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PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *DETARIUM MICROCARPUM*

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ABSTRACT

Detarium microcarpum is generally utilized for the treatment of various diseases in Northern Nigeria. Using the spectrophotometer technique against 1,1-dipenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2), the phytochemical and antioxidant free radical rummaging behavior of the ethanolic leaves concentrate of this plant was examined in vitro. Free radical scavenging activity of the plant extract against DPPH and H_2O_2 was focus subordinate with IC_{50} estimation of 4.84 and 2.77 separately as contrasted and those of standard ascorbic acid with IC_{50} estimation of 2.93 and 1.72 individually. In this research, it was discovered that the ethanolic extract of *D. microcarpum* contains high phenolic content that may

represent the powerful action against DPPH and H_2O_2 radicals. This scavenging action is presumably due to the presence of hydroxyl bunch joined to the aromatic ring structures and accordingly help to extinguish the radicals. The presence results show that leaves extract of *D. microcarpum* has strong in-vitro antioxidant activity because of the presence of phenolic mixtures. The findings suggest that ethanolic extract of *D. microcarpum* possess natural antioxidant property that could be used for treating several free-radical mediated diseases and also for herbal formulations.

KEYWORDS: In vitro, antioxidant activity, phytochemicals screening, $IC_{50,}$ *Detarium microcarpum,* Ascorbic acid.

1.0 INTRODUCTION

D. microcarpum is an African tree which belongs to family Fabaceae. Bushes that grow up to 15 m tall in damp regions can also grow up to 25 m tall. *D. Microcarpum* is a Fabaceae family tree from Africa. Bushes that grow up to 15 m tall in damp regions can also grow up to 25 m tall. Shoots can grow up to 1.5 m 2 m in size during normal growth. It sprows in the stormy season from September/May to November (July) to September/November; the tree leaves and grows new leaves in spring. It germinates between September, May and November during the stormy season (July); the tree leaves and gives new leaves in the Spring.[1] Wellspring of a fix or treatment for specific illnesses.[2,3] The act of conventional medication in the field of science and the treatment of illnesses and diseases is quickly expanding the universe of science. The leaves are utilized to treat weakness, loss of motion, meningitis, cramps.[4] Leaf powder is utilized in the treatment of loose bowels in Africa.[5,6] In Senegal, seeds are utilized as an option in contrast to intestinal sickness. As indicated by Phytomedicinally, portions of plants or pieces are utilized to treat sickness in an individual. It is a restorative plant broadly utilized for stomach issues, skin issues, snake chomps, colds and different sicknesses.[7] Today there is a developing interest in bioactive synthetic accumulates dependent on plants.[8] Numerous bioactive synthetics are segregated and read for restorative capacity. In the course of recent many years, the drug business has put vigorously in drug and compound examination worldwide with an end goal to locate the most strong medications, as opposed to only a couple new medications.[9] Plants have effectively breezed through the business testing assessment.[10]

Cell reinforcements assume a significant function as wellbeing assurance specialists. Logical proof recommends that cell reinforcements decrease the danger of ongoing sicknesses

including malignant growth and coronary illness.[4,5] The primary wellsprings of cancer prevention agents are normally happening in grains, products of the soil. Plants found in cell reinforcements, for example, nutrient C, nutrient E, carotenes; phenolic acids and so on have been known to lessen the danger of infection. Most cell reinforcement mixes in the basic eating regimen are found in plant sources and fall into different classes.[1,2] Cell reinforcements are substances that relax free extremists and their activities. There are normal cancer prevention agent chemicals, for example, Superoxides dismutase and disulfides, which retain a riddling frame in every cell, are glutathione peroxidase, reductase glutathione and thioredoxine thiols.[9] Alphatocophherols are a cell reinforcing chains that prevent free radicals from being spread. Therefore, this study aims mainly to examine the phytochemical components of the leaves of *Detarium microcarpum* as well as to establish the antioxidant function and IC_{50} values of the extract.

3.0 MATERIALS AND METHODS

3.1 Materials: The materials used for the current study are summarized in **table 1.**

Table 1: List of Materials and Reagents.

1. Pestle and mortar
2. Electric blender
3. Shaker water bath
4. Dragendorff's reagent
5. Alcoholic KOH
6. 1% HCL and dilute HCL
7. 15% $FeCl_3$ solution
8. Chloroform
9. Dilute ammonia solution
10. Distilled water
11. Methanol
12. Sprit
13. Ethanol
14. Hydrogen peroxide
15. Phosphate buffer (pH 7.5)
16. Acetate
17. Ascorbic acid
18. Mayer's reagent
19. Molish reagent
20. Fehling's solution
21. Filter papers

2.2 METHODS

2.2.1 DPPH Scavenging activity

DPPH checking may be a well-known technique widespread in natural studies of inhibitor products. One in every of the explanations is that this method is incredibly sensitive Associate in Nursingd simple. This test relies on the concept that a gas donor is an antioxidant. It measures compounds that are powerful monitors.[12] The antioxidant result is proportional to the disappearance of the foremost wide used method thanks to its simplicity and color ever-changing from purple to yellow following the formation of DPPH in obtaining hydrogen to the antioxidant. This reaction is ratio in regard to the amount of hydrogen acid atoms.[13] Dried extracts obtained during this way are used to test the antioxidant activity using various in vitro forms.

3.0 SAMPLE COLLECTION AND PREPARATION OF PLANT EXTRACT

The leaves of the plants were air dried underneath the shade and cut into small pieces using a pestle and mortar then ground into a powder using an electrical blender. 200 grams of fine herbs are added to 500ml of distilled water or 70% ethanol in separate 1.5 L barrels at room temperature. The same mixture was applied in hot water bath for 18 hours. The content is filtered through a fabric cloth then a Whatman filter paper. Filters were extracted from water and the resulting extract was placed in separate clean bottles and stored in the refrigerator at $4^{o}C$.[14]

4.0 PHYTOCHEMICAL SCREENING

4.1 Molish test (Test for Carbohydrates)

Molish test (General test for Carbohydrates): 2g of extracted plant dissolved in 5ml of 70% alcohol in an exceedingly test tube more or less 3ml of this resolution was given to a different test tube; a couple of H_2SO_4 drops are fastidiously inserted to create all-time low layer. The red alter the interface indicates the presence of carbohydrates.[15]

4.1.2 Fehling Test (Sugar Reduction Test)

The leaves plant extraction resolution was prepared by dissolving 0.1g of leaves extracting in 5ml of water. 2ml of equal mixture of Fehling A and B solutions was supplemental to concerning 5-6ml of the extraction solution and therefore the mixture was stewed in an exceedingly water bathtub for five minutes. The redness of the bricks indicates the presence of free sugar. If the higher than check was not available, 0.2g of the drug solution was hydrolyzed in water by boiling with 5ml of dilute acid for 5 minutes. 5ml of 10% hydroxide

was added to dilute solution A and B and stewed. The redness of the bricks indicates the presence of a reduced sugar content.[16]

4.2 Test for Flavanoids

4.2.1 Ferric Chloride

The mixture (0.5g) is boiled in H_2O and filtered. In 2ml filtrate, few drops of 10% ferric chloride solution was added. Appearance of a greenish-blue or Violet color is a sign of the presence of a synthetic resin hydroxyl radical.[17]

4.2.2 Sodium hydroxide

The extracted leaves (0.5g) are dissolved in distilled and filtered water. Within the filtrate, 10% liquid sodium hydroxide 2ml (NaOH) was added to obtain a yellow color. The change in color from yellow to colorless upon addition of dilute acid (HCl) indicates presence of flavonoids.[17]

4.3 Test for Alkaloids

Around 2ml of the extract is mixed with 1 ml of 1% HCl, stewed in an exceedingly water bathtub with three drops of Wagner, Mayer and Dragendorffs chemical reagent included.

The orange precipitate appearance is suggestive of alkaloid presence. The leaves (0.5g) which have been removed are diluted in the bath and filtered by 5ml of 10% liquid hydrochloric acid (HCl). The filtrate (3.5 ml) is taken in test tubes and divided into three components. 3 drops of dragendroff reagent have been applied to the first test tube. The creation of a bright red orange provided a positive result. 3drops of Mayers reagent were added in the second test tube. Strong results have been achieved with curly skin formation as a sign of alkaloid involvement. 3 drops of Wagner's reagent have been applied to the third test tube. A confirmed result was obtained by the formation of dark brown ice as an indicator of alkaloids.[17,18]

4.4 Test for Saponins

2 grams of extracts stewed in 10ml of water, filtered and used for the following tests.

Frothing test: The filtrate (5ml) is mixed with 10ml of water into a test tube. The test tube is suspended and vigorously shaken for 5 minutes. The occurrence of a bubble column that persists for more than 5 minutes indicates the presence of saponins.[19]

Fehling's Test: The filtrate 2ml is mixed with 2.5ml of equal volumes of Fehling's solutions A and B and then heated. Appearance of a brick-red precipitate is taken as the presence of saponins.[20]

4.5 Test for Tannins

2mL of the extract solution was mixed with 2mL of 15%$FeCl_3$ solution. The appearance of blue-black precipitate indicated the presence of tannins within the sample.

4.6 Test for Steroids: within the extract (2g), 2ml of acetic anhydride was added and stewed well in ice. Next, H_2SO_4 is fastidiously added. Color changes from violent to blue and dark blue may be a fashionable indication of steroids eg: aglycone part of cardiac glucoside.[21]

4.7 Test for Triterpenoid: To 0.5g of the extract, 5ml of ethanol was added followed by addition of 1ml of carboxylic acid anhydride. Subsequently during this, 1ml of Conc. H_2SO_4 is carefully placed next to the tube to create the lower layer. The colour change from pink to violence indicates the presence of triterpenoids.[21]

Test for Salkwoski: The extract (0.5 g) is dissolved in 2ml of chloroform. H_2SO_4 is fastidiously placed next to the tube to create the lower layer. A reddish-brown or yellow color at the interphase indicates the presence of a steroids ring.[21]

5.0 DETERMINATION OF DPPH RADICALS SCAVENGING ACTIVITY

The DPPH is freely stable and can be converted into stable diamagnetic particles by electron and hydrogen radicals.[22,23] The radical effect of DPPH decreases to 517 nm caused by inhibitors.[24] It is a result of the reduction of the radical DPPH. The reaction between antioxidant molecules and radicals has resulted in a substantial loss of gas supply due to the reduction in radical DPPH depletion caused by antioxidants. The transition of color from purple to yellow can be observed. Therefore, DPPH is commonly used for the assessment of antioxidant activity as a substratum.[25] The results show that gas is released to reduce radicals in the corresponding reduction agent before they react to their antioxidant target with hydrogen donors.[26] With suitable reduction agents, radicals react to DPPH, electrons are synthesized and, according to the electron quantities taken, the resolution leaves color stoichiometrically based.[27]

6.0 STATISTICAL ANALYSIS

The descriptions of this analysis are presented as standard deviations. Statistical analysis where applicable was performed using variants (ANOVA) followed by Tukey's post test with the help of IBM Statistical Package software for Social Scientist (SPSS 20). The difference is considered significant when P <0.05. Halfmaximal inhibitory (IC_{50}) filters were calculated on basis of % inhibition compared to the exclusion of a random curve for each extract.

$$\% = [(Ab\text{-}Ab) / Ab\ control \times 100]$$

7.0 RESULTS AND DISCUSSION

The leaves extract of *D. microcarpum* are widely used for their diuretic and antioxidant properties.[28] This wide application forms for the in vitro antioxidant and phytochemicals screening of the plant leaves extract. Increases in the body of freely radicals and ROS without a sufficient good anti-oxidant defence, which are connected to pathogic diseases such as heart diseases, cancer, brainery and diabetes mellitus, lead to oxidative stress.[29] It has been found in the present study that the leaves extract of *D. microcarpum* contains high phenol levels that are responsible for the strong activity against radicals DPPH and H2O2[30] (Table 3 and table5). The findings, on the other hand, revealed that leaves extracts of *D. Microcarpum* scavenged DPPH to varying levels(fig 1). The leaves extracts showed excellent IC_{50} values of 4.84 in comparison to standard ascorbic acid which has IC_{50} of 2.93(table 4). DPPH is a stable free radical inheriting a characteristic low-purple colour; antioxidants can contribute to its loss of the usual deep violet colour, which can lead to the absorption of proton from antioxidants by 517 nm.[31] The color degree is thus an indicator of the antioxidant scavenging potential of the extracts. Of course, the extract from the leaves had the most effective DPPH scarcity, as its IC50 values show (Table 3) (fig 2). The antioxidant activity of phenolic compounds and flavonoids in biological systems, as single oxygen scavengers as well as freer radicals have been reported to be associated.[32,33] Due to its ability to penetrate biological membranes, hydrogen peroxide is a particularly targeted and highly significant reactive oxygen species. However, by reacting with Fe2+ and presumably Cu2+ ions, it may be toxic if converted into hydroxyl radical in the cell.[34] This test demonstrates the ability of *D.microcarpum* to stop the reaction mixture of hydrogen peroxide[35] (fig 2 and table 6). It was found that the plant extract behaviors were nearly identical to reference compounds. This may be because phenolic compounds are present, which donate electron to H2O2 and thus neutralize the electron to water.[36] Literature reviews indicate that triterpenoids, hormones, steroides, flavonoids, and alkaloids are found in these species.[37]

Table 2: Result for Phytochemical Screening.

Phytochemical	Result
Carbohydrates	+
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	+
Steroids	+
Triterpenoids	+
Cardioglycoside	+

Key: +: Presence, - : Absence

Table 3: DPPH Scavenging Activity of the Plant Extract.

Sample	Concentration (mg/ml)	% Inhibition	IC_{50}	r^2
Plant Extract	0.2	0.064±0.005	4.84	0.8314
	0.4	0.063±0.006		
	0.6	0.067±0.006		
	0.8	0.099±0.009		
	1.0	0.034±0.005		

Table 4: DPPH Scavenging Activity of the Ascorbic Acid.

Sample	Concentration (mg/ml)	% Inhibition	IC_{50}	r^2
Ascorbic Acid	0.2	0.216±0.004		
	0.4	0.195±0.004	2.93	0.644
	0.6	0.212±0.003		
	0.8	0.027±0.006		
	1.0	0.085±0.005		

Table 5: Hydrogen Peroxide Scavenging Activity of the Plant Extract.

Sample	Concentration (mg/ml)	% Inhibition	IC_{50}	r^2
Plant Extract	0.2	0.509±0.006		
	0.4	0.530±0.003	2.77	0.7325
	0.6	0.578±0.008		
	0.8	0.644±0.005		
	1.0	0.607±0.005		

Table 6: Hydrogen Peroxide Scavenging Activity of the Ascorbic Acid.

Sample	Concentration (mg/ml)	% Inhibition	IC_{50}	r^2
Ascorbic Acid	0.2	0.622±0.003		
	0.4	0.676±0.003	1.72	0.9722
	0.6	0.702±0.008		
	0.8	0.737±0.006		
	1.0	0.758±0.002		

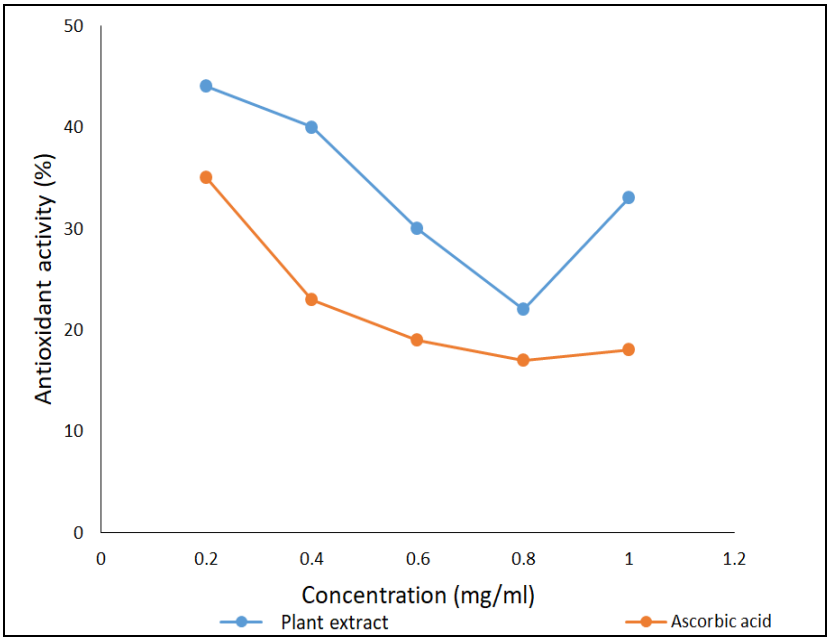

Fig 1: A Plot percentage DPPH Scavenging Activity of Plant Extract and Ascorbic acid against its concentration.

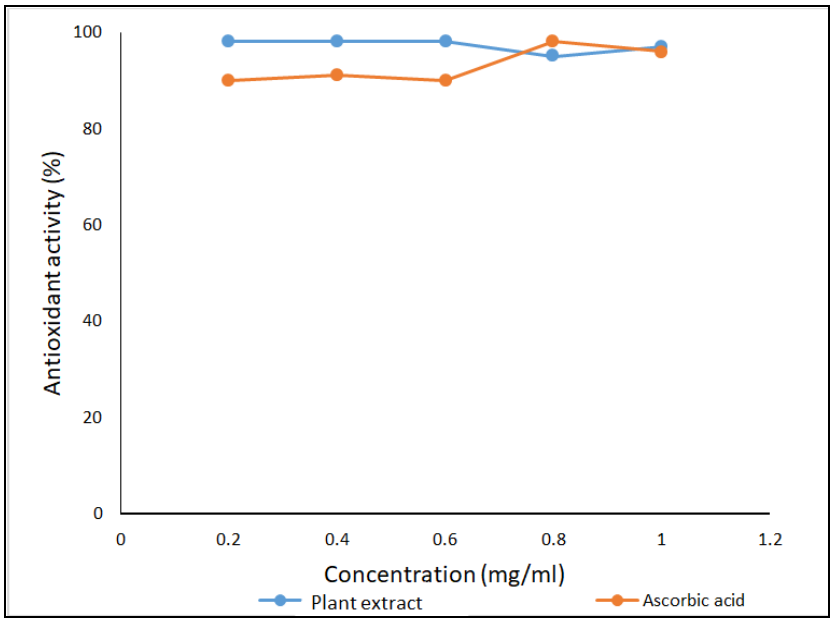

Fig 2: A Plot percentage Hydrogen Peroxide (H_2O_2) Scavenging Activity of Plant Extract and Ascorbic acid against its concentration.

CONCLUSION

In conclusion, present results demonstrate that leaves extract of *D. microcarpum* has in vitro antioxidant activities due to the present of phenolic compound. *D. microcarpum* is being used traditionally for curing various ailments arising due to free radicals. The findings of this research justified the use of *D. microcarpum* in folkloric medicines and is a source of natural

antioxidants. The present study has gone some way towards enhancing our understanding of antioxidant activity of *D. microcarpum*, and lays the groundwork for future research into *D. microcarpum*. However, further investigations and experimentations are strongly recommended to isolate and characterize the active principle(s) of the plant extracts and estimate the therapeutic efficacy using animal models.

Authors contribution

All authors contributed extensively to the work presented in this paper.

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Conflict interest

The authors declare no conflict of interest.

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