2 radical by *X. nilotica* extract could be attributed to the phenolics

compounds present which might have donated electron to the radical, thus reducing it to water. Furthermore, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant capacity

Total Antioxidant Capacity: Using Phosphomolybdenum

A concentration dependent total antioxidant capacity of *X. nilotica* stem-bark extract was observed following the phosphomolybdenum method (Fig. 4). The result reveals that the plant *Xeromphis nilotica* reduced Mo(IV) to Mo(V) at acidic pH, and subsequent formation of a green phosphate-Mo (V) complex. The antioxidant capacity observed was at the range between 26.513 and 187.01mg/g AAE for concentration series of 62.5 to 1000 µg/ml. Thus this result reveals that the plant *Xeromphis nilotica* has the capacity to scavenge free radicals.

Antioxidant activity of plant material depends on the presence of its bioactive compounds mainly polyphenols, carotenoids, and vitamin E (Rahman et al., 2015). This suggests that the concentration of the bioactive compounds present in the extract is attributable to showed antioxidant activity. Thus, there is a positive correlation between extracts concentration and its antioxidant ability. In this study, the reducing ability of the extracts to convert Mo (IV) to green phosphate-Mo (V) was expressed in mg of ascorbic equivalent. The extract showed a good total antioxidant activity that increased with accumulative concentration (figure 4). Our results comply with the data published elsewhere (Oktay et al., 2003) and suggest that the antioxidant capacity could be attributed to bioactive components present in the extract. However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing capacity as well as radical scavenging (Yildirim et al., 2000). Our results indicate that *X.nilotica* stem-bark extract contains significant amounts of phyto-constituents such as flavonoids, saponins, phenolic compounds. Flavonoids and phenolic compounds have good antioxidant potentials and the mechanism of action of flavonoids is through scavenging or chelation, while phenolic compound are important because of their hydroxyl groups which confer scavenging ability (Long et al., 2021).

CONCLUSION

The results obtained in the present study indicated that *X. nilotica* stem-bark extract exhibited free radical scavenging activity against hydrogen peroxide and DPPH, as well as total antioxidant capacity. The antioxidant activity of *X. nilotica* stem-bark extract might be attributed to its polyphenolic content and other phytochemical constituents. The extract was capable of scavenging DPPH and hydroxyl peroxide radicals in a concentration dependent manner. This studies demonstrate that the *X. nilotica* stem-bark extracts has potential antioxidant capacity. The findings of the present study suggested that *X. nilotica* stem-bark extract could be a potential source of natural antioxidant that would have great importance as therapeutic agents in preventing or slowing oxidative stress related degenerative diseases.

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