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Research Article

IN VIVO ANTIOXIDANT CAPACITY AND ENZYMES OF INFLAMMATION INHIBITORY ACTIVITY OF N-BUTANOL FRACTION FROM *BOSWELLI DALZIELII* HUTCH (BURSERACEAE)

**Konaté Kiessoun^{1, 2, 4*}, Metuor Dabiré Amana³, Mamounata Diao¹,
Alain Souza⁴ and Mamoudou H. Dicko¹**

¹Laboratory of Food Biochemistry, Enzymology, Biotechnology and Bioinformatic,
University of Ouagadougou, 03 P.O.Box: 848, Ouagadougou 03, Burkina Faso

²University of Dedougou, Burkina Faso. Training and Research Unit in Applied and Technological Sciences,
P.O. Box 176, Dedougou, Burkina Faso

³Laboratory of Biology and Genetic Molecular, 01 P.O. Box 1091, Ouagadougou 01,
Burkina Faso, 03 BP 7021, Ouagadougou 03, Burkina Faso

⁴Laboratory of Animal Physiology, Electrophysiology and Pharmacology, Faculty of Sciences,
University of Science and Technology of Masuku, Franceville, Gabon

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ABSTRACT

Introduction: *Boswelli dalzielii* Hutch (Burseraceae) is a plant widely founded in the tropical region of Africa. The present study evaluated the *in vivo* antioxidant and enzymes of inflammation inhibitory profiles of n-Butanol fraction of stem bark from *Boswelli dalzielii*. **Material and Methods:** The *in vivo* antioxidant effects were evaluated using serum catalase, superoxide dismutase and thiobarbituric acid reactive substance assay methods. About enzyme of inflammation inhibitory activity, A5-LOX inhibitory, Hyaluronidase inhibitory and Xanthine oxidase inhibitory were determined. **Results:** Concerning the effects of n-Butanol fraction on the body weight of the treated rats. Fraction did not produce any significant ($P > 0.05$) difference in body weight gain in treated rats when compared to the negative control. About the *in vivo* antioxidant effect of n-Butanol fraction on rats, it is noticed that the fraction produced a dose dependent decrease in the MDA levels in the serum. The serum MDA level of the group treated with 300 mg/kg of n-Butanol was significantly ($p \leq 0.5$) lower when compared to other treatment groups and the negative control group. Fraction also produced a dose dependent increase in the serum level of catalase activity. The serum catalase activity of the group treated with 300 mg/kg of n-Butanol was significantly ($p \leq 0.5$) higher when compared to other treatment groups and the negative control group. Furthermore, fraction produced dose dependent increase in the serum level of superoxide dismutase activity. The serum superoxide dismutase activity of the groups treated with n-Butanol fraction were significantly ($p \leq 0.5$) higher when compared with the negative control group. **Conclusions:** These findings suggest that of stem bark from *Boswelli dalzielii* possess a potent antioxidant activity which may be responsible for some of its reported inflammatory activity and can be used as antioxidant supplement.

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INTRODUCTION

Medicinal plants have always been used and still remain a major source in the treatment of number of diseases including inflammatory and oxidative-stress associated chronic diseases. Oxidative stress and inflammation are two linked pathophysiological processes; stimulated by abnormal

production of free radicals and proinflammatory mediators causing extreme cellular damage which involves in the pathogenesis of human chronic diseases. Antioxidants act as a defence mechanism that protect against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system¹. Reactive oxygen species not only are produced naturally in cell following stress or respiration but

*Corresponding author: Konaté Kiessoun

Laboratory of Food Biochemistry, Enzymology, Biotechnology and Bioinformatic, University of Ouagadougou, 03 P.O.Box: 848, Ouagadougou 03, Burkina Faso

also have been reported to be produced by radiation, bacterial and viral toxin, smoking, alcohol, and psychological or emotional stress. Oxygen free radicals induced damage due to peroxidation to bio-membranes and also to DNA, which leads to tissue damage, thus cause occurrence of a number of diseases and biochemical disorders. Antioxidants neutralize the effect of free radicals through different ways and may prevent the body from various diseases. The antioxidants in biological system can be either enzymatic or nonenzymatic. The enzymatic antioxidants include catalase, superoxide dismutase, and glutathione which catalyze neutralization of many types of free radicals². Moreover, the excess of reactive oxygen species (ROS) generated will lead to inflammation by stimulating cytokines and activation of pro-inflammatory enzymes such as lipoxygenase, hyaluronidase, inducible nitric oxide synthase and xanthine oxidase³. Lipoxygenases are capable of generating lipid mediators such as leukotrienes and prostaglandins, which can provoke several inflammatory diseases such as bronchial asthma, allergic rhinitis, cardiovascular diseases, rheumatoid arthritis and certain types of cancer⁴. Hyaluronidase will lead to degranulation of mast cells and release inflammatory mediators leading to several pathological conditions including rheumatoid arthritis⁵. Xanthine oxidases also play a major role in the metabolic disease called gout, which is closely associated with inflammation and some other inflammatory mediated diseases due to the formation of free radicals during the catalytic function of the enzyme. It is evident that these pro-inflammatory enzymes play an important role in the pathogenesis of inflammation via different pathways. Hence, inhibition of these enzymes is considered as targets for the management of diseases associated with oxidative stress and inflammation⁶.

Anti-oxidants also play an important role in the management of inflammation. The efficacy of antioxidants and anti-inflammatory drugs derived from medicinal plants in the management of inflammatory diseases has been extensively documented. In this concern, medicinal plants are considered as valuable sources of potential therapeutic agents. A number of modern drugs have been isolated from medicinal plants based on the traditional use. There is an emerging interest in the use of natural products mainly those derived from medicinal plants in therapeutic applications⁷. In Burkina Faso, this Burseraceae is traditionally used against many metabolite diseases such as inflammation, cardiovascular diseases⁸. However, No so much scientific data is available hence, in this research article attempt has been made to focus the experimental work on *in vivo* antioxidant and enzymes inhibition of inflammation of n-Butanol fraction of *Boswelli dalzielii*.

MATERIAL AND METHODS

Plants materials

The vegetable materials (Fresh stem barks) of *Boswelli dalzielii* Hutch (Burseraceae) were collected in August 2014 in Dedougou, 230 Km West of Ouagadougou, capital of Burkina Faso. This plant was botanically identified by Dr. Traoré Lassina from the plants Biology Department of the University of Koudougou.

Animals Handling

Swiss NMRI mice (25-30 g) and Wistar albino rats (120-170 g) of both sexes were used for all tests. All animals were housed in cages under controlled conditions of 12 h light/and 12 h without light and 25°C. They received pellets of food enriched with 20% protein and water ad libitum. They were deprived of food for 15 h (but with access to drinking water) and weighed before the experiments. *In vivo* studies were carried out in accordance with guidelines for care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals⁹.

Extraction and fractionation

Fifty grams of powdered plant material was extracted with 80% aqueous acetone (500 mL) in 1/10 ratio (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavapor R-200, Switzerland) at approximately 40 °C. The aqueous extracts were subjected to sequential liquid-liquid extraction with n-hexane, dichloromethane, ethyl acetate and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain n-hexane fraction (n-HF), dichloromethane fraction (DCMF), ethyl acetate fraction (EAF) and n-butanol fraction. The different fractions were freeze-dried by Telstar Cryodos 50 freeze-dryer. The fraction residues were packed in waterproof plastic flasks and stored at 4 °C until use. After quantitative tests, it revealed that n-butanol fraction contents possess the best fraction than the other. Therefore, *in vivo* antioxidant and enzymes of inflammation inhibition properties should be evaluated with this fraction.

In vivo antioxidant profiling

Study design

Albino Wistar rats (120-170 g) of both sexes were randomly divided into four groups of five animals each. Group 1 served as the control and received 0.4 mL of distilled water. Group 2 received 75 mg/kg of n-Butanol fraction. Group 3 received 150 mg/kg of n-Butanol fraction, and group 4 received 300 mg/kg of n-Butanol fraction. The animals were dosed daily for 21 days and were observed daily for changes and other signs of toxicity and death throughout the period of study. Twenty-four hours after the last treatment, blood obtained through direct cardiac puncture was used to assay for *in vivo* antioxidant activity of n-Butanol fraction.

Experimental Methods

Serum Preparation

The blood used for serum preparation was collected via direct heart puncture with 21 G needle attached to 5 mL syringe, following mild chloroform anaesthesia of the rats. The serum was prepared using standard method as described by¹⁰. Briefly, the method used is as follows. Blood was allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes and serum was harvested.

Determination of the Lipid Peroxidation (LPO) in Serum

The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in

serum by the modified method as described by¹¹. The serum (50 µL) was deproteinized by adding 1 mL of 14% trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 g for 10 min, the absorbance of the colored product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56×10^5 mol/L/cm) using the formula, $A = \Sigma CL$, where A = absorbance, Σ = molar coefficient, C = concentration, and L = path length. The results were expressed in nmol/mg of protein.

Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase activity was assayed according to the method of¹². In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of enzyme providing 50% inhibition of NBT reduction. Results are expressed as U/mL.

Estimation of Catalase Activity

The catalase activity in serum was determined using the modified method as described by¹³. Briefly, the method is as follows: serum (10 µL) was added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of $0.041 \text{ mM}^{-1} \text{ cm}^{-1}$ was used to calculate catalase activity.

Enzyme inhibitory activity

Arachidonate 5-lipoxygenase (A5-LOX) inhibitory assessment

A5-LOX inhibitory activity of n-Butanol fraction was determined by a modified spectrometric method¹⁴. Fraction was assayed at the concentration of 200 µg/mL. Briefly, sodium phosphate buffer (110 µL, 100 mM, pH 8.0), Fraction dissolved in methanol (10 µL), and A5-LOX solution (55 µL) were incubated for 10 min at 25 °C followed by the addition of linoleic acid solution (25 µL, 0.08 mM). Absorbance was measured at $\lambda = 234$ nm for 10 min at 25 °C. Percentage inhibition of A5-LOX was determined by comparison of reaction rates of fraction relative to control using the formula $(E - S)/E \times 100$, where E and S are activities of the enzyme with and without fraction, respectively. Baicalein was used as the reference standard.

Hyaluronidase inhibitory assessment

Hyaluronidase inhibitory activity of n-Butanol fraction was evaluated by a spectrometric method with modifications¹⁵. Fraction was assayed at the concentration of 200 µg/mL. Fraction (50 µL) was incubated with hyaluronidase enzyme solution (10 µL) at 37 °C for 10 min followed by the addition of calcium chloride (12.5 mM, 20 µL) and re-incubation at 37 °C for 10 min. Sodium hyaluronate (50 µL) was added to the reaction mixture and incubated at 37 °C for 40 min followed by the addition of Sodium hydroxide (0.9 M, 10 µL) and Sodium

borate (0.2 M, 20 µL) and incubation at 100 °C for 3 min. p-Dimethylaminobenzaldehyde (PDMAB), (50 µL, 67 mM) was added to the reaction mixture and incubated at 37 °C for 10 min. Absorbance was measured at $\lambda = 585$ nm. Percent enzyme inhibition was calculated as given below, compared to the control. Tannic acid was used as the reference standard.

$$\text{Inhibition (\%)} = \frac{[(\text{Abs. control} - \text{Abs. sample})/\text{Abs. control}] \times 100}{100}$$

Xanthine oxidase inhibitory assessment

Xanthine oxidase inhibitory activity of n-Butanol fraction was determined by a kinetic method¹⁶ with slight modifications. Fraction was tested at the assay concentration of 200 µg/mL. Briefly, sodium phosphate buffer (150 µL, 50 mM, pH 7.4), fraction (10 µL) and xanthine oxidase solution (10 µL) were incubated at 25 °C for 10 min. The reaction was then initiated with the addition of xanthine solution (0.1 mM). Absorbance was monitored with the change of absorbance at $\lambda = 295$ nm for 15 min at 25 °C. Percentage inhibition of xanthine oxidase was calculated using the formula $(E - S)/E \times 100$, where E is the activity of enzyme without fraction and S is the activity of enzyme with fraction. Allopurinol was used as the reference standard.

Statistical analysis

The data were expressed as Mean ± Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p < 0.05$ and linear regression) was carried out with XLSTAT 7.1.

RESULTS

The effects of n-Butanol on the body weight of the treated rats

The result of the effects of n-Butanol fraction on the body weight of the treated rats is presented in Table 1. Fraction did not produce any significant ($P > 0.05$) difference in body weight gain in treated rats when compared to the negative control.

Table 1 The effects of n-Butanol fraction on the body weight of the treated rats.

Group	Day 0	Day 7	Day 14	Day 21
Distilled water 10mL/kg	152.18±5.07	168.12±4.54	172.71± 7.32	176.18±10.31
n-BF 75mg/kg	140.20±1.54	154.52± 2.80	166.21± 18.21	170.26±10.31
n-BF 150mg/kg	153.52±4.21	157.26±7.61	168.18±10.31	172.22±9.31
n-BF 300mg/kg	154.54±7.36	163.58±9.31	170.37±1.54	174.20±4.01

No statistical difference ($P > 0.05$) compared with control group.

The in vivo antioxidant effects of n-Butanol in rats

The results of the in vivo antioxidant effect of n-Butanol fraction on rats are presented in Table 2. The fraction produced a dose dependent decrease in the MDA levels in the serum. The serum MDA level of the group treated with 300 mg/kg of n-Butanol was significantly ($p \leq 0.5$) lower when compared to other treatment groups and the negative control group. Fraction also produced a dose dependent increase in the serum level of catalase activity. The serum catalase activity of the group treated with 300 mg/kg of n-Butanol was significantly ($p \leq 0.5$) higher when compared to other treatment groups and the negative control group. Furthermore, fraction produced dose dependent increase in the serum level of superoxide dismutase

activity. The serum superoxide dismutase activity of the groups treated with n-Butanol fraction were significantly ($p \leq 0.5$) higher when compared with the negative control group.

Table 2 The in vivo antioxidant effects of n-Butanol fraction in rats (mean \pm SEM).

Groups	Catalase ($\mu\text{mol}/\text{mg}$ protein)	MDA (nmol/mg protein)	SOD (unit/mL)
Distilled water 10 mL/kg	22.71 \pm 10.54	0.116 \pm 0.03	20.00 \pm 0.58
n-BF 75 mg/kg	30.22 \pm 4.09	0.109 \pm 0.01	23.00 \pm 0.54*
n-BF 150 mg/kg	44.18 \pm 11.31	0.095 \pm 0.05	27.00 \pm 0.00*
n-BF 300 mg/kg	121.69 \pm 13.71*	0.022 \pm 0.05*	31.00 \pm 0.54*

P < 0.05 are statistically significant when compared to distilled water treated group.

Enzyme inhibitory activities of n-Butanol fraction

The results revealed that, n-Butanol fraction had the highest A5-LOX inhibitory activity (Figure 1). The activity of n-Butanol was found to be significantly different from the positive control baicalein, which showed a strong dose dependent activity against A5-LOX enzyme activity.

Concerning hyaluronidase inhibitory activity, we noticed that n-Butanol fraction showed moderate activities (Figure 1) compared to the reference standard tannic acid and good, comparable activities when compared with the reported activity of indomethacine (50%, 500 $\mu\text{g}/\text{mL}$) a clinical drug in use against inflammation. n-Butanol fraction which showed the highest xanthine oxidase inhibitory activity and we notice a dose dependent inhibitions (Figure 1).

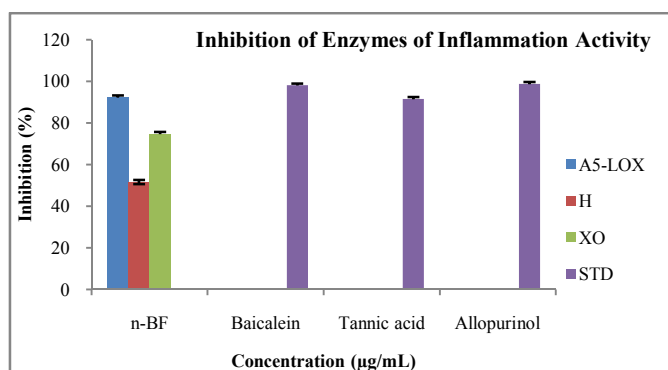


Figure 1 Arachidonate 5-lipoxygenase (A5-LOX), Hyaluronidase (H) and Xanthine oxidase (XO) Inhibitory Activities of n-Butanol Fraction (n-BF) of stem bark from *Boswellia dalzielii* Hutch (Burseraceae) and Standards.

Inhibition (%) of A5-LOX, Xanthine Oxidase and Hyaluronidase is recorded at 200 $\mu\text{g}/\text{mL}$ assay concentrations respectively. Results are presented as mean \pm SE (N = 3). Different letters indicate significant difference ($p < 0.05$)

DISCUSSION

Traditionally, herbal medicines with antioxidant properties have been used for various purposes and epidemiological data also points at prevalent acceptance of use of these agents. Nowadays, research in the field of natural products has increased considerably in finding naturally occurring antioxidants to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity. Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing cellular damage in the biological system¹⁷. The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body also relies on external sources, primarily the diet, to

obtain the rest of the antioxidants it needs¹⁸. These exogenous antioxidants are commonly called dietary antioxidants and those are found in fruits, vegetables, and grains¹⁹. The *in vivo* antioxidant assay showed that the extract increased the activity of serum superoxide dismutase (SOD) and catalase and decreased the serum level of TBARS. Catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production²⁰. The SOD catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite²¹. The increased serum activities of catalase and SOD as observed in this study suggest that the extract has an *in vivo* antioxidant activity and is capable of ameliorating the effect of ROS in biologic system²². Also, ROS react with all biological substance; however, the most susceptible ones are polyunsaturated fatty acids. Reactions with these cell membrane constituents lead to lipid peroxidation (LPO)²³. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptor²⁴. Thiobarbituric acid reactive substance (TBARS) levels were measured as a marker of LPO and malondialdehyde (MDA) production. Malondialdehyde is an endogenous genotoxic product of enzymatic and ROS-induced LPO whose adducts are known to exist in DNA isolated from healthy human being²⁵. In our study, the level of TBARS in the extract treated groups decreased in a dose dependent manner when compared to control. This decrease in the TBARS levels may indicate increase in the activities of glutathione peroxidase and hence inactivation of LPO reactions²⁶. Some of the phytochemical constituents of the extract may be responsible for the antioxidant activity as demonstrated in our study. Flavonoids or bioflavonoids are a ubiquitous group of polyphenolic substances which are present in most plants, concentrated in seeds, fruit skin or peel, bark, and flowers²⁷. Numerous studies have shown that flavonoids possess potent antioxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals²⁸. A recent study attributed the pharmacological activities of flavonoids to their potent antioxidant activity⁴. Concerning anti-inflammatory potential, our results could be justified by the presence of polyphenols contains in the fraction. In effect, it well known that the stem bark of *Boswellia dalzielii* has been traditionally used to treat rheumatoid arthritis, which is mediated by inflammation²⁹. According to previous studies, The extracts of this plant are known to possess a broad range of pharmacological activities including anti-inflammatory properties⁸. Therefore, from the findings of this experiment it can be concluded that the fraction have a noticeable anti-inflammatory effect against inhibition of enzyme of inflammation *in vivo*.

CONCLUSION

We have demonstrated that the fraction of *Boswellia dalzielii* showed potent anti-inflammatory and radical scavenging activity. This fraction can be explored for its applications in the prevention of free radical related diseases. The overall activity is due to the type of phenolics groups present and may be some unidentified antioxidants and anti-inflammatory compounds. The fraction allows us to conclude that it is a good candidate for

further studies of activity-monitored characterisation to identify their active components.

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