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## ***In-Vitro* Antioxidant Activity for Terminalia Pallida and Boswellia Ovalifoliolata**

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### ABSTRACT

*Terminalia pallida* brandis and *Boswellia ovalifoliolata* most commonly found plants in the south India. Methanolic and nHexane extract of *Terminalia pallida* roots and *Boswellia ovalifoliolata* roots were analysed for antioxidant activity. The *In-vitro* methods for antioxidant activity are based on the principle of inhibition of free radicals. Present study provides focus on the reducing power assay and the free radical scavenging activity of both the plants in both the extracts. It was observed that *Terminalia pallida* roots and *Boswellia ovalifoliolata* roots have demonstrated dose dependent reducing property.

**Keywords:** Methanolic extract, n-Hexane extract, Reducing power assay, Free radical scavenging activity.

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## INTRODUCTION

Animals' survival on this earth has been made possible only because of the imperative role played by plants. Plants and animals coexist together and their enslavement on each other gives importance to living life. Medicinal plants exist even before human being made their life on the earth<sup>1,2</sup>. *Terminalia pallida* is herb belongs to the family Combretaceae. *Terminalia pallida* is semi-evergreen tree species<sup>3</sup>. *Boswellia ovalifoliolata* (Burseraceae) is a narrow endemic and endangered deciduous tree species<sup>4</sup>. The *In-vitro* methods for antioxidant activity are based on inhibition of free radicals. Samples are added to a free radical-generating system and inhibition of the free radical action is measured. This inhibition is related to antioxidant activity of the sample. Methods vary greatly as to the generated radical, the reproducibility of the generation process and the end point that is used for the determination. Even though, *In-vitro* methods provide a useful indication of antioxidant activities, data obtained from *In-vitro* methods are difficult to apply to biological systems and do not necessarily predict a similar *In-vivo* antioxidant activity. All the methods developed have strengths and limitations and hence a single measurement of antioxidant capacity usually is not sufficient. A number of different methods may be necessary to adequately assess *In-vitro* antioxidant of a specific compound or antioxidant capacity of a biological fluid. In the present study METP, HETP, MEBO and HEBO used for their *In-vitro* antioxidant activity using two standard methods the reducing power assay and the free radical scavenging activity. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the formula.

$$I\% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Where,  $A_{\text{blank}}$  = Absorption of blank,  $A_{\text{Sample}}$  = Absorption of sample

## MATERIALS AND METHOD

### Collection and Authentication

The roots of *Terminalia pallida* Brandis and *Boswellia ovalifoliolata* most widely found in the India. The plant was collected from Ananthagiri forest region, Vishakhapatnam District, Andhra Pradesh, India. The plant species were authenticated by Dr. Madhava Chetty, Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The voucher specimen numbers are 934 and 2231 for *Terminalia pallida* Brandis and *Boswellia ovalifoliolata* Bal & Henry respectively.

### Extraction

The fresh roots were collected, cleaned, shade dried at room temperature for a week and powdered using mixer grinder. 25 gms of the coarse powder was continuously extracted with 100 ml of methanol for 18 hrs at 60°C using Soxhlet apparatus. The powder was successively extracted with n-Hexane. All extracts were filtered through a Whatman no.1 filter paper. Thereafter, the extracts were concentrated using rotary flash evaporator (50°C) to 30ml volume. And finally 30 ml extracts were concentrated to constant weight in vacuum oven at 30 to 50°C. The evaporated residues with constant weight were stored prior to analysis in dark at 4°C. Methanolic residue of *Terminalia pallida* was given name as METP and n-Hexane residue of *Terminalia pallida* was given name as HETP. Methanolic residue of *Boswellia ovalifoliolata* was given name as MEBO and n-Hexane residue of *Boswellia ovalifoliolata* was given name as HEBO <sup>5</sup>.

### Reducing Power Assay

Different concentrations (50µg, 100µg, 150µg, 200µg and 250 µg) of the METP, HETP, MEBO and HEBO separately in 1 ml of distilled water were mixed in to the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. Following incubation, 2.5 ml of 10% trichloro acetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the results averaged. Increased absorbance of the reaction mixture indicated the increased reducing power <sup>6</sup>.

The % reducing power was calculated by using the formula:

$$\% \text{ increase in absorbance} = \frac{\text{control OD} - \text{test OD}}{\text{control OD}} \times 100$$

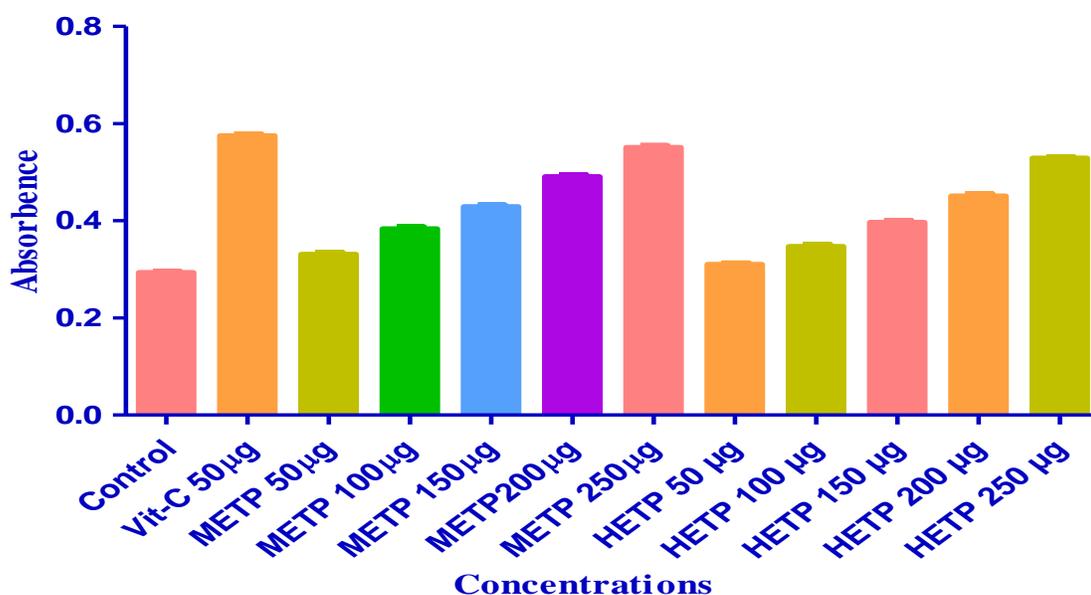
### Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al.* Stock solutions of EDTA (1mM), FeCl<sub>3</sub> (10 mM), ascorbic acid (1mM), H<sub>2</sub>O<sub>2</sub> (10mM) and deoxyribose (10mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml of EDTA 0.01 ml of FeCl<sub>3</sub>, 0.1 ml of H<sub>2</sub>O<sub>2</sub>, 0.36 ml of deoxyribose, 1.0 ml of METP, HETP, MEBO and HEBO (50–250 µg/ml), 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1hr. About 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA to develop the pink chromogen, measured at 532 nm. Sodium metabisulphite used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage decrease in absorbance was calculated <sup>7</sup>.

## RESULTS AND DISCUSSION

### Reducing Power Assay

Reducing power assay is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this method antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. The reducing power was determined according to the method of Oyaizu<sup>6</sup>. It was observed that METP, HETP, MEBO and HEBO have demonstrated dose dependent increase in the reducing property. Whereas, 50 $\mu$ g ascorbic acid (Std) had 96.25% reducing property. METP and HETP at 250  $\mu$ g had shown 87.95% and 80.44% reducing property. The results are summarized in Table 1 and graphically depicted in Figure 1. However MEBO and HEBO at 250  $\mu$ g had shown 84.69% and 78.42% reducing property. The results are summarized in Table 2 and graphically depicted in Figure 2.



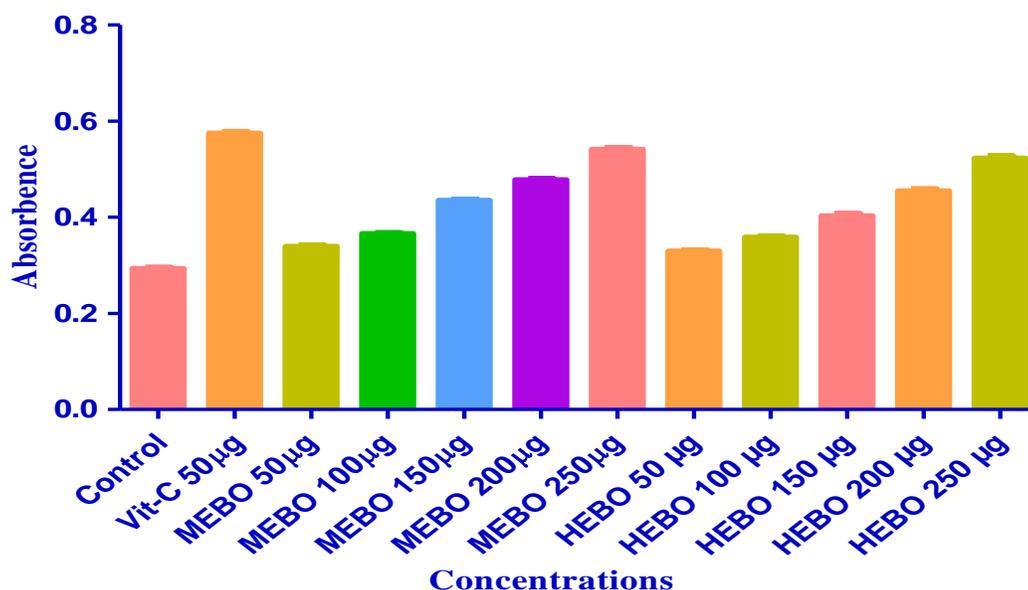
**Figure 1: Reducing Power Assay Activity of METP and HETP**

**Table 1: Reducing Power Assay Activity of METP and HETP**

Groups	Absorbance Mean $\pm$ SEM	% Increase
Control	0.293 $\pm$ 0.002	---
Ascorbic acid 50 $\mu$ g	0.575 $\pm$ 0.003 ***	96.25%
METP 50 $\mu$ g	0.331 $\pm$ 0.003 ***	12.87%
METP 100 $\mu$ g	0.383 $\pm$ 0.004 ***	30.72%
METP 150 $\mu$ g	0.429 $\pm$ 0.003 ***	46.31%

METP 200 µg	0.491 ± 0.003 ***	67.47%
METP 250 µg	0.551 ± 0.003 ***	87.95%
HETP 50 µg	0.310 ± 0.002 ***	5.70%
HETP 100 µg	0.415 ± 0.004 ***	18.43%
HETP 150 µg	0.396 ± 0.004 ***	35.29%
HETP 200 µg	0.451 ± 0.004 ***	53.92%
HETP 250 µg	0.529 ± 0.003 ***	80.44%

Values are the mean ± S.E.M., n=3, \*\*\*Significant at p<0.001 compared to control. Std: Ascorbic acid, METP- Methanolic extract of *Terminalia pallida*, HETP: n-Hexane extract of *Terminalia pallid*



**Figure 2: Reducing Power Assay Activity of MEBO and HEBO**

**Table 2: Reducing Power Assay Activity of MEBO and HEBO**

Groups	Absorbance Mean ± SEM	% Increase
Control	0.293 ± 0.002	---
Ascorbic acid 50µg	0.575 ± 0.003 ***	96.25%
MEBO 50µg	0.339 ± 0.002 ***	15.82%
MEBO 100 µg	0.366 ± 0.002 ***	24.79%
MEBO 150 µg	0.435 ± 0.002 ***	48.55%
MEBO 200 µg	0.478 ± 0.002 ***	63.08%
MEBO 250 µg	0.541 ± 0.003 ***	84.69%
HEBO 50 µg	0.329 ± 0.002 ***	12.41%
HEBO 100 µg	0.359 ± 0.002 ***	22.40%
HEBO 150 µg	0.403 ± 0.004 ***	37.50%
HEBO 200 µg	0.455 ± 0.003 ***	55.23%
HEBO 250 µg	0.523 ± 0.004 ***	78.42%

Values are the mean  $\pm$  S.E.M., n=3, \*\*\*Significant at  $p < 0.001$  compared to control. Std: Ascorbic acid, MEBO- Methanolic extract of *Boswellia ovalifoliolata*, HEBO: n-Hexane extract of *Boswellia ovalifoliolata*

### Hydroxyl Radical Scavenging Activity

In biochemical systems, superoxide radical and  $H_2O_2$  react together to form the hydroxyl radical,  $OH^\bullet$ , which can attack and destroy almost all known biochemicals. Phenylhydrazine when added to erythrocyte hosts cause peroxidation of endogeneous lipids and alteration of membrane fluidity. This peroxidation damage to erythrocytes is probably initiated by active oxygen species like  $O_2^\bullet$ ,  $OH^\bullet$  and  $H_2O_2$  which are generated in solution from auto-oxidation of phenyl hydrazine. This forms the basis of this experiment<sup>7</sup>. It was observed that METP, HETP, MEBO and HEBO have demonstrated dose dependent increase in the hydroxyl radical scavenging activity. 50  $\mu$ g Sodium metabisulphite (Std) had 65.38% scavenging activity. However METP and HETP at 250  $\mu$ g had shown 59.30% and 57.54% of significant scavenging activity. The results are summarized in Table 3 and graphically depicted in Figure 3. However MEBO and HEBO at 250  $\mu$ g had shown 61.75% and 54.84% of significant scavenging activity. The results are summarized in Table 4 and graphically depicted in Figure 4.

**Table 3: Hydroxyl Radical Scavenging Activity of METP and HETP**

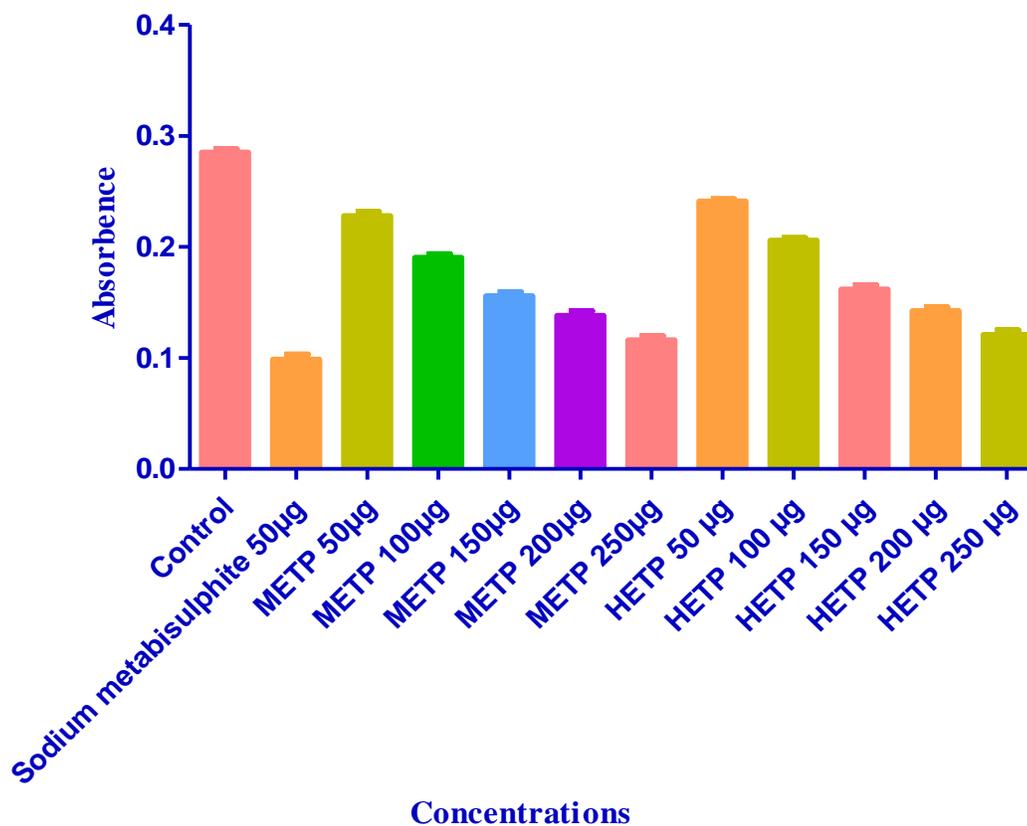
Groups	Absorbance Mean $\pm$ SEM	% Inhibition
Control	0.285 $\pm$ 0.002	- - -
Sodium metabisulphite 50 $\mu$ g	0.098 $\pm$ 0.004***	65.38%
METP 50 $\mu$ g	0.228 $\pm$ 0.003***	20.00%
METP 100 $\mu$ g	0.190 $\pm$ 0.003***	33.23%
METP 150 $\mu$ g	0.155 $\pm$ 0.003***	45.37%
METP 200 $\mu$ g	0.138 $\pm$ 0.004***	51.58%
METP 250 $\mu$ g	0.116 $\pm$ 0.003***	59.30%
HETP 50 $\mu$ g	0.241 $\pm$ 0.002***	15.44%
HETP 100 $\mu$ g	0.206 $\pm$ 0.002***	27.72%
HETP 150 $\mu$ g	0.161 $\pm$ 0.003***	43.26%
HETP 200 $\mu$ g	0.142 $\pm$ 0.003***	50.07%
HETP 250 $\mu$ g	0.121 $\pm$ 0.002***	57.54%

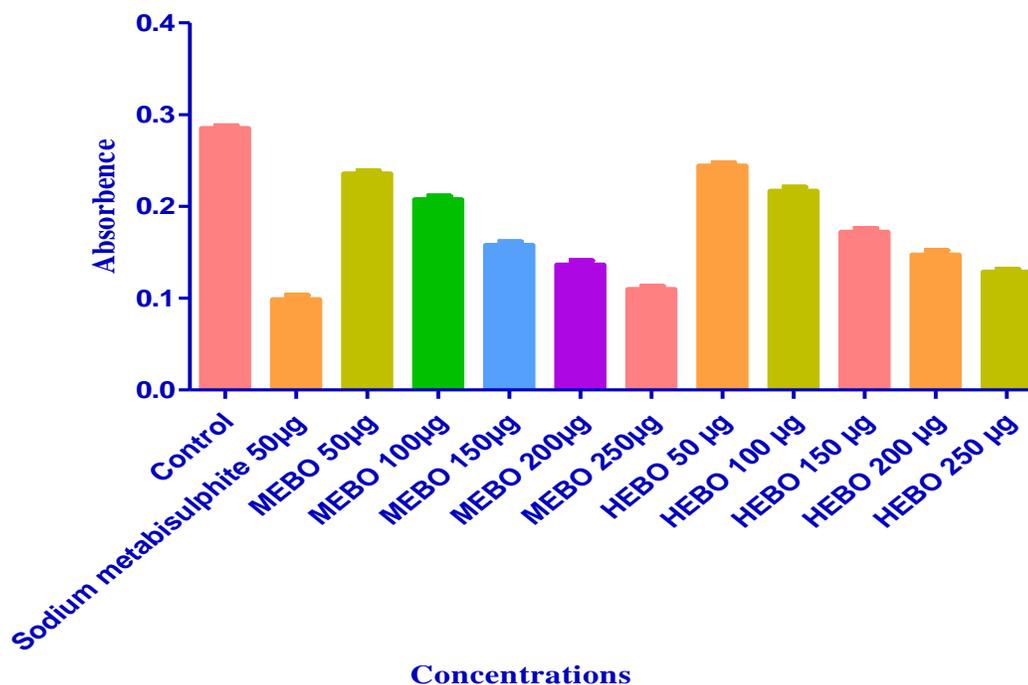
Values are the mean  $\pm$  S.E.M., n=3, \*\*\*Significant at  $p < 0.001$  compared to control. Std: Ascorbic acid, METP- Methanolic extract of *Terminalia pallida*, HETP: n-Hexane extract of *Terminalia pallid*

**Table 4: Hydroxyl Radical Scavenging Activity of MEBO and HEBO**

Groups	Absorbance Mean $\pm$ SEM	% Inhibition
Control	0.285 $\pm$ 0.002	- - -
Sodium metabisulphite 50 $\mu$ g	0.098 $\pm$ 0.004***	65.38%
MEBO 50 $\mu$ g	0.235 $\pm$ 0.002***	17.30%
MEBO 100 $\mu$ g	0.207 $\pm$ 0.003***	27.12%
MEBO 150 $\mu$ g	0.157 $\pm$ 0.003***	44.67%
MEBO 200 $\mu$ g	0.136 $\pm$ 0.004***	52.18%
MEBO 250 $\mu$ g	0.109 $\pm$ 0.003***	61.75%
HEBO 50 $\mu$ g	0.244 $\pm$ 0.003***	14.28%
HEBO 100 $\mu$ g	0.216 $\pm$ 0.004***	23.96%
HEBO 150 $\mu$ g	0.172 $\pm$ 0.004***	39.65%
HEBO 200 $\mu$ g	0.147 $\pm$ 0.004***	48.32%
HEBO 250 $\mu$ g	0.128 $\pm$ 0.002***	54.84%

Values are the mean  $\pm$  S.E.M., n=3, \*\*\*Significant at p<0.001 compared to control. Std: Ascorbic acid, MEBO- Methanolic extract of *Boswellia ovalifoliolata*, HEBO: n-Hexane extract of *Boswellia ovalifoliolata*

**Figure 3: Hydroxyl Radical Scavenging Activity of METP and HETP**



**Figure 4: Hydroxyl Radical Scavenging Activity of MEBO and HEBO**

#### Statistical Analysis

The values are expressed as Mean  $\pm$  SEM. The data was analysed by using one way ANOVA followed by Tukey multiple comparison tests using Graph pad prism software. Statistical significance was set at  $P \leq 0.05$ .

#### CONCLUSION

It is clear that METP, HETP, MEBO and HEBO have dose dependent increase in the reducing property as well as hydroxyl radical scavenging activity. Most of the antioxidants found to have hepatoprotective activity<sup>6</sup>. Thus methanolic and n-Hexane extract of roots of *Terminalia pallida* brandis and *Boswellia ovalifoliolata* can be further evaluate for the presence or absence of hepatoprotective activity.

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