

## ANTIMICROBIAL EFFECTS OF LEAF EXTRACTS FROM *OXALIS CORYMBOSA* AGAINST PATHOGENIC BACTERIAL AND FUNGAL ISOLATES

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### ABSTRACT

Medicinal plants are known to possess repertoire of bioactive compounds with antimicrobial, anti-inflammatory, anticancer and antioxidant activity. Oxalidaceae, a well known versatile medicinal plant family from India, has been shown to encompass potential of multitude of bioactive ingredients. Though much emphasis has been given on the potential of *Oxalis Corniculata*, there are other species in the genus which may serve as promising candidate for source of bioactive metabolites. *Oxalis Corymbosa*, is an important medicinal plant and the present work was undertaken to explore the potent inhibitory activity its leaf extract against pathogenic bacterial and fungal isolates. Interestingly, leaf extract of *Oxalis Corymbosa*

exhibited much potent antibacterial activity with maximum inhibition in acetone and ethyl acetate extract. Further, phytochemical analysis provides a detailed insight of the probable presence of phytochemical constituents in leaf extract. These findings for the first time highlights the prospective of leaf extract of *Oxalis Corymbosa* against pathogenic bacteria and fungi. Further studies are warranted to pave the path of exploring the synergistic effects with antibiotics as treatment modality.

**KEYWORDS:** *Oxalis Corymbosa*, Antibacterial, Antifungal.

### INTRODUCTION

Ancient literature of Ayurveda and Unani medicine contains plethora of iterations for the benefits of herbal drugs for human consumption. Owing to the pleotropic effect of the synthetic compounds, there is an elevated magnitude of side effects associated with these

drugs. Thus, harnessing of the medicinally important novel bioactive compounds from the plants has been the forefront of ethnopharmacology. Though multitude of attention has been given towards pharmacological screening of compounds from medicinally important plants, still a plethora of plants with immense medicinal value remains to be explored.<sup>[1,2,3]</sup>

There is a global rise in microbial infectious diseases associated mortality due to appearance of resistance in microbes against the prevalent antibiotics. Due to muddled use of anti-microbial drugs, pathogenic bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* etc. have developed resistance against prevalent drugs. Medicinal plant extracts have shown to be potent bioresource of novel anti-microbial agents at comparatively low cost. Oxalidaceae, a well known versatile medicinal plant family from India, has been shown to encompass potential of multitude of bioactive ingredients. Members of *Oxalidales* include annuals, perennial herbs, lianas, shrubs, and trees of both temperate and tropical regions.<sup>[4]</sup> *Oxalis corniculata* 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 plant is rich in niacin, vitamin C and  $\beta$ -carotene. The juice of the plant is given in jaundice and in stomach troubles.<sup>[5]</sup> The juice of the plant, mixed with butter, is applied to muscular swellings, boils and pimples.<sup>[6]</sup> *Oxalis corniculata* is also used as an antiseptic, refrigerant, diaphoretic, diuretic and anti diabetic. It is used as complementary medicine in wound healing, anemia, dyspepsia, cancer, piles, dementia and convulsions. Plants from *Oxalis* genus have been documented to possess anti-helminthic, anti-inflammatory, astringent, depurative, diuretic, stomachic and styptic activity. It is also used in the treatment of influenza, fever, urinary tract infections, enteritis, diarrhoea, traumatic injuries and sprains.<sup>[7,8,9]</sup>

Bioactive potential of *Oxalis corniculata* has been attributed to the presence medicinally viable compounds like tannins, palmitic acid, a mixture of 8 oleic, linoleic, linolenic and stearic acids. Interestingly, methanolic and ethanolic extracts of the plant showed presence of glycosides, phytosterols, phenolic compounds, flavonoids, proteins (12.5%), amino acids and volatile oil. The extracts showed significant antibacterial activity against *Xanthomonas* and human pathogenic bacteria. Though the antibacterial activity of extracts against human pathogenic bacteria was moderate compared to the standard streptomycin, methanol extract exhibited an increased antibacterial activity against plant pathogenic bacteria.<sup>[10]</sup>

The medicinal action of plants is attributed to the respective species or groups and their ecological niche which is contemplated by the taxonomically diverse combination of secondary products from plant of different species in same genus. Thus, with an increased concern of the rise in bacterial infections and appearance of antibiotic resistant bacteria *oxalis* genus can serve as a pool of medicinal plants to provide novel bioactive agents. The present study was undertaken to investigate the antimicrobial potential of *Oxalis Corymbosa* against a spectrum of pathogenic microorganisms. Further, a preliminary biochemical investigation was carried out to hypothesize the phytochemical constituent of the plant.<sup>[10,11]</sup>

## 2.0 MATERIALS AND METHODS

**2.1 Collection of plant Sample:** The area under investigation for ethno medicinal studies falls in Dehradun, Uttarakhand. The temperature in summer may reach up to 45°C and in winter below 5°C (up to 2°C). The average annual rainfall is 1065 mm. The forest is of tropical dry deciduous type covering an area of 2447 Sq. km (Dense Forest 1078 SQ. Km., open Forest – 1369 SQ. Km). Plant collection was followed by washing, cutting properly, drying in normal temperature, crushed and weighed before loading in the soxhlet extractor. Fresh plants that were easily accessible on a regular basis from the site of collection were used for extracting different active compounds. The plant body were crushed into fine particles in order to obtain a large surface area for solvent extraction.<sup>[12]</sup>

## 2.2 Extraction of active principles

**2.2.1 Soxhlet extraction:** Soxhlet extraction is an amendment of simple percolation where a small volume of hot liquid is made to percolate through a column repeatedly. The extraction process in soxhlet is conducted continually until complete extraction is making the effects. The compound may be isolated from extracted liquid after distillation. The menstrum of soxhlet assembly is filled with about 90g of dried leaves.

**2.2.2 Solvent used for extraction:** The solvents were used for extraction purposes in sequential manner based on increasing polarity for dissolving different components present in root. The boiling points of solvents are given in Table 1.

**2.3 Microbial cultures:** The cultures of bacteria and fungi isolated, identified and maintained in the Department of Microbiology, SGRRITS, Patel Nagar, Dehradun, were used for the present study. In the present study *Staphylococcus aureus*, *Escherischia coli*, *Bacillus subtilus*, *Pseudomonas aeroginosa*, *Seretia sp*, *Pseudomonas sp*, *Cocci sp*, *Strptobacilli sp*

*and Bacillus sp* were used as target causative microorganisms for bacterial infections and *Alternaria sp*, *Rhizopus sp*, *Penicillium sp*, *Aspergillus niger*, *Cladosporium sp*, *Fusarium sp*, *Mucor sp* were used as fungal targets. The microbial strains were grown on nutrient broth/potato dextrose broth at 37°C for 16h- 18h and were maintained on nutrient agar/potato dextrose agar slants at 4°C. The bacterial suspensions were standardized using McFarland turbidity standards.

**2.4 Preparation of 0.5 Mcfarland standards:** 0.5 ml of 0.048M BaCl<sub>2</sub> (1.17% w/v BaCl<sub>2</sub>) was added to 99.5 ml of 0.18M H<sub>2</sub>SO<sub>4</sub> (1% w/v) with constant stirring. The O.D. of the solution was recorded; ideal range is kept at 0.08-0.1 at 625 nm (1.5x10<sup>8</sup> cells/ml). Standard was stored in amber color bottle to prevent it from light at room temperature. Standard was briskly vortexed on a vortex mixer prior to use. (NCCLS, 1997)

**2.5 KB disc diffusion assay:** Discs of 5mm size were used with 20µl of loading capacity. Samples were prepared, loaded on sterile discs and dried under aseptic conditions. Plates containing Nutrient/Rose Bengal Agar media were swabbed with 0.5 McFarland adjusted 16-18 hour old culture of the test organisms. Sample loaded discs were then placed on the swabbed media plates and incubated at 37°C overnight for 24h. Solvent loaded disc served as control. Diameter of zone of inhibition was measured to decipher the antibacterial and antifungal of the extracts.

**2.6 Preliminary Phytochemical Evaluation:** The extracts obtained were subjected to preliminary phytochemical screening following the standard protocols.<sup>[13]</sup>

**Test for steroids and alkaloids:** One gram of the test substance was dissolved in a few drops of acetic acid, acetic anhydride, warmed and cooled under the tap water and drop of concentrated sulphuric acid were added along the sides of the test tube. Presence of green colour indicates the presence of Steroids. Small fractions of solvent free extract was stirred with a few drops of diluted HCl and filtered, the filtrate was tested for following colour tests: Mayer's Test: - Mayer's reagent (potassium mercuric iodide) was added to the test solution. It gives green colour precipitate.

**Test for flavonoids:** Shinado's test: Test solution was dissolved in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red coloration confirms the presence of Flavonoids.

**Test for triterpenoids:** Noller's test: Test solution was warmed with Tin and Thionylchloride. Purple coloration indicated the presence of Triterpenoids.

**Test for tannins:** The test solution was mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of Tannins.

**Test for saponins:** The substance shaken with water, foamy lather formation indicated the presence of saponins.

**Test for quinones:** To the test solution, sodium hydroxide was added. Blue green or red colour indicated the presence of quinone.

**Test for coumarin:** To the test sample 10% of sodium hydroxide and chloroform were added. Formation of yellow colour indicated the presence of coumarin.

#### **Test for protein**

Biuret test: To the test solution the Biuret Reagent (40% sodium hydroxide and diluted copper sulphate) was added. The blue reagent turned violet in the presence of proteins.

**Test for sugars and gum:** The test solution was mixed with equal volume of Fehling's A and B solutions and heated in water bath. Formation of red colour was the indication of the presence of sugar. To the test solution, 5 ml of water was added with shaking. Formation of swells or adhesives indicates the presence of gum.

### **3.0 RESULTS**

#### **3.1 Percentage yield of extracts from *Oxalis corymbosa* using different solvents**

350g of dried plant material was extracted by using a Soxhlet assembly for various solvents as mentioned before. Assembly was run for maximum of 3 days for each sample at indicated temperature. It was found that the yield of extract was maximum with ethyl acetate (40%) followed by benzene (35%), chloroform (34%), Petroleum ether (32%) and acetone extracts (30%) (Fig.1).

**3.2 Distillation of the extracts:** Distillation was carried for all the 5 solvent extracts to obtain a crude plant extracts. All the five samples were distilled at the indicated temperature for 45 minutes each. Maximum crude plant extract was obtained from ethyl acetate (40 ml) and

minimum from chloroform (25 ml) (Table 2). Temperature of distillation was chosen as per the boiling points of respective solvent.

### 3.3 Antimicrobial potential of different polar solvent extracts of *Oxalis corymbosa* against pathogenic bacteria

The antibacterial activity of extracts which was done using disc diffusion method showed inhibition in Inhibition of Zone in Diameter (IZD) against various extracts and bacteria. The acetone extract showed range of antibacterial activity with inhibition zone diameter as *Serratia sp.* (11mm), *Staphylococcus aureus* (21mm), *Escherechia coli* (16mm), *Bacillus subtilus* (14mm), *Sachharomyces* (9mm), *Pseudomonas aeruginosa* (17mm), *Cocci sp.* (9), *Pseudomonas sp.* (13mm), *Bacillus sp.* (14mm) and *Streptobacili sp.* (9mm). The antibacterial activity of ethyl acetate extract was less compared to acetone extract but followed the same pattern. The extract showed *Serratia sp.* (6mm), *Staphylococcus aureus* (9mm), *Escherechia coli* (8mm), *Bacillus subtilus* (6mm), *Sachharomyces* (6mm), *Pseudomonas aeruginosa* (12mm), *Cocci sp.* (5mm), *Pseudomonas sp.* (9mm), *Bacillus sp.* (18mm) and *Streptobacili sp.* (22mm) of inhibitory zone diameter (Fig. 2). The benzene extract showed *Serratia sp.* (4mm), *Staphylococcus aureus* (6mm), *Escherechia coli* (6mm), *Bacillus subtilus* (5mm), *Sachharomyces* (6mm), *Pseudomonas aeruginosa* (3mm), *Cocci sp.* (3mm), *Pseudomonas sp.* (5mm), *Bacillus sp.* (3mm), *Streptobacili sp.* (6mm) inhibition zone diameter. The chloroform extract showed *Serratia sp.* (5mm), *Staphylococcus aureus* (6mm), *Escherechia coli* (3mm), *Bacillus subtilus* (3mm), *Sachharomyces* (8mm), *Pseudomonas aeruginosa* (4mm), *Cocci sp.* (8mm), *Pseudomonas sp.* (3mm), *Bacillus sp.* (6mm) and *Streptobacili sp.* (4mm) of inhibition zone diameter. The petroleum ether extract showed *Serratia sp.* (3mm), *Staphylococcus aureus* (8mm), *Escherechia coli* (7mm), *Bacillus subtilus* (10mm), *Sachharomyces* (3mm), *Pseudomonas aeruginosa* (7mm), *Cocci sp.* (3mm), *Pseudomonas sp.* (2mm), *Bacillus sp.* (6mm) and *Streptobacili sp.* (5mm) of inhibition zone diameter (Fig. 2).

### 3.4 Antimicrobial potential of different solvent extracts of *Oxalis corymbosa* against pathogenic fungus:

As multitudes of infections are caused due to fungal infections, the efficacies of all the extracts were test against the pathogenic fungal species. The acetone extract showed range of antibacterial activity with inhibition zone diameter as *Alternaria sp.* (12mm), *Aspergillus (green) sp.* (10mm), *Penicillium sp.* (16mm), *Aspergillus sp.* (13mm), *Cladosporium sp.* (8mm), *Fusarium sp.* (15mm), *Mucor sp.* (12mm) and *Rhizopus sp.*

(13mm). The ethyl acetate extract showed *Alternaria sp.* (9mm), *Aspergillus (green) sp.* (10mm), *Penicillium sp.* (10mm), *Aspergillus sp.* (11mm), *Cladosporium sp.* (5mm), *Fusarium sp.* (12mm), *Mucor sp.* (8mm) and *Rhizopus sp.* (10mm) of inhibition zone diameter. Both acetone and ethyl acetate extracts showed more activity compared to other solvent extracts. The benzene extract showed *Alternaria sp.* (4mm), *Aspergillus (green) sp.* (2mm), *Penicillium sp.* (4mm), *Aspergillus sp.* (3mm), *Cladosporium sp.* (1mm), *Fusarium sp.* (1mm), *Mucor sp.* (4mm) and *Rhizopus sp.* (1mm) of inhibition zone diameter. The chloroform extract showed *Alternaria sp.* (2mm), *Aspergillus (green) sp.* (6mm), *Penicillium sp.* (1mm), *Aspergillus sp.* (1mm), *Cladosporium sp.* (6mm), *Fusarium sp.* (3mm) and *Mucor sp.* (4mm) of inhibition zone diameter. The chloroform extract had no effect on *Rhizopus sp.* The petroleum ether extract showed *Alternaria sp.* (1mm), *Aspergillus (green) sp.* (3mm), *Penicillium sp.* (4mm), *Cladosporium sp.* (5mm), *Fusarium sp.* (1mm), *Mucor sp.* (5mm) and *Rhizopus sp.* (3mm) of inhibition zone diameter (Fig. 3). The petroleum ether extract had no effect on *Aspergillus sp.*

**3.4 Minimum Inhibitory Concentration (MIC) analysis:** Since the acetone extract showed the maximum anti-microbial activity, it was further taken to enumerate the minimum inhibitory concentration. The MIC of different acetone extract was determined by measuring the zone of inhibition of growth around the disc. Highest zone of inhibition at minimum concentration indicated the measure of MIC value.

**3.5 Preliminary Phytochemical Evaluation:** Preliminary phytochemical evaluation of different extracts was conducted to decipher the presence of probable active compounds. The analysis of *Oxalis corymbosa* conferred the presence of steroids, tannins, alkaloids and proteins were present in all the three extracts. Apart from the above mentioned constituents triterpenoids, saponins and coumarin were found to be present in both petroleum ether and ethyl acetate. Sugar and Gum were also present in ethyl acetate extract (Table. 4).

Table 1. Solvents used for extraction with boiling point temperature.

S. no.	Solvent	Formula	Boiling Point
1	Petroleum ether	C <sub>6</sub> H <sub>14</sub>	60 <sup>0</sup> C - 80 <sup>0</sup> C
2	Benzene	C <sub>6</sub> H <sub>6</sub>	80 <sup>0</sup> C - 80.1 <sup>0</sup> C
3	Acetone	C <sub>3</sub> H <sub>6</sub> O	56.2 <sup>0</sup> C
4	Ethyl acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	77.1 <sup>0</sup> C
5	Chloroform	CHCl <sub>3</sub>	61.2 <sup>0</sup> C

Table 2. Yield of leaf extracts from *Oxalis corymbosa* in different solvents.

S. no	Volume of sample	Temperature	Duration (min)	Amount of distillate (ml)	Amount of crude extracts(ml)
1	Acetone extracts	56.2 <sup>0</sup> C	45	75	30
2	Benzene extracts	70-75 <sup>0</sup> C	50	102	35
3	Chloroform	61 <sup>0</sup> C	45	108	25
4	Ethyl acetate	77 <sup>0</sup> C	55	120	40
5	Petroleum ether	60-80 <sup>0</sup> C	45	95	38

Table 3. MIC of acetone extracts from leaves of *Oxalis corymbosa* against pathogenic bacteria isolates.

S. No.	Strains	Zone of Inhibition at indicated concentrations (Diameter in mm)				
		100%	50%	25%	12.5%	6.25%
1	<i>Serratia sp.</i>	13	10	9	11	6
2	<i>Staphylococcus aureus</i>	12	10	6	2	3
3	<i>Eschrechia coli</i>	14	15	10	6	10
4	<i>Bacillus subtilis</i>	15	0	12	10	6
5	<i>Saccharomyces sp.</i>	10	6	8	7	10
6	<i>Pseudomonas aeruginosa</i>	19	16	14	18	18
7	<i>Cocci sp.</i>	10	11	10	18	11
8	<i>Pseudomonas sp.</i>	16	8	11	9	9
9	<i>Bacillus sp.</i>	14	10	8	6	8
10	<i>Streptobacilli</i>	10	12	7	2	6

Table. 4. Preliminary phytochemical determination of *Oxalis corymbosa* leaf extract in different solvent systems.

Phytochemicals	Petroleum ether	Acetone	Ethyl acetate
<b>Steroids</b>	+	+	+
<b>Alkaloids</b>	+	+	+
<b>Flavonoids</b>	-	-	-
<b>Triterpenoids</b>	+	-	-
<b>Tannins</b>	+	+	+
<b>Saponins</b>	+	-	+
<b>Quinone</b>	-	-	-
<b>Coumarin</b>	+	-	+
<b>Protein</b>	+	+	+
<b>Sugar</b>	+	-	+
<b>Gum</b>	-	-	+

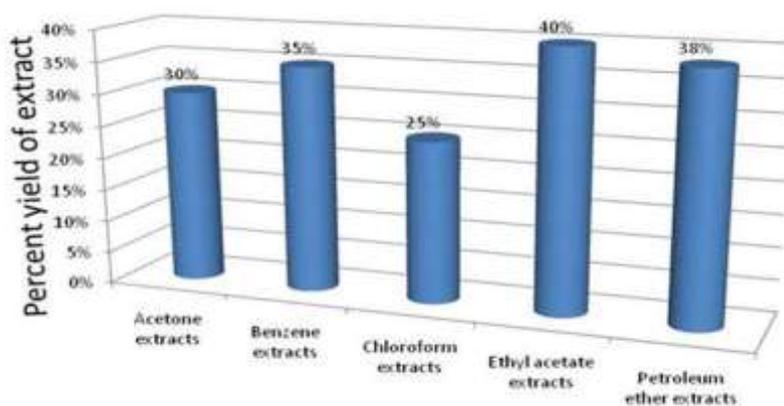


Figure 1. Bar diagram representing percentage yield of plant extracts

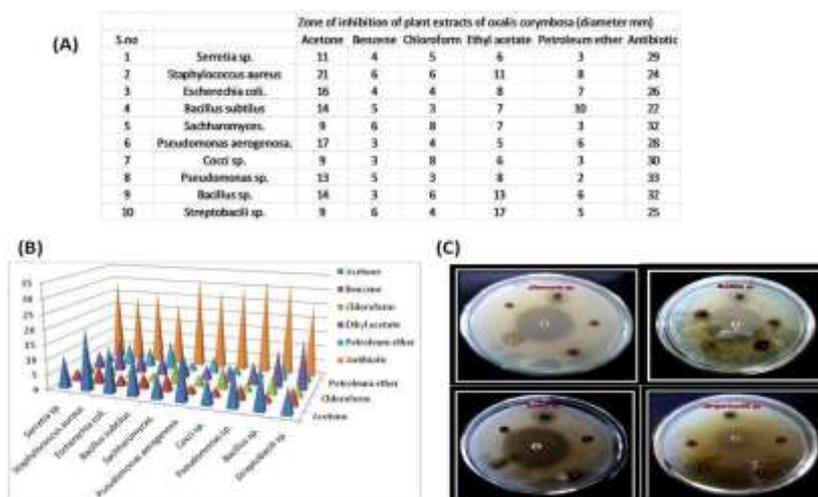
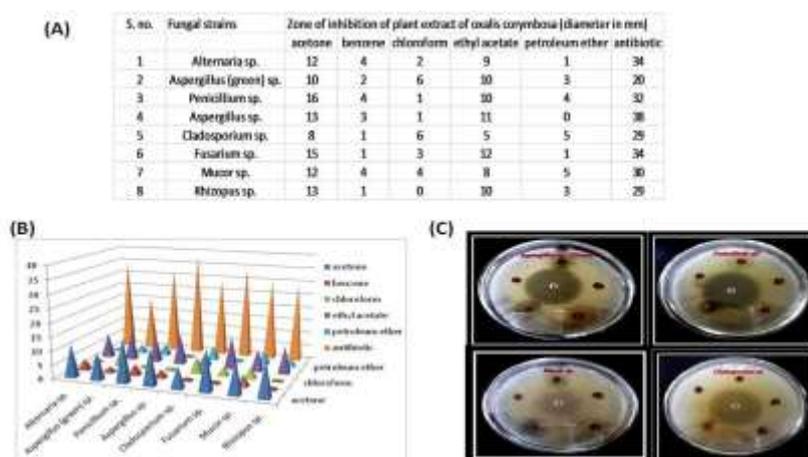


Figure 2. Antibacterial efficacy of different solvent based extracts of *Oxalis corymbosa*. (A, B & C) Represents the inhibition zone diameter of different solvent extracts of *Oxalis corymbosa*



**Figure 3. Antifungal efficacy of different solvent based extracts of *Oxalis corymbosa*. (A & B) Represents the inhibition zone diameter of different solvent extracts of *Oxalis corymbosa***

#### 4.0 DISCUSSION

The progress in new antimicrobial agents to multidrug resistant pathogens for the treatment of various infectious diseases is of increasing interest. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. Therefore different plant extracts of many plants have been used locally against various pathogens, which showed positive results. Medicinal plants are the most prominent source of natural products against various common infectious microbes. The appearance of multidrug-resistant infectious microbes, high cost of synthetic compounds, and uninvited side effects of certain drugs necessitated the new epoch to search for the novel bioactive agents from alternative and low cost sources like medicinal plants. Pharmacological screening of the medicinal plants may serve as the repository for developing the novel agents. The key decisive factor for the selection of plant for such a study is documented use as therapeutics in indigenous medicinal preparations. The traditional Indian medicinal system mentions herbal remedies for the treatment of variety of diseases.

Antimicrobial agents of plant origin are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects associated with synthetic antibiotics. Presence of plethora of secondary metabolites effective as single entity or in combination underlines the beneficial medicinal effects of plant. In plants, prevalent compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, phenol compounds, flavonoids, steroids, resins and fatty acids gums which are capable of producing definite physiological action on body. *Oxalis*, one of the most resourceful genus in terms of medicinal

properties, belongs to the family Oxalidaceae with about 500 species, distributed in America, Africa, Europe and Asia.<sup>[14]</sup> *Oxalis corniculata* is a well-known plant in India and is one of the most versatile medicinal plants having a wide spectrum of biological activity.<sup>[15,16]</sup> *Oxalis* species mainly *corniculata* have been documented to possess plethora of activities including anti-inflammatory, antiseptic, anti-diabetic and anti-helminthic. It is used in traditional medicines for the treatment of diarrhea, influenza and enteritis. It showed the presence of glyoxylic acid, oxalic acid, pyruvic acid, isovitexin, vitexin-2-O-beta-D-glucopyranoside, glycolipids; vitamin C; phospholipids; fatty acids, alpha and beta tocopherols. Plethora of studies has been focused on *Oxalis corniculata*, whereas multiple medicinally important species remain to be explored for bioactive agents.<sup>[13]</sup>

The present work was undertaken to investigate the bioactive potential of *Oxalis Corymbosa* against pathogenic bacteria. Interestingly, leaf extracts of *Oxalis Corymbosa* showed moderate to better antimicrobial activity in different solvent systems. Major antimicrobial potential was seen in acetone and ethyl acetate extract. Both the extracts showed potent antibacterial and anti-fungal activity against pathogenic bacteria and fungus respectively. The minimum inhibitory concentration was determined for the most active acetone extract, which showed potent antibacterial activity against the spectrum of test pathogenic bacteria. Further, to understand the phytochemistry of the plant extract rendering the antimicrobial activity, phytochemical evaluation of the active extracts were performed. The phytochemical evaluation of the extracts showed the presence of steroids, tannins, alkaloids and proteins in all the tested extracts could be correlated with the observed antimicrobial activity. Triterpenoids, saponins and coumarin were observed in petroleum ether and ethyl acetate.

In conclusion, the study provides a detailed insight of the antibacterial action of *Oxalis corymbosa* extract against pathogenic bacteria and fungus. Our findings may help in identifying *Oxalis corymbosa* as a storehouse of potent bioactive compounds of medicinal value in oxalidaceae family. The finding further warrants investigation to decipher the active ingredient underlying the antimicrobial action.

**5.0 CONFLICT OF INTEREST** There is no actual or potential conflict of interest.

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