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Research Article

Phytochemical Studies and *In Vitro* Antioxidant Properties of *Ziziphus mauritiana* Fruit Extract.

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ABSTRACT

The present study was aimed at investigating the phytochemical and antioxidant properties of *Ziziphus mauritiana* fruit. Qualitative phytochemical studies were carried out which revealed the presence of flavonoids, saponins, glycosides, steroids, terpenoids and tannins. The antioxidant properties of the extract was also evaluated using various in vitro assays.The IC50 value of 2,2-diphenyl-2-picryl hydrazyl (DPPH) was found to be 338.45µg/ml. Reducing power assay was another in vitro model used which showed antioxidant potential by reducing ferrous ion which was concentration dependant. Measurement of total phenolic compounds was found to be 402.31±53.6mg Gallic Acid Equivalent/100g.

Key words: *Ziziphus mauritiana*, phytochemical, antioxidant properties

INTRODUCTION

Medicinal plants are plants that have at least one of their parts (leaves, seeds, stems, roots, fruits, foliage e.t.c) used for therapeutic purpose[1]. Recently, medicinal plants have become important for the treatment of different diseases such as diabetes, atherosclerosis, cancer, malaria, anemia[2]. So many of the present day drugs are known to be isolated from natural sources and there isolation were based on the information about the uses of the agents in folk lore medicine[3]. Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of either herbal preparations or conventional drug of plant origin[4]. *Ziziphus mauritiana*, a tree of *Rhamnacea* family is distributed in the Sahelian region of Africa, warm-temperate and subtropical regions throughout the world[5]. The tree of *Ziziphus mauritiana* is 7-12m in height and the trunk is 30cm in diameter. Branches are slender and downy, bearing paired brown spines. It is a fast growing tree with an average bearing life span of 25 years[6]. The fruit have interesting contents of carbohydrates, minerals (calcium, phosphorus, iron), β-Carotene, Vitamin C and bio-flavonoid. *Ziziphus mauritiana* fruit are used for processing different products particularly in India which provides products like traditional bread, cakes, cream and beverage[7]. The powdered fruit is used as famine food in Niger republic[8]. Major scientific interest has focused on the vitamin C and bio flavonoid content of the pulp, considering their biological and synergetic properties (maintenance of capillary walls, antibacterial and prevention of allergies)[9]. Antioxidants are molecules that can delay or prevent an oxidative reaction[10]. They play an important role in the protection of cells and tissues against free radical mediated tissue injury[11]. Many plants often contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannins and can be utilized to scavenge excess free radicals from human body[12].

An antioxidant is a molecule that inhibits the oxidation of free radicals. Free radicals initiate oxidation reactions. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agent such as thiols, ascorbic acid, or polyphenols[13].

Phytochemicals are naturally occurring, biologically active chemical compounds in plants. In plants phytochemicals act as a natural defense system for host plants and provide color, aroma and flavor[14]. More than 4000 of these compounds have been discovered to date. Phytochemicals are protective and disease – preventing particularly for some forms of cancer and heart diseases[15]. The most important action of these chemicals with respect to human beings is somewhat similar in that they function as antioxidants that react with the free oxygen molecules or free radicals in the body[16].

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade.

Plant Sourcing, Identification and Processing: The fruit of *Ziziphus mauritiana* plant were collected from Wamakko Local Government Area, Sokoto State, Nigeria. The sourced plant was identified and authenticated by a systemist in the Botany unit, Department of Biological science, Usmanu Danfodiyo University, Sokoto, Nigeria.

The fruits were shade dried and pulverized into powder using wooden pestle and mortar to separate the fruit from the seed. The powdered fruit was then filtered with the aid

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of 1mm2 sieve and stored until required for use in a plastic desicator[17].

Plant Extraction: One hundred (100g) of the powdered fruit of the plant was extracted with Methanol (1600ml) at room temperature (250C) for 48 hours. The extract was filtered using Whatman filter paper (No 1) and concentrated using the solvent completely under reduced pressure. The filtrate was concentrated to dryness using rotary evaporator.

Phytochemical Analysis

The methods[18,19,20,21] were used.

The extract was evaluated qualitatively for the presence of flavonoids, alkaloids, saponins, tannins, glycosides, cardiac glycosides, anthraquinones, saponin glycosides, flavonols, flavonoid aglycones, flavonoid glycosides, phlobatannins, steroids and terpenoids.

Test for Flavonoids: 2g of the air-dried powdered plant material was boiled for 7-10 minutes in 20ml distilled water and filtered. The filtrate was acidified with few drops of dilute HCl. Aliquot of the filtrate (5ml) was made alkaline (PH 10) with Sodium hydroxide. A yellow color developed which indicated the possible presence of flavonoid compounds.

5ml aliquot of the filtrate was separately shaken with 5ml of amyl alcohol. The presence of faintly yellow alcoholic upper layer indicated the presence of flavonoid aglycones. 10ml aliquot of the filtrate was separately shaken with 5ml amyl alcohol in a small separating funnel. The aqueous lower layer was separated and boiled with 10ml of concentrated HCl for two (2) minutes. The acidic solution was cooked, divided into two portions and treated as follows:

The first portion was shaken with amyl alcohol. A yellow coloration was produced which indicated the presence of flavonoid glycosides.To the other portion, few pieces of magnesium metal was added, no red coloration produced which indicated the absence of flavonols.

Test for Saponins: 2g of the powdered extracts was placed into a beaker, 20ml of water was added and was heated to boil for 3 minutes. The extract was filtered while hot and was allowed to cool. The following test was applied to the filtrate.

Frothing with Water, the extract (1ml) was placed in a test tube and was shaken strongly. The whole tube was filled with froth that lasted for several minutes which indicated the presence of saponins.

Emulsifying Properties, the extract (5ml) was diluted to 10ml with distill water and 5ml of the mixture was placed in a test tube. 5ml of olive oil was added and shaken violently for half a minute. A thick white emulsion was formed that remain stable for half an hour indicating the presence of saponins.

Test for Anthraquinones: The extract (5g) was boiled with 10ml aqueous H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml benzene. The benzene layer was separated and half of its own volume of 10% ammonia solution was added. No pink, red or violet color formed which indicated the absence of anthraquinones.

Test for Alkaloids: The extract (0.5g) was stirred with 5ml of 10% aqueous HCl on a steam bath for 20 minutes. It was cooled and filtered. The filtrate was used for the following test. 1ml of the filtrate was treated with few drops of Mayer's reagent. No creamy precipitate formed which indicated the absence of alkaloids in the extracts.1ml of the filtrate was treated with few drops of Wagner's reagent. No reddish brown precipitate formed which indicated the absence of alkaloids.

Test for Glycosides: 10ml of 50% H_2SO_4 was added to 1ml of the extracts in a test tube. The mixture was heated in a boiling water for 15 minutes. A 10ml of Fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed which indicated the presence of glycosides.

Test for Cardiac Glycosides: To 1ml of the extracts, 2ml of 3.5% ferric chloride was added and allowed to stand for one minute. 1ml of concentrated H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface with the upper layer turning green to blue indicated the presence of cardiac glycosides.

Test for Saponin Glycosides: To 2.5ml of extracts, 2.5ml of Fehling solution A and B was added. A bluish green precipitate formed indicated the presence of saponin glycosides.

Test for steroids and terpenoids: Powdered fruit plant (5g) was extracted by maceration with 50ml of 95% ethyl alcohol, filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and then filtered. The filtrate was divided into two equal portions. The first portion of the solution was mixed with 2ml of hydrochloric acid carefully so that hydrochloric acid form a lower layer. A reddish brown color at the interface indicated the presence of steroids. The second portion was mixed with 1ml of acetic anhydride, followed by addition of 1ml of concentrated H_2SO_4 down the wall of the tube to form a layer underneath. The formation of reddish – violet color at the junction of the two liquids and a green color in the chloroform layer indicated the presence of terpenoids.

Test for Tannins: Freshly prepared 10% KOH (1ml) was added to 1ml of the extract. A dirty white precipitate was observed which indicated the presence of tannins. Two drops of 5% $FeCl_3$ was added to 1ml of the extracts. A greenish precipitate indicated the presence of tannins.Test for phlobatannins: Extract (1ml) was added to 1% HCl. No red precipitate formed which indicates the absence of phlobatannins.

Triterpenes: Extract (1ml) was added to 5 drops of acetic anhydride and a drop of concentrated H2SO4 added. The mixture was then steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. The absence of blue – green color indicates the absence of triterpenes

In Vitro antioxidant Assay: The following methods were used to evaluate the in vitro antioxidant assay.

DPPH Free Radical Scavenging Activity

The method of [22] was employed.

Preparation of standard solution: Required quantity of ascorbic acid was dissolved in methanol to give the concentration of 5, 10, 20, 30, 40 and 50µg/ml.

Preparation of test sample: Stock solutions of samples were prepared by dissolving 10mg of dried methanolic extracts in 10ml of methanol to give concentration of 1mg/ml

Preparation of DPPH solution: 4.3mg of DPPH was dissolved in 3.3ml methanol. It was protected from light by covering the test tubes with aluminum foil.

Procedure for estimation of DPPH scavenging activity: 150µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 516nm for control reading.

Different volume levels of test sample (100, 120, 140, 160, 180 and 200µl) were screened and made 200µl of each dose level by dilution with methanol up to 3ml.150µl DPPH solution was added to each test tube and absorbance taken at 516nm in UV – visible spectrophotometer using methanol as blank.

The free radical scavenging activity was calculated using the following equation.

$$\% \text{ antiradical activity} = \frac{\text{control absorbance - sample absorbance}}{\text{control absorbance}} \times 100$$

Each experiment was carried out in triplicate and result are expressed as mean % antiradical activity + SD

Reducing Power Assay (FRAP)

The method of[23] was employed.

Preparation of standard solution: 10mg of ascorbic acid was dissolved in 10ml of distilled water. Dilutions of this solution with distilled water were prepared to give the concentration of 5, 10, 20, 40 and 50µg/ml.

Preparation of test sample: 10mg of the test samples was dissolved in 5ml of methanol and volume were made up to 10ml with phosphate buffer. Separately all the samples were diluted in 10ml volumetric flask with phosphate buffer to give (100, 500, 1000, 2000 and 3000µg/ml concentration.

Procedure for reducing power assay: 2 ml of each sample and standard solutions were spiked with 2.5ml of 1% potassium ferricyanide solution. The mixture was kept at 500C in water bath for 20 minutes.

After cooling, 2.5ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 minutes. 2.5ml of distilled water and 1ml of 0.1% ferric chloride and kept for 10 minutes. Control was prepared in similar manner excluding the sample. The absorbance of the resulting solution was measured at 700nm.

Total Phenol Content: The total phenol content was determined according to the method of[24] using Folin-Ciocalteu's reagent.

Procedure: A 1:10 dilution of aqueous extracts of the sample was oxidize with 2.5ml of 10% Folin-Ciocalteu's reagent (v/v) and neutralized by 2.0ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 450C. After cooling the absorbance was measured at 565nm. The total phenol content was subsequently calculated as gallic acid (10mg/100ml) equivalent C=CV/M

RESULTS

The results in Table 1 revealed the presence of various medicinally active phytoconstituents. Test for flavonoids, flavonoid aglycones, flavonoids glycosides, saponins, cardiac glycosides, saponin glycosides, steroids, terpenes and tannins were positive in the extract.

The result as shown in table 2 above for the assay of DPPH free radical scavenging activity are expressed in percentage antiradical activity. The analysis on the table above showed that the radical scavenging activity of methanolic extract of *Ziziphus mauritiana* increases with increasing in concentration which showed the ability of the extract to scavenge free radicals. The present result postulated that the extract of *Ziziphus mauritiana* reduces the DPPH radical to corresponding hydrazine when it reacts with hydrogen donor in antioxidant principles.

DISCUSSION

From the results obtained from table 4 above, the antioxidant potentials of *Ziziphus mauritiana* extract was estimated for their ability to reduce ferric ions. This was observed from the yellow color of the test solution that changed to blue. Higher absorbance of *Ziziphus mauritiana* extracts was observed in test sample with higher concentration. This higher absorbance of the reaction mixture indicated higher reducing ability which increased with increasing amount of fractions. The reducing power of the extracts may be due to the biologically active compounds in the extract which posses potent donating abilities. Table 1 shows the qualitative phytochemical constituents of fruit extract of *Ziziphus mauritiana*. Tests for flavonoids, saponins, glycosides, steroids, terpenoids and tannins were present in the extract. Flavonoids, phenolic acids and some terpenoids have been reported to posses antioxidant activities in different mechanism which gave the induction of different antioxidant estimated parameter[25].

The in vitro antioxidant assay as shown on table 3 indicated that the IC50 value of DPPH was 338.45µg/ml. The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant screening of plant extract. The method employing the stable 2, 2-diphenyl-l-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and convenience[26]. The present study showed that the methanolic fruit extract of *Ziziphus mauritiana* demonstrated H-donor activity by a means of decrease in absorbance as the concentration of the sample is been increased. The increase in percentage inhibition as concentration increased is a clear indication of free radical scavenging activity of the extract.

CONCLUSION

From the present investigation it can be concluded that *Ziziphus mauritiana* fruit can be used as a potent antioxidant as depicted by the results of the in vitro studies. Phytochemical screening of the extracts reveals the presence of flavonoids, saponins, glycosides, steroids, terpenoids and tannins. Thus, these antioxidant potential of the plant extract used may be due to the presence of these phytoconstituents.

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