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ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF THE METHANOLIC LEAVES EXTRACT OF *B RUFESCENS* (LAM)

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ABSTRACT

Interest in natural products as a source for innovation in drug discovery and agrochemicals is still growing worldwide. Natural products, whose immense diversity has been appreciated for many years, may become in a rich source of novel chemical structures. The present study was conducted to investigate the *in-vitro* antioxidant (DPPH assay) and phytochemical screening of methanol extract of *B rufescens* (leaves). The methanol extract of *B. rufescens* (leaves) was tested for antioxidant screening for their free radical scavenging properties using 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), while propyl galate was used as standard antioxidant and phytochemical screening. The methanol extract of *B rufescens* (leaves) antioxidant activity was (81 ± 0.04 RSA%) in comparison to the control of propyl galate levels (88 ± 0.07RSA%) and Preliminary phytochemical screening of the leaves of *B rufescens* revealed that the plant contain triterpenes, alkaloids, tannins, saponins and flavonoids. Negative results were recorded for glycosides and coumarins. Hence, the results obtained in the present study indicate that *B rufescens* have promising antioxidant indicates that the plant could be promising agent in scavenging free radicals and treating diseases related to free radical reactions.

Keywords: Antioxidant (DPPH-assay), Phytochemical, *B rufescens* (leaves).

INTRODUCTION

Recently in many African countries comprehensive research was conducted on medicinal plants for the treatment of different diseases and conditions, such as diabetes, malaria, anemia and cancer.

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The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor *et al*., 2005). Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the

potential of local plants against these disabling diseases (Amaral *et al*., 2006; Koko *et al*., 2008).

The medicinal properties of plants have been inve stigated, in the light of recent scientific developments, thro ugh out the world due to their potent pharmacological activ ities and economic viability. A great number of aromatic, s picy, medicinal and other plants contain chemical compoun ds, exhibiting antioxidant properties. Source of natural anti oxidants are primarily, plant phenolics that may occur inall parts of plants such as fruits, vegetables, nuts, seeds, leave s, roots and barks (Pratt, 1990). Many of theseantioxidant compoundspossess anti-inflammatory, antiatherosclerotic, antitumor, anticarcinogenic,, antibacterial or antiviral activities to a greater or lesser extent (Sala *et al*, 2002). Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolic are increasingly of interest in the food industry, because they r etard oxidative degradation of lipids and thereby improve t he quality and nutritive value of food (Kahkonen *et al.*, 1999**;** Rice *et al*, 1995**).**

The plant *B rufescens* Lam is a scandent shrub or small tree belonging to the giant family Leguminosae, subfamily Leguminosae-caesalpiniodeae; usually 1-3 m high, sometimes reaching 8 m; often scraggy, stunted and multi-stemmed. Bark ash-grey, smooth, very fibrous and scaly when old, slash pink, twigs arranged in 1 plane like a fishbone, with thornlike, lignified, lateral shoots, 10 cm long. The leafs are very small, bilobate almost to base, with semi-circular lobes, glaborous, with long petioles, greyish-green, less than 3 cm long. Flowers are greenish-yellow to white and pale pink, petals 5, spathulate, 15-20 mm long; stamens 10, filaments hairy at the base. Fruits aggregated, long, narrow pods, twisted, up to 10 cm long, glaborous, obliquely constricted, shining dark red-brown, with 4-10 seeds each (Burkill, 1995).

The plant is deciduous in the drier area and evergreen in the wetter area, often found in the dry Savannah region, especially near streams or river banks; occurring throughout West Africa and extends across Africa up to Sudan. It has wide array of medicinal and socio-cultural uses. Several *B* species are utilized as folk medicines worldwide, including Africa, Asia, South America and Central America, An extract of the root is used as an astringent or antipyretic in local medicine. Leaves and fruit are applied for the treatment of diarrhea, dysentery and ophthalmic diseases. The bark of the roots and trunk is used to cure chest complaints, syphilis and other venereal diseases, leprosy, diarrhea and dysentery and to reduce fever (Ayensu, 1978). The present study was conducted to investigate the antioxidant activity and phytochemical screening of *B rufescens* (leaves) in Sudan.

MATERIALS AND METHODS

Plant materials

The *B rufescens* (leaves) were collected from central Sudan between January and February 2015. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI).

Preparation of crude extracts

Extraction was carried out for the leaves of *B rufescens* plant by using overnight maceration techniques according to the method described by (Harbone, 1984). About 50 g were macerated in 250 ml of methanol for 3 h at room temperature with occasional shaking for 24 h at room temperature, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration, the solvent was then removed under reduced pressure by rotary evaporator at 55°C. Each residue was weighed and the yield percentage was calculated then stored at 4°C in tightly sealed glass vial ready for use. The remaining extracts which is not soluble by successively extracted by methanol using the previous technique. Extracts kept in deep freezer for 48 h, then induced in freeze dryer (Virtis, USA) they were until completely dried. The residue was weighed and the yield percentage was calculated. The extracts were kept and stored at in 4°C required.

Antioxidant activity of B rufescens extract

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method by (Shimada *et al*., 1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as 300 µm. The extract was dissolved in DMSO (500µg/ml. concentration), while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and propyl gallate (PG). All tests and analysis were run in triplicate.

Phytochemical Screening

Phytochemical screening is of great importance in providing us with information about chemicals found in the plant in term of their nature and range of occurrence. This information would enable us to correlate between the nature and range of occurrence of these chemicals and biological assays held to investigate a certain bioactivity of the mentioned plant. In this study the preliminary phytochemical screening was conducted according to (Haborne, 1984).

Preparation of the Extracts

10 mg of the powdered leaves of plant were refluxed with 100 ml of ethanol 80% for 4 hours. The cool solution was filtered and enough ethanol 80% was passed through the volume of the filtrate 100 ml. This prepared extract (PE) was used for the various tests.

Test for Unsaturated Sterols and Triterpenes

10 ml of the prepared extract (PE) was evaporated to dryness on a water bath and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with 20 ml of chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. 5 ml of chloroform solution was mixed with 0.5 ml acetic anhydride followed by two drops of conc. Sulphuric acid. The gradual appearance of green, blue pink to purple color was taken an evidence of the presence of sterol (green to blue) and or triterpenes (pink to purple) in the sample (Harborne, 1984).

Test for Alkaloids

7.5 ml of (PE) was evaporated to dryness on a water bath. 5 ml of HCl (2N) was added and stirred while heating on the water bath for 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent were added. While to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes was tanked as presumptive evidence for the presence of alkaloids (Harborne, 1984).

Test for Flavonoids

17.5 ml of the (PE) was evaporated to dryness on a water bath, cooled and the residue was defatted with petroleum ether and the defatted residue was dissolved in 30 ml of ethanol (80%) and filtered. The filtrate was used for the following tests: (A) To 3 ml of the filtrate in a test tube 1 ml of 1% aluminum chloride solution was in methanol was added. Formation of yellow color indicated the presence of Flavonoids, (Flavones and / or chalcone). (B) To 3 ml of the filtrate in a test tube 1 ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of the Flavonoids compounds (flavones or flavanones) chalcone and/or flavonol. (C) To 2 ml of the filtrate 0.5 ml of magnesium turnings were added. Producing of defiant color to pink or red was taken as presumptive evidence that flavanones were present in the plant sample (Harborne, 1984).

Test for Tannins

7 ml of the (PE) was evaporated to the dryness on water bath. The residue was extracted several times with n-hexane and filtered. The insoluble residue was stirred with 10 ml of saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to 10 ml with more saline solution. 5 ml of this solution was treated with few drops of gelatin salt reagent. Formation of immediately precipitate was taken as an evidence for the presence of tannin in plant sample. To another portion of this solution, few drops of ferric chloride test reagent were added. The formation of blue, black or green was taken as an evidence for the presence of tannins.

Test for Saponins

1 g of the original dried powdered plant material was placed in a clean test tube. 10 ml of distilled water was added and the tube was stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of (honeycomb). The appearance of honeycomb, which persisted for least an hour, was taken as an evidence for the presence of Saponins.

Test for Anthraquinone Glycosides

10 g of the powdered plant sample were boiled with 10 ml of 0.5N KOH containing 1 ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with10 ml of benzene. 5 ml of the benzene solution was shaken with 3 ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of Anthraquinones was indicated if the alkaline was found to have assumed pink or red color.

Test for Coumarins

3 g of the original powdered plant sample was boiled with 20 ml of distilled water in a test tube and filter paper was attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of coumarone was indicated if the spot has found to be absorbed the UV light.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program 2007.

RESULTS AND DISCUSSION

The leaves of *B rufescens* family (Leguminosae) were screened for antioxidant screening for their free radical scavenging properties using 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), while propyl galate was used as standard antioxidant and phytochemical screening.

Antioxidant activity of B. rufescens(leaves) extract

This table indicates the antioxidant DPPH of methanol extract of *B. rufescens* (leaves), propyl gallate was used as standard drug level. The tested antioxidant activity gave (81 ± 0.04 RSA %) in comparison to the control of propyl galate levels gave (88 ± 0.07 RSA %).

As shown in Table (1), the results of antioxidant activity *B rufescens* (leaves) showed high antioxidant activity against the DPPH free radical (81.04 RSA%). It is reported that the phenolic compounds constitute a major group of compounds that acts as primary antioxidants (Hatano*et al.*, 1989). Literature reports showed that there is high correlation between antioxidant activity and phenolic compounds (Odabasoglu *et al.*, 2004). The reducing

capacity of compounds could serve as indicator of potential antioxidant property (Meir *et al*., 1995).

Phytochemical analysis of B rufescens (leaves)

The Phytochemical analysis of crude methanolic extract performed by the method described earlier and then and then analyzed for phytocompounds like steroids or terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins and anthraquinone preliminary analyzed and present in the (Table 2).

The Phytochemical analysis of crude methanolic extract of *B rufescens* performed by the method described earlier and then analyzed for phytocompounds like steroids or terpenoids, alkaloids, flavonoids, coumarins, saponins, tannins and anthraquinone preliminary analyzed in the present study.

Phytochemical screening of the leaves of *B rufescens* revealed that the plant contain triterpenes, alkaloids, flavonoids, tannins and saponins (Table2). Negative results were recorded for Anthraquinone glycoside and Coumarins It is reported that the phenolic compounds constitute a major group of compounds that acts as primary antioxidants (Hatano *et al.,* 1989).The free radical-scavenging activity of different extract was determined by the DPPH, method and the results are shown in (Tables 2). The DPPH radical is one of the most widely used and stable chromogen compound used to evaluate the antioxidant activity of the tested compounds. This result similar to (Aliyu *et al.*, 2009) who found high antioxidant activity in the same part of *B rufescens* leaves extract.

Table 1. Antioxidant activity of *B rufescens* (leaves)

No	Name of plant	Part	%RSA* ± SD (DPPH)
1	***B rufescens***	**leaves**	81 ± 0.04
2	***Control**	**PG**	88 ± 0.07

Key: RSA* = Radicals scavenging activity ***Control = P.G** = Propyl Gallate .

Table 2. Preliminary Phytochemical Screening analysis ofB *rufescens* (leaves) extract

No.	Tested	*B. rufescens*
1	Triterpenes	+
2	Alkaloids	+
3	Flavonoids	+
4	Tannins	+
5	Saponins	+
6	Anthraquinone glycoside	_
7	Coumarins	_

+ = Present -- = Absent.

CONCLUSION

From complete investigation about antioxidant and pharmaceutical screening studies of *B rufescens* leaves it can be recommended that extracts could be used as a easily available foundation of natural antioxidants, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, inflammation, etc., diabetes swelling.Further studies are needed on the isolation and elucidation of their chemical structures of antioxidant components.

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CONFLICT OF INTEREST

None

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