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Phytochemical analysis, anti microbial and anti-oxidant potential of oxalis *Latifolia* Kunth

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Abstract
Oxalis latifolia Kunth. (Family: Oxalidaceae) is a well-known species in India and it is one of the most multipurpose medicinal plants having a wide spectrum of biological activity. The preliminary phytochemical analysis of *Oxalis latifolia* Kunth was carried out by a standard techniques and the results revealed the presence of tannins, alkaloids, reducing sugar, protein, phenols, saponins, flavonoids and glycosides. Total content of phenol and total content of flavonoid in methanolic extract was evaluated by means of the method named Folin aluminum trichloride method respectively. The antimicrobial screening was carried out using the following organisms; Eshericha coli, Bacillus subtilis, Salmonella typhi, Aspergillus niger and Aspergillus fumigates. The free radical scavenging activity of *Oxalis latifolia* Kunth was studied by its ability to reduce the DPPH, a stable free radical. The DPPH inhibition of leaf extracts are shown in Fig-3. The maximum scavenging activity was seen at a concentration of 512 µg/ml and lowest was at 2µg/ml. The DPPH scavenging activity was ranging from 4.3 ± 1.1% to 66.8 ± 1.06%. The result of the present study indicated that the extracts of the leaves of the *Oxalis latifolia* have several phytochemical constituents who possess the antibacterial and strong antioxidant activities.

Keywords: Antimicrobial; *Oxalis latifolia Kunth*; Phytochemicals; Organisms; Ethnomedicine

1. Introduction
India is known for its customary medicinal systems of medicine such as Ayurveda, Siddha and Unani. Therapeutic systems are found mentioned even in the early Vedas and other scriptures. The Ayurvedic perception appeared and developed between 2500 and 500 BC in India (Subhose *et al.*, 2005) [1]. The factual meaning of Ayurveda is "science of life," because early Indian system of healthcare focused on views of man and his illness. It has been pointed out that productive health means metabolically well-balanced human beings. Ayurveda is also known as the "science of prolonged existence" because it offers a whole system to live a long healthy life. It provides programs to rejuvenate the body through diet and nutrition. It offers treatment methods to heal many common diseases such as food allergies, which have few modern treatments. Conversely, one should be aware that Ayurvedic nutrition is not a "magic bullet" system but requires the complete involvement of the patient to succeed. An interactive method is user-friendly and educational. It inculcates the patient to become responsible and self-empowered. Ayurveda is not a nutritional method for those looking for an escape or excuse to further mistreat their body or mind. It is a system for empowerment, a system of liberty and long life.

Oxalis latifolia Kunth. (Family: Oxalidaceae) is a well-known species in India and it is one of the most multipurpose medicinal plants having a wide spectrum of biological activity. It is commonly known as broadleaf, it is native to Mexico and parts of Central and South America. This is a perennial herb growing from a system of small bulbs and spreading via stolen. There is no stem. The leaves arise on long petioles from ground level, each made up of three widely heart-shaped leaflets about 4.5 centimeters wide. The inflorescence is an collection of numerous flowers, each with five pink petals. Broad leaf wood sorrel, an excellent plant in the nature having composition of all the essential constituents that are required for normal and good health of humans (Anil Kumar K. Kuntal Das 2010) [2] Herb is a good appetizer, removes kapha, vata, and piles; astringent cures dysentery and diarrhoeas, skin diseases and quarten fevers. An infusion of the small leaves is externally used to remove warts and opacities of cornea. The leaves are anti-inflammatory, refrigerant and antiscorbutic (Kirtikar K. R., Basu B. D. 1988) [3].

Taxonomic Classification

Kingdom:	Plantae
Clade	Angiosperm
Clade	Eudicots
Clade	Rosids
Order:	Oxalidales
Family:	Oxalidaceae
Genus:	Oxalis
Species:	*O. latifolia* Kunth.

2. Materials and methods
2.1. Collection of plant: The plant was obtained from the local tropical region of Thirumoorthy hills Tamil Nadu, India during the month of January. The plant *Oxalis* is easily growing and available in the tropical and subtropical regions. The plant species is authenticated from Botabical Survey of India (BSI) Southern region Coimbatore, Tamil Nadu.

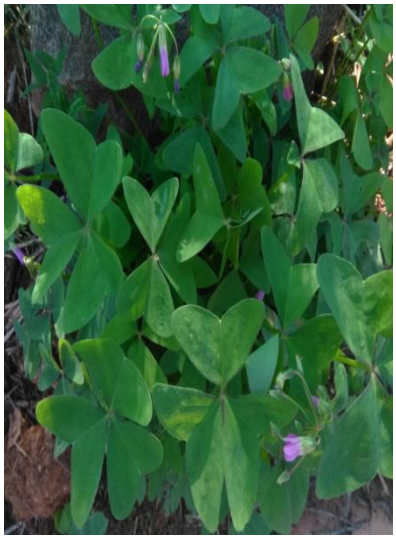

Fig 1: *O.latifolia*

Fig 2: Drying process

Fig 3: gathering of plants by author

2.2 Preparation of plant extract

The plant material were washed, shade dried and powdered using mixer grinder. The powdered material (10 g) was extracted with 100 ml of selected organic solvents (aqueous, chloroform, ethyl acetate, methanol and ethanol) using soxhlet apparatus and filtered through Whatmann No. 1 filter paper. The filtrate was concentrated and dried under reduced pressure and controlled temperature. The concentrated extracts of the leaves were stored in small vials at -20º C and used for further analysis.

2.3 Qualitative phytochemical analysis of the plant extract

(Peach and Tracey, 1995; Raaman, 2006) The phytochemical screening ethanol, methanol, ethyl acetate, chloroform and petroleum extracts of plant sample was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures. To identify the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides, phenols, carbohydrates and proteins

a. Alkaloids (Meyar's reagent): 1.3 g of mercuric chloride was dissolved in 60 ml distilled water and 5.0 g of potassium iodide in 10 ml of water. After mixing up of these two solutions, this solution mixture is diluted to 100 ml with distilled water. To 1.0 ml of leaf extract, few drops of reagent were added. Formation of white or pale yellow precipitate showed the presence of alkaloids.

Phenols (Ferric chloride test)

To 1.0 ml of leaf extract 2.0 ml of distilled water, followed by a few drops of 10 % aqueous $FeCl_3$ solution was added. Appearance of blue or green colour indicates the occurrence of phenols.

b. Flavonoids: In the test tubes containing 0.5 ml of leaf extract, 5-10 drops of dilute HCl and small piece of zinc or magnesium were added and the solution was boiled for a few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.

c. Tannins (Ferric chloride test): To 1- 2 ml of leaf extract, few drops of 5 % aqueous $FeCl_3$ solution was added.

A bluish black colour, which disappears on the addition of a few ml of dilute H_2SO_4 was followed by the formation of a yellowish brown precipitate.

d. Saponines: In a test tube containing about 5.0 ml of leaf extract, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth was formed and it showed the presence of saponins.

e. Terpenoids (Salkwski reaction): 5.0 ml of leaf extract was mixed in 2.0 ml of chloroform and concentrated H_2SO_4 (3.0 ml) was carefully added to form a layer. A reddish brown coloration in the inter phase formed to show positive results for the presence of terpenoids.

F. steroids (Libermann-Burchard's test): To 1.0 ml of leaf extract, 1.0 ml of conc. H_2SO_4 was added, followed by the addition of 2.0 ml of acetic anhydride solution. A greenish colour developed and turned blue indicates the presence of steroids.

g. carbohydrates(Benedict's test): 173 g of sodium citrate and 100 mg of sodium carbonate was dissolved in 500 ml of water. To this solution 17.3 g of copper sulphate dissolved in 100 ml of water was added. To 0.5 ml of the leaf extract, 5.0 ml of Benedict's reagent was added and boiled for 5 minutes. Formation of a bluish green colour showed the presence of carbohydrates.

h. Glycosides: A small amount of leaf extract was dissolved in 1.0 ml of water and then an aqueous sodium hydroxide solution was added. Appearance of a yellow colour indicates the occurrence of glycosides.

i. proteins (Biuret's test): To 1.0 ml of leaf extract, 5-8 drops of 5 % sodium hydroxide solution were added, followed by one or two drops of 1 % copper sulphate. Formation of pink or purple colour confirmed the presence of proteins and amino acids.

2.4. Determination of total phenol content (TPC) and total flavonoid content (TFC)

2.4.1 Total phenol content (TPC)

The total phenolic content was analyzed using the Folin – Ciocalteu colorimetric technique tailored by (Velioglu *et al.*, 1998; Chlopicka *et al.*, 2012) with slight modifications. An aliquot of 0.3 ml of leaf extract was mixed with Folin – Ciocalteu phenol reagent (2.20 ml). After 5 min, 6% sodium carbonate (2.20 ml) was added and the mixture was permitted to rest at room temperature for about 70 min. The absorbance of the mixture was calculated at 725 nm. Standard calibration curve for gallic acid at the range of 0–200 mg/ml was prepared in the same model and results were expressed as mg gallic acid equivalent (GAE) per gram of extract

2.4.2 Determination of total flavonoids

Total flavonoid content (TFC) was determined using the aluminum colorimetric technique (Chang *et al.*, 2002; Stankovic, 2011) with slight modifications using quercetin as the standard. A calibration curve of quercetin was prepared in the range of 0–200 mg/ mL. Briefly, extract (0.5 ml) and standard (0.5 ml) were positioned in different test tubes and to each 10% aluminum chloride (0.1 ml), 1 M of potassium acetate (0.1 ml), 80% methanol (1.5 ml) and distilled water (2.8 ml) were added and then mixed thoroughly. A blank was prepared in the same technique where 0.5 mL of distilled water was used instead of the sample or standard, and the amount of aluminum chloride was replaced by distilled water. All test tubes were incubated at room temperature for 30 minutes. The absorbance was recorded at 415 nm. The concentration of flavonoid was articulated as mg quercetin equivalent (QE) per gram of extract (GE)

2.5. Antimicrobial activities

a. Test microorganisms

The test organisms used were clinical isolates *viz., Bacillus Subtilis, Escherichia coli and Salmonella paratyphi and Klebsiella.* The human fungal pathogens like *Aspergillus fumigates and A.niger* which were obtained from Department of Microbiology, J.S.S. College of arts and science Nilgiri. The bacterial and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively.

The bacterial and fungal cultures were maintained on nutrient broth (NB) at37°C and fungus was maintained on Potato dextrose agar (PDA) at 28°C.

Table 1: Composition of Nutrient agar medium and of PDA medium

Nutrient agar medium	**Peptone**	**5.0 g**	PDA medium	**Potato**	**200.0 g**
	Beef extract	3.0 g		Dextrose	20.0 g
	Agar	15.0 g		Agar	15.0 g
	Distilled water	1000 ml		Distilled water	1000 ml
	pH	7.0		pH	6.2

b. Preparation of Inoculum

The gram positive bacteria *Escherichia coli, Bacillus Subtilis* and gram negative bacteria *Salmonella paratyphi* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min. pellet was hovering in twice distilled water and the cell density were standardized spectro-photometrically (A_{610} nm). The fungal inoculums *Aspergillus fumigates* and *A. niger* were prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore thickness of each fungal species was adjusted with spectrophotometer (A_{595}nm) in order to obtain a ultimate concentration of approximately 10^5 spores/ml.

c. Anti-bacterial Activity (Anonymous, 1996)

The samples were tested by the well diffusion method. Different concentration of the extracts (30, 60, 90 µg/ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Chloramphenicol (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm.

d. Antifungal Activity (Taylor *et al.*, 1995)

The antifungal activity was tested by well diffusion method. The potato, dextrose sugar and agar plates were inoculated with each fungal culture (6-10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 100 µg concentrations of the synthesized silver nanoparticles were placed on test organism-seeded plates. Chloramphenicol, penicillin (10 µg well 1) used as positive control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

2.6. Antioxidant activity

A. DPPH Assay

The free radical scavenging activity of the leaf extract was measured using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) as described by (Blois 1958). Briefly, to 1ml of different concentrations of methanolic leaf extract, 1ml of DPPH 0.1 mM (millimolar = 10^{-3}mol/L) was added. The mixture was mixed and left to stand for 30 min in the dark and the

absorbance was recorded at 517 nm. An equal amount of DPPH and Methanol served as control. The experiment was done in triplicate. Ascorbic acid was used as standard control. The percentage scavenging was calculated using the following formula, DPPH Scavenging effect (%) = (Acontrol-Asample) x 100

B. ABTS radical scavenging

The ABTS radical scavenging assay of leaf extract was carried out using the standard protocol described by (Pellegrini *et al.*, 1999) with slight modifications. Stock solutions of ABTS (7mM) and potassium sulphate (140 mM) in water were prepared, and mixed together to a final concentration of 2.45 mm potassium persulfate. This mixture was left to react overnight (12-14 hrs) in the dark, at room temperature. The solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. To 20µl of the various concentrations (2-1000 µg/ml) of test compound, 2.0 ml of diluted ABTS solution added and the absorbance was taken after 6 min. For the control, methanol was used instead of the test compound. Ascorbic acid was used as standard.

3. Result and Discussion

Table 2: Qualitative analysis of phytoconstituents present in different solvent extract of *Oxalis latifolia*

Phytochemicals	Aqueous	Ethanol	Methanol	Ethyl acetate	Chloroform
Alkaloids	-	+	+	+	++
Phenols	+	+	+	+	+
Flavonoids	-	++	++	+	+
Tannins	+	+	+	+	+
Saponins	+	+	+	-	+
Terpenoids	-	+	-	+	+
Steroids	-	+	+	-	+
Carbohydrates	++	++	+	+	+
Glycosides	-	-	-	+	-
protein	++	+	+	+	+
Coumarone	-	-	-	-	-

+ = present in small concentration; ++ = present in moderately high concentration; +++ = present in very high concentration; - = absent.

3.1. Total phenol and Flavonoid content

The present results revealed that the total phenol contents of methanolic leaf extract of *Oxalis latifolia* (Kunth.) is *63.43* ± 2.62 mg GAE/g dry weight. This value is somewhat greater than that of the total phenol content of other species of same genera. Among polyphenols, flavonoids are of great importance because they help the human body to fight against diseases. The ability of flavonoids to act as potent antioxidants depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure. They are abundantly found in plants as their glycoside (Rajanandh and Kavitha, 2010). Present investigation reveals that, total flavonoid content for the leaf *Oxalis latifolia* Kunth *72.73* ± 2.37 mg QE/g dry weight. These values are comparable or slightly higher than total flavonoid content of the same family and other medicinal plants. Hence, this species is medicinally important (Table 3).

Table 3: Showing Estimation of total phenolic and flavonoid contents

Leaf extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Oxalis latifolia Kunth.	*63.43* ± 2.62	*72.73* ± 2.37

3.2. Antimicrobial activities

3.2.1 Antibacterial assay of *Oxalis latifolia* Kunth.

The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm. both the extract showed almost similar result. However when compared methanol extract, the more inhibition zone found in aquas leaf extract. Minimum inhibition zone found in 30 µl concentration of methanol extract while maximum found at 90 µl concentration of aquas extract. (Table 4)

Table 4: Diameter of inhibition zone (mm) of Extracts of *Oxalis latifolia*

S. No	Pathogenic bacteria	Methanol extract			Aqua's extract			Standard (Chloramphenicol)
		Zone of inhibition (mm)			Zone of inhibition (mm)			
		30 µl	60 µl	90 µl	30 µl	60 µl	90 µl	
1.	*Escherichia coli* (+)	04	06	08	02	04	09	10
2.	*Bacillus Subtilis*(+)	02	05	08	04	06	09	10
3.	*Salmonella paratyphi*(-)	04	07	09	05	08	10	10
4.	*Klebsiella nemoniae* (-)	04	08	10	05	09	10	10

The experiment was conducted in triplicates (n=3)

3.2.2 The anti-fungal activity of *Oxalis latifolia*

The anti-fungal activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm. minimum zone of inhibition found at 30 µl of both methanol and aqua's extract while maximum activity found at 90 µl of aquas extract. (Table 5)

Table 5: Diameter of inhibition zone (mm) of Extracts of *Oxalis latifolia*

S.No	Pathogenic fungus	Methanol extract			Aquas extract			Standard (Chloramphenicol)
		Zone of inhibition (mm)			Zone of inhibition (mm)			
		30 µl	60 µl	90 µl	30 µl	60 µl	90 µl	
1.	*Aspergillus fumigatus*	02	06	08	02	07	10	10
2.	*Aspergillus niger*	02	05	08	04	07	11	11

3.3. Antioxidant activity

The free radical scavenging activity of *Oxalis latifolia* Kunth was studied by its ability to reduce the DPPH, a stable free radical. The DPPH inhibition of leaf extracts are shown in Fig-3. The maximum scavenging activity was seen at a concentration of 512 µg/ml and lowest was at 2µg/ml. The DPPH scavenging activity was ranging from 4.3 ± 1.1% to 66.8 ± 1.06%. The reduction in the number of DPPH molecule can be correlated with the available number of hydroxyl groups. Hence the significant scavenging activity may be due to the presence of hydroxyl groups present in the extracts. (Table 6 & fig 4)

Table 6: DPPH AND ABTs Scavenging activity of the ethanol extract of *Oxalis latifolia*

DPPH Scaving Activity EC_{50} (µg mL^{-1}) [a]				ABTS radical scavenging activity	
S.NO	Concentration (µg/ml)	***Oxalis latifolia***	(standard)Vitamin C	***Oxalis latifolia***	(standard) Vitamin C
1	02	4.3 ± 1.1	26.3 ± 1.06	9.60 ± 0.67	25.3 ± 1.09
2	04	5.95 ± 0.08	37.95 ± 0.65	9.0 ± 0.56	37.95 ± 0.75
3	08	8.25 ± 0.15	41.25 ± 0.36	15.90 ± 095	40.25 ± 0.66
4	16	8.8 ± 0.20	80.3 ± 0.54	19.95 ± 0.25	49.3 ± 0.54
5	32	9.92 ± 0.28	85.9 ± 0.20	38.20 ± 1.35	60.9 ± 0.26
6	64	18.1± 0.74	88.0± 1.02	40.40 ± 0.40	78.90± 1.05
7	128	30.6± 0.26	86.6± 1.26	52.75 ± 1.09	79.6± 1.26
8	256	49.2± 0.88	86.0± 1.60	66.6± 0.28	75.0± 1.68
9	512	66.8 ± 1.02	84.46 ± 1.62	55.09± 0.64	70.46 ± 1.42
10	1000	65.1± 1.2	85.32± 0.82	67.7± 0.88	69.32± 0.09

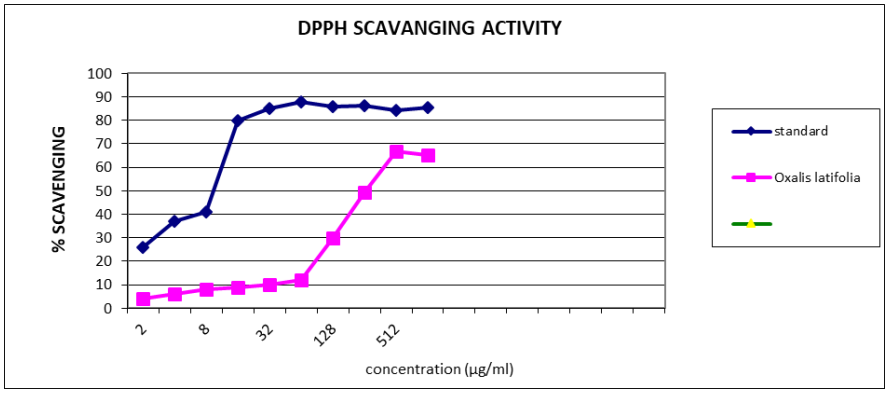

Fig 4: DPPH radical scavenging activity of different concentrations of Oxalis latifolia leaf, extracts

In the ABTS assay, the maximum scavenging activity of *Oxalis latifolia* Kunth was seen at a concentration of 1000 µg/ml and lowest was at 2µg/ml. The scavenging activity was ranging from 9.0±0.56% to 67.7± 0.88%. This broad range of antioxidant activity may be accredited to the wide variety of bioactive compounds like phenolics, flavonoids, tannins etc, present in the plant. (table 6 and fig 5)

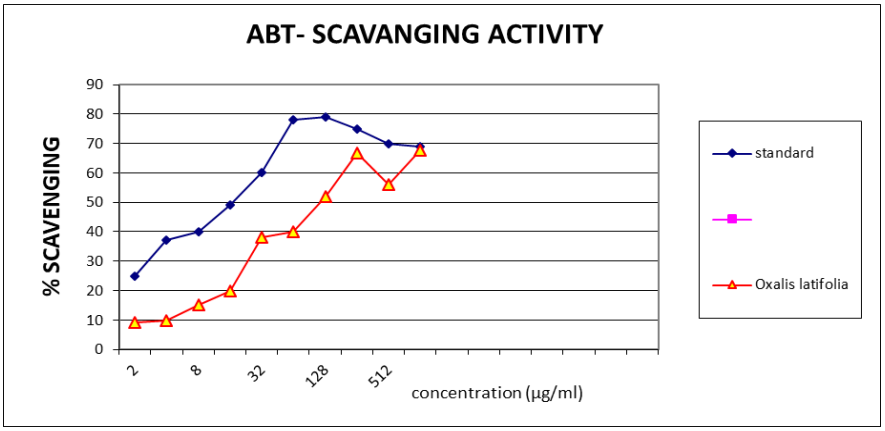

Fig 5: ABT radical scavenging activity of different concentrations Oxalis latifolia of leaf, extracts

Conclusion

The results of different studies were indicated that the methanolic extract of leaves of *Oxalis latifolia* can be used as a medicinal supplement in pharmaceutical industry for treatment of various diseases. It is easily accessible source of natural antioxidant. The *in vitro* studies indicate that leaf extracts of *O. latifolia* shows good source of secondary metabolites Which showed natural antioxidants and antibacterial activities? Therefore, further research need to be carried out to isolate and identify the important compounds present in the plant extracts.

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