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Investigation of antioxidant activity of the ethanol extract of the resin exudates of trunk bark of *Boswellia dalzielii* Hutch (Burseraceae)

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Abstract

Antioxidant plays an important role in inhibiting and scavenging free radicals, thus, providing protection to human against inflammation diseases. Now the modern research is directed towards "Natural antioxidants" from the herbal plants due to safe therapeutic. The aim of the present research was to evaluate the antioxidant activity *in vitro* of the 95 % ethanol extract of the resin exudates of trunk bark of *Boswellia dalzielii* (EERBD). In addition, quantitative estimation of tannin content and phenolic content was also measured by colorimetric methods, using vanillin and Folin–Ciocalteu reagent, respectively. The antioxidant activity were determined by using different in vitro models including 2, 2-diphenyl-1-picrylhydrazyl (DPPH*), superoxide radical scavenging capacity and ferric ion reducing antioxidant power (FRAP) assays. The dosage of both phenols and tannins of EERBD revealed content in total phenols of 147 µg EAG/g Ex and total tannins of 0,0038 mg/g Ex respectively. Furthermore the EERBD exhibited the highest activity to DPPH scavenging activity with an IC50 value of 178.12 mg /mL and a FRAP was found to be 147,85 mg/mL for ascorbic acid and then showed the strong antioxidant activity. The strongest antioxidant activity of 95 % ethanol extract could be due to the presence of phenols. Thus, the screening of the ethanol extract for potential antioxidants as source of drugs for several diseases especially inflammation and cancers is illustrated. The assays performed pointed out the EERBD to be a rich source of natural antioxidant components.

1. Introduction

Human body is characterized by continuous production of free radicals and other reactive oxygen species due to aerobic metabolism. Oxidative stress occurs when redox homoeostasis within the cell is altered [1]. If the production of free radicals exceeds the antioxidant capacity of a living system, it is responsible for some degenerative diseases like inflammation, atherosclerosis, cancer and rheumatoid arthritis [2]. Furthermore, free radicals cause oxidative damage to macromolecules in the body, such as lipids, proteins and nucleic acids. Against these radicals, mammalian cells possess intracellular defenses such as super oxide dismutase, catalase and glutathione peroxidase which protects the cells against excessive free radicals [3]. Antioxidants are substances that mop up free radicals and prevent them from causing cell damage. Plants contain antioxidant compounds that function as free radical scavengers, reducing agents and quenchers of singlet oxygen formation [4] and are less

toxic as compared to their synthetic analogues like Butylated Hydroxyanisole (BHA) and Butylated Hydroxyltoluene (BHT), which have been reported to be carcinogenic and cause liver damage [5].

Boswellia dalzielii (Family: Burseraceae) is a tree plant, abundantly found in northern Cameroon, where the Mafa speaking people refer to it as *tenguété*. It has a smooth pale brown bark that is particularly separated by papery plates ragged [6, 7]. *B. dalzielii* is a very tall tree (more than 13 meters high), producing small fragrant and aromatic white lowers. The plant products (such as the resin) and different parts of the plant are widely employed in traditional medicine. Since a long time, the resin has been used throughout history to treat some maladies as a stomachic [8]. In West Africa, the bark has been reported to be used for the treatment of fever, rheumatism and gastro-intestinal problems [9]. In India, the traditional Ayurvedic medical system refers to the use of the gum extracted from *B. dalzielii*, which is recommended for arthritic and inflammatory conditions, gastric disorders, pulmonary diseases and skin ailments. It is also reported to have a strong action on the nervous system and reduces phlegm, asthmatic attack and stops vomiting [6]. The aim of the present study was to investigation of antioxidant activity of the ethanol extract of the resin exudates of trunk bark of *B. dalzielii*.

Although numerous studies have shown the medicinal values of this plant, there still remains ample scope for further in depth research. To the best of our knowledge, there is no published report of antioxidant potential of the ethanol extract of the resin exudates of trunk bark of *B. dalzielii*. Therefore, we report in the present study the in vitro antioxidant potential of EERBDby different procedures.

2. Materials and methods

2.1. Plant collection and authentication

The resin of *B. dalzielii* was collected from it local area of Mandaka Village, which is located at the Far North Region part of Cameroon during the month of December, 2017. The plant was identified by Professor Mapongmetsem Pierre-Marie, a Botanist in the Biological Sciences, Faculty of Science, University of Ngaoundéré, Cameroon and confirmed by comparison with a sample preserved at Cameroon National Herbarium where a voucher specimen (N° 20532/SRF-CAM, Yaoundé) has been deposited there.

2.2. Chemicals and reagents

Ethanol, Folin-Ciocalteu reagent, anhydrous sodium carbonate (Na_2CO_3), gallic acid, crystalline aluminum chloride, crystalline sodium acetate, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, Butylated Hydroxy Toluene (BHT), ascorbic acid. These reagents used for the investigation were of analytical grade.

2.3. Preparation of the plant extract

The resin of *B. dalzielii* Hutch (100 g) was extracted with 95 % ethanol (1000 mL) (v/v) by the maceration process for 24 h with intermittent shaking. The mixture was filtrated through Whatman sizes 1 filter paper. The filtrate obtained was concentrated at 40 °C using a HEILDOLPH brand (Germany) rotary evaporator. The concentrate kept in air tight bottle in a refrigerator at 4 °C when needed.

2.4. Quantification of polyphenols

2.4.1. Determination of the total phenolic content

The amount of total concentration of phenol was quantified in the EERBD by the Folin-Ciocalteu colorimetric to Talla *et al.* [10]. Gallic acid was used as standard for total phenolic content (TPC) estimation. The test consists of determining the oxidation of phenolic groups using a mix of phosphomolybdic($H_3PMO_{12}O_{40}$) and phosphotungstic acids ($H_3PW_{12}O_{40}$) in base medium, producing blue acids of tungsten and molybdenum. Shortly, 20 µL of the EERBD (10 g/mL) was combined with 200 µL of Folin–Ciocalteu reagent (diluted ten times) which was diluted with distilled water. After 3 min reaction, 400 µL of Na_2CO_3 solution (20 %, w/v) was added in to the mixture and vortexed. The mixture was allowed to stand for 20 min at 40 °C with intermittent shaking. After this reaction time, the test tube was incubated in 25 °C in the darkness during 30 min. The absorbance was then read

at 765 nm using the UV- visible spectrometer. Gallic acid was used as standard and plotted standard curve with various concentrations of gallic acid. The total phenolic present in the ethanol extract was expressed in mg equivalents gallic acid equivalents (GAE) per 100 mL EERBD and was calculated on the basis of the calibration curve of gallic acid.

2.4.2. Determination of the tannin condensed content

Total tannin content in EERBD was measured using the vanillin assay described by Ba et al. [11] with slight modifications to achieve optimum conditions for determination of the total tannin content. Shortly, a volume of 500 µL of ethanolic extract stock solution was mixed to 1000 µL of vanillin (prepared with methanol) solution (4 %, w/v) followed of 750 µL of concentrated HCl, and the solution was homogenized with vortex. Then, the sample mixture solution was kept at 25 °C in a dark at room temperature for 15 min. The absorbance at 500 nm was measured against the blank (methanol-acid mixture). Catechin was used as standard, positive control. The inhibitions of total tannin in percent (% T) were calculated according to the following equation:

$$T\ (\%) = 5,\ 2.10^{-2} \times DO \times V/P$$

Where % T= percentage of condensed tannin set,
$5,\ 2.10^{-2}$= constants expressed in Cyanidine equivalents,
DO= optical density,
V= volume of the extract used,
P= sample weight.

2.5. DPPH radical-scavenging activity

The antioxidant activity of the EERBD was evaluated by the 2, 2-diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging protocol, were performed according to Talla *et al*. (10) reviewed and adapted to the laboratory conditions. Briefly, 0, 5 mL of extract at different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL in methanol) were respectively added with 1 milliliter of freshly prepared DPPH methanolic stock solution (20 mg / L). The mixtures were thoroughly vortex-mixed and left to stand for 15 min in the dark at 37 ºC for any radical-antioxidant reaction to occur. Vitamin C was used as the antioxidant standard at concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. Then, the absorbance of these mixtures was read against a bank (without DPPH) at 517 nm at different time intervals until the reaction reached stability. The control experiment with a solution composed of 500 µL of pure methanol and 1000 µL of $DPPH^{\bullet}$ was used. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The inhibitions of DPPH radical in percent (% Inhibition) were calculated according to the following equation:

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

Where A_{blank} is the absorbance value of the control reaction (containing all reagents except the sample) and A_{sample} is the absorbance value of the EERBD or the standard, ascorbic acid. The extract concentration providing 50 % inhibition (IC50 values) of radical scavenging activity was calculated through interpolation of linear regression analysis and expressed as mg/mL.

2.6. Ferric reducing antioxidant power(FRAP)activity

The Ferric reducing antioxidant power procedure was followed to a modified method described by Bougandoura and Bendimerad [12]. Various concentrations of EERBD (50 to 1000 µg per mL) were 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 ($FeCl_3$) was prepared freshly in some distilled water and then the absorbance of reaction mixture was measured at 700 nm using a spectrophotometer (UV-VIS). Higher absorbance

of the reaction mixture indicates an important reducing power. As positive control, ascorbic acid was usedand results of total antioxidant content were expressed as absorbance reading.

2.7. Statistical analysis

Data obtained for each set of experiments were pooled and analysis was done using SPSS version. A single-factor Analysis of ANOVA (variance test) with a Dunnett's post-hoc was determined. The differences in the test- versus control-values were considered to be statistically significant at the 5 % (P-values < 0.05). The values are given as the mean ± standard deviation.

3. Results and discussion

In this study, ethanolic extract was chosen because on polarity basis, it is the nearest to the preparation used traditionally. Alcohol solvents are more capable of increasing the permeability of cell walls and facilitating the extraction of a greater number of polar molecules of both medium and low polarity [13, 14].

3.1. Determination of the total phenolic content

Phenolic is a kind of polyphenols that can be divided into tannin, propanoid and flavonoid. Phenolic compounds which may contribute directly to antioxidative action [15]. The total phenolic content (TPC) of the ethanol extract determined by the Folin-Ciocalteu method, was found to be 147 µg EAG/g Ex using the regression equation of calibration curve of Gallic acid: Y =11.67x + 0.008, R^2 = 0.995, where x is the absorbance and y is the tannic acid equivalent (GAE).

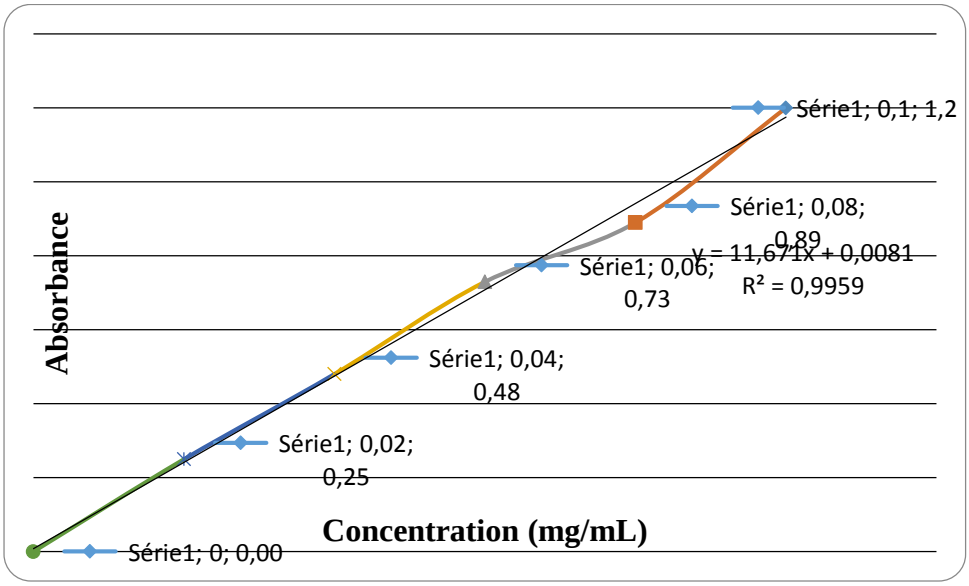

Figure 1: Standard curve of gallic acid

These results indicated the richness of the tested plant in phenolic. This is clear when Phenolic compound is natural antioxidant originates and have been found as strong antioxidants towards hindering the influence of free radicals and ROS, which is the basis of several chronic human infections [16].

3.2. Determination of the total tannin content

This method is based on the ability of vanillin to react with tannic units in the presence of acid.The percentage of total tannin content of the EERBD was 0.0038 mg/g Ex from total antioxidants content. The values presented as mean ± SD of three measurements.The quantity of this compound is important in justifying the antioxidative properties of the EERBD.

3.3. Free radical scavenging activity (DPPH)

The DPPH radical scavenging assay is usually recognized as one of the easiest assay to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible method [17], and to test the ability of compounds to act as free radical scavengers or hydrogen donors. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. The principle of this assay is usually laid on the hydrogen/electron donating ability of the antioxidant compounds to the reactive free and stable radical, DPPH for stabilization that resulted in the decolourization of the violet coloured DPPH solution [18].The ethanolic extract exhibited a strong scavenging activity against DPPH radical with IC50 values of 178.12 mg /mL, which is comparable to that of the ascorbic acid (179.12 mg / mL). To the best of our knowledge, there are no reports in the literature concerning the antioxidant activity of EERBD. The present results suggest that the extracts are apparently good free radical scavengers (especially of those of peroxy type) and probably have the ability to inhibit autoxidation of lipids and could thus be beneficial in the treatment of various diseases where lipid peroxidation is an important mechanism for pathogenesis [19]. The high capacity in ethanolic extract might be attributed to the presence of phytochemicals such as phenolic compounds, and tannins presented in our previous results, with high contents [20]. The found results could be explain the important ability of this extract to scavenging free radical such as ROS, inhibiting lipid peroxidation, avoiding DNA damage and prevent carcinogenesis processes [21].

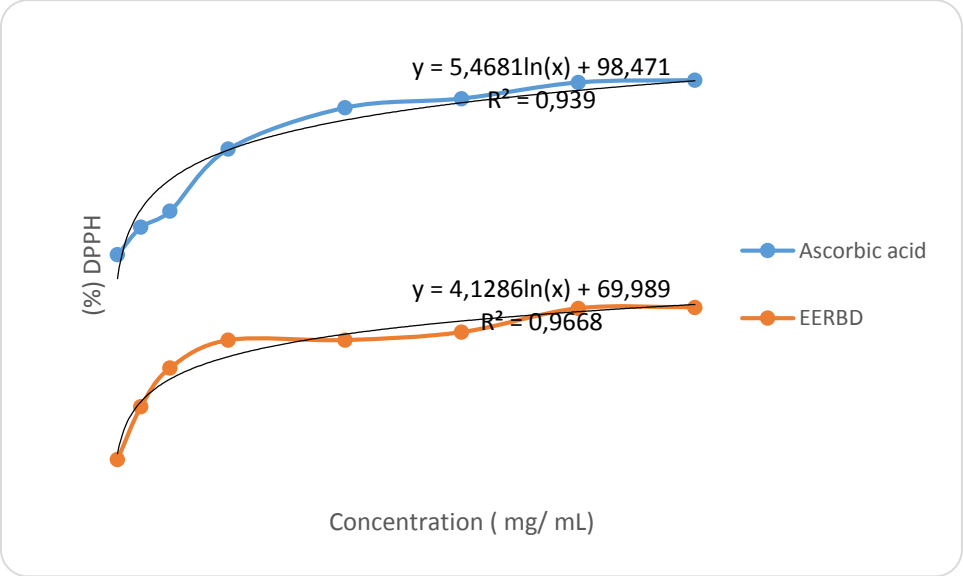

Figure 2: DPPH radical scavenging activity (% inhibition Vs concentration)

3.4. Ferric reducing/antioxidant power assay (FRAP)

Ferric Reducing Antioxidant Power (FRAP) assay is a quantitative assay for measuring the antioxidant potential within a sample. The iron colorimetric probe complex develops a dark blue color product upon reduction [22]. The ferric reducing antioxidant power (FRAP) assay measures the reducing ability of antioxidants against the oxidative effects of reactive oxygen species. This assay is responsible for the reduction of Ferric iron (Fe^{3+}), by electron-donating antioxidants present in the extract, to its ferrous form (Fe^{2+}). The addition of $FeCl_3$ to the ferrous form led to the formation of blue colored complex. Increasing absorbance indicates an increase in reductive ability. The reducing properties associated with the presence of compounds exert their action by breaking the free radical chain through donating a hydrogen atom [23]. The results of FRAP assays suggested that the ethanol extract possessed a strong antioxidant activity of 147.85 mg/mL. Furthermore, this activity increase progressively by increasing the concentration of extracts, this observed activity was dose-dependent. The ability of extract to reduce iron (FRAP) suggests that they contain compounds that are electron donors, which can react with free radicals to convert them to more stable products and terminate radical chain reaction [24].

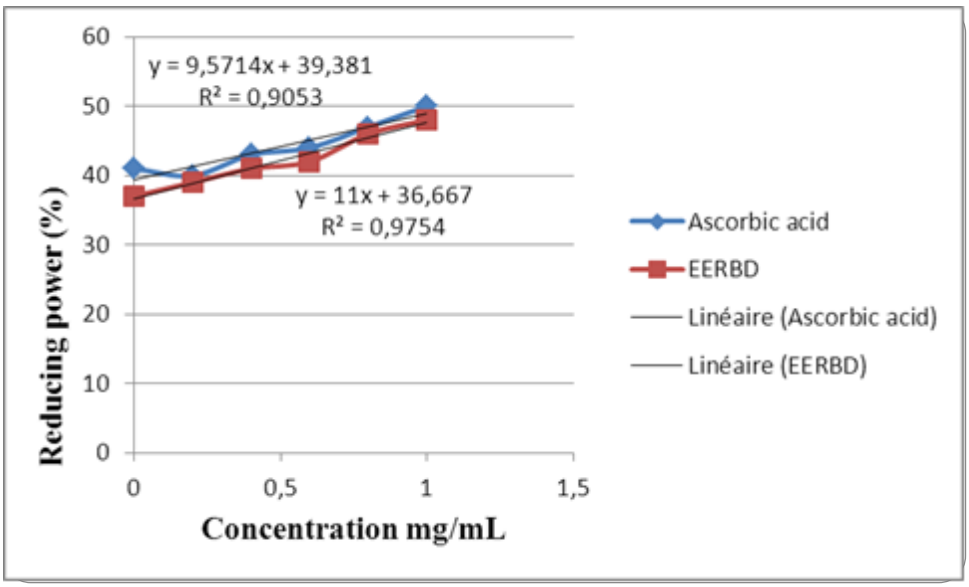

Figure 3: Reducing power assay

Conclusion

In this study, the antioxidant capacity of EERBD was determined by the in vitro antioxidant activity by DPPH radical-scavenging and Ferric reducing antioxidant power assays. Ascorbic acid at similar mass concentration was served as positive control. The ethanol extract of the resin showed a strong antioxidant activity by scavenging DPPH and FRAP methods with an IC50 value of 179.12 mg/mL and 147.85 mg/mL respectively. Furthermore, the extract was found to contain relatively high levels of total phenolic and tannins, which play a major role in controlling oxidation generated by free radicals. The results of the study showed that the ethanol extract of the resin exudates of trunk bark of *Boswellia dalzielii* has can be used as a prospective source of natural antioxidant.Therefore, further studies are needed to determine the mechanism behind the antioxidant activity of this plant.

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