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COMPARATIVE EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *OXALIS TETRAPHYLLA* CAV. AND *OXALIS LATIFOLIA* KUNTH – AN *IN VITRO* APPROACH

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ABSTRACT
The present investigation has been undertaken to study the preliminary phytochemical screening, antioxidant and anti-inflammatory activity of *Oxalis tetraphylla* and *Oxalis latifolia* bulb. The different extracts such as petroleum ether, chloroform, ethyl acetate, methanol and Hot water were subjected to quantification (total phenolics, tannin, flavonoids) and *in vitro* antioxidant assay (DPPH, ABTS, FRAP, Superoxide radical scavenging assay, Phosphomolybdenum, reducing power assay and Anti-Inflammatory activity (Membrane stabilization assay). Among the various solvents, ethyl acetate extract of both the samples displayed maximum total phenolic (609.79 mg GAE/g extract), tannin (601.42 mg GAE/g extract) and flavonoid contents (156.31 mg RE/g extract). Results of *in vitro* antioxidant studies revealed that the ethyl acetate extract of bulb possessed an efficient 2,2-diphenyl-1-picryl-hydrazyl (DPPH•) (IC_{50} 14.97 µg/mL), ABTS (57465.28 µM TE/g extract), FRAP (595.55 mM Fe (II)/mg extract), superoxide (32.44%) radical scavenging activities, Phosphomolybdenum (168.86 mg AAE/g extract), Reducing power possesses higher activity and Membrane stabilization assays (80.85%) activity. Therefore, the results indicate that the bulb of *O. tetraphylla* and *O. latifolia* can serve as a potential preliminary phytochemical, antioxidant and anti-inflammatory activity.

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

INTRODUCTION

Oxidative stress is a mechanism that increases the production of reactive oxygen species (ROS) in the body (Rahmat *et al*., 2012). These are a series of metabolic by products that are involved in degenerative and pathological processes in the human body (Han *et al*., 2016). The Over production of ROS in the body could disturb cellular redox balance, resulting in cell injury or apoptosis (Pizzino *et al*., 2017). Some of the relevant ROS are hydroxyl (OH), superoxide anion ($O2^{\cdot}{}_2^{-}$), peroxyl ($ROO^{\cdot}$), alkoxy ($RO^{\cdot}$), hydrogen peroxide (H_2O_2), and hypochloride (HOCl). Besides these, reactive nitrogen species like nitric oxide (NO) are also important. These trigger the oxidative damage of tissues and organs, could lead the development of various diseases, such as cancer, atherosclerosis, diabetes, chronic inflammatory disease, cardiovascular disease and Alzheimer (Shoham *et al*., 2008; Babbar *et al*., 2011).

Antioxidants are substances that inhibit oxidation of an oxidizable substrate when present at low concentration in comparison with those of the substrate antioxidant neutralises the effectiveness of free radicals, which are safely interacted with the free radicals and theses form chain reaction before vital molecules get damaged. Phenolics, flavanoids, vitamins (E and C), numerous minerals (Cu, Mn, Zn, Se and Fe) and glutamine are one of the most widely recognized antioxidants (Abhishek *et al.,* 2013). Free radicals are constantly produced in living system and they are detoxified by antioxidants. However, they can cause oxidative damage to important cellular compartments when present in excess. Indeed, consumption of foods as a source of antioxidants has been reported to have health-promotion and disease prevention effects (Olamide *et al.,* 2017). Inflammation is a body's response that disturbs the homeostasis caused by infection, injury or trauma resulting in systemic and local effects Inflammation is the first line of defence against pathogens can contribute to all phases of tumor genesis including tumour initiation, promotion and metastasis (Syed Murthuza, and Manjunatha, 2018). *Oxalis tetraphylla* and *O. latifolia* belonging to Oxalidaceae family is a stem less herb of cosmopolitan distribution found abundantly in agricultural farms, gardens, lawns etc. The bulbs are reported to cure various disorders such as paralysis, stomach disorder and it also acts as thirst reliever. Thus these plants are taken for phytochemical and pharmacological study.

MATERIALS AND METHODS

Collection and identification of plant materials

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

Preparation of plant extracts

The powdered bulbs (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 using hot water with constant stirring for 24h and the water extract was also filtered using Whatman No. 1 filter paper. The different solvent extracts were concentrated by rotary vacuum evaporator (Model; Evator E11) and then air dried.

Extract recovery percentage

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

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

Preliminary qualitative phytochemical analysis

The different extracts of *O. latifolia* and *O. tetraphylla* bulbs 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 and mucilages according to standard methods (Raaman, 2006).

Determination of total phenolic

The amount of total phenolics was determined with the folin-ciocalteu reagent using the method described by Parimelazhagan, 2016. A standard curve was plotted using Gallic acid as a standard. 100 µL of sample was dissolved in 500µL of folin-ciocalteu reagent and 100 µL of distilled water. The solutions were mixed and incubated at room temperature for 1min. After 1 min, 2500 µl 5% sodium carbonate (Na_2Co_3) solution was added. The final mixture was vortexed and then incubated for 40min. in the dark at room temperature. The absorbance of all samples was measured at 725 nm using UV – Visible Spectrophometer and the results were expressed as Gallic acid equivalents per gram of extract (GAC/g Extract).

Determination of total tannin

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. 100 mg of polyvinyl polypyrrolidone (PVPP) was weighed and 500 µL each of distilled water and 500 µL sample extracts was added. The content was vortexed and kept in the test tube at 4^oC for 4h. Then the sample was centrifuged at 4000 rpm for 10 min. at room temperature and the supernatant was collected. The phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolic on a dry matter basis. From the above result, the tannin content of the sample was calculated (Parimelazhagan, 2016).

Tannins = Total phenolics - Non tannin phenolics

Determination of total flavonoids

The flavonoids contents of the extracts were quantified according to the method described by Parimelazhagan, 2016. Initially, 500 µL of all the extracts were taken in different test tubes. To each extract, 2mL of distilled water was added. Then 150 µL of 5% $NaNo_2$ was added to all the test tube followed by incubation at room temperature for 6 min. After incubation, 150µL of $AlCl_3$ (10%) was added to all the test tubes including the blank. All the test tubes were incubated for 6 min. at room temperature. Then 2 mL of 4% NaOH was added, which was made upto 5mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 min. at room temperature. 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.

***IN VITRO* ANTIOXIDANT ASSAYS**

DPPH radical scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH Parimelazhagan, 2016. A methanol solution of the sample extract at various aliquots (20-100 µL) was added to 3mL of 0.1 mM methanolic solution of DPPH and allowed to stand for 30min. at 27°C. The absorbance of the sample was measured at 517 nm. Methanol was served as blank and solution without extract served as control. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula, more significantly, the Ic_{50} of the extracts were also calculated.

ABTS radical scavenging activity

The total antioxidant activity of the samples was measured by ABTS radical cation decolourization assay (Parimelazhagan, 2016). ABTS was produced by reacting 7mM ABTS aqueous solution with 2.4mM potassium persulfate in the dark for 12-16h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30^oC to give an absorbance at 734 nm of 0.7 ±0.02. The stock solution of the sample extracts were dilution such that after addition of different aliquots into assay, they produced between 20% and 80% inhibition of the blank absorbance after the addition of 1 mL of diluted ABTS solution, different concentrations of sample or trolox standards in ethanol, absorbance was measured at 30°C exactly blanks were also run in each assay. Aliquot determinations were made at each dilution of the standard, and the percentage inhibition was calculated an absorbance of 734 nm, and it was plotted as a function of trolox concentration. The unit of antioxidant activity (TAA) is defined as the concentration of trolox having equivalent antioxidant activity expressed as µMol/g sample extract on dry matter.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of different extracts of samples were estimated according to the procedure described by Parimelazhagan, 2016. The ferric –reducing antioxidant power (FRAP) regent contained 2.5 mL of 20 mMol/L TPTZ (2,4,6- tripyridyl-s-triazinic) solution in 40 mMol/L HCl plus 2.5 mL of 20 mMol/L $FeCl_3$ $6H_2O$ and 25 mL of 0.3 Mol/L acetate buffer (pH 3.6). FRAP regent (900 µL), prepared freshly and incubated at 37°C, was mixed with 90 µL distilled water and 10µL of test sample or methanol (for the regent blank) incubated at 37°C for 30 min in a water bath at the end of incubation the absorbance reading

were taken immediately at 593nm. The FRAP value is expressed as mMol Fe (II) equivalent/mg extract.

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 (Parimelazhagan, 2016). About 3 mL of reaction mixture containing 50 mM sodium phosphate buffer (pH-7.6), 20 µg riboflavin, 12 mM EDTA and 0.1 mg NBT was added to 100 µL sample solution, BHT and rutin. Reaction was started by illuminating the reaction mixture with samples for 90 seconds. The illuminated reaction mixture without sample was used as the negative control. Immediately after illumination, the absorbance was measured at 590 nm against the blank (unilluminated reaction mixture without plant sample). The scavenging activity on superoxide anion generation was calculated by the following formula.

Phosphomolybdenum assay

The antioxidant activity of sample was evaluated by the green phosphomolybdenum complex formation (Parimelazhagan, 2016). A triplicate of 200 µL of sample solution(1mM in dimethyl sulfoxide) was combined in 4 mL vial with 1 mL of reagent (0.6 M sulfuric acid 28 mM sodium phosphate and 4mM ammonium molybdate). The vials capped and incubated in a water bath at 95°C for 90 min. After cooling the vials, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as grams of ascorbic acid equivalents per gram samples (AEAC).

***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 (Shinde *et al.*, 1999). Blood was collected from retina of Wistar albino rats and 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 pensionin 10% (v/v) isosaline were made. The reaction mixture (4.5mL) contained 1 mL phosphate buffer (pH 7.4), 2mL 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 560nm. Percent membrane stabilization activity was calculated by the following formula:

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

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

The qualitative phytochemical screening of bulb of *O. tetraphylla* and *O. latifolia* for major primary and secondary phytochemicals were carried out and the results were shown in Table 1. The results revealed that the primary metabolites such as carbohydrates, proteins and amino acids showed positive result in all the extracts of both the samples.

The results of the qualitative phytochemical screening showed the presence of all the phytochemical constitution of the present study such as 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 1: Preliminary Phytochemical Analysis of *O. tetraphylla* and *O. latifolia*

Sample	*O. tetraphylla*					*O. latifolia*				
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	-	-	+	+	++	-	-	+	+	++
Phenol	++	+	++	+	+	+	+	++	+	+
Flavonoid	+	+	++	++	+	+	+	+	++	+
Glycoside	+	+	+	+	+	+	+	+	+	+
Flavonos 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

Quantification of total phenolic content

The content of extractable phenolic compounds in the *O. tetraphylla* and *O. latifolia* bulb extracts were determined through a linear gallic acid standard curve (y = 0.009x + 0.006; R^2 = 0.994). The amount of total phenolics present in various extracts were statistically analyzed and presented in Table 2. Among the different solvent extracts, both the ethyl acetate bulb extracts of *O. tetraphylla* (609.79mg GAE/g extract) and *O. latifolia* content (421.41 mg GAE/g extract) registered maximum phenolic content when compared with other extracts. The phenolics, the major group of secondary metabolites, are associated with the various pharmacological activities like antimicrobial, antiviral, anti-allergic, anti-inflammatory and anti-carcinogenic (Mohammed and Atik, 2011). In human diets, the plant polyphenols are antioxidants rich compounds which are most essential to exist positive correlation between the total phenolic contents and the antioxidant activities of the plant extracts (Dutta and Ray, 2015).These polyphenols are reported to have abundant amount of antioxidant which are included in human diet.

TABLE 2: Total phenolic, Flavonoid and tannins contents of *O. tetraphylla* and *O. latifolia* bulb extracts

Samples	Extracts	Total Phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)	Flavonoid (mg RE/g extract)
O. tetraphylla	Petroleum ether	125.45 ± 4	123.38 ± 4.07	21.22 ± 1.89
	Chloroform	123.43 ±2.31	119.36 ± 2.16	49.07 ± 1.74
	Ethyl acetate	609.79 ± 6.12	601.42 ± 5.36	156.31 ±5.22
	Methanol	178.48 ± 4.37	117.23 ± 4.37	6.09 ± 2.49
	Water	159.29 ± 2.31	152.99 ± 2.64	3.02 ± 1.31
O. latifolia	Petroleum ether	36.56 ± 3.81	35 ± 3.33	8.72 ± 2.11
	Chloroform	95.15 ± 3.03	93.77 ± 4.13	36.35 ± 4.38
	Ethyl acetate	421.41 ± 2.31	416.5 ± 3.33	119.91 ± 4.01
	Methanol	187.57 ± 4.54	182.26 ± 4.69	6.53 ± 1.65
	Water	106.26 ± 4.37	103.37 ± 5	3.2 ± 1.31

GAE – Gallic Acid Equivalents: RE – Rutin Equivalents

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

Quantification of total tannins content

Tannins are water soluble polyphenols present in many plants and have also been recognized as antioxidants. The amount of tannins presents in different extracts of *O. tetraphylla* and *O. latifolia* bulb is represented in Table 2. It can be observed that for all the extracts studied both ethyl acetate extract of *O. tetraphylla* (601.42 mg GAE/g extract) and *O. latifolia* (416.5 mg GAE/g extract) had highest amount of tannin content. Natural tannins has high amount of ortho-di-hydroxy group which are said to be agents of powerful antioxidant (Aggarwal and Shishodia, 2006).

Quantification of total flavonoid content

The amount of total flavonoids of different extracts of *O. tetraphylla* and *O. latifolia* bulb is shown in Table 2. The estimation of total flavonoid content in *O. tetraphylla* bulb revealed that the ethyl acetate extract possesses the maximum (156.31mg RE/g extract). Flavonoids, a group of phenolic compounds well known for their antioxidant and pharmacological activity it is been reported as derived from tyrosine, phenylalanine, and malonate, these also have an important role to have anti inflammatory and anti cancer property (Hertog *et al*., 1993; Sharma, 2006). Moreover, over-accumulation of flavonoids could enhance oxidative and drought tolerance phenomenon in Arabidopsis (Nakabayashi *et al*., 2014).

***IN VITRO* ANTIOXIDANT ASSAYS**

DPPH$^{\cdot}$ radical scavenging activity

Radical scavenging activity of several natural products has been estimated using DPPH radical scavenging assay. The result on free radical scavenging activity of the different solvent extracts along with the reference standards Rutin and BHT are shown in Fig. 1.

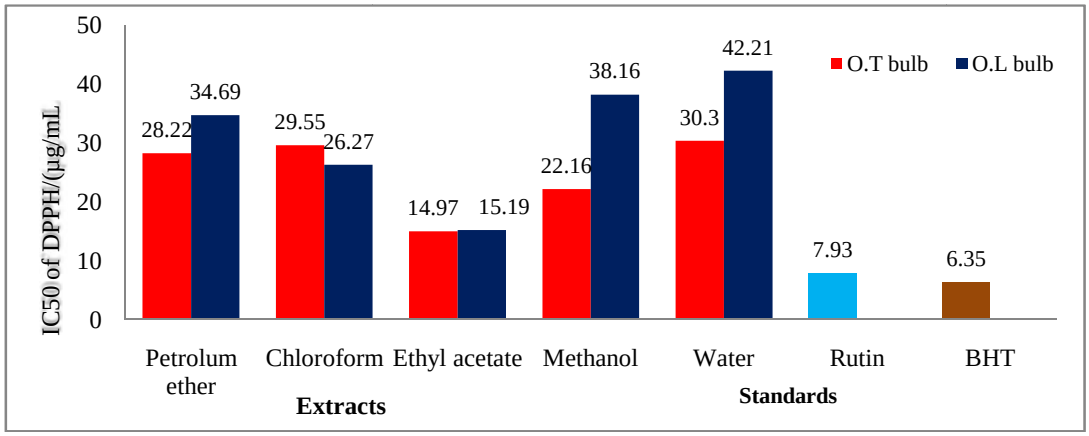

FIGURE 1: DPPH radical scavenging activity of *O. tetraphylla* and *O. latifolia* bulb extracts

The ethyl acetate extract of *O. tetraphylla* bulb showed the highest radical scavenging activity (14.97µg/mL), The IC_{50} value of Rutin and BHT, used as positive control in this study. Free radicals are produced due to oxidative stress that they play a major role in cellular damage which becomes a major cause for much chronic disorder. In DPPH the free radical are neutralized to stable diamagnetic molecule after accepting an electron or hydrogen radical (Sochor *et al*., 2010).

Total Antioxidant Activity by $ABTS^{\bullet+}$ Radical Cation Assays

ABTS•+ scavenging activity of different extracts of *O. tetraphylla* and *O. latifolia* bulb and standards are presented in Table 3. The both ethyl acetate extract of *O. tetraphylla* (57465.28 µM TE/g extract) and *O. latifolia* (56770.83 µM TE/g extract) showed the highest radical scavenging activity (57465.28 µM TE/g extract). The compounds are soluble in both aqueous and organic solvents, which are not affected by ionic strength and can be used to measure the antioxidant capacity and so the hydrophilic and lipophilic compounds in plant samples (Roginsky and Lissi, 2005). Phenolic compounds have a high antioxidant capacity due to low redox potential as these compounds can react with cation radicals due to its thermodynamic property (Osman *et al*., 2006). The phenolic compounds are not affected by ionic strength since these are soluble in both aqueous and organic solvent the antioxidant capacity can be measured for ABTS assay indicated that the extracts of *O. tetraphylla* possess strong hydrogen donating ability and could serve as free radical scavengers by acting as primary antioxidants when they are ingested along with nutrients.

Ferric reducing antioxidant power assay (FRAP)

The natural antioxidants possess ability to donate electrons to reactive radical cation, reducing them to more stable and un-reactive compound which is said to be the basis of ferric reducing power assay (FRAP). Table 3 shows the results of FRAP analysis of *O. tetraphylla* and *O. latifolia* bulb extract. The results revealed that the ferric reducing capacity of both ethyl acetate extract of *O. tetraphylla* (595.55 mM Fe (II)/mg extract) and *O. latifolia* (548.14 mM Fe (II)/mg extract) exhibited a high reducing power activity. The ferric reducing antioxidant power assay is used to measure the antioxidant effect of any substance in the reaction medium by means of its reducing ability. (Siddhuraju *et al*., 2002) have reported that low and high molecular phenolics are associated with antioxidants to scavenge free radicals. It has also been proved that the potential antioxidants through *in vitro* ferric-reducing antioxidant power assay increased the total antioxidant capacity of blood plasma (Serafini *et al*., 2003). Thus the ferric reducing power of different extracts of *O. tetraphylla* reveals that there are compounds which have high affinity to the ferrous ions and thereby quench them through redox reactions.

Superoxide radical scavenging activity

Superoxide radical scavenging activity was estimated based on the capacity of the samples to improve the aerobic photochemical reduction of nitroblue tetrazolium (NBT) in the presence of riboflavin. All the solvent extracts of *O. tetraphylla* (32.44%) and *O. latifolia* (32.28%) bulb showed significant scavenging activity on the superoxide radicals in a dose dependent manner (Table 3). Superoxide radical acts as a precursor of more reactive oxygen species like hydrogen peroxide, hydroxyl and singlet oxygen and is known to be a very harmful species to cellular components (Lee *et al*., 2004). Although they cannot directly initiate lipid oxidation, superoxide radical anions are potent precursors of highly reactive species such as hydroxyl radical and thus the study of scavenging of this radical is important (Kannat *et al*., 2007). Since the ethyl acetate extract of bulb of *O. tetraphylla* showed appreciable percentage of scavenging activity against superoxide radical, it can be used against adverse effects caused by superoxide radical in the body. The active principles in the plant extracts may eliminate the radical by its reduction to attain the octant stage or through the formation of water molecule.

TABLE 3: ABTS radical cation scavenging activity, Ferric reducing antioxidant power assay and superoxide radical scavenging activity of *O. tetraphylla* and *O. latifolia* bulb extracts

Samples	Extracts	ABTS (µM TE/g extract)	FRAP mM Fe (II)/mg extract)	Superoxide % of inhibition
O. tetraphylla	Petroleum ether	23020.83± 813.56	144.19± 3.91	18.68± 0.49
	Chloroform	24479.17± 908.10	225.18± 4.21	22.35± 0.33
	Ethyl acetate	57465.28± 60.14	595.55± 8.34	32.44± 0.15
	Methanol	54513.89± 394.36	234.56± 2.6	24.89± 0.55
	Water	55208.33± 275.59	237.53± 2.3	26.01± 0.47
O. latifolia	Petroleum ether	17256.94± 334.84	40.74± 5.35	8.01± 0.39
	Chloroform	29652.78± 420.98	136.54± 3.7	11.85± 0.31
	Ethyl acetate	56770.83± 104.16	548.14± 6.32	32.28± 0.42
	Methanol	54236.11± 159.11	399.25± 5.25	23.53± 0.52
	Water	46145.83± 275.59	61.97± 4.27	24.96± 0.71
Standard	Rutin	130104.2± 1041.66	492.83± 4.46	-
	BHT	127152.8± 1591.17	566.91± 3.5	94.2± 0.1
	BHA			94.7± 0.25

TE – Trolox Equivalents; Fe (II) - Ferric Equivalents.
Values are mean of triplicate determination (n=3) ± standard deviation

Phosphomolybdenum assay

The phosphomolybdenum assay was used to determine the total antioxidant capacity of *O. tetraphylla* and *O. latifolia* bulb and the results are presented in Fig 2. However, the results are estimated from linear ascorbic acid standard curve (y=0.146 x−0.096; R^2=0.984).The better antioxidant capacity was revealed by both ethyl acetate extract of *O. tetraphylla* (168.86 mg/g) and *O. latifolia* (94 mg/g). Absolute *O. tetraphylla* ethyl acetate extract was found to be more efficient solvent for extracting the Phosphomolybdenum assay. Being simple and independent of other antioxidant measurements commonly employed, the application of assay was extended to plant extracts (Prieto *et al*., 1999). Since the antioxidant activity is expressed as the number of equivalents of ascorbic acid, the total antioxidant capacity observed for the extracts of *O. tetraphylla* can be correlated with its free radical scavenging activity. The reduction of Mo (VI) to Mo (V) by the bulb extracts of *O. tetraphylla* may be due to the electron transfer or hydrogen ion transfer by the bioactive compounds, specifically phenolics and flavonoids present in the respective plant parts.

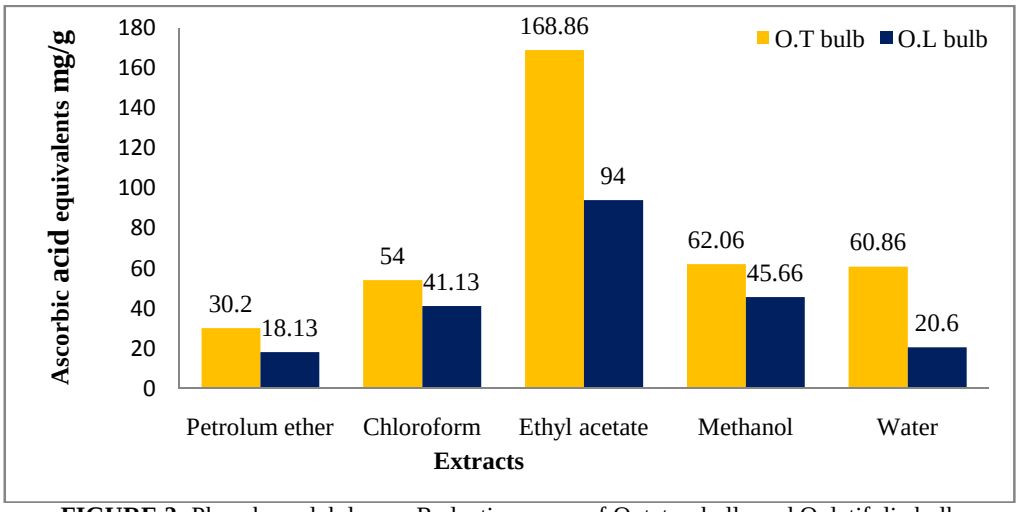

FIGURE 2: Phosphomolybdenum Reduction assay of O. tetraphylla and O. latifolia bulb
Values are mean of triplicate determination (n=3) ± standard deviation

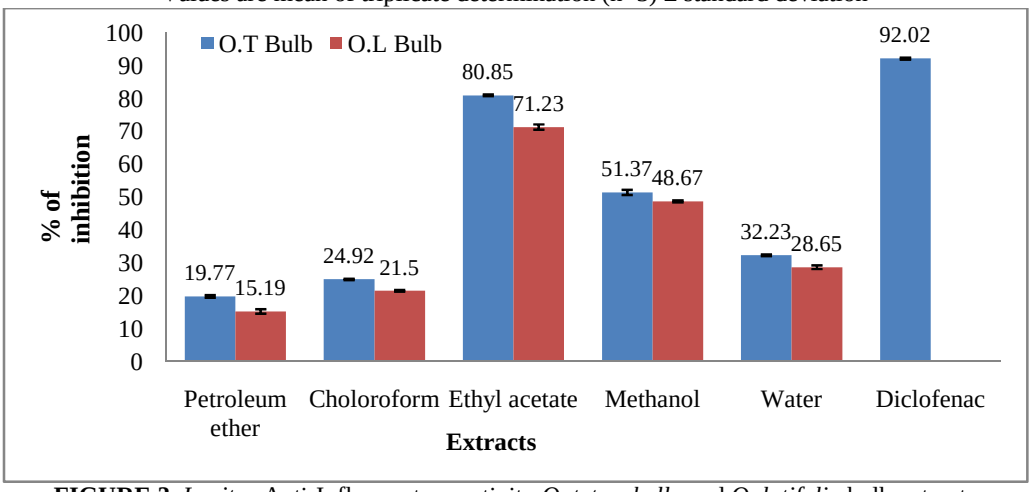

FIGURE 3. *In vitro* Anti-Inflammatory activity *O. tetraphylla* and *O. latifolia* bulb extracts
Values are mean of triplicate determination (n=3) ± standard deviation

***In vitro* Anti-Inflammatory activity**

During inflammation the lysosomal enzymes are produced and these take responsibilities to induce many diseases. Acute and chronic disorders are very much related to the extracellular enzyme activity (Middleto *et al.*, 2000). The results were expressed as percentage inhibition of extracts the anti-inflammatory activities of both ethyl acetate extracts of *O. tetraphylla* (80.85%) and *O. latifolia* (71.23%) are shown in Fig 3.

Inflammatory mediators have been reported to affect the erythrocytes whose formation is triggered by injuries and foreign particles. The significant protection of cell membranes against injurious substances by interfering with the release of phospholipases were the compounds are present with membrane stabilizing properties and these set of the formation of inflammatory mediators. Here, Animal (Rat) red blood cells are exposed to hypotonic medium which causes membrane lysis and oxidation of haemoglobin. The haemolytic effect of hypotonic solution is associated with excessive accumulation of fluid inside the cell resulting in the rupturing of its membrane which causes secondary damage through free radical induced lipid peroxidation (Rahman *et al*., 2015). As indicated by the previous results, the extracts of *O. tetraphylla*

exhibited good antioxidant property also in addition to its anti-inflammatory property. This suggests that the plant could serve as an excellent anti-inflammatory agent analogously reducing the oxidative stress.

CONCLUSION

From the results it could be concluded that the ethyl acetate extract of *O. tetraphylla* bulb may be a potential source of natural antioxidant revealed from various *in vitro* antioxidant assays. 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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