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Research Paper

IN VITRO ANTIOXIDANT ACTIVITY AND ANTI INFLAMMATORY ACTIVITY OF METHANOLIC LEAF EXTRACT OF *BOSWELLIA SERRATA*

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Phytochemical analysis, *in vitro* antioxidant activity and *in vitro* inflammatory activity of leaf extract of *Boswellia serrata* were studied. The methanolic leaf extract (methanolic) was tested for the presence of phytochemicals. *In vitro* antioxidant activity of extract was evaluated by using DPPH free radical (1, 1-diphenyl-2-picryl-hydrazyl) scavenging assay, nitric oxide assay and reducing power methods and anti inflammatory activity was evaluated by HRBC method. Phytochemical screening reveals the presence of saponins, tannins, anthraquinones, terpenoids, and flavonoids. The extract of *Boswellia serrata* had shown good DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity. Ascorbic acid was used as standard antioxidant and positive control. The DPPH radical scavenging activity of the extract was increased with the increasing concentration, the reducing power of extract was carried out with ascorbic acid as a standard reducing agent. The methanolic extract of *Boswellia serrata* exhibited higher scavenging and reducing power and anti-inflammatory activity. All the analysis was made with the use of UV-VIS Spectrophotometer (Hitachi U2000, India). These results were an indication of antioxidant potential of the *Boswellia serrata* leaf extract and may be responsible for some of the therapeutic uses.

Keywords: Antioxidant activity, Anti inflammatory activity, *Boswellia serrata*

INTRODUCTION

Plants have been used as alternative remedy for the treatment of various ailments since ancient times. In recent years considerable research has been progressed in the exploitation of medicinal plants, in the treatment of various stress related disorders caused by metabolism of oxygen leads to generation of free radicals. Free radicals are atoms or groups containing one unpaired electron, making them highly reactive. The potentially reactive derivatives of oxygen are known as reactive oxygen species (ROS) (e.g., superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide radicals), which induces oxidative damage to various biomolecules including, proteins, lipids, lipoproteins and DNA and also reported to be

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involved in the numerous diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases, inflammatory diseases and also in the ageing process (Halliwell and Gutteridge, 1985). In order to protect against free radicals, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/ reductase) and exogenous (vitamins C and E, α-carotene, uric acid) defences; yet these defense systems are not sufficient in critical situations (oxidative stress and UV exposure, etc). In recent years, there has been growing interest in alter-native therapies and the therapeutic use of natural products, especially those derived from plants (*Goldfrank et al.*, 1982; Vulto and Smet, 1988; Mentz and Schenkel, 1989). Therapeutic potential of plants is mainly due to the presence of bioactive compounds. Among different bioactive compounds poly phenols are antioxidants responsible for the prevention of chronic diseases and health care (Sati *et al*., 2010). Antioxidants are vital substances because they can protect the body from the damage caused by free radicals. Antioxidants exert their effect by scavenging the free radicals (i.e. reactive oxygen species (ROS) or reactive nitrogen species) universally present in biological systems (Wilson, 1988).

Boswellia serrata (*Burseraceae*) also known as "Indian frankincense" is a native of India especially available in Rajasthan, Madhya Pradesh and Andhara Pradesh. Traditionally the plant is reported to have antiulcer activity, which is used for treatment of various ailments such as arthritis. Extracts of *Boswellia serrata* have been clinically studied for osteoarthritis and joint function, particularly for osteoarthritis of the knee (Kimmatkar *et al*., 2003). Positive effects of Boswellia in some chronic inflammatory diseases including rheumatoid arthritis, bronchial asthma, osteoarthritis, ulcerative colitis and Crohn's disease have been reported (Ammon, 2010). Boswellic acid, an extract from *Boswellia serrata*, has been studied for anti-neoplastic activity, especially in experimental primary and secondary brain tumors, indicating potential efficacy from *in vitro* (Xiufeng *et al*., 2009; Jian and Rui dong, 2009) and limited clinical research (Flavin, 2007). *Boswellia serrata* has potent analgesic and anti-inflammatory effects that can reduce the pain and inflammation of joints (Memon, 1971). In view of several drawbacks of synthetic compounds, preparations of plant origin have received increasing attention.

MATERIALS AND METHODS

Collection of Plant Material

Leaves of *Boswellia serrata* were collected from Tirumala hills, Andhra Pradesh, India. The leaves were shade dried and ground into powder with the aid of blender and stored in air tight bottles at room temperature till use.

Preparation of Extract

The powder was extracted sequentially with methanol using cold process followed the method of (Boakye-Yiadom, 1979). In the cold percolation, 20 g of the dried leaf powder was weighed out, transferred into a beaker, and 100 ml of solvent was added. The mixture agitated and allowed to extract at laboratory temperature for 48 h. The mixture was then filtered in a flask, using Whatman's No 1 filter paper. The filtrate was evaporated at 40^{o}C on a hot plate. The concentrated extracts were then allowed to cool and stored in a sterile bottle for further analysis.

Preliminary Phytochemical Analysis

Phytochemical analysis was performed using the methods described by Harbone (1973) and Trease and Evans (1983) were used to identify alkaloids, saponins, tannins, flavonoids, terpenoids and glycosides.

In Vitro Antioxidant Assays

The antioxidant activity of methanolic leaf extract was determined by different *invitro* methods such as, DPPH free radical scavenging assay, nitric oxide assay and reducing power methods. The extract dissolved in methanol (0.5 mg/ml). All the assays was carried out in triplicate and average value were recorded.

DPPH Free Radical Scavenging Activity

The effect of extract on DPPH radicals were estimated according to the method of Blois (1958) with minor modifications. 4 ml (0.004% w/v) of DPPH solution was mixed with 1.0 ml of extract (in methanol). The reaction mixture was vortex-mixed thoroughly and incubated at room temperature in the dark for 30 min. Reduction in the absorbance of the mixture was measured at 517 nm using ascorbic acid as a control. Scavenging of DPPH radicals by the extract was calculated using the following formula:

% DPPH free radical scavenging =

$$\frac{(\text{Absorbance of control-Absorbance of test})}{\text{Absorbance of control}} \times 100$$

where Abs control is the absorbance of DPPH and Abs- test is the absorbance of the DPPH radical + sample extract/standard. The half maximal inhibitory concentration (IC50) values denoted the concentration of sample required to scavenge 50% of DPPH free radicals.

Nitric Oxide Scavenging Assay

The nitric oxide radical scavenging activity of extract was determined using the method of Sreejayan and Rao (1997) with minor modifications. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions determined by the Griess reagent. 2 ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations (25 to125 µg/ml). The mixture was incubated at 25ºC. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm. Ascorbic acid was used as standard. The amount of nitric oxide radicals scavenged was calculated as described in DPPH assay.

Reducing Power Assay

The reducing power was determined by the method of Oyaizu (1986). Various concentrations of the extract (25 to 125 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (1%) (2.5 ml). The mixture was incubated at 50^{0}C for 20 min. Aliquots of trichloroacetic acid (10%) (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.1%) (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (25 to125 µg/ml) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

In Vitro Anti-inflammatory Activity

Human red blood cell method was used for the estimation of anti-inflammatory activity *in vitro* (Azeem *et al*., 2010). Blood was collected from healthy volunteers and were mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by spectrophotometer at 560 nm. The percentage of hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage Protection = 100 – (OD sample/OD Control) x 100

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Phytochemical screening reveals that methanolic extract showed the presence of Alkaloids, Terpenoids, Saponins and Flavonoids (Table 1).

DPPH Free Radical Scavenging Activity

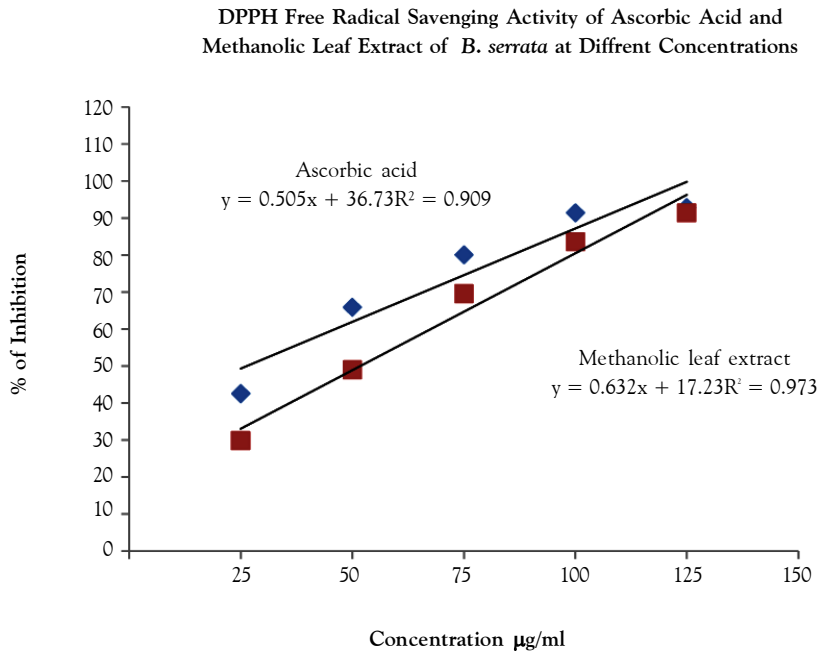
The scavenging activity of methanolic extract of *Boswellia serrata* and ascorbic acid on the DPPH radical was illustrated in Figure 1. Methanolic extract has significant scavenging effect on DPPH, it was increased with the increasing concentration from 25-125 µg/ml but the scavenging activity of the extract was lower than that of standard. Figure 1 represents the percentage of inhibition of ascorbic acid and methanolic extract of leaves of *Boswellia serrata.* IC 50 values are of ascorbic acid and methanolic leaf extract were found to be 26.02 µg/ml and 54.06 µg/ml respectively. Basically, a higher DPPH radical-scavenging activity was associated with a lower IC50value. DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay (Bozin

Table 1: Phytochemical Screening of Methanolic Leaf Extract of *B. serrata*

S.No.	Phyto Constituents	Methanolic leaf extract
1.	Alkaloids	+
2.	Terpenes and steroides	+
3.	Tannins	+
4.	Saponins	+
5.	Flavonoids	+
6.	Carbohydrates	+
7.	Glycosides	+

Note: – = Negative (absent); + = Positive (present).

Figure 1: DPPH Free Radical Savenging Activity Methanolic Leaf Extract of *B.serrata*



et al., 2008). This assay was known to give reliable information concerning the antioxidant ability of the tested compounds (Huang and Prio, 2005).

Nitric Oxide Scavenging Activity

Methanolic extract of *Boswellia serrata* exhibited good nitric oxide scavenging activity. Aqueous extract of both the plants inhibited nitrite formation in concentration dependent manner. This may be due to the presence of antioxidant principles in the extract, which complete with oxygen to react with nitric oxide. The results were expressed as percentage (%) inhibition exhibited by the test substances and the standard (Figure 2) IC50 value was calculated in each case. It was observed that, the percentage inhibition was increased with the increase in concentration of the extract. IC50 value for scavenging of Nitric oxide by the methanolic extract of *B.serrata* was found to be 62.12 µg/ml. While for ascorbic acid it was found to be 47.10 µg/ml.

Reducing Power Assay

The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir *et al*., 1995). Reducing power was to measure the reductive ability of antioxidant, and it was evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts (Gulcin *et al*., 2003). Methanolic extract of *Boswellia serrata* exhibited good reducing power was summarized in (Figure 3). High absorbance indicates high reducing power. Reducing power of the methanolic extract was dose dependent. These results corroborate with the findings of Gülçin *et al.* (2003) and Noriham *et al*. (2004), who demonstrated antioxidative activity on *Pimpinella anisum* seed extracts and four types of Malaysian plants. The EC 50 values were found to be 121.0 µg/ml and 95.0 µg/ml of ascorbic acid and methanolic leaf extract of *B. serrata*

Figure 2: Nitric Oxide Savenging Activity of Methanolic Leaf Extract of *B. serrata*

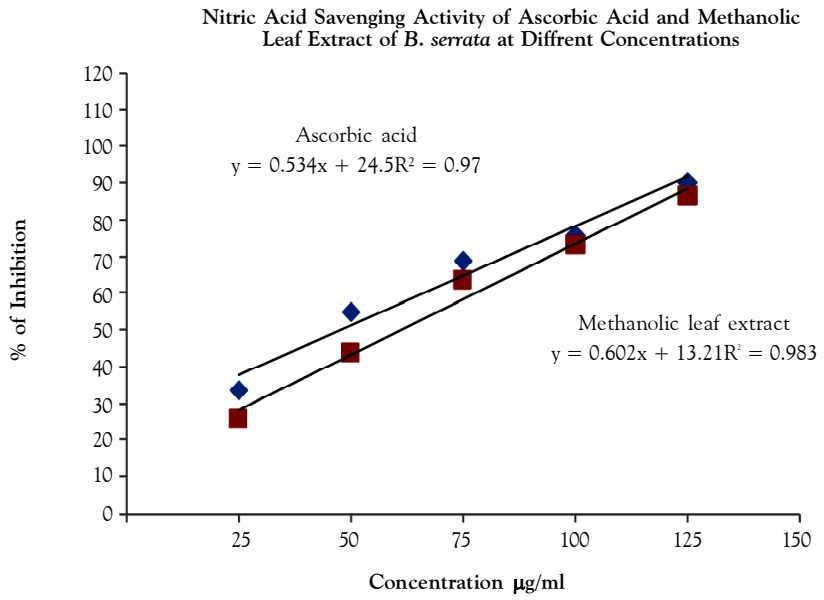
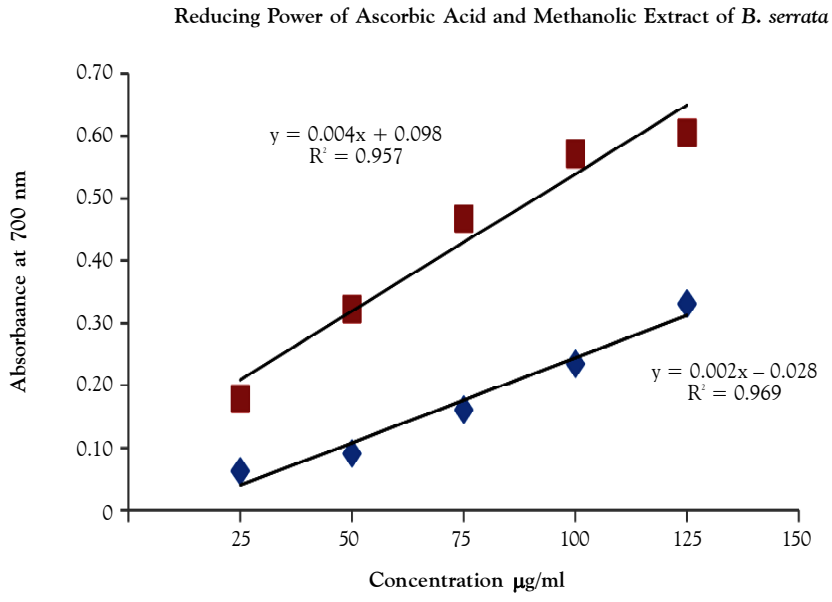


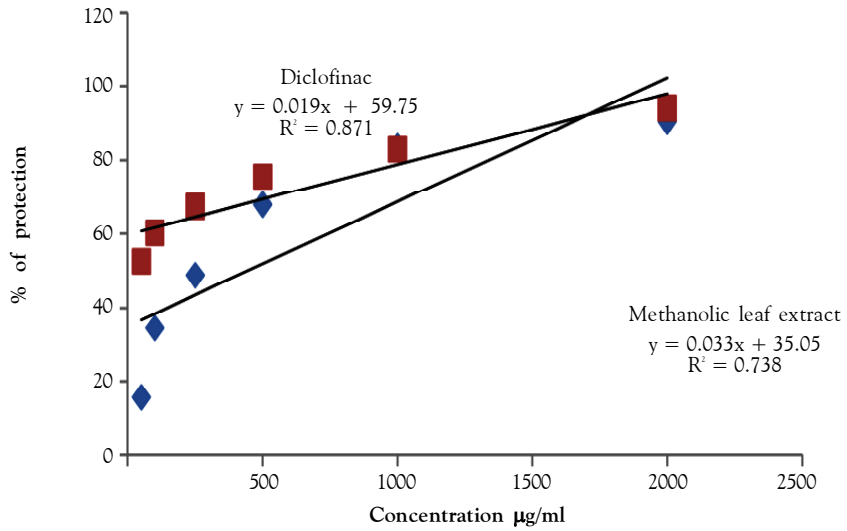
Figure 3: Reducing Power Assay of Methanolic Leaf Extract of *B. serrata*



In Vitro Anti-Inflammatory Activity

B.serrata extracts at different concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) showed significant stabilization towards HRBC membranes. The percentage protection of methanolic extract was higher at 2000 µg/ml. (Figure 4). The anti-inflammatory effect of this extract may be due to the presence of flavonoids and saponins. Flavonoids and steroids show remarkable anti-inflammatory activity by inhibiting

Figure 4: *In Vitro* Anti-inflammatory Activity of Methanolic Leaf Extract of *B. serrata*



the cox and lox systems [Robet *et al*., 2001; Tapas *et al*., 2008). The correlation between presence of flavonoids and their membrane stabilizing ability was also observed (Sankari *et al*., 2009).

CONCLUSION

This study reveals that, the methanol fraction prepared from *B. serrata* leaves contains high amounts of total phenolics and total flavonoids and it exhibited strong reducing power and antioxidant activity and antiinflamatory activity. Thus, methanol seems to be most promising solvent for extraction and isolation of natural antioxidative compounds form *B. serrata* leaves. Further studies in isolation of individual phenolic compounds particularly flavonoids in this fraction and its effect on antioxidant in animal models are needed to evaluate their potential benefits.

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