In vitro Studies on antioxidant and anti-inflammatory activity of *Oxalis latifolia* Kunth *and Oxalis tetraphylla* Cav. Petiole

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Abstract

In the present study, Qualitative Phytochemical Screening, evaluation of antioxidant activity and in vitro anti-inflammatory activity of various solvent extracts of *O. latifolia* and *O. tetraphylla* petiole. The powdered petiole samples were subjected for the extraction by using different solvents such as petroleum ether, chloroform, ethyl acetate, mthanol and hot water and the extracts were subjected to quantification of total phenolics, tannin and flavonoids. The *in vitro* antioxidant assay (DPPH, ABTS, FRAP, Superoxide radical scavenging assay, Phosphomolybdenum, reducing power assay) and Anti-Inflammatory activity assay (Membrane stabilization assay were also undertaken). Among the different extracts of *O. latifolia* and *O. tetraphylla* petiole were studies, the ethyl acetate extract of *O. tetraphylla* depicted the maximum amount of phenolics (159.39 mg GAE/ g extract) tannin (150.43 mg GAE/ g extract) and flavonoids (179.57 mg RE/g extract) and also registered highest DPPH% (IC_{50}: 10.19 µg/mL), ABTS% (57256.94 µg TE/g extract), FRAP (318.51 mM Fe (II)/mg extract), Superoxide (43.63%) radical scavenging activity, Phosphomolybdenum (81 mg AAE/g extract), Reducing power. Anti-inflammatory studies showed that *O. tetraphylla* ethyl acetate extract possesses higher activity and Membrane stabilization assays (80.85%). The present study provides evidence that ethyl acetate extract of *O. tetraphylla* petiole possesses excellent in vitro antioxidant and anti-inflammatory activity, which can be a potential source of natural antioxidants and should be further exploited for its use in clinical medicine.

Keywords: *Oxalis tetraphylla*, *Oxalis latifolia,* phytochemical screening, antioxidant and anti-inflammatory activity.

I. Introduction

Plants are the most valuable gifts of nature and they play an important role in all kinds of food and medicine. From antique time to modern times it is impossible to produce medicine without plants. Plants produce treasured source of natural active constituents that are very much useful in maintaining human health and to treat many ailments [1]. The usage of medicinal plants and human diseases go hand by hand and this interaction is as old as mankind. The effectiveness of medicinal plants considerably contributes to the discovery of new medicinal properties that can be regularly prescribed for good health. In most of the medicinal plants known to mankind, the phytochemical constituents are not known completely [2].

Natural products that are present in plants are safer than the synthetic drug molecules. Now a days, these compounds are widely recognized by the pharmaceutical industries for their broad structural diversity and the pharmacological activities [3-4].

Reactive oxygen species (ROS) and free radicals are formed during normal physiological events in a healthy organism. The over production of ROS during abnormal physiological coditions leads to oxidative stress which resulted oxidative modifications of the cellular membranes. The regular intake of antioxidant rich substances strengthens and acts as defender against ROS [5]. Free radicals and ROS are constantly generated in the living system were responsible for widespread damage to tissues resulted various chronic degenerative diseases, and also lead to extensive lysis [6]. In present days, synthetic drugs play a major role and are also used for scavenging the free radicals, which protect against oxidative stress. However, these synthetic drugs cause adverse side effects [7]. On seeing the other side of the coin, the alternative of synthetic drugs being the consumption of natural antioxidants from natural plant sources, food supplements and traditional medicines which can protect the human system without causing adverse side effects [8]. Phytotherapy researchers have taken steps in identifying the plants with high antioxidant activity which will be very much useful in protection against oxidative stress.

Oxalis latifolia and *O. tetraphylla* belonging to Oxalidaceae family is a stem less herb of cosmopolitan distribution found abundantly in agricultural farms, gardens, lawns etc. The Oxalis species are reported to cure various disorders such as paralysis, stomach disorder and it also acts

as thirst reliever. The present work has been undertaken to identify the *in vitro* antioxidant property and anti-inflammatory activity study of *Oxalis latifolia* and *O. tetraphylla* and this work has been reported for the first time.

2. Materials and methods

2.1. Collection and identification of plant materials

Petiole of the *Oxalis latifolia* and *O. tetraphylla* were collected during the month of May 2017, from The Nilgiris, the Western Ghats, Tamil Nadu, India. The taxonomic identities of the plants were confirmed from Botanical Survey of India (BSI), Southern Regional Centre, Coimbatore. The petioles were washed under running tap water to remove the surface pollutants and air dried under shade for 15 days. The dried petiole were homogenised into a fine powder using pulveriser and stored for further studies.

2.2. Chemicals

2, 2 - diphenyl - 1 - picryl - hydrazyl (DPPH), potassium persulphate, 2,2′ -azinobis (3-ethyl benzothiozoline) −6- sulfonic acid diamonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), butylated hydroxy toluene (BHT), rutin, gallic acid, tannic acid, ferrous chloride, ferric chloride, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra-acetic acid (EDTA), N-(1-naphthyl) ethylene diamine dihydrochloride, sodium nitroprusside, ferulic acid, caffeic acid, quercetin, p-coumaric acid, chlorogenic acid, catechin, naringenin and trans- cinnamic acid, etc. were purchased from Himedia (Mumbai), SRL (Mumbai) and Sigma Aldrich (USA). All the chemicals and solvents used were of analytical grade.

2.3. Preparation of plant extracts

The powdered petiole (70g) were packed in small thimbles and separately extracted with organic solvents (400ml) such as petroleum ether, Chloroform, Ethyl acetate and Methanol in the increasing order of their polarity using Soxhlet apparatus, each time before extracting with the next solvent, the thimbles were air dried. Finally, the material was macerated by using hot water with constant stirring for 24h and the water extract was also filtered through Whatman No. 1

filter paper. The different solvent extracts were concentrated by rotary vacuum evaporator (Model; Evator E11) and then air dried.

2.4. Extract recovery percentage

The amount of extract recovered after successive extraction from dried plant powder (petiole) was weighed and the percentage yield was calculated by the following formula

$$\text{Extract recovery percentage} = \frac{\text{Amount of extract recovered (g)}}{\text{Amount of plant sample (g)}} \times 100$$

2.5. Preliminary qualitative phytochemical analysis

The different extracts of *O. latifolia* and *O. tetraphylla* petioles were analyzed for the presence of major phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, flavonoids, glycosides, flavanol glycosides, cardiac glycosides, phytosterols, fixed oils & fats, gums & mucilages according to standard methods [9].

2.6. Quantification Assays

2.6.1. Determination of total phenolics

The amount of total phenolics was determined with the folin-ciocalteu reagent using the method described [10]. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation, the absorbance was read at 725 nm. The results were calculated by using standard calibration curve of gallic acid (R^2 =0.9236) and expressed as gallic acid equivalents per gram of extract (GAC/g Extract).

2.6.2. Determination of total tannins

The total phenolics contain both tannin and non-tannin phenolics. The amount of tannins was calculated by subtracting the non-tannin phenolics from total phenolics. The method described [10], was used for determination of non-tannin phenolics. The analyses were also performed in triplicates and the results were expressed in tannic acid equivalents (TAE).

Tannins = Total phenolics - Non tannin phenolics

2.6.3. Determination of total flavonoids

The flavonoids contents of the extracts were quantified according to the method described [11]. The pink colour developed because of the presence of flavonoids was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated in Rutin equivalents (RE).

2.7. In vitro antioxidant assays

2.7.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The antioxidant activity of the extracts were determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, as described [11]. The absorbance of the sample, standards (Rutin and BHT) and control were measured at 517 nm. Methanol was served as blank. Radical scavenging activity of the samples was expressed as IC_{50} value which is the concentration of the sample required to inhibit 50% of DPPH concentration.

2.7.2. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Activity

The total antioxidant activity of the sample extracts were measured by ABTS radical cation decolourization assay [11]. The absorbance of samples and standards (BHT and Rutin) were measured at 734 nm against the ethanol. The results were expressed as the μM Trolox equivalent per g extracts.

2.7.3. Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of different extracts of samples were estimated according to the procedure described [11]. The absorbance of the blue colour developed by the sample and standards (BHT and Rutin) were read immediately at 593 nm. The FRAP value is expressed as mM Fe (II) equivalent/mg extract.

2.7.4. Superoxide radical scavenging activity

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin–light–NBT system [11]. The

absorbance of the sample and standards (BHT and Rutin) read immediately at 590 nm. The percentage inhibition of superoxide anion generation was calculated by the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of treated sample}}{\text{Absorbance of control}} \times 100$$

2.7.5. Phosphomolybdenum assay

The antioxidant activities of sample extracts were evaluated by the green phosphomolybdenum complex formation [11]. The absorbance of the sample and standards (BHT and Rutin) were measured at 695 nm. The results reported are mean values expressed as grams of ascorbic acid equivalents per gram samples.

2.7.6. Reducing power assay

The reducing power of sample extracts was determined according to the method [11]. The absorbance of the sample and standards (Tannic acid) were measured at 700 nm.

2.8. *In vitro* anti-inflammatory activity

2.8.1 *In vitro* anti-inflammatory activity by membrane stabilization method

Alsever's solution was prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in distilled water and then the solution was sterilized [12]. Blood was collected from retina of Wistar albino rats. The collected blood was mixed with equal volume of sterilized alsever's solution. The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed 3 times with isosaline (0.9%, pH-7.2) and as us pensionin 10% (v/v) isosaline were made. The reaction mixture (4.5 mL) contained 1 mL phosphate buffer (pH-7.4) , 2 mL Hyposaline (0.45%), 1 mL plant extract (1 mg/mL) and 0.5 mL RBC (Red blood cells) suspension. Diclofenac sodium was used as the reference drug. Reaction mixture without plant sample was used as control and phosphate buffer served as blank. The assay mixtures were incubated at 37˚C for 30 min. and centrifuged again. The haemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm. Percent membrane stabilization activity was calculated by the formula:

Absorbance of control – Absorbance of treated sample

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control}}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. Extract recovery percentage

The Extract recovery percentage of *Oxalis latifolia* and *O. tetraphylla* petiole extracts from different solvents are presented in Table 1. Amoung the various solvents, the methanol extracts showed maximum yield for both *O. tetraphylla* (13.67%) and *Oxalis latifolia* (10.98%) sample of the present.

Table1. Extract recovery percentage of *Oxalis latifolia* and *O. tetraphylla*

S. No.	Solvents	Yield Percentage	
		O. latifolia	*O. tetraphylla*
1	Petroleum ether	4.47	5.50
2	Chloroform	9.58	6.98
3	Ethyl acetate	2.97	3.58
4	Methanol	**10.98**	**13.67**
5	Hot water	6.64	9.77

3.2. Qualitative Phytochemical Screening

The qualitative phytochemical screening of petiole of *O. latifolia* and *O. tetraphylla* for major primary and secondary phytochemicals were carried out and the results were shown in Table 2. The results of the qualitative phytochemical screening showed the presence of all the phytochemical constitution of the present study such as carbohydrates, alkaloid, saponins, phenol, flavanoid, Flavonol glycosides, cardiac glycosides, phytosterol, fixed oils and fats, gums and mucilage in both the samples. The +++ sign indicates high concentration of particular secondary metabolites which was indicated by the high intensity of the colour developed.

Table 2: Preliminary Phytochemical Analysis of *O. latifolia* and *O. tetraphylla*

Sample	*O. latifolia*	*O. tetraphylla*

Extract / Phyto Chemical	P.E	C.F	E.A	M	H.W	P.E	C.F	E.A	M	H.W
Carbohydrate	+	+	+++	++	+	+	+	+++	+++	++
Protein	-	+	+	++	+	-	+	+	+	+
Amino acid	-	-	+++	++	+	-	-	+++	++	+
Alkaloids	+	+	+++	+	+	+	+	+++	+	+
Saponins	-	-	+	+	++	-	-	+	+	+++
Phenolic	++	+	++	+	+	+	+	++	+	+
Flavonoid	-	+	++	++	+	-	+	+	++	+
Glycoside	+	+	+	+	+	+	+	+	+	+
Flavonol glycosides	+	-	++	+	+	+	-	+	+	+
Cardiac glycoside	++	+	+	+	+	++	+	+	+	+
Phytosterol	+	++	++	++	+	+	++	++	+	+
Fixed oils & fats	++	+	+	+	+	++	+	++	+	+
Gums & mucilages	+	+	+	+	+	+	+	++	+	+

(+): Presence of chemical compound, (-): Absence of chemical compound

(+) < (++) < (+++): Based on the intensity of characteristic colour

P.E- Petroleum ether, C.F- Chloroform, E.A- Ethyl acetate, M-Methanol, H.W- Hot Water

3.3. QUANTIFICATION ASSAYS

3.3.1. Determination of total phenolics and tannin content

Total phenolic content has been reported to be directly associated with antioxidant activity. These compounds are known as powerful chain-breaking antioxidants [13-14]. The

amount of total phenolics of different extracts of *O. latifolia* and *O. tetraphylla* petiole were analyzed and shown in Table 3. The total phenolics were found to be higher in ethyl acetate extract of both *O. latifolia* (119.32 mg GAE/g extract) and *O. tetraphylla* (159.39 mg GAE/ g extract). The results revealed that the *O. tetraphylla* ethyl acetate extract contains the highest total phenolic content and thus it possesses better antioxidant potential sample.

Table 3: Total phenolic and Tannins contents of *O. latifolia* and *O. tetraphylla* petiole extracts

Samples	Extracts	Total Phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)
O. latifolia	Petroleum ether	50.63 ± 2.91	43.3 ± 2.68
	Chloroform	45.92 ± 5.56	41.03 ±4 .08
	Ethyl acetate	**119.32 ± 3.08**	**111.91 ± 2.99**
	Methanol	114.61 ± 1.54	105.05 ± 1.51
	Water	78.92 ± 4.2	72.92 ± 4.09
O. tetraphylla	Petroleum ether	54.34 ± 3.03	42.78 ± 3.77
	Chloroform	51.31 ± 5.24	46.12 ± 3.39
	Ethyl acetate	**159.39 ± 3.64**	**150.43 ± 3.4**
	Methanol	85.65 ± 2.1	76.88 ± 1.27
	Water	94.41 ± 3.24	87.74 ± 3.93

GAE – Gallic Acid Equivalents: RE – Rutin Equivalents

Values are mean of triplicate determination (n=3) ± standard deviation.

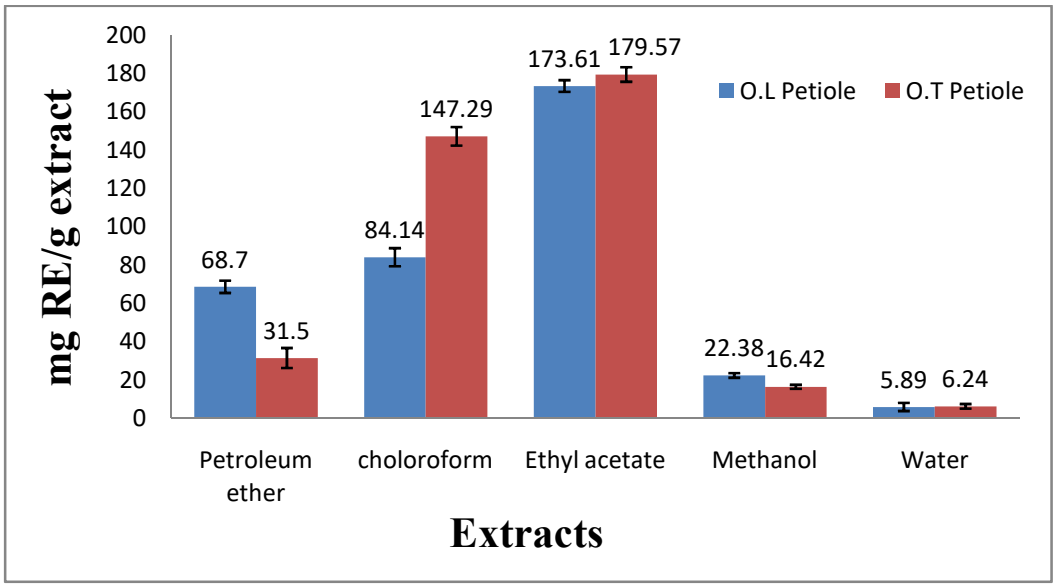
Tannins are water soluble polyphenols present in many plants and have also been recognized as antioxidants. The amount of tannins present in different extracts of *O. latifolia* and *O. tetraphylla* petiole is represented in Table 3. The tannins were found to be higher in ethyl acetate extract of petiole of *O. latifolia* (111.91 mg GAE/g extract) and *O. tetraphylla* (150.43 mg GAE/ g extract). The results revealed that the *O. tetraphylla* ethyl acetate extract contains the highest tannin content. Tannin components were suggested to be anti-carcinogenic and have been shown to reduce the mutagenic activity. Mutagens produce oxygen free radicals for interaction with cellular macromolecules [15]. The anti-carcinogenic and anti-mutagenic

potentials of tannins may be connected to their anti-oxidative properties, which are important in protecting against cellular oxidative damage [16].

3.3.2. Determination of flavonoids Content

Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals which are implicated in several diseases [17]. The flavonoid contents present in different extracts of *O. latifolia* and *O. tetraphylla* petiole is represented in Fig 1. Among all the extracts, the ethyl acetate extract of both the samples have appreciable amount of flavonoid contents (*O. latifolia* 173.61mg RE/g extract and *O. tetraphylla* 179.57 mg RE/g extract). whereas methanol, chloroform, petroleum ether and hot water extracts also registered with appreciable flavonoid content. The flavonoid content in the petiole of *O. tetraphylla* was found to be higher than *O. latifolia* in ethyl acetate and chloroform extracts. Flavonoids suppresses reactive oxygen formation, chelate trace elements involved in freeradical production, scavenge reactive species and upregulate and protect antioxidant defenses [18].

Fig 1: Flavonoid contents of *O. latifolia* and *O. tetraphylla* petiole extracts



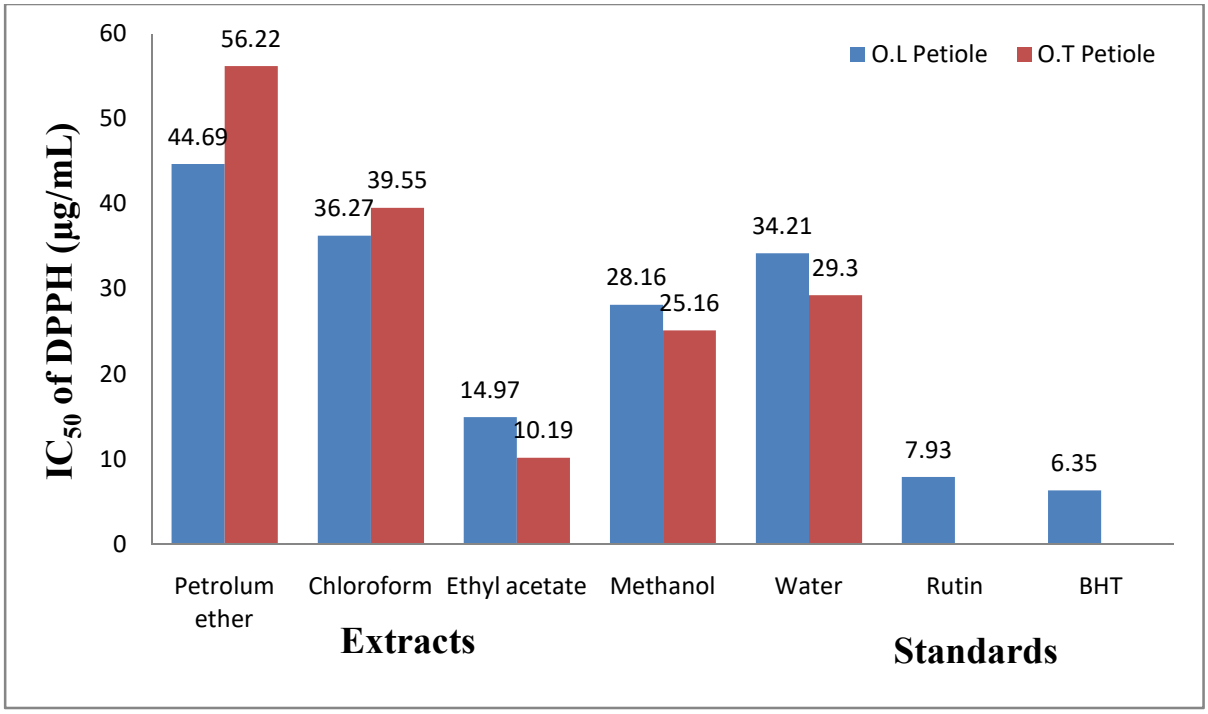
Values are mean of triplicate determination (n=3) ± standard deviation

3.4. *In vitro* antioxidant assays

3.4.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

DPPH is a free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [19]. The DPPH radical scavenging activities of *O. latifolia* and *O. tetraphylla* petiole are shown in Fig 2. In this assay, the commercial antioxidants BHT and rutin were used as standards. The ethyl acetate extracts of both *O. latifolia* (14.97µg/ml) and *O. tetraphylla* (10.19 µg/ml) showed better IC_{50}values for DPPH radical scavenging activities compared to other solvent extracts. The IC_{50}value of standard natural antioxidant rutin and synthetic antioxidant BHT was found to be 7.93 and 6.35 µg/ml respectively.

Fig 5: DPPH radical scavenging activity of *O. latifolia* and *O. tetraphylla*



Values are mean of triplicate determination (n=3) ± standard deviation

The antioxidant activities against DPPH were correlated with the concentration, chemical structures and polymerization degrees of organic antioxidants. In fact, numerous studies have indicated that plant extracts rich in phenolic compounds are capable of complexing with and stabilizing transition metal ions, rendering them unable to participate in metal-catalyzed initiation and hydro peroxide decomposition reactions [20].

3.4.2. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Activity

The TEAC (Trolox Equivalents Antioxidant Capacity) was measured using the improved $ABTS^{+}$ radical decolourisation assay; one of the most commonly employed methods for antioxidant capacity, which measures the ability of a compound to scavenge ABTS cation radical. The results were expressed as µg TE (Trolox Equivalents)/g of extract. The results of ABTS cation radical scavenging activities of *O. latifolia* and *O. tetraphylla* petiole are shown in Table 4. The ethyl acetate extracts showed higher radical scavenging activities in both *O. latifolia* (55138.89 µg TE/g) and *O. tetraphylla* (57256.94 µg TE/g).

The ABTS assay has been employed as an index that reveals the antioxidant activity of test samples [21]. This shows that *O. tetraphylla* extract possess a good ability to scavenge the ABTS radical.

3.4.3. Ferric reducing antioxidant power assay (FRAP)

FRAP is a simple inexpensive assay and may offer presumed index of antioxidant activity. The FRAP assay measures the ability of antioxidants to reduce the ferric 2,4,6-tripyridyl-S-triazine complex [Fe(III)-(TPTZ) 2]$^{2+}$ to intensely blue coloured ferrous complex [Fe(II)-(TPTZ) 2]$^{2+}$ in an acidic medium [22]. Generally, the reducing properties are linked with the presence of compounds which exert their action by breaking free radical chain by donating a hydrogen atom [23]. The results showed (Table 4) that the ferric reducing capacity of ethyl acetate petiole extract was much higher in *O. tetraphylla* (318.51 mM Fe(II)/mg extract).

3.4.4. Superoxide radical scavenging activity

Although superoxide anion is a weak oxidant, it ultimately produces powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [24]. The extracts were found to be an efficient scavenger of superoxide radical generated in riboflavin- NBT- light system *in-vitro*. The scavenging activity of ethyl acetate extract of both *O. latifolia* (27.73%) and *O. tetraphylla* (43.63%) respectively. These results clearly suggest that the antioxidant activity of the plant *O. tetraphylla* also related to scavenge the superoxide radicals.

Table 4: ABTS radical cation scavenging activity, Ferric reducing antioxidant power assay and superoxide radical scavenging activity of *O. latifolia* and *O. tetraphylla* petiole extracts

Samples	Extracts	ABTS (µg TE/g extract)	FRAP mM Fe (II)/mg extract)	Superoxide % of inhibition
O. latifolia	Petroleum ether	18715.27 ± 1.27	20.49 ± 3.72	8.89 ± 0.2
	Chloroform	22673.61 ± 1.32	28.14 ± 5.92	17.31 ± 1.44
	Ethyl acetate	**55138.88 ± 0.7**	**224.44 ± 3.22**	**27.73 ± 0.88**
	Methanol	48368.05 ± 1.1	168.14 ± 1.86	22.49 ± 1.52
	Water	30902.77 ± 2.02	46.91 ± 3.08	25.55 ± 1.02
O. tetraphylla	Petroleum ether	25625 ± 1.12	17.03 ± 1.48	14.95 ± 2.06
	Chloroform	43680.55 ± 1.16	29.87 ± 3.5	28.2 ± 1.07
	Ethyl acetate	**57256.94 ± 0.78**	**318.51 ± 2.22**	**43.63 ± 2.03**
	Methanol	54513.88 ± 0.12	185.51 ± 4.52	25.97 ± 0.91
	Water	40868.05 ± 0.26	176.04 ± 5.55	35.63 ± 0.53
Standard	Rutin	130104.2 ± 1041.66	492.83 ± 4.46	8.89 ± 0.2
	BHT	127152.8± 1591.17	566.91 ± 3.5	94.7 ± 0.25
	BHA			94.2 ± 0.1

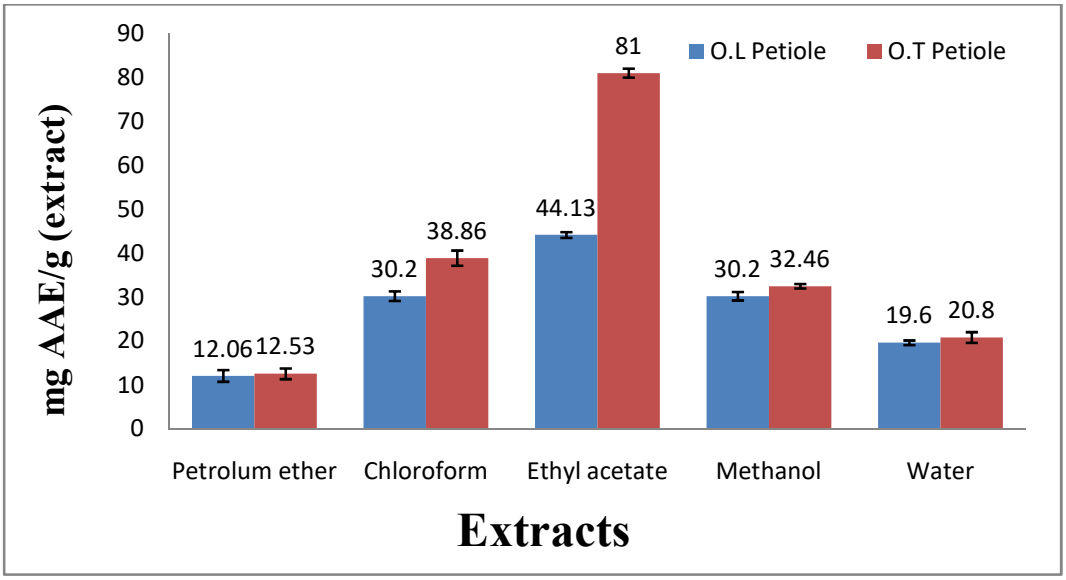
TE – Trolox Equivalents; Fe (II) - Ferric Equivalents.

Values are mean of triplicate determination (n=3) ± standard deviation

3.4.5. Phosphomolybdenum assay

The phosphomolybdenum method has been widely used in the assessment of total antioxidant activity of plant extracts, natural compounds and foods [25]. The total antioxidant capacity of different solvent extracts of *O. latifolia* and *O. tetraphylla* petiole were analyzed and shown in Fig 3. The better antioxidant capacity was shown by ethyl acetate extract of *O. latifolia* (44.13 mg AAE/g extract) and *O. tetraphylla* (81 mg AAE/g extract). The results revealed that the *O. tetraphylla* ethyl acetate extract contains the highest phosphomolybdenum and thus it possesses better antioxidant potential than the other extracts.

Fig 3: Phosphomolybdenum Reduction assay of *O. latifolia* and *O. tetraphylla*



Values are mean of triplicate determination (n=3) ± standard deviation

3.4.6. Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the presence of reductants (antioxidants) in tested samples would result in the reduction of Fe^{3+} ferricyanide complex to the ferrous form. The measurement of the reductive ability, from Fe^{3+} to Fe^{2+} transformation [26]. The reducing powder of *O. latifolia* and *O. tetraphylla* petiole extracts was investigated (Fig- 4 & 5) and shows the reductive capabilities of different solvent extracts. The results were compared with Tannic acid. Increase in absorbance of the reaction mixture indicated the reducing power of the samples. Much highr reducing power was found in ethyl acetate extract of *O. tetraphylla* petiole.

Fig 4: Reducing power assay of *O. latifolia* petiole extract

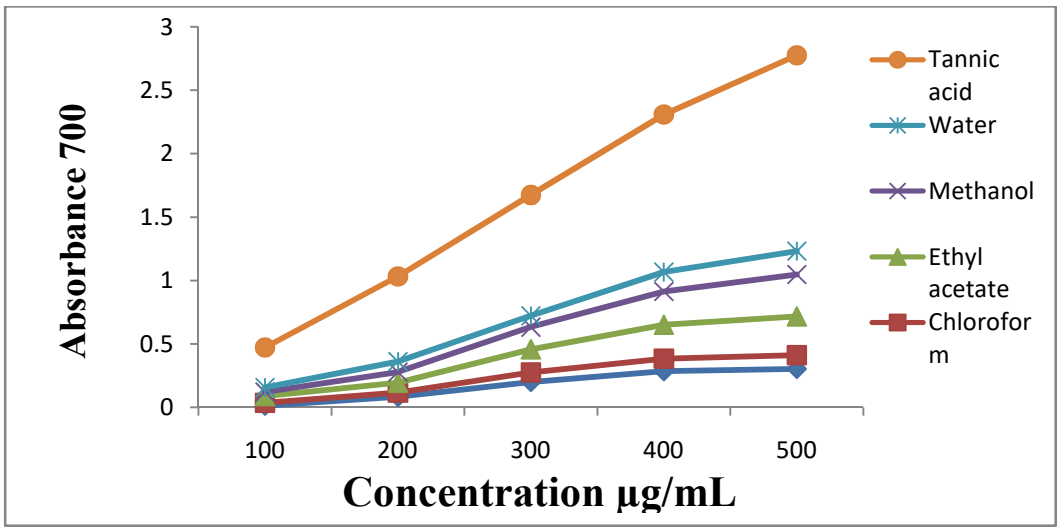
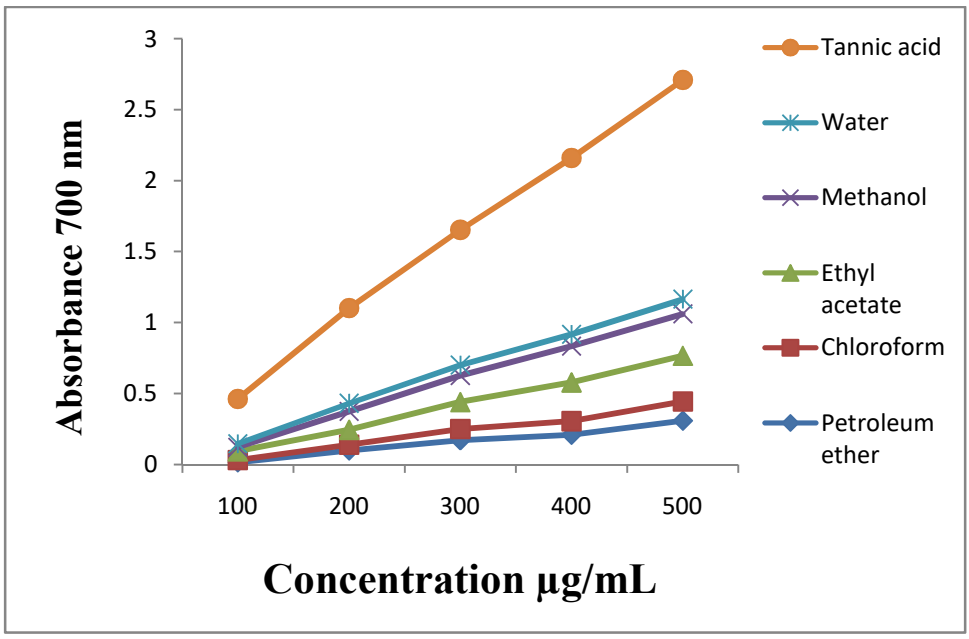


Fig 5: Reducing power assay of *O. tetraphylla* petiole extract

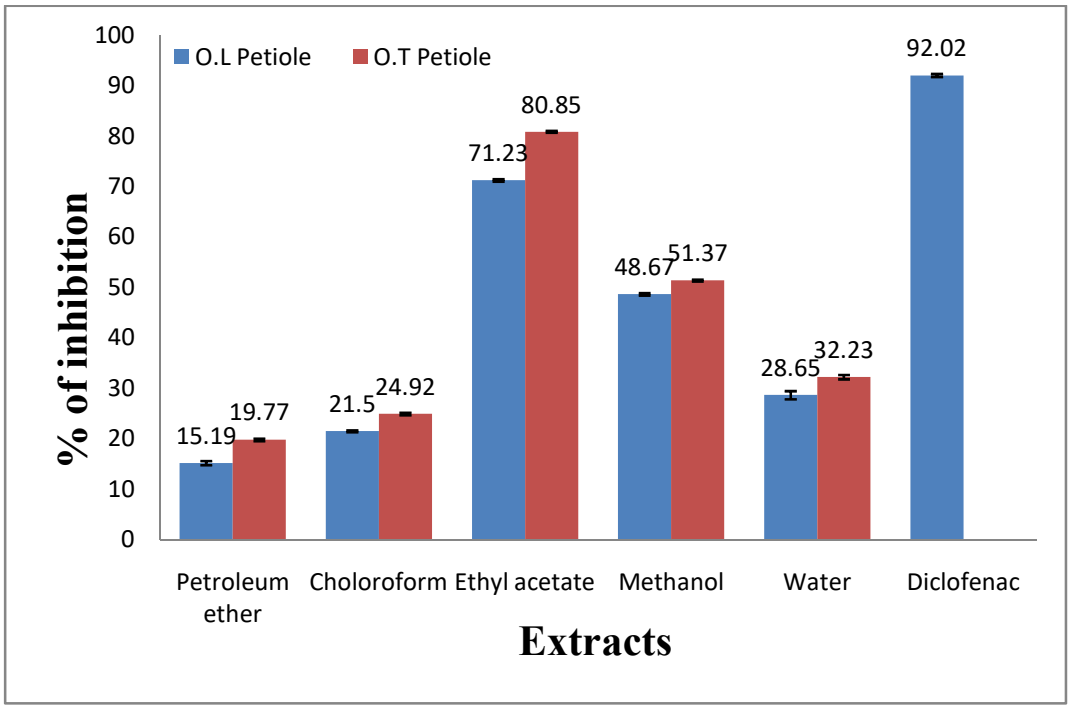


3.5. *In vitro* Anti-Inflammatory activity

The lysosomal enzymes released during inflammation produced a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The results were expressed as percentage inhibition of extract. The results of anti-inflammatory activities of petiole extracts of *O. latifolia* and *O. tetraphylla* are shown in Fig 6. Among the

various extracts of the present study, ethyl acetate extract of both *O. latifolia* (71.23%) and *O. tetraphylla* (80.85%) showed higher inhibition activity.

Fig 6: *In vitro* Anti-inflammatory activity of *O. latifolia* and *O. tetraphylla*



The extract exhibited membrane stabilization by inhibiting hypotonicity-induced lyses of the erythrocyte membrane [27], and its stabilization implies that the extract may stabilize lysosomal membranes. Stabilization of liposomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as microbial enzymes and proteases, which cause further tissue inflammation and damage by extracellular release [28]. The extract may inhibit these processes, which may stimulate or enhance the efflux of these intracellular components [29].

4. CONCLUSION

From the results it could be concluded that the ethyl acetate extract of *O. tetraphylla* petiole exhibited a strong antioxidant activity in the following antioxidant assays such as DPPH, ABTS, FRAP, Superoxide radical scavenging assay, Phosphomolybdenum, reducing power assay and Anti-Inflammatory activity (Membrane stabilization assay) of which *O. tetraphylla* petiole exhibited highest radical scavenging activity and have been recognised to have highest hydrogen donating ability and metal ion chelating capability. Further studies are warranted for

the isolation and identification of individual bioactive compounds and also *in vivo* studies are needed for understanding their mechanism of action as an antioxidant and anti- Inflammatory drug which can be a cost effective and reliable source of medicine for the human welfare.

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