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EVALUATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY OF 70% ETHANOLIC EXTRACT OF BARK OF *BOSWELLIA OVALIFOLIOLATA LINN.*

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ABSTRACT

Objective: To evaluate the *in-vitro* antioxidant capability of the 70% ethanolic extract of the bark of *Boswellia ovalifoliolata Linn.*

Method: *In-vitro* antioxidant activity was evaluated by studying total phenol content , flavonoid content, DPPH radical scavenging activity, nitric oxide scavenging activity, hydrogen peroxide scavenging activity, reducing power, super ion scavenging activity and metal chelating activity. Gallic acid, ascorbic acid & Butylated hydroxy toluene & quarcetin were used as standard for all the experiments.

Results: IC50 values observed for studying total phenol content, flavonoid content, DPPH radical scavenging activity, nitric oxide scavenging activity, hydrogen peroxide scavenging activity, reducing power, super ion scavenging activity and metal chelating activity were determined to be 44.8, 41.75, 223.94, 259. 24,289.63, 271.78, 287.53 & 283.59. Each experiment was carried out in triplicate and results are expressed as mean ± SD.

Conclusions: The results clearly indicate that the 70% ethanolic extract of bark of *Boswellia ovalifoliolata* of the study species was effective in scavenging free radicals and has the potential to be a powerful invitro antioxidant.

Key words: *In-vitro* antioxidant activity, *Boswellia ovalifoliolata Linn*

1. INTRODUCTION

Free radicals have been implicated in the causation of several problems like asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory disease, liver disease, muscular degeneration and other inflammatory process[1]. Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal conditions; they are scavenged and converted to non -reactive species by different intracellular enzymatic and non-enzymatic antioxidant system [2]. Reactive oxygen species, causing damage to DNA, proteins and lipids have been associated with carcinogenesis, coronary heart disease, and many other health problems[3].

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Reactive oxygen species produced by sunlight, ultraviolet, ionizing radiation, chemical reactions and metabolic processes were considered as important factors in the etiology of several pathological conditions[4].Over production or an ineffective elimination of ROS may induce oxidative stress and cause damage to all types of biomolecules such as proteins, lipids and nucleic acids[5].Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems [6]. When generation of ROS over takes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to a number of physiological disorders[7]. Plants are the primary sources of naturally occurring antioxidants for humans. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules.

The most common reactive oxygen species (ROS) include superoxide (0^{2-}) anion, hydrogen peroxide (H_20_2),peroxyl (ROO^-) radicals and reactive hydroxyl (OH^-) radicals . The nitrogen derived free radicals are nitric oxide (NO) and per oxy nitrite anion (ONOO). Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. They may reduce the energy of the free radical or suppress radical formation or break chain propagation or repair damage and reconstitute membranes[8]. Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and or activators of anti oxidative defense enzyme system to suppress the radical damages in biological systems [9-10]. In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance [11-13].

Natural antioxidants present in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which react with other molecule by taking or giving electrons, and involved in many pathological conditions [14]. Currently available synthetic antioxidants like BHT, butylated hydroxyl anisole (BHA) and tertiary butylated hydroquinone's have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants [15].

India is a botanical garden of the world and a goldmine of well recorded and traditionally well practiced knowledge of medicinal plants. More than 6000 plants in India including endemics are used in traditional folk and herbal medicine representing about 75% of them medicinal need of the third world countries [16]. India represents 7% of the worlds flora and about 40,000 species of flowering plants and endemic to India [17]. 40 hotspots of Indian flora are located with concentration of endemics as a major criteria .The study area Seshachalam hill range is a part of 11th hotspot of India[18].

The Seshachalam hill range is situated between $79^0 19^1$and $79^0 23^1$ East longitudes and $13^0 37^1$to $13^0 43^1$ North longitudes. *Boswellia ovalifoliolata* belongs to the family Burseraceae and is included in the Andhra Pradesh Red data book as a critically threatened plant [19].The leaves, bark and gum which are highly medicated. The plant is a deciduous and medium sized tree which can grow up to 10 m tall with imparipinnate, ,alternate leaves & crowded at the end of the branches. Branchlets, veins and rachis are reddish in colour. The plant bark is grayish, smooth and papery exfoliating into thin slakes. Flowers are cream coloured to greenish white in monochasial cymes.

Flowers with 10 stamens, alternatively long and short are inserted on the disc. Fruit is trigonous drupe with 3 pyrines each with one seed. Seeds are winged and compressed [20]. The studies revealed that the Boswellia ovalifoliolata consist of variety of secondary metabolites. It contains Alkaloids -, flavonoids, Steroid, Phenols, saponins, tannins & amino acids [21]. The Boswellia ovalifoliolata is to treat number of ailments such as ulcer, diabetic, jaundice, inflammation & arthritis. No reports are available on the *In vitro* antioxidant activity of Boswellia ovalifoliolata. Hence, the present study focuses on the scientific investigation of *in vitro* antioxidant activity of Boswellia ovalifoliolata.

Boswellia ovalifoliolata

Fig.1: *Boswellia ovalifoliolata* BAL & Henry: (Indian herbal Pharmacopoeia, 2002)

2. MATERIALS AND METHODS

2.1. *Chemicals and Standards*

DPPH (1,1-diphenyl-2-picryl hydrazyl), Gallic acid, ascorbic acid, Follin-Ciocalteu's phenol reagent and BHT (Butylated hydroxyl toluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (petroleum ether , chloroform, ethyl acetate, ethanol,), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride, hydrochloric acid, copper sulphate, aluminium chloride, lead acetate, acetic acid & ammonia from Merck (Pvt.) Ltd. (Germany).

2.2. Plant material

The fresh bark of Boswellia ovalifoliolata were collected in December 2009 from Seshachalam hills of Tirupathi, Andhra pradesh (India) and authenticated by Dr. K. Madhav Chetty, Assistant Professor, Taxonomist, Dept. of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh [22]. A voucher specimen has been kept in our laboratory for future reference

2.3 Preparation of extract

The whole plant was cleaned, air dried and grounded into powdered. The dried powdered plant material was passed through sieve 40 and stored in air tight containers. The powdered material was subjected to successive solvent extraction using petroleum ether, benzene, chloroform, ethyl acetate, ethanol & water. Initially 100 gms of crude powder was taken and packed in a packing paper. This pack was placed in a soxhlet extractor for extraction with different solvents i.e. (Petroleum ether, chloroform, ethyl acetate, ethanol & water) and the temperature was adjusted as per the solvent been used in the extraction. The extract is then concentrated and dried under reduced pressure. The percentage yield obtained was calculated and reported [23 – 24].

2.4. Preliminary phytochemical analysis

The preliminary phytochemical studies were conducted on the active extracts using standard procedures adopted by Harborne (1973) and Gibbs (1974). Preliminary phytochemical analysis on plant extracts was performed using the following chemicals and reagents: flavonoids (Mg metal and HCl), phenolics (FeCl3), protein and amino acid (Millon's and Ninhydrin reagent), alkaloids (Mayer and Dragendorff's reagent), saponins (Foam test), phytosterols, triterpenoids (Liebermann- Burchard Test) and carbohydrates (Fehling's solution A and B) [25-27].

2.5. Determination of antioxidant activity

The antioxidant activity was evaluated by eight ways which are as follows;

2.5.1. Total poly phenol content determination

Total phenolic contents in the extracts were determined by the Folin-Ciocalteu reagent method[28]. 1 ml of the plant extracts/standard of different concentration solution was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (7.5% sodium carbonate) of sodium carbonate[28]. The tubes were vortexed for few seconds and allowed to stand for 30 min at 20°C for color development. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. Absorbance of samples and standard were measured at 765 nm using spectrophotometer against blank. A typical blank solution contained the solvent used to dissolve the plant extract. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent of dry extract. All determination was performed in triplicate

2.5.2. Determination of total flavonoids content

Aluminum chloride colorimetric method was used for flavonoids determination[29]. 1 ml of the plant extracts/standard of different concentration solution was mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank. Quarcetin was used as standard for the calibration curve.

Total flavonoid content of the extracts and fractions were expressed as mg quercetin equivalents (QE) per gram of sample (mg/g).

2.5.3. Free radical scavenging activity (DPPH method)

The scavenging activity for DPPH free radicals was measured[30]. Ethanolic solution of the sample extract at various concentrations (50, 100, 150, 200 and 250 µg/mL) was added separately to each 5 mL of 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27^0c. After incubation, the absorbance of each solution was determined at 517 nm using spectrophotometer. Ascorbic acid & BHT (butylated hydroxy toluene) was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} * 100$$

IC_{50} value is the concentration of the sample required to scavenge the 50% DPPH free radical.

2.5.4. Nitric oxide scavenging capacity assay

Nitric oxide scavenging assay was carried out using sodium nitroprusside[31]. This can be determined by the use of the Griess illosvoy reaction. 2 ml of 10 mM sodium nitro prusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub-fraction at various concentrations and the mixture was incubated at 25°C for 150 min.

From the incubated mixture, 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 min. Finally, 1.0 ml naphthyl ethylene diamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 min.

The absorbance was measured at 546 nm. A typical blank/control solution contained the same solution mixture without plant extract or standard. The absorbance of the blank/control solution was measured at 546 nm. The percentage inhibition was calculated according to the following equation:

$$\text{(\%) of inhibition} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} * 100$$

2.5.5. Scavenging of hydrogen peroxide

Scavenging activity of extract and its sub-fractions were evaluated by hydrogen peroxide[32]. 1 ml of various concentrations of the extract, sub-fractions and standards in ethanol was added to 2 ml of hydrogen peroxide solution in phosphate buffered saline (PBS, pH 7.4). Then finally the absorbance was measured at 230 nm after 10 min. Ascorbic acid and BHT were used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance were read at 230 nm using a spectrophotometer. The percentage inhibition was calculated according to the following equation:

Hydrogen peroxide scavenging activity (%) was calculated as:

$$\frac{Ao - AS}{Ao} X\ 100$$

Where, AO = absorbance of Control;
AS = absorbance of sample

2.5.6 Reducing power capacity assessment

Assay of reducing power was carried out by potassium ferricyanide method[33]. 1 ml of extract and its sub-fractions (final concentration 5 to 200 g/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. To this mixture, 2.5 ml of trichloro acetic acid was added which was then centrifuged at 3000 rpm for 30 min. Finally, 2.5 ml of the supernatant solution was collected and mixed with 2.5 ml of distilled water and 0.5 ml ferric chloride and absorbance was measured at 700 nm. Ascorbic acid and butylated hydroxy toluene (BHT) were used as standard and phosphate buffer as blank solution. The reducing power capacity assessment was calculated as:

$$\frac{Ao - AS}{Ao} X\ 100$$

Where, AO = absorbance of Control;
AS = absorbance of sample

2.5.7. Superoxide Anion Radical Scavenging Activity

Superoxide Anion Radical Scavenging activity[34] was measured with some modifications. The various fractions of plant extracts were mixed with 3 ml of reaction buffer solution (pH, 7.4) containing 1.3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction solution was illuminated by exposure to 30 W fluorescent lamps for 20 minutes and the absorbance was measured at 360 nm using a spectrophotometer. Ascorbic acid & BHT was used as positive control and the reaction mixture without any sample was used as negative control. The superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{Ao - AS}{Ao} \times 100$$

Where, AO = absorbance of Control;
AS = absorbance of sample

2.5.8. Metal chelating activity

The chelating of ferrous ions by the extract of stem of Boswellia *ovalifoliolata* was estimated by the method[35]. Various concentrations of the extracts viz., 50, 100, 150, 200, 250 and 300 µg/mL of sample was added with 1 ml of 2mM $FeCl_2$ separately. The reaction was initiated by the addition of 5 mM ferrozine (1ml). Absorbance was measured at 562nm after 10min. Ascorbic acid and BHT was used as standards

$$\text{Chelating activity (\%)} = \frac{\text{Control OD - Sample OD}}{\text{Control OD}} * 100$$

2.6. Statistical analysis

Data were expressed as mean ± SD. Linear regression analysis was used to calculate the IC_{50} value. Student's t-test was used for the comparison between two means for the possible significant interrelation. Data were considered statistically significant only when P value < 0.05.

RESULTS

Successive Solvent Extraction

The powdered material was subjected to soxhlation using petroleum ether, chloroform, ethyl acetate, ethanol & water by successive solvent extraction method based on the increasing order of polarity of solvent. 100 gms of crude powder was taken and packed in a packing paper. This pack was placed in a soxhlet extractor with different solvents i.e. (Petroleum ether, chloroform, ethyl acetate, ethanol, water) and the temperature was adjusted as per the solvent been used in the extraction. The percentage yield obtained was calculated and reported.

Table 1: **Phytochemical screening *of Boswellia Ovalifoliolata***

S NO	Phyto constituents	Extracts				
		Pet ether	Chloroform	Ethyl acetate	Ethanol	Water
1	Alkaloids	-	-	-	+	-
2	Reducing sugars	-	-	-	-	+
3	Tannins	-	-	+	+	+
4	Saponins	+	+	-	+	+
5	Flavonoids	+	+	+	+	-
6	Terpenoids	+	+	+	+	+
7	Cardiac glycosides	+	+	+	+	+
8	Carbonyl	+	+	+	+	+
9	Amino acids	-	-	-	+	+
10	Steroids	-	-	+	+	+
11	Phenols	-	+	-	+	-

Percentage of yield of Boswellia Ovalifoliolata

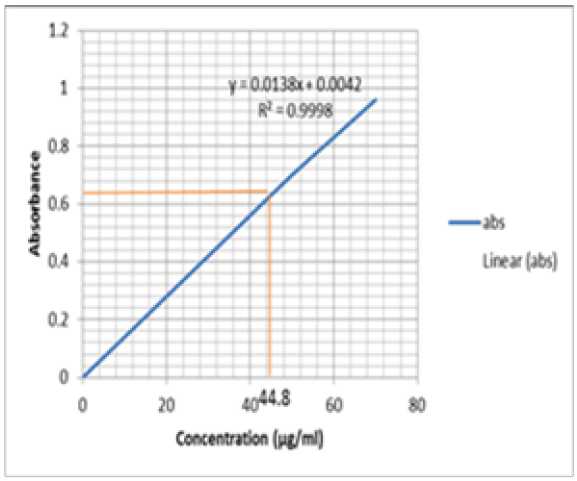
S NO	Parameters	Pet ether	Chloroform	Ethyl acetate	Ethanol	Water
1	Consistency	Waxy	Oily	Oily	Viscous	Viscous
2	Color	Greenish	Light green	Brownish	Raddish black	Cream
3	% of yield	3.704%	3.36%	4.96%	8.60%	5.24%

Determination of total phenol content

Graph -1 show the total phenol content of ethanolic extract of bark of Boswellia ovalifoliolata compared to the calibration curve of gallic acid. The total phenol content was found remarkable in ethanolic soluble fraction to be 44.8 mg gallic acid equivalent/g extract.

Y = mx +c

Y= Absorbance of ethanolic extract of bark of Boswellia ovalifoliolata, m = slope,
X = concentration, c = constant.

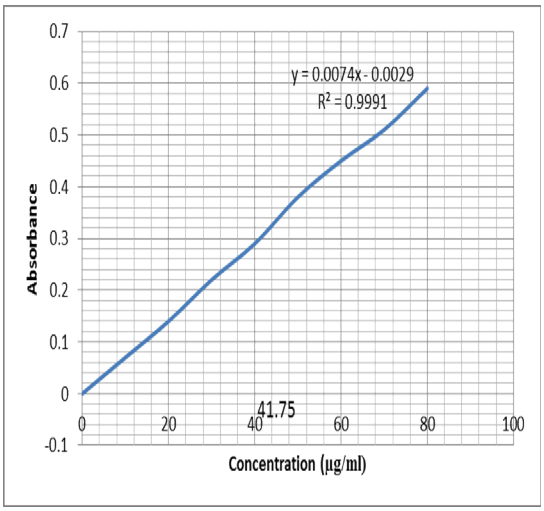


Determination of total flavonoid content

Graph - 2 shows the total antioxidant capacity of ethanolic extract of bark of Boswellia ovalifoliolata compared to the calibration curve of quercetin. The total flavonoid content was found in ethanolic soluble fraction of bark of Boswellia ovalifoliolata to be 41.75 mg quercetin equivalent/g extract.

Y =mx +c

Y= Absorbance of ethanolic extract of bark of Boswellia ovalifoliolata, m = slope,
X = concentration, c = constant.



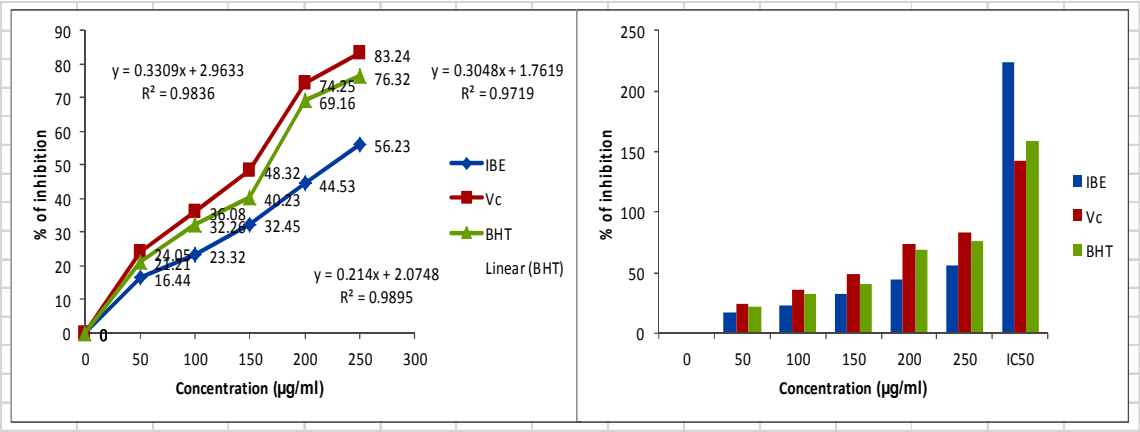
DPPH radical scavenging activity

The radical scavenging capacity based on DPPH assay was determined and the results are shown in Table 1 for the species, Boswellia ovalifoliolata. The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentrations of the extract from 50 -250 ug/mL.

The percentage of inhibition of the DPPH radical was varying from 16.44% (in 50 ug/ml of the extract to 56.23% (in 250 µg /mL of extract). The IC_{50} value of the ethanolic extract of this species was determined to be 223.94ug/ml. The extracts in all concentrations showed the percentage of inhibition of free radicals when compared to standard drugs – Ascorbic acid and BHT. DPPH radical scavenging activities of Boswellia ovalifoliolata shown in table.

Table – 1- DPPH radical scavenging activity of ethanolic extract of *Boswellia Ovalifoliolata*

Con µ/ml	Boswellia ovalifoliolata Bal	Ascorbic acid	BHT
50	16.44±0.65	24.05±0.76	21.21±0.98
100	23.32±0.96	36.08±0.79	32.26±0.74
150	32.45±0.72	48.32±0.98	40.23±1.11
200	44.53±1.32	74.25±1.26	69.16±1.36
250	56.23±1.64	83.24±1.51	76.32±1.62
IC50	223.94	142.14	158.26



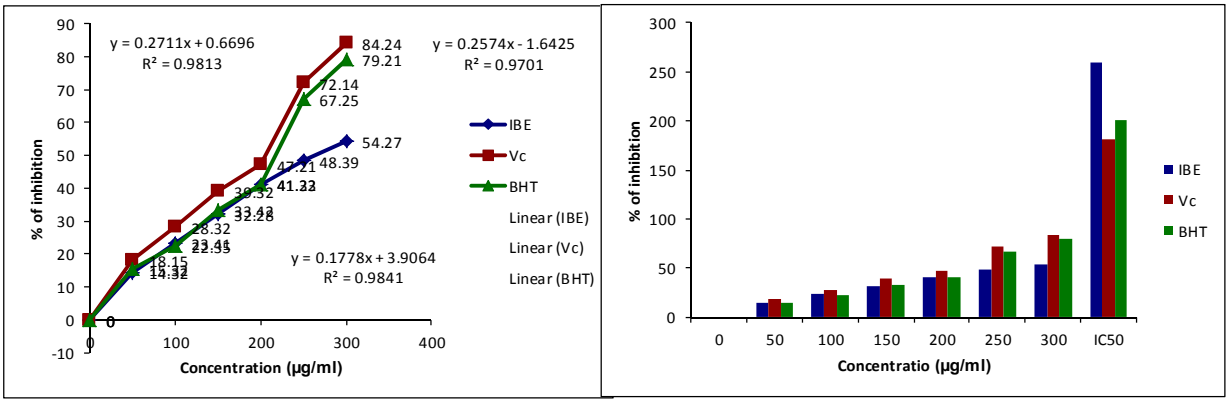
Values are expressed as Mean ± SD. (n=3).

NO Scavenging activity

NO is a very unstable species and reacting with oxygen molecule producing stable nitrate and nitrite which can be estimated by using Griess reagent. NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of Boswellia ovalifoliolata decreased the amount of nitrite generated from the decomposition of SNP in vitro. The scavenging of NO by the extracts was increased in dose dependent manner. The percentage of inhibition of the NO radical was varying from 14.32% (in 50 µg g/ml of the extract to 54.27% (in 300 µg /ml of extract). The IC50 value of the ethanolic leaf extract of this species was determined to be 259.24 µg /ml. The extracts in all concentrations showed the percentage of inhibition of free radicals when compared to standard drugs BHT and ascorbic acids.

Table -2 NO Scavenging activity of ethanolic extract of *Boswellia Ovalifoliolata*

Con µ/ml	Boswellia ovalifoliolata Ba	Ascorbic aci	BHT
50	14.32±0.85	18.15±0.78	15.32±0.89
100	23.41±0.83	28.32±0.58	22.35±0.59
150	32.28±1.54	39.32±1.90	33.42±1.62
200	41.32±1.87	47.21±1.53	41.23±1.29
250	48.39±1.95	72.14±2.05	67.25±1.98
300	54.27±1.22	84.24±1.43	79.21±1.06
IC50	259.24	181.96	200.63



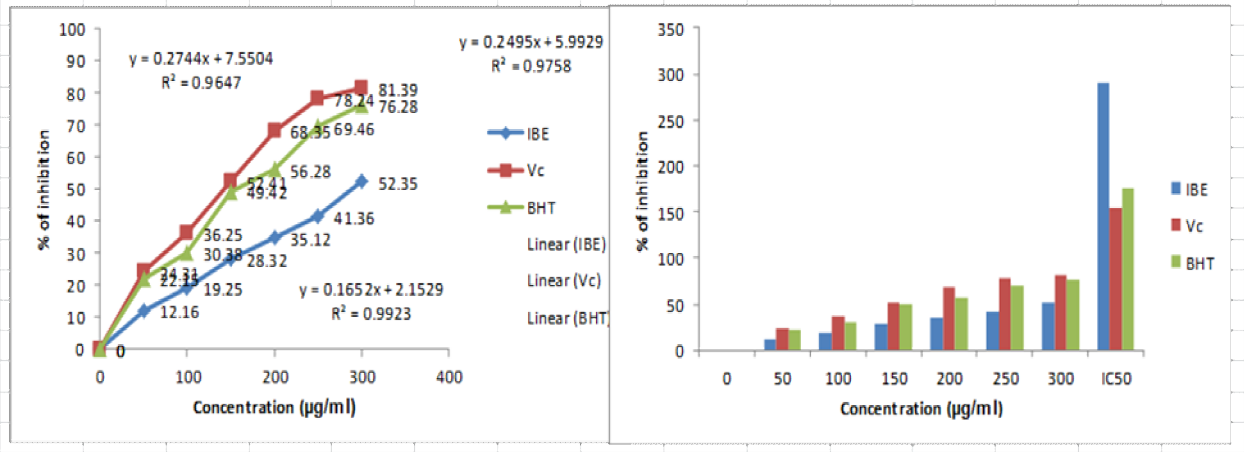
H_2O_2 Scavenging activity

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects. Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is very important for protection of food systems.

Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. The percentage of inhibition of the H_2O_2 was varying from 12.16% (in 50 µg/ml of extract) to 52.35% (in 300 µg/mL extract). The IC50 value of the ethanolic leaf extract of the study species was 289.63 µg /ml. All the concentrations of sample extracts showed higher percentage of inhibition of H_20_2 Scavenging power to the respective concentration of standard drug, BHT & ascorbic acid.

Table - 3 H_2O_2 Scavenging activity* of ethanolic extract of *Boswellia Ovalifoliolata

Con µ/ml	Boswellia ovalifoliolata Bal	Ascorbic acid	BHT
50	12.16±0.67	24.31±0.87	22.15±0.93
100	19.25±0.84	36.25±0.69	30.38±0.62
150	28.32±1.78	52.41±0.82	49.42±0.72
200	35.12±1.37	68.35±1.62	56.28±1.27
250	41.36±1.21	78.24±1.29	69.46±1.49
300	52.35±1.58	81.39±1.53	76.28±1.83
IC50	289.63	154.69	176.38

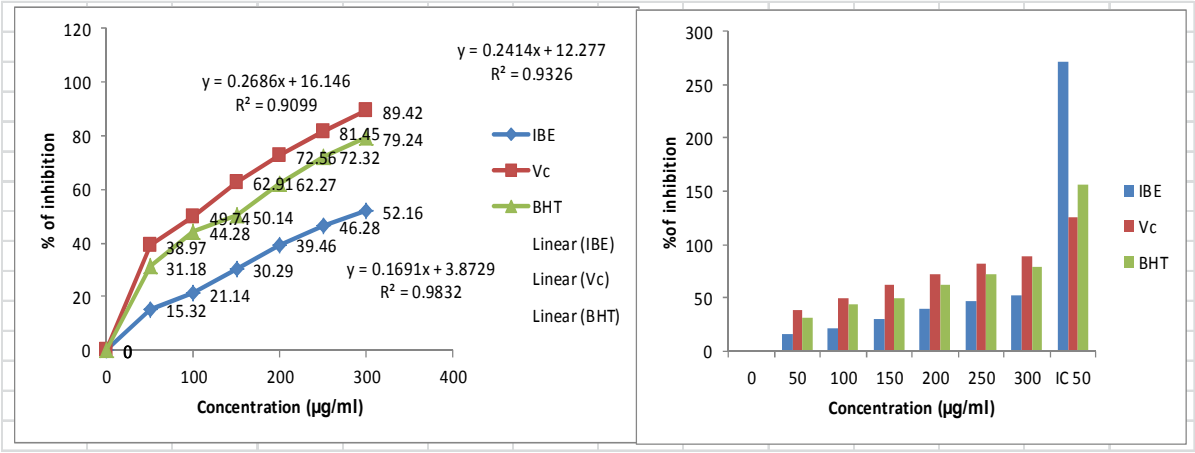


Reducing power assay

The results of antioxidant activity of the bark extract of *Boswellia Ovalifoliolata* based on reducing power were presented in Table 4. The scavenging activity was determined to be increased with the increase in the concentration of extract from 50 to 300 µg /ml. The percentage of inhibition of the reducing power was varying from 15.32% (in 50 µg /ml of extract) to 52.16% (in 300 µg/ml extract). The IC_{50} value of the ethanolic extract of the study species was 271.78 µg /ml. All the concentrations of sample extracts showed reasonable percentage of inhibition of reducing power to the respective concentration of standard drug, BHT & ascorbic acid.

Table – 4 Reducing power activity of ethanolic extract of *Boswellia Ovalifoliolata*

Con µ/ml	Boswellia ovalifoliolata Bal	Ascorbic acid	BHT
50	15.32±0.65	38.97±0.87	31.18±0.98
100	21.14±0.72	49.74±1.98	44.28±0.76
150	30.29±0.99	62.91±1.56	50.14±1.21
200	39.46±1.23	72.56±1.37	62.27±1.39
250	46.28±1.95	81.45±1.82	72.32±1.91
300	52.16±2.12	89.42±1.76	79.24±1.56
IC 50	271.78	126.03	156.26

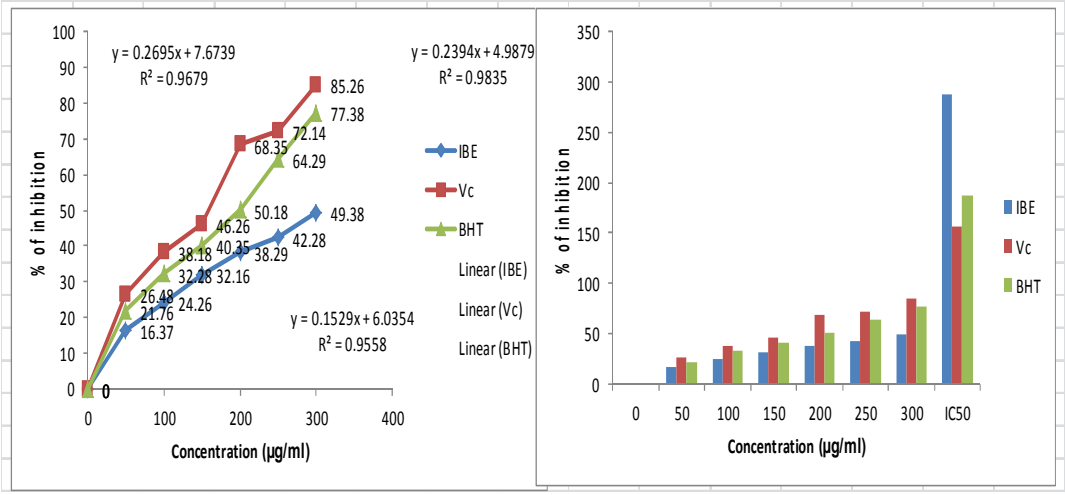


Superoxide anion radical scavenging activity

Percentage inhibition of superoxide radical generation was determined and compared with same doses of BHT & ascorbic acid in Table - 5. The percentage inhibition of superoxide generation by 50- 300 µg/ml concentration of *Boswellia Ovalifoliolata* was in Table -5. The IC50 value of the ethanolic extract of the study species was to be 287.53 µg /ml. The ethanolic extract showed good activity as when compared to standards. This may be due to the presence of polyphenols

Table-5 Superoxide anion radical scavenging activity of ethanolic extract *of Boswellia Ovalifoliolata*

Con µ/ml	Boswellia ovalifoliolata Bal	Ascorbic acid	BHT
50	16.37±1.15	26.48±0.86	21.76±0.92
100	24.26±0.97	38.18±0.89	32.28±0.85
150	32.16±0.82	46.26±1.12	40.35±0.99
200	38.29±1.23	68.35±1.45	50.18±1.14
250	42.28±1.43	72.14±1.46	64.29±1.86
300	49.38±1.66	85.26±1.53	77.38±1.59
IC50	287.53	157.05	188.02

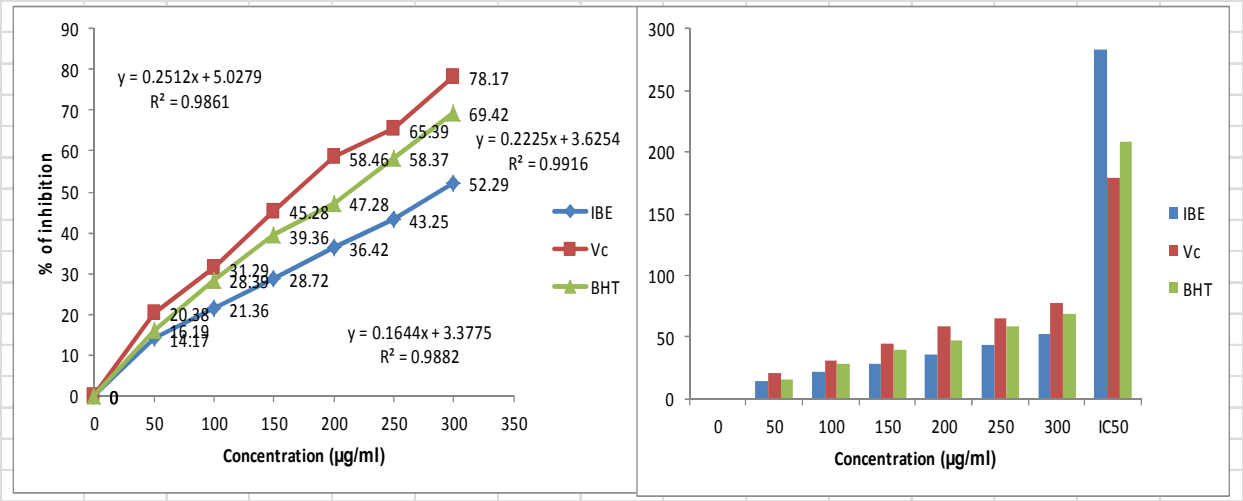


Metal chelating activity

The results of antioxidant activity of the extract of *Boswellia Ovalifoliolata* based on metal chelating activity are given in Table 6. As observed in DPPH and hydroxyl radical scavenging assays, the percentage of metal chelating activity was determined to be sample concentration dependent and it was increasing with the increase in the concentration of extract from 50 to 300 µg / ml. The percentage of inhibition of the metal chelation was varying from 14.17% (in 50 µg/mL of extract) to 52.29% (in 300 µg /ml extract). The IC50 value of the ethanolic of extract of the *Boswellia Ovalifoliolata* species was 283.59 µg /ml. The percentage of inhibition of free radicals by various concentrations of sample were less than the respective concentration of standard drug & BHT

Table -6 Metal chelating activity of ethanolic extract of *Boswellia Ovalifoliolata*

Con µ/ml	Boswellia ovalifoliolata Bal	Ascorbic acid	BHT
50	14.17±0.88	20.38±0.87	16.19±0.91
100	21.36±0.69	31.29±0.86	28.39±0.97
150	28.72±1.78	45.28±0.84	39.36±1.23
200	36.42±1.56	58.46±1.13	47.28±1.56
250	43.25±1.65	65.39±1.37	58.37±1.63
300	52.29±1.69	78.17±1.79	69.42±1.89
IC50	283.59	179.02	208.42



DISCUSSION:

Oxidants, commonly known as "free radicals," is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals, which are the harmful byproducts generated during normal cell aerobic respiration in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology[36]. An over production of these reactive species can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free radical formation[37]. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. They are the first line of defense against free radical damage and are critical for maintaining optimum health[38].

Therefore, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and/or the propagation of oxidative diseases[39].

The most common antioxidants present in vegetables and part of the plants are vitamins C, vitamin E, Carotenoids, phenols and flavonoids. *In vitro* antioxidant activity of the ethanolic extract leaves of *Boswellia ovalifoliolata* was investigated in the present study by total phenol content, flavonoid content ,DPPH, nitric oxide scavenging activity , hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity , super oxide ion scavenging activity and metal chelating assays. The antioxidative effect is mainly due to phenolic components, such as phenolic acids, phenolic diterpenes and the presence of hydroxyl groups[40].

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[41].

Graph -1 show the total phenol content of ethanolic extract of bark of Boswellia ovalifoliolata compared to the calibration curve of gallic acid. The total phenol content was found remarkable in ethanolic soluble fraction to be 44.8 mg gallic acid equivalent/g extract. Graph - 2 shows the total flavonoid content ethanolic extract of bark of *Boswellia ovalifoliolata* compared to the calibration curve of quercetin. The total flavonoid content was found in ethanolic soluble fraction of bark of *Boswellia ovalifoliolata* to be 41.75 mg quercetin equivalent/g extract is due to the presence of flavonoids.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 - 1 diphenyl - 2 - picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug[42]. In the present study, the ethanolic extract of leaves of *Boswellia ovalifoliolata* had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample from 50 - 250 ug/mL. The percentage of inhibition of the DPPH radical was varying from 16.44% (in 50 ug/ml of the extract to 56.23% (in 250 µg /mL of extract). The IC_{50} value of the ethanolic extract of this species was determined to be 223.94ug/ml.

Nitric oxide (NO) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, smooth muscle relaxation, inhibition of platelet aggregation and regulation of cell mediated toxicity and antitumor activities. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NO. May lead to tissue damage. Suppression of released NO may be partially attributed to direct NO scavenging activity, as the extracts of *Boswellia ovalifoliolata* decreased the amount of nitrite generated from the decomposition of SNP in vitro. The scavenging of NO by the ethanolic extract of leaves of *Boswellia ovalifoliolata* was increased in dose dependent manner from 50 to 300 ug/ml under aerobic conditions.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells[43]. The results showed that extracts and fractions of *Boswellia Ovalifoliolata* had an effective H2O2 scavenging activity. The percentage of inhibition of the H_2O_2 was varying from 12.16% (in 50 µg/ml of extract) to 52.35% (in 300 µg/mL extract). The IC50 value of the ethanolic leaf extract of the study species was 289.63 µg /ml.

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Over production of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT (Nitro-blue tetrazolium) resulting in the formation of blue formazan. *In-vitro* super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. The super oxide radical reduces NBT to a blue colored formazan that can be measured at 360 nm. This compound donated their electrons to the superoxide and scavenges them to prevent their further interaction with NBT followed by inhibition of formation of blue colored formazan product[44]. The percentage inhibition of superoxide generation by 50- 300 µg/ml concentration of *Boswellia Ovalifoliolata* was in Table -5. The IC50 value of the ethanolic extract of the study species was to be 287.53 µg /ml. The presence of transition metal ions in a biological system could catalyze the Haber-Weiss and Fenton type reactions, resulting in the generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which results in the suppression of OH generation and inhibition of peroxidation processes of biological molecules[45].

The metal ion scavenging effect was increasing with the increase in the concentration of the extracts from 50-300 ug/mL. The high metal ion scavenging activity of the ethanolic extract of leaves of *Boswellia ovalifoliolata* is probably due to the chelating agents, which form sigma bonds with the metal and effective as secondary antioxidants because they reduce the redox potential, thereby the oxidized form of the metal ion[46].

The results of the present study indicate that the ethanolic extract leaves of Boswellia Ovalifoliolata has significant antioxidant activities which is comparable to that of the standard drugs ascorbic acid & BHT. Thus, the ethanolic extract of leaves of *Boswellia Ovalifoliolata* as promising natural sources of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free-radical-mediated diseases.

CONCLUSION

The study was performed to evaluate the invitro anti oxidant activity of ethanolic extract of leaves of *Boswellia Ovalifoliolata.* The results obtained from all the eight methods indicates the significant antioxidant activity through total phenol content, total flavonoid content , ,DPPH scavenging activity, NO Scavenging activity, H_2O_2 Scavenging activity, Reducing power assay, Superoxide anion radical scavenging activity & metal chelation activity. In the present study, it is found that ethanolic extract of *Boswellia Ovalifoliolata* has showed concentration dependent free radical scavenging activity and this antioxidant effect may be due to the presence of higher content of alkaloids, flavonoids, steroids, glycosides, tannins and phenols. The results were compared with standard references Gallic acid, quercetin, ascorbic acid and BHT. Further research investigations may be carried out to isolate the actual phytoconstituents responsible for antioxidant activity.

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