Evaluation of antioxidant potentials of ethanol stem bark extract of *Boswellia dalzielii* and its phytochemical screening

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Abstract

Healing with traditional herbal remedies is invaluable in avoiding the toxicity of synthetic drugs. The ethanol stem bark extract of *Boswellia dalzielii* (Burseraceae) was chosen for the experiment to estimate the phytochemical constitution and their antioxidant activity using ferric reducing power assay (FRAP) and 2,2-diphenyl-1-picrylhydrazylhydrazide (DPPH) radical scavenging activities and 50% inhibitory concentration (IC₅₀) was calculated and compared with the standard synthetic drug ascorbic acid. Phytochemical screening was used to check the presence of pharmacologically active compounds known as phytochemicals in the stem bark extract. In addition, total phenolic (TP), total flavonoids (TF) and antioxidant activities of ethanol stem bark extract of *Boswellia dalzielii* (ESBD) were determined. Results of the phytochemical profile of the extract, analyzed exhibited that the ESBD contains several secondary metabolites. The total phenolic and flavonoid contents were found to be 22.08 mg gallic acid equivalents (GAE) per 100 gram extract and 0.034 mg rutin equivalents (RE) per 100 g extract, respectively. For antioxidant activity, the ESBD showed the good scavenging activity against DPPH with IC₅₀ value of 34.65 µg/mL and the highest reducing power with IC₅₀ value of 18.37 µg/mL. These values are comparable to those of the ascorbic acid as standard for DPPH scavenging activity and reductive potential. The results obtained in this research demonstrated in that ESBD contains phytochemicals of biological and pharmacological importance and has antioxidant capacity which can be utilized to alleviate the symptoms of chronic and degenerative diseases.

1 Introduction

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen that are mainly produced by the mitochondrial electron transport chain as by-products of the common cellular metabolism¹. The overproduction of reactive oxygen species and its consequent oxidative stress can cause oxidative damage to cellular macromolecules including lipids, proteins, and DNA as well as modulation of anti-inflammatory systems and antioxidants². Antioxidants, present in low concentrations compared to those of an oxidisable substrate, have the capacity in preventing or slowing the oxidation reactions and have been recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease³. In this communication, many antioxidant agents such as

butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) used in the delay or prevention of oxidative stress^{4,5}. However, the use of synthetic antioxidants is associated with the toxic due to presence of higher amount of preservatives⁶. To compensate for the side effects of synthetic products and their toxicities, scientists are faced with the obligation to use of herbal medicine. Furthermore, according to the statistics, nearly 65-80% of the world population in developing countries, because of the poverty and because of the lack of access to the modern medicine, depends essentially on the traditional medicine for their health care primary sector⁷.

Boswellia dalzielii (*B. dalzielii*) is a tree plant of the savannah forest recognizable by its papery bark peeling off in a ragged manner⁸ and belongs to the family of Burseraceae. It is commonly

called Frankincense tree in English Language⁹, *tenguété* in Mafa Language (Far North Region, Cameroon). The phytochemicals screening of the *B. dalzielii* stem bark revealed the presence of various bioactive components of which alkaloids, phenolic compounds, tannins, flavonoids, and saponins.

The plant has several medicinal uses. The stem bark is boiled and is used to treat rheumatism, venereal diseases and gastrointestinal disorders⁹. In the Republic of Benin, a decoction of the bark is used for the treatment of angina, dysentery and haemorrhage¹⁰. Our previous studies showed that the ethanol extracts of the resin exudates of stem bark from North Cameroon to possess anti-inflammatory activity¹¹. The fresh bark is eaten to induce vomiting and relieve symptoms of giddiness, palpitation and take taken internally for gastrointestinal troubles⁸. The root decoction boiled along with *Hibiscus sabdariffa* is used for the treatment of syphilis. This study was therefore carried out to evaluate the phytochemical profile and antioxidant potentials of ethanol extract from the stem bark of *B. dalzielii*, validate its traditional pharmacopoeia use for this purpose.

2 Materials and methods

2.1 Plant material

The plant specimen i.e. fresh stem bark of *B. dalzielii* was collected from its natural habitat in local area of Mbé Village (Adamawa Region), Cameroon during the month of March, 2019. The plant material was taxonomically identified by a plant taxonomist, Professor Pierre-Marie Mapongmetsem, attached to the Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, Cameroon. The plant was deposited by comparison with a sample preserved at Cameroon National Herbarium under the registration number 20532/SRF-CAM (Yaoundé, Cameroon).

Fig 1: Plant of *Boswellia dalzielii*

2.2 Preparation of plant material

The fresh stem bark of *B. dalzielii* were cleaned properly, cut into small pieces and air-dried at room temperature (25°C) for about two weeks avoiding direct sunrays on it and the material became

well dried size reduced into coarse powder with mortar and pestle. The extract was obtained by maceration method. Shortly, the dry powder of the stem bark (10 g) was macerated in 100 mL of cold 95% ethanol (1: 10, v/v) in laboratory temperature for seven days. After decantation, the supernatant was filtered, concentrated at 55°C by evaporating in a vacuum rotary evaporator (HEILDOLPH®). The concentrated was stored in a dry, sterile bottle in the refrigerator for different phytochemical components and evaluation for antioxidant potentials.

2.3 Chemicals

Gallic acid, ascorbic acid, Folin-Ciocalteu reagent, aluminum chloride, ferric chloride (FeCl_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH), rutin, sodium acetate (Na_2CO_3), potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$), and trichloroacetic acid were purchased from Sigma-Aldrich (Yaoundé, Cameroon). All chemicals and reagents were of analytical grade.

2.4 Qualitative phytochemicals screening

Chemical tests for the screening and identification of bioactive components or pharmacologically active compounds known as phytochemicals in freshly prepared extract of *B. dalzielii* stem bark were performed using the conventional procedures^{12, 13}.

2.4.1 Test for phenolic compounds

The reaction to iron chloride (FeCl_3) detected the presence of phenolic compounds. At 1 mL of *B. dalzielii* stem bark solution, add one to three drops of 10 % iron III chloride (FeCl_3) solution. The appearance of a white or green coloration is a positive test.

2.4.2 Test for tannins

The tannins were detected from 2 mL of the *B. dalzielii* stem bark solution and a few drops of the 1 % of FeCl_3 solution are added to the solution (prepared with methanol). After stirring the extract, the color turns blue-black in the presence of gallic tannins and greenish brown in the presence of catechish tannins.

2.4.3 Test for flavonoids

The test consists of adding 1 mL of the *B. dalzielii* stem bark solution, 1 mL of concentrated hydrochloric acid (HCl) and some cups of Mg and 1 mL of isoamylic alcohol. Leave on for 3 minutes and watch the color change. The presence of flavonoids is confirmed by intense red, orange, dew or purple red coloration.

2.4.4 Test for alkaloids

0.5 g of the *B. dalzielii* stem bark was diluted to 10 mL with acid alcohol, boiled, and filtered. To 5 ml of the filtrate, 2 mL of dilute ammonia and 5 ml of chloroform was included and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Mayer's

reagent) or reddish brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

2.4.5 Test for triterpenoids/steroids

Steroids and triterpenoids were sought by Liebermann-Burchard's reaction. A 0.5 mL solution of the *B. dalzielii* stem bark solution is added to 0.5 mL of acetic anhydride and 0.5 mL of concentrated sulphuric acid. The appearance of a blue-green or purple pink ring produced immediately indicates the presence of steroids or triterpenoids.

2.4.6 Test for saponins

To *B. dalzielii* stem bark solution, 5 mL of ethanol extract was added in a graduated test tube. Then, the solution was shaken vigorously and observed for a stable persistent froth. Formation of a layer of foam that persists for fifty minutes indicated the presence of saponins.

2.5 Determination of total phenolic content

Total phenolic content in the stem bark of *B. dalzielii* was determined by using Folin-Ciocalteu reagents, a method based on a colorimetric oxidation/reduction reaction with analytical grade gallic acid as the standard¹⁴. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. Shortly, 0.1 mL of plant extract solution was prepared in methanol and was introduced into test tubes. After this, 2.5 mL of 10 % (v/v) Folin-Ciocalteu reagent and 2 mL of 20 % Na₂CO₃ solution was added to the mixture. The tubes containing the mixture were shaking thoroughly for 15 sec. After mixing, mixture incubated for 30 min at room temperature (25°C) to develop blue colour. After being kept in total darkness for 30 minutes, the absorbance was measured at 765 nm using a UV-2100 Spectrophotometer (UNICO®) against a blank.

Gallic acid is the standard used to establish the curve calibration from which the concentration of the total polyphenols of the extract is calculated. The results were expressed as gallic acid equivalents (GAE) mg/ 100g of extract.

2.6 Determination of total flavonoids content

The aluminum chloride colorimetric assay was used for total flavonoids contents determination. The crude sample was prepared by liquefying 10 mg of the extract in 10 mL of the solvent to yield a concentration of 1 mg/mL. A volume of 0.5 mL of the *B. dalzielii* stem bark solution was taken in test tubes to which, 1.5 mL of aluminum chloride (10%, w/v) previously prepared in ethanol, 0.1 mL of potassium acetate (1M), and 2.8 mL of distilled water were placed in vials and kept in the dark for 40 minutes at room temperature. After 40 minutes incubation at room temperature, the absorbance of the reaction mixture was measured at 415 nm. A yellow color indicated the presence of flavonoids. The amount of total flavonoids compounds in extract was expressed as mg RE per 100 gram weight of extract. All the determinations were carried out in triplicates

2.7 DPPH radical scavenging assay

To determine the radical scavenging ability, the protocol reported by¹⁵, was used with minor modifications. Briefly, freshly prepared methanol solution of DPPH (1 mL) was placed in a test tube was added to sample (0.5 mL) containing various concentrations in clean test tubes. The crude sample was prepared by liquefying 1 mg of the extract in 1 mL of the solvent to yield a concentration of 1 mg/mL. The mixture was vortexed thoroughly and incubated in the dark place at ambient temperature (25°C ± 2°C) and was measured by spectrophotometer at 517 nm after 30 min for any radical-antioxidant reaction to occur. A blank solution was prepared containing the same amount of methanol and DPPH. The inhibitions of DPPH radical in percent (% Inhibition) were calculated according to the following equation:

$$\% \text{ Inhibition of DPPH} = [(A_{\text{Blank}} - A_{\text{Sample}}) / A_{\text{Blank}}] \times 100$$

Where A_{blank} is the absorbance value of the control reaction (containing all reagents (DPPH + methanol) except the sample) and A_{sample} is the absorbance value of the *B. dalzielii* stem bark solution or the standard, ascorbic acid (DPPH radical + sample). One synthetic antioxidant, Vitamin C (ascorbic acid) was used as positive control. The IC₅₀ or 50% inhibitory concentration (also called EC₅₀ for Efficient concentration 50), is the concentration of the tested sample needed to reduce 50% of the radical DPPH. IC_{50s} are calculated graphically by the linear regressions of Straights drawn; percentages of inhibition based on different concentrations of ethanol extract tested.

2.8 Ferric reducing antioxidant power (FRAP) activity

The Ferric reducing antioxidant power procedure was followed to a method described by¹⁶ with minor modifications. The *B. dalzielii* stem bark solution was taken in various concentrations (0 to 1000 µg per mL) from the stock solution and was mixed with 2 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2 mL of 1% potassium ferricyanide in a test tube. After incubation in water bath at 50 °C for 20 min, 2 mL of 10% trichloroacetic acid was added to the mixture to stop the reaction, and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2 mL) was mixed with 2 mL distilled water and 0.5 mL of 1% ferric chloride (FeCl₃) was prepared freshly in some distilled water and then the absorbance of reaction mixture was measured at 700 nm using a UV-2100 Spectrophotometer (UNICO®). Higher absorbance of the reaction mixture indicates an important reducing power. As positive control, ascorbic acid was used and results of total antioxidant content were expressed as absorbance reading. The measurements were done in triplicate.

2.9 Statistical analysis

The results were given as mean ± SEM. The significance of difference between the control and treated groups were determined using one-way analysis of variance (ANOVA)

followed by Dunnett's post hoc. P values < 0.05 were taken to be statistically significant.

3 Results and discussion

The basic phytoinvestigations of extract for their main constituents (phytocompounds) are vital because the active ingredients of many drugs are these secondary metabolites found in plants. Phytochemical screening of the *B. dalzielii* stem bark revealed the presence of various bioactive components of which alkaloids, phenolic compounds, tannins, flavonoids, and saponins. In contrast, the steroids/terpenoids and glycosides were not detected. The result of phytochemical test is summarized in Table 1.

Table 1: Phytochemical screenings of ethanol stem bark extract of *Boswellia dalzielii*

Phytochemical	Results
Alkaloids	+
Phenolic compounds	+
Flavonoids	+
Tannins	+
Saponins	+
Triterpenes /steroids	-
Glycoside	-

+ = Present, - = Absent

These classes of phytochemicals are known to possess a variety of biological activities including antioxidant, anti-inflammatory, and anticancer activities. These preliminary results constitute a scientific basis which justifies the use of the stem bark of *B. dalzielii* in the traditional remedies¹⁷.

Phenolic compounds are major plant secondary metabolite, which have several biological functions including antioxidant activity. The total phenolic content of *B. dalzielii* stem bark was measured by using the Folin–Ciocalteu colorimetric method with gallic acid as a comparison. These methods are based on formation molybdenum-blue from oxidation with a phenolic group¹⁸. In *B. dalzielii* stem bark extract, the phenolic content is 22.08 mg gallic acid equivalents (GAE) per 100 gram of extract. The absorbance of various dilutions of gallic acid was found as standard curve equation with $y = 0.8747x + 0.0418$, $R^2 = 0.98$ (Fig. 2). Analysis of the phenolic contents in this extract revealed contained the maximum phenolic content in terms of gallic acid equivalents. Phenolic compounds from plants are secondary metabolites which, due to their ability, act by absorbing and neutralizing free radicals, decomposing peroxides, and quenching the activity of reactive oxygen species¹⁹.

Therefore, in the present study; total flavonoid content present in extract was estimated using the aluminum chloride colorimetric

method. In this extract, the flavonoid content ranged from 0.034 mg rutin equivalents (RE) per 100 g of extract. The absorbance of Rutin was found as standard curve equation with $y = 3.0495x - 0.0093$, $R^2 = 0.98$ (Fig. 3). Phenolic compounds have antioxidant properties because of their ability to trap free radicals and reactive oxygen species, the process is radical²⁰.

Fig 2: Calibration curve of gallic acid

Fig 3: Calibration curve of Rutin

Oxidative stress is defined as a profound imbalance of the balance between pro oxidants and antioxidants in favour of the former; this imbalance is either due to an exaggerated production of oxidizing agents or from an exaggerated defense mechanisms^{21, 22}. The DPPH assay is a very convenient method for screening small antioxidant molecules because the reaction can be analyzed by simple spectrophotometric assays²³. From the result, table 1 was shown the free radical scavenging of the ethanol extract of *Boswellia dalzielii* stem bark compare to ascorbic acid used as positive control.

It was observed that at a concentration of 1 mg/mL, the scavenging activity of ethanol extract reached 97.87%, while the standard drugs used (i.e. ascorbic acid) were 96.78%. The IC₅₀ value for ethanol extract and ascorbic acid are 34.65 and 18.37 µg/ml respectively. The result indicates that the ethanol extract of *Boswellia dalzielii* stem bark is capable of scavenging the free radicals and prevent the initiation of free radicals by stabilizing them to participate in any deleterious reactions. *Boswellia dalzielii* stem bark was a strong antioxidant comparable to vitamin C antioxidant power and was in good agreement with the number of phenolic compounds, a major category of plant-derived compounds (Fig 4).

Fig 4: DPPH Radical Scavenging Activity

The reducing power method reflects the electron donation ability of the antioxidant present in the extract to convert the Fe^{3+} into Fe^{2+} . The amount of Fe^{2+} complex was measured at 700 nm, and the increase in absorbance indicates the increase in the reducing power activity²⁴. Generally in this assay the reducing power increased when the concentration of *B. dalzielii* stem bark solution increased and also the standard, which was represented as absorbance at 700 nm versus sample concentration on plot. The IC_{50} value for ethanol stem bark extract is 18.37 $\mu\text{g/mL}$. The antioxidant capacity revealed in vitro of the stem bark can be directly related. The reducing power of plant extract is probably due to its richness in polyphenols but also to the structure of the plant, which can be used as an electron donor. Therefore, the action of free radicals and reactive oxygenated species at the origin of many diseases and food deterioration and spoilage²⁵ can be countered by polyphenols from plants (Fig 5)^{26, 27}.

Fig 5: Ferric reducing antioxidant power (FRAP) activity

4 Conclusion

The present study showed that the ethanol extract of stem bark of *B. dalzielii* contains the main chemical groups such as tannins, saponins, phenolics compound, alkaloids and flavonoids would be responsible for noticed antioxidant activities. In general, the antioxidant activity of medicinal plants is associated with total phenolic content. The ability of ethanol stem bark extract of *B. dalzielii* to act as hydrogen donors is indicated by their ability to scavenge DPPH free radicals, is comparable to that of ascorbic acid. Further research is required to fully investigate the mechanisms responsible for these observed responses.

5 Author's contributions

Author JV carried out literature review wrote the protocol and collected the plants sample. Author SDS was responsible for statistical work, calculations in addition to manuscript proofing and carried out discussion of the present study. All the two authors read and approved the final manuscript.

6 Acknowledgements

Nil.

7 Conflict of interest statement

We declare that we have no conflict of interest.

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