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Vol. 7(29), pp. 2025-2032, 8 August, 2013
DOI 10.5897/AJPP12.593
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Evaluation of comparative free-radical scavenging capacity, and antioxidant activities of methanolic extracts of leaf, stem, and roots of *Operculina turpethum*

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Accepted 17 June, 2013

The aim of this study was to evaluate the free-radical scavenging capacity and antioxidant activity of methanolic extracts of *Operculina turpethum* leaves (MEOTL), stem (MEOTS), and roots (MEOTR) *in vitro* methods. The antioxidant activity was evaluated by reducing power, 2, 2- diphenyl-1-picrylhydrazyl radical (DPPH) photometric assay, nitric oxide free radical scavenging, iron chelation, hydrogen peroxide (H_2O_2) scavenging activities. The total phenolic content was determined by the Folin-Ciocalteu method. The percent reduction and IC_{50} values were calculated using standard methods. The MEOTS and MEOTR exhibited scavenging activity lower than gallic acid at similar concentrations in Folin-Ciocalteu method. The IC_{50} values of the ascorbic acid, MEOTL, MEOTS, and MEOTR were found to be 46.67 ± 1.67, 50.01 ± 2.13, 72.63 ± 2.76 and 85.0 ± 3.55 µg, respectively in DPPH assay. The IC_{50} values of the ascorbic acid, MEOTL, MEOTS, and MEOTR were found to be 14.32 ± 1.45, 18.15 ± 2.23, 20.80 ± 1.88 and 23.71 ± 2.67 µg, respectively in reducing power method. Among all extracts, MEOTS has higher antioxidant activity. Further investigations are needed to identify the lead molecule and to elucidate the structure and exact mechanism of action for their antioxidant activity.

Key words: Antioxidant, diabetes, 2, 2- diphenyl-1-picrylhydrazyl, *Operculina turpethum,* reactive oxygen species.

INTRODUCTION

Reactive oxygen species (ROS) are responsible for various cellular anomalies like protein damage, deactivation of enzymes, alteration of DNA and lipid per oxidation which in turn leads to pathological condition like carcinogenesis, reperfusion injury, rheumatoid arthritis, diabetes, etc. The regular intake of antioxidants seems to limit or prevent the dangerous effects caused by ROS. Thus, to maintain cellular health, it is important to have a specific and effective antioxidant that scavenges multiple types of free radicals so that it can be used in multiple

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diseases. Based on the efficiency of free radical scavenging, the compounds are classified into strong, moderate and weak antioxidants (Gramza et al., 2010). Naturally, there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence, there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials. That is why plants with antioxidant properties are becoming more and more popular all over the world (Indu and Thiraviam, 2010). To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to neutralize free radicals (Pullaiah, 2007).

Operculina turpethum, which is the commonly known as trivit belonging to family Convolvulaceae, is a large stout perennial twinner with milky juice and fleshy branched roots (Shankaraiah et al., 2012). It also has anthelmintic, expectorant, antipyretic, hepatoprotective (Riaz et al., 2009), anti-inflammatory, purgative and anti-diabetic properties (Shankaraiah et al., 2011, 2012). It contains a wide variety of phytoconstituents, which are useful in treatment of different ailments and includes glycosidic resin, coumarins, beta-sitosterol, and essential oils (John, 1988). It is reported to be highly medicinal and is cultivated occasionally in gardens as an ornamental plant. The aim of our study was to evaluate antioxidant activity of methanolic extracts of leaf, stem and roots of *O. turpethum in vitro* models.

MATERIALS AND METHODS

Reagents and chemicals

Agarose, ascorbic acid, butylated hydroxyl anisole (BHA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid were purchased from Sigma-Aldrich, USA. 2, 2'-azino-*bis* (3 ethylbenz – thiazoline – 6 – sulfonic acid) (ABTS) was purchased from Fluka chemicals Pvt. Ltd, India. Ammonium hydroxide, benzene, dimethyl sulfoxide (DMSO), ethanol, ethyl acetate, Folin-Ciocalteu's phenol reagent, formic acid, ferric chloride, methanol, sodium hydroxide, and potassium ferricyanide were purchased from SRL chemicals Pvt. Ltd, India. Aluminum chloride ($AlCl_3$) and sodium nitrite ($NaNO_2$) were purchased from SD Fine Chemicals, India. Ammonium per sulfate (APS) and hydrogen peroxide solution (H_2O_2; 6% w/v) was purchased from Loba Chemie, India. Bromophenol blue, fetal bovine serum (FBS), trichloroacetic acid and trypsin-EDTA were purchased from Hi-Media chemicals Pvt. Ltd, India.

Collection of plant material

O. turpethum (L.) Silva Manso plant material was collected from local areas of Vijayawada, Andhra Pradesh, India. Its parts were botanically authenticated by Prof. S. V. Raju, Taxonomist, Department of Botany, Kakatiya University, Warangal, Andhra Pradesh, India. The herbarium was maintained in the Department of Pharmacognosy and Phytochemistry, Vaagdevi College of Pharmacy, Hanamkonda. *O. turpethum* stem was washed under tap water and were efficiently dried under shade for about one week and protected from deterioration. The shade dried stem was grinded made into powder with the help of a laboratory mixer. These were efficiently dried under shade for about one week and protected from deterioration and then ground and made into powder.

Preparation of plant

O. turpethum leaves stem and roots were washed under tap water and were efficiently dried under shade for about one week and protected from deterioration. The shade dried leaves, stem and roots were ground made into powder with the help of a laboratory mixer. These were efficiently dried under shade for about one week and protected from deterioration and then ground and made into powder.

Extraction

Soxhlation

The stem, root material was weighed (100 g) and using successive solvent extraction process (soxhlet apparatus) with methanol for 6 h. After completion of soxhlation process the liquid extract was collected and concentrated under reduced pressure below 50°C, until a soft mass was obtained in it and was dried and kept in a desiccators.

Maceration

The leaf material was weighed (250 g) and methanol was extracted at room temperature in a glass container for 3 days. The material was stirred from time to time to ensure proper extraction. After 3 days, the contents of the container were filtered through muslin cloth and the filtrate was concentrated under reduced pressure below 50°C, until a soft mass was obtained and then preserved in a desiccators.

Antioxidant activity methods

Reducing power method

About 2 ml of each sample and standard solutions were spiked with 2.5 ml of 1% potassium ferricyanide solution. This mixture was kept at 50°C in water bath for 20 min. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min. About 2.5 ml of supernant was mixed with 2.5 ml of distilled water and 1 ml of 0.1% ferric chloride and kept for 10 min. Control was prepared in similar manner without samples. The absorbance of resulting solution was measured at 700 nm (Carol et al., 2010).

DPPH free radical scavenging activity

About 150 ml of DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 516 nm for control reading. Different volume levels of test sample (20, 40, 60, 80 and 100 µl) were screened and 100 µl of each dose level by dilution with methanol up to 3 ml was made. About 150 ml of DPPH solution was added to each test tube (Soni et al., 2006). Absorbance was measured at 516 nm in UV-visible spectrophotometer (Shimadzu,

UV-1800, Japan) after 15 min using methanol as a blank. The percent reduction and IC_{50} were calculated and the free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

Antiradical activity (%) = Control absorbance - Sample absorbance/Control Absorbance × 100

Each experiment was carried out in triplicate.

Nitric oxide free radical scavenging activity

Sodium nitroprusside (10 mg) in phosphate buffer saline was mixed with different volume levels of test sample (10, 20, 30, 40, 50 and 100 µl) and 100 µl of each dose level by dilution with methanol was made. The solution was incubated at room temperature for 150 min. The same reaction mixture without the extract but equivalent amount of methanol served as control. After the incubation period 5 ml of Griess reagent was added. The absorbance was taken in UV-visible spectrophotometer at 546 nm (Shreedhara et al., 2010). Ascorbic acid was used as positive control. The percent reduction and IC_{50} were calculated. Each experiment was carried out in triplicate.

Iron chelation activity

Iron chelation activity is a measure of antioxidant activity. Different concentrations of the extract and ascorbic acid solution each as 2 ml in 5% v/v methanol were incubated with methanolic O-phenanthroline solution (1 ml, 0.05% w/v) and ferric chloride solution (2 ml, 200 M) at ambient temperature for 10 min. After incubation, the absorbance of solutions was measured at 510 nm. The experiments were performed in triplicate.

Hydrogen peroxide (H_2O_2) scavenging activity

Hydrogen peroxide scavenging activity of plant extract was determined using a modification of the method. About 4 mM solution of H_2O_2 was prepared in phosphate-buffered saline (PBS, pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorbance at 230 nm. Plant extract corresponding to 50, 100, 150, 200, and 250 µl of 1 mg/ml plant extract stock solution in 4 ml distilled water were added to 0.6 ml hydrogen peroxide in PBS solution. Absorbance of H_2O_2 was determined at 230 nm (Muntasir, 2010). Absorbance was determined 10 min later against a blank solution similar to that mentioned earlier.

H_2O_2 Scavenging (%) = Absorbance of control – Absorbance of test / Absorbance of Control × 100

Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteau method. The extract samples (0.5 ml of different dilutions) were mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, 250, and 300 µg/ml solutions of gallic acid in methanol:water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent which is a common reference compound.

Data analysis

All statistics were calculated using Graph Pad Prism 5.0 software (San Diego, CA, USA). Pharmacokinetic parameter values for groups were compared using analysis of variance with Tukey's and Dunnett's tests for multiple comparisons. The p value less than 0.05 were considered significant.

RESULTS

Reducing power method

The reducing power assay is a convenient and rapid screening method for measuring the anti oxidant potential. The reduction ability, that is, "Fe^{3+} to Fe^{2+} transformation" by measuring absorbance at 700 nm is as shown in Figure 1. The reduction ability was found to be increased with increasing concentrations of methanolic extracts of *Operculina* leaves (MEOL), stem (MEOS), and roots (MEOR) as shown in Table 1. MEOL, MEOS, and MEOR exhibited reducing power when compared with ascorbic acid at similar concentrations. The IC_{50} values of the ascorbic acid, MEOL, MEOS, and MEOR were found to be 14.32 ± 1.45, 18.15 ± 2.23, 20.80 ± 1.88, and 23.71 ± 2.67 µg, respectively.

DPPH free radical scavenging activity

Free radical scavenging activity of ascorbic acid, methanolic extracts of *Operculina turpethum* leaves (MEOTL), stem (MEOTS), and roots (MEOTR) were performed by DPPH method. The reduction capability of the DPPH radical is determined by the decrease in its absorbance. Figure 2 Shows the concentration-absorbance profile of ascorbic acid, MEOTL, MEOTS, and MEOTR. The MEOTL scavenging activity is higher (Table 2) when compared with other extracts. The IC_{50} values of the ascorbic acid, MEOTL, MEOTS, and MEOTR were found to be 46.67 ± 1.67, 50.01 ± 2.13, 72.63 ± 2.76 and 85.0 ± 3.55 µg, respectively.

Nitric oxide scavenging activity

The nitric oxide scavenging of ascorbic acid, MEOTL, MEOTS, and MEOTR were carried out after incubation for 150 min with different concentrations. The methanolic and stem and root extracts exhibited scavenging activity lower than ascorbic acid at similar concentrations (Figure 3). Here, methanolic stem extract scavenging activity is higher when compared with other extracts as shown in Table 3. The IC_{50} values of the ascorbic acid, MEOTL, MEOTS, and MEOTR were found to be 16.10 ± 1.57, 16.45 ± 1.89, 16.10 ± 1.24 and 16.90 ± 1.66 µg, respectively.

Iron chelation scavenging activity

The scavenging activity of iron chelation by ascorbic acid,

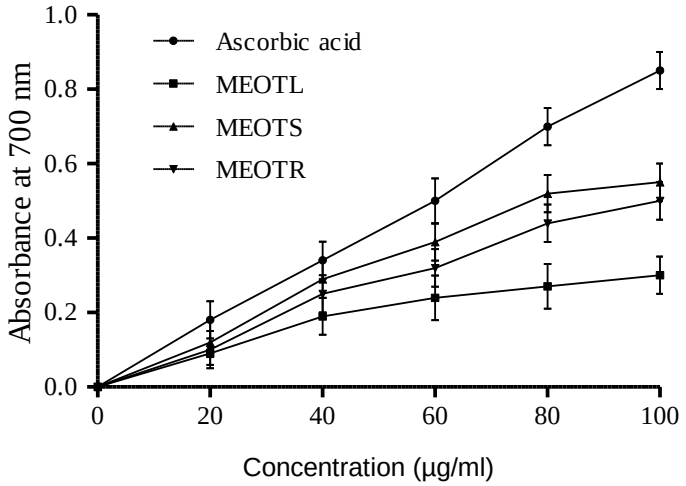

Figure 1. Absorbance of ascorbic acid, MEOTL, MEOTS and MEOTR in reduced scavenging activity.

Table 1. Percent inhibition of ascorbic acid, MEOTL, MEOTS, and MEOTR by reduced scavenging method.

Concentration (µg/ml)	Ascorbic acid	MEOTL	MEOTS	MEOTR
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
20	83.33 ± 4.78	66.66 ± 4.35	75.56 ± 5.23	70.28 ± 4.96
40	91.17 ± 5.23	84.21 ± 4.12	89.65 ± 4.70	88.94 ± 5.26
60	94.13 ± 6.41	87.50 ± 3.66	92.30 ± 6.89	90.62 ± 4.13
80	95.71 ± 5.34	88.88 ± 5.87	94.23 ± 6.21	93.18 ± 6.87
100	96.47 ± 6.22	90.03 ± 6.11	94.54 ± 5.25	94.31 ± 5.67

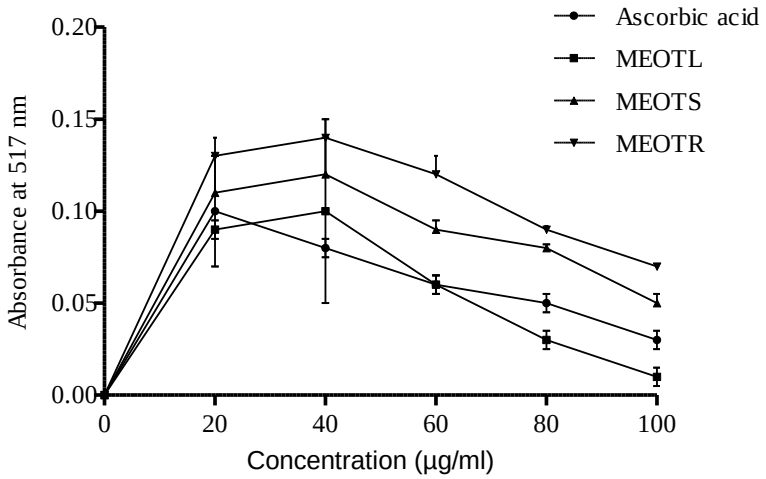

Figure 2. Absorbance of ascorbic acid, MEOTL, MEOTS and MEOTR in DPPH scavenging activity.

MEOTL, MEOTS, and MEOTR were carried out. The methanolic and stem and root extracts exhibited scavenging activity lower than ascorbic acid at similar concentrations (Figure 4). The methanolic stem extract

Table 2. Percent inhibition of ascorbic acid, MEOTL, MEOTS, and MEOTR by DPPH scavenging activity method.

Concentration (µg/ml)	Ascorbic acid	MEOTL	MEOTS	MEOTR
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
20	37.52 ± 3.67	16.32 ± 2.45	31.25 ± 3.71	18.75 ± 2.45
40	50.76 ± 4.61	13.61 ± 2.11	25.34 ± 2.49	12.57 ± 1.34
60	62.50 ± 5.78	19.58 ± 1.56	43.75 ± 3.06	25.76 ± 2.56
80	68.75 ± 4.20	31.74 ± 3.70	50.68 ± 3.55	43.75 ± 3.78
100	81.25 ± 5.77	48.05 ± 3.22	68.75 ± 6.13	56.25 ± 4.06

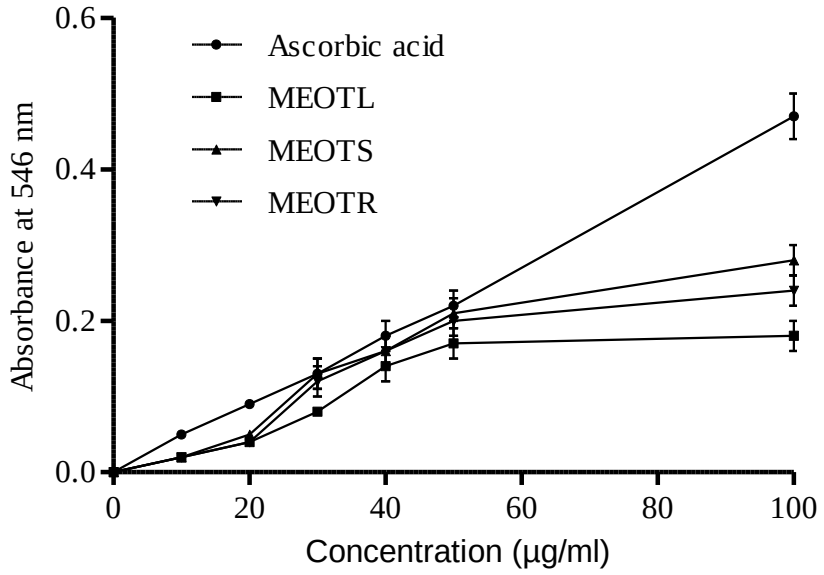

Figure 3. Absorbance of ascorbic acid, MEOTL, MEOTS and MEOTR in nitric oxide scavenging activity

Table 3. Percent inhibition of ascorbic acid, MEOTL, MEOTS, and MEOTR by nitric oxide scavenging activity method.

Concentration (µg/ml)	Ascorbic acid	MEOTL	MEOTS	MEOTR
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	40.41 ± 2.45	-50.78 ± 2.53	-50.31 ± 2.42	-50.24 ± 3.84
20	66.66 ± 4.71	25.35 ± 2.11	40.40 ± 3.13	25.67 ± 2.55
30	76.92 ± 5.09	62.50 ± 3.56	76.92 ± 4.50	75.75 ± 4.22
40	83.33 ± 5.22	78.57 ± 5.62	81.25 ± 5.47	81.25 ± 5.24
50	86.36 ± 4.81	82.35 ± 6.34	85.71 ± 4.30	85.78 ± 5.92
100	93.61 ± 5.04	83.33 ± 5.21	89.28 ± 5.79	87.50 ± 4.77

scavenging activity is higher when compared with other extracts as shown in Table 4. The IC_{50} values of ascorbic acid, MEOTL, MEOTS, and MEOTR were found to be 30.33 ± 2.56, 50.42 ± 4.37, 55.56 ± 4.68 and 67.89 ± 6.35 µg, respectively.

Hydrogen peroxide scavenging activity

The scavenging activity of hydrogen peroxide by ascorbic acid, MEOTL, MEOTS, and MEOTR exhibited scavenging activity higher than ascorbic acid at similar

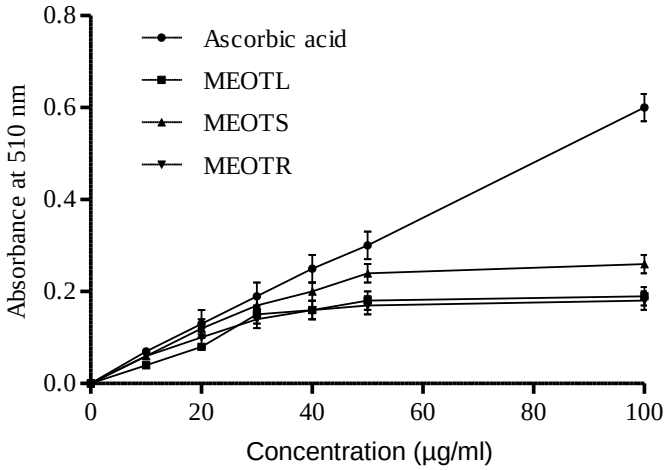

Figure 4. Absorbance of ascorbic acid, MEOTL, MEOTS and MEOTR in Iron chelation scavenging activity.

Table 4. Percent inhibition of ascorbic acid, MEOTL, MEOTS, and MEOTR by Iron chelation scavenging activity method.

Concentration (µg/ml)	Ascorbic acid	MEOTL	MEOTS	MEOTR
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	14.28 ± 3.21	-50.22 ± 3.54	0 ± 0	0 ± 0
20	53.84 ± 3.67	25.55 ± 3.12	50.74 ± 3.56	40.65 ± 2.45
30	68.42 ± 5.20	60.19 ± 4.67	64.70 ± 2.06	57.14 ± 4.21
40	76.45 ± 5.51	62.58 ± 4.22	70.34 ± 4.20	62.52 ± 4.87
50	80.23 ± 6.39	66.66 ± 5.81	75.87 ± 6.94	64.70 ± 4.33
100	90.39 ± 6.45	68.42 ± 5.24	76.92 ± 5.43	66.66 ± 3.65

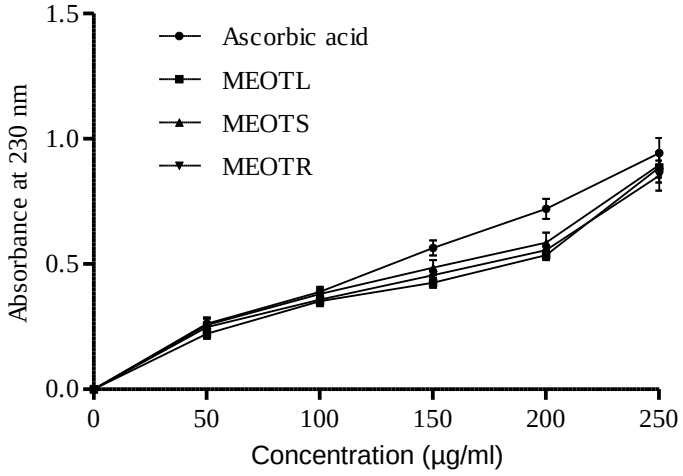

Figure 5. Absorbance of ascorbic acid, MEOTL, MEOTS and MEOTR in hydrogen peroxide scavenging activity.

concentrations. The results suggested that methanolic stem extract scavenging activity was higher (Figure 5) when compared with other extracts depicted in Table 5. The IC_{50} values of the ascorbic acid, MEOTL, MEOTS,

Table 5. Percent inhibition of ascorbic acid, MEOTL, MEOTS, and MEOTR by hydrogen peroxide scavenging activity method.

Concentration (µg/ml)	Ascorbic acid	MEOTL	MEOTS	MEOTR
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
50	27.78 ± 2.56	25.05 ± 2.45	28.68 ± 2.12	29.07 ± 2.46
100	41.25 ± 3.53	39.72 ± 3.21	42.52 ± 3.02	41.96 ± 3.76
150	59.80 ± 3.78	47.96 ± 3.73	54.24 ± 4.28	53.34 ± 5.70
200	76.45 ± 5.34	60.38 ± 5.62	65.40 ± 4.36	65.18 ± 4.66
250	100.12 ± 6.57	93.15 ± 2.10	97.45 ± 4.26	96.25 ± 3.57

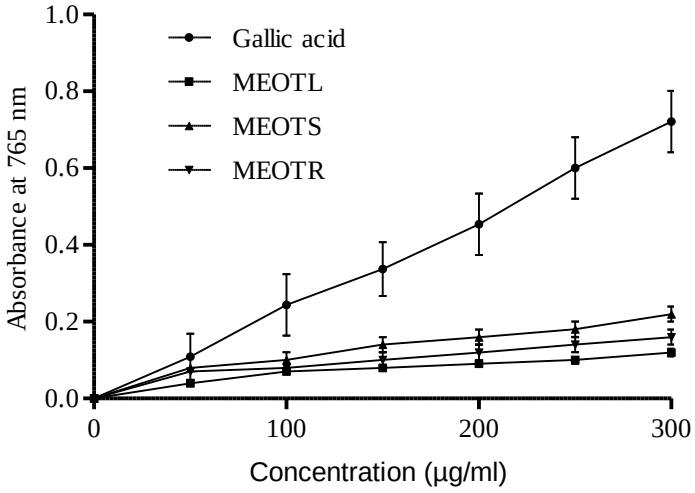

Figure 6. Absorbance of ascorbic acid, MEOTL, MEOTS and MEOTR in total phenolic activity.

and MEOTR were found to be 123.24 ± 8.36, 129.2 ± 10.47, 129.9 ± 9.58 and 137.74 ± 11.43 µg, respectively.

Total phenolic activity

The total phenolic content of MEOTL, MEOTS, and MEOTR were performed. The methanolic extracts of stem and root exhibited scavenging activity lower than gallic acid at similar concentrations as shown in Figure 6. Based on the results obtained from the method followed, the methanolic stem extract scavenging activity was higher when compared with extracts as sown in Table 6. The IC_{50} values of the ascorbic acid, MEOTL, MEOTS, and MEOTR were found to be 22.83 ± 2.57, 70.13 ± 5.78, 61.13 ± 4.62 and 80.72 ± 6.23 µg, respectively.

DISCUSSION

For many centuries, plants have been used throughout the world as drugs and remedies for treatment of various diseases as they have great potential for producing new drugs of great benefit to humankind. There are many approaches to search for new biologically active principles in higher plants. Natural flora has gained its attention in the treatment of common cold to dreadful diseases, namely, AIDS, cancer, etc, such plants are called medicinal plants which have curative properties due to the presence of various complex chemical substances of different composition, namely, grouped as alkaloids, glycosides, corticosteroids, terpenoids, isoflavanoids, steroids, etc (Shankaraiah et al., 2012).

O. turpethum is a perennial plant with milky juice belongs to family Convolvulaceae. This plant is widespread in old tropics from East Africa to North Australia, and is common in Godavari, Andhra Pradesh, India (John et al., 2005). It is widely distributed in tropical Africa and Asia. In India, it is found in damp and it occurs almost throughout India up to an altitude of about 1000 m. It is sometimes grown in gardens for its beautiful flowers. It is rare on open sandy soils. It is occasionally cultivated in India. Traditionally, *O. turpethum* root is prescribed in the treatment of snake bite and scorpion sting, but it is not an antidote to either snake-venom or scorpion-venom. In constipation, it is an effective laxative. It is used in periodic fevers and in the treatment of anemia accompanied by spenomegaly. It is also used to

Table 6. Total phenolic content of ascorbic acid, MEOTL, MEOTS, and MEOTR by total phenolic activity method.

Concentration (µg/ml)	Gallic acid	Leaf P.E + Meth	Stem Meth	Root Meth
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
50	81.65 ± 4.61	50.45 ± 4.23	75.32 ± 4.78	71.42 ± 5.46
100	91.80 ± 6.35	71.42 ± 4.76	80.12 ± 5.24	75.26 ± 6.33
150	94.06 ± 6.87	75.36 ± 6.22	85.71 ± 6.01	80.87 ± 6.94
200	95.59 ± 5.80	77.77 ± 5.02	87.55 ± 6.27	83.33 ± 5.32
250	96.66 ± 6.32	80.21 ± 6.34	88.88 ± 5.83	85.71 ± 5.29
300	97.22 ± 4.87	83.33 ± 5.25	90.90 ± 6.34	87.50 ± 5.66

relieve flatulence and colic and in the treatment of obesity to decrease fat. It is used to treat dropsy, dyspepsia with constipation and flatulence, gout and rheumatism, and other inflammations (Suresh et al., 2006). Several studies reported the antioxidant activity of these plants including *Zingiber officinale* (Atef et al., 2013), *Cinnamomum longepaniculatum* (Cui et al., 2013), and *Boerhaavia diffusa* (Jitender et al., 2013).

In the present study, the MEOTL, MEOTS, and MEOTR were evaluated for antioxidant activity. The percent reduction and IC_{50} values were calculated using standard methods. The percent reduction values are summarized in Tables 1 to 6. Finally, the present study results suggested that among all extracts, MEOTS has higher antioxidant activity.

Conclusion

The findings reported by the comparison of antioxidant activity of MEOTL, MEOTS, and MEOTR. The stem methanolic extract has higher antioxidant activity than other extracts. Although, promising results have been obtained, more concentrated efforts are still needed for the characterization of the comparison of antioxidant activity with MEOTL, MEOTS, and MEOTR. Further investigations are needed to identify the lead molecule and to elucidate the structure and exact mechanism of action for their antioxidant activity.

ACKNOWLEDGEMENTS

The authors are grateful to the management of Vaagdevi College of Pharmacy, Warangal for providing necessary facilities. The authors are very grateful to N. Venkateswarlu, President and P. Lakshmana Rao, Secretary of Siddhartha Academy General and Technical Education for their encouragement. The authors thank Dr. G. Devalarao, Pricipal and Dr. Buchi. N. Nalluri, Director for PG studies and Research of KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada for their support.

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