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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *OXALIS CORNICULATA* AGAINST HUMAN PATHOGENS

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ABSTRACT

Plants are the major sources of new medicines and thus in-vitro antibacterial activity and preliminary phytochemical screening of the weed plant *Oxalis corniculata* was performed to find out its therapeutic potential. Aqueous, methanol, chloroform and hexane extracts of sample powdered were prepared. These extracts were tested against standard gram positive bacterial strains *Staphylococcus epidermidis*, *Bacillus cereus* and standard gram negative bacterial strains *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumonia* and *Escherichia coli*. The agar well diffusion method was used to evaluate the antibacterial activity of the prepared extracts. The results obtained showed the broad spectrum activity of the aqueous and methanol extracts of *Oxalis corniculata* and inhibited the growth of both standard gram positive bacterial strains and standard gram negative bacterial strains. The bacterial growth showed dose-dependent inhibition. The diameter of zone of inhibition of aqueous and methanol extracts were similar to that of zone of inhibition of tetracycline disc used against the pathogenic bacterial strains. Chloroform extract of plant showed little antibacterial activity while in case hexane the activity observed is negligible as compared to other. The Preliminary Phytochemical screening was performed on aqueous and methanol extract and the results revealed the presence of carbohydrates, reducing sugar, proteins, sterols, acidic compounds, alkaloids, tannins, phenolic compounds, flavonoids, cardiac glycosides in both aqueous and methanol extract of the plant. The findings of the present study indicated that the extracts of the leaves of the *Oxalis corniculata* have several phytochemical constituents who possess the antibacterial activity.

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INTRODUCTION

Nature has been a source of medicinal agents from thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicines (Saini *et al.*, 2009). According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Farnsworth *et al.*, 1985). In India, Herbal medicines have been the basis of treatment and cure for various diseases in methods practiced such as Ayurveda, Unani and Siddha. Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attributes. Antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious disease. In spite of rapid development in methods of organic synthesis in laboratories, medicinal plants continue to play a significant role in modern medicine due to their inherent distinct chemical and biological properties.

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In nature a plant is able to synthesize complex molecules, namely alkaloids, terpenoids, tannins, saponins, glycosides etc from simple ones through highly specific reaction mechanisms that they use for defence and communication. It is difficult and expensive to duplicate such synthesis in laboratory. The compounds synthesized by the plants play an important role as medicinal and pharmaceutical agents not only as purified isolates and extractives but also as lead compounds for synthetic optimization. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times (Farombi 2003). Eighty percent of medicinal drugs originate in plants. Although many plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. That means there are many important drugs yet to be discovered. The plant *Oxalis corniculata* (creeping wood sorrel) also called procumbent yellow sorrel belongs to family *oxalidaceae*. It is very popular perennial herb that is distributed worldwide. The leaves of wood sorrel are quite edible with a tangy taste (Lee Allen Peterson, 1977). The entire plant is rich in vitamin-C. The vitamin C supplementation effects on brain

acetyl cholinesterase and neurotransmitter levels and treated in dementia induced by scopolamine in animals (Lee *et al.*, 2001). *Oxalis corniculata* also used in wound healing activity (Taranalli *et al.*, 2004), and Abortifacient antimplantation (Sharangouda and Patil *et al.*, 2007). It is known to cure dysentery, diarrhea and skin diseases (Kirtikar and Basu, 1975). The juice of the *Oxalis corniculata* plant is given in stomach trouble, used to relieve the intoxication produced by Datura, as a refrigerant. The extract of the plant is applied in case of scorpion sting; fresh leaves of *Oxalis corniculata* are crushed and are used to stop bleeding from wounds (Mir 2000). The raw fresh leaves are crushed and directly applied on skin to treat eczema (Abinash, 2006). Anti fungal activity (Iqbal *et al.*, 2002) relaxant activity (Achola *et al.*, 1995) were also tested. Other traditionally used includes anaemia, dyspepsia, cancer, piles, dementia, convulsionis (Madhava Chetty *et al.*, 2008). Ethanolic extract from the leaves of *Oxalis corniculata* have significant nematocidal properties (Qarar *et al.*, 1998). The methanolic extract of *Oxalis corniculata* significantly shown memory enhancing agent in corticosterone induced dementia (Yalla Reddy *et al.* 2010). *Oxalis corniculata* is used for the treatment of aphthae. (Hebbar *et al.*, 2004). It is also used for giddiness, diarrhea and dysentery, juice of leaves applied to open wound relieves pain, paste of ground leaves and raw onions applied to forehead for intense headache (Singh, 1986). The plant is also used for amenorrhea (Kong *et al.*, 1986) bile diseases and as diuretics (Neuyem *et al.*, 1993). It is also as antidote against datura poisoning (Ameenah *et al.*, 1993). *Oxalis corniculata* when used in combination with other plant extract it gives synergist effects to cure rheumatism (Libman *et al.*, 2006). It is recommended to use in urinary inflammation and suggested to use as carminative (Al-Qurain, 2009). The alcoholic and petroleum ether extract of whole plant of *Oxalis corniculata* showed significant wound healing activity in rats (Taranalli *et al.*, 2004). Considering the vast potential of plant as a source for antimicrobial drug, a systematic investigation was undertaken to screen the weed plant *Oxalis corniculata* for their antibacterial activity and phytochemical analysis. The major factor for the survival and persistence of weed is their ability to resist pathogens in their environment by producing beneficial secondary metabolites thus they may be the potential source of antimicrobial compounds.

MATERIALS AND METHODS

Plant Material and Chemicals

The plant *Oxalis corniculata* was collected from different places in and around Lucknow (U.P.) India. The collected plant was identified at Department of Botany, Lucknow University, Lucknow (U.P.) India. The fresh disease free leaves of identified plant was washed thoroughly 2-3 times with running tap water and once with sterile water, shade dried and was powdered with the help of pestle and mortar. The fine particles were separated and stored in clean container until used for extraction. All the chemicals and standard antibiotics were purchased from Hi-Media Laboratories, Mumbai, India; and all the solvents used were of analytical grade.

Extraction Methodology

10g powdered sample was dissolved with each solvent i.e. 100ml of distilled water (aqueous), methanol, chloroform and hexane in a separate volumetric conical flask, plugged with cotton wool and then the flasks were kept on a rotary shaker at

180rpm at a temperature of 25+10C for 24 hours so that the bioactive constituents in the leaf powder extracts out into the solvent of different polarity. These extracts were filtered through a Whatmann filter paper No 1. This process was repeated for three times, than total extracts of three times for each solvent collected separately and then dried using rotavapour, (Buchi model no. R-250) under reduced pressure of 20-50 kPa and the rotation was set to 125 rotation/min (rpm) the temperature range was between 35- 40°C. They were dissolved in DMSO and stored at 4°C until used for the evaluation of antibacterial property.

Microbial strains

The aqueous and organic extracts of *Oxalis corniculata* were tested against standard gram positive microbial strains *Staphylococcus epidermidis* (NCIM 2493), *Bacillus cereus* (NCIM 2150) and standard gram negative strains *Enterobacter aerogenes* (NCIM 5139), *Pseudomonas aeruginosa* (NCIM 5029), *Salmonella typhimurium* (NCIM 2501), *Klebsiella pneumonia* (NCIM 2957), *Escherichia coli* (NCIM 2065). These standard strains were collected from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune. Stock cultures were maintained on nutrient agar slants. Subculture was done once a month to maintain their viability and to check for their purity.

Inoculum's preparation

Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient broth achieve optical densities corresponding to 2.0-10 colony forming units (CFU/ml) (Kloucek *et al.*, 2005).

Agar well diffusion assay

The agar well diffusion method was used to test the antimicrobial activity of prepared extracts (Okeke *et al.*, 2001) (Perez *et al.*, 1990.). All media plates (9 cm in diameter) were prepared with nutrient agar. One hundred µL of each diluted standardized microbial suspension were inoculated on nutrient agar plates using sterile cotton. The inoculums were allowed to dry for 5 min. The well (7 mm in diameter) was cut from the agar to produce a total of five wells per each agar plate. For test, alternate cups were filled with 25, 50, 75, and 100µl of the each extracts and 100µl 10% DMSO using microtiter pipette. The plates were kept at room temperature for 15-20 min to allow the diffusion of the extracts solution. The plates were then incubated in the upright position at 37°C for 18 hours. Two replicates were carried out for each extract against each of the test organism.

The above procedure was followed with each solvent extracts (aqueous, methanol, hexane and chloroform) of *Oxalis corniculata*. 10% DMSO was used as negative control. Standard antibiotics discs (Hi-media Laboratories) tetracycline (10mcg/disc) and Ampicillin 10 mcg/disc were included in the assay as positive control to compare its effect on test organisms with the plants extracts. After incubation at 37°C for 24 h, all plates were examined for any zones of growth inhibition and the diameter of these zones were measured by Hi Antibiotic Zone scale (Hi-media Laboratories).

Preliminary Phytochemical screening

Preliminary Phyto-chemical screening were carried out on the aqueous and methanol samples of extracts using standard procedures as described by Sofowara (1993), Trease and Evans (1989) Harborne (1973) Kokate and Ali (1998) to identify the major phytoconstituents *i.e.* alkaloids, steroids, flavonoids, tannins, saponins, phenolic compounds, acidic compounds, and cardiac glycosides. The triplicate samples were taken for analysis.

RESULTS AND DISCUSSION

The results of the antibacterial activity by agar well diffusion method were given in Table 1. The antibacterial activity was tested on the basis of the magnitude of zone of inhibition (in mm). The results of the antibacterial activity suggested that, the aqueous and methanol extracts of the *Oxalis Corniculata* showed significant activity. Effectiveness of the extracts was clearly seen as per increasing order of the polarity index *i.e.* water (10.2) > methanol (5.1) > chloroform (4.1) > and Hexane (0.1) Snyder (1978).

2065). The results of the diameter of zone of inhibition of standard antibiotics discs were given in Table 2. The comparative analysis of results of zone of inhibition of extracts and antibiotic disc was presented through graph Figure 1.

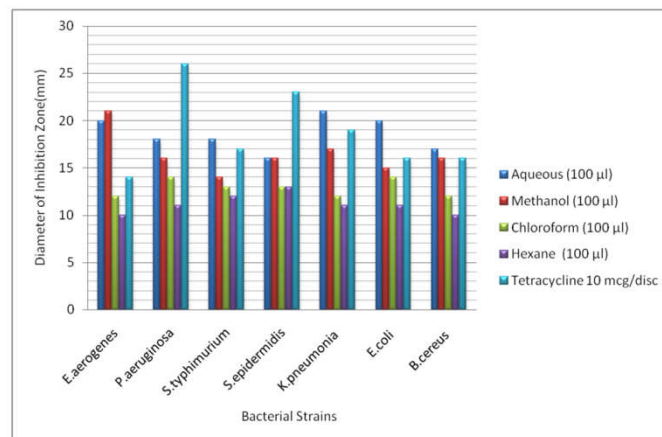


Figure 1. Comparative analysis of results of zone of inhibition of extracts and antibiotic disc

Table 1. Diameter of inhibition zone (mm) of Extracts of *Oxalis corniculata*

Bacterial strains	Diameter of inhibition zone (mm) of Extracts of <i>Oxalis corniculata</i>															
	Aqueous Extract				Methanol Extract				Chloroform Extract				Hexane Extract			
	25 µl	50 µl	75 µl	100 µl	25 µl	50 µl	75 µl	100 µl	25 µl	50 µl	75 µl	100 µl	25 µl	50 µl	75 µl	100 µl
<i>E. aerogenes</i>	13	15	18	20	11	14	15	21	-	10	11	12	-	-	-	10
<i>P. aeruginosa</i>	12	14	15	18	13	15	15	16	-	-	12	14	-	-	10	11
<i>S. typhimurium</i>	13	14	16	18	10	11	13	14	-	10	11	13	-	-	11	12
<i>S. epidermidis</i>	12	14	15	16	11	14	15	16	-	-	10	13	-	-	10	13
<i>K. pneumonia</i>	13	15	19	21	11	14	15	17	-	-	10	12	-	-	-	11
<i>E. coli</i>	13	16	19	20	10	13	14	15	-	10	11	14	-	-	10	11
<i>B. cereus</i>	11	13	14	17	10	11	12	16	-	-	10	12	-	-	-	10

Table 2. Diameter of inhibition zone of standard antibiotics discs

Bacterial strains	Diameter of inhibition zone of standard antibiotics discs	
	Ampicillin (10 mcg/disc)	Tetracycline (10mcg/disc)
<i>E.aerogenes</i>	21	14
<i>P.aeruginosa</i>	-	26
<i>S. typhimurium</i>	-	17
<i>S. epidermidis</i>	22	23
<i>K.pneumonia</i>	-	19
<i>E.coli</i>	16	16
<i>B.cereus</i>	21	16

Table 3. Phytochemical Screening

S. No.	Compounds	Aq.	Meth.
1.	Carbohydrates	++	+++
2.	Reducing Sugar	++	++
3.	Proteins	+++	++
4.	Sterols	++	+
5.	Acidic Compounds	-	-
6.	Alkaloids	+	++
7.	Tannins	+++	+++
8.	Saponins	-	-
9.	Phenolic Compounds	+++	+++
10.	Flavonoids	++	+++
11.	Cardiac glycoside	++	++
12.	Resins	-	-

This simply reflects the presence of the polar compounds of the plant responsible for the activity. The bacterial growth showed dose-dependent inhibition as extracts used in evaluating activity in different concentrations 25µl, 50 µl, 75µl and 100µl. The aqueous and methanol extracts of the selected plant showed broad spectrum activity and inhibited the growth of both gram positive microbial strains *Staphylococcus epidermidis* (NCIM 2493), *Bacillus cereus* (NCIM 2150) and standard gram negative strains *Enterobacter aerogenes* (NCIM 5139), *Pseudomonas aeruginosa* (NCIM 5029), *Salmonella typhimurium* (NCIM 2501), *Klebsiella pneumonia* (NCIM 2957) and *E. coli* (NCIM

The diameter of zone of inhibition of aqueous and methanol extracts were similar to that of zone of inhibition of tetracycline disc used against pathogenic bacteria. The results of zone of inhibition of aqueous extract against *Enterobacter aerogenes* (20mm), *Klebsiella pneumonia* (21mm) and *Escherichia coli* (20mm) was more than that found with tetracycline antibiotic disc (14mm, 19mm and 16mm). The results of zone of inhibition of methanol extract against *Enterobacter aerogenes* (21mm), *Klebsiella pneumonia* (17mm), *Escherichia coli* (15mm) and *Bacillus cereus* (16mm) which was also very close to the zone of inhibition found in tetracycline antibiotic disc

(14mm, 19mm, 16mm and 16mm). Chloroform extract of plant showed little antibacterial activity while in case hexane the activity observed is negligible as compared to other. Thus the Preliminary phytochemical screening was performed on aqueous and methanol extract of the plant to investigate the possible constituents responsible for the antibacterial activity.

The results of the Preliminary phytochemical screening were given in the Table 3. The results of the phytochemical screening revealed the presence of carbohydrates, reducing sugar, proteins, sterols, acidic compounds, alkaloids, tannins, phenolic compounds, flavonoids, cardiac glycosides in both aqueous and methanol extract of the plant. Saponins and Resins are not detected in the tests. The results are in parallel to the findings of the previously reported study of *O. corniculata*. It was revealed earlier that the antibacterial activity of the methanol and ethanol extract was due to the presence of phenolic compounds. (Raghavendra *et al.*, 2006), *O. corniculata* had a positive antibacterial activity in water extract (Unni *et al.* 2009), 80% ethanol extracts of *O. corniculata* shows antibacterial activity (Valsaraj *et al.*, 1997) and *O. corniculata* exhibited significant antibacterial effect to a certain degree (Reena *et al.* 2009). *Oxalis corniculata* leaves having three major C-glycosyl flavones as are reported. These are iso orientin, isovitexin and swertisin etc., (Hiroki Mizokami *et al.*, 2008). Phytochemical investigations of *Oxalis corniculata* Linn have revealed the presence of tannins, palmitic acid, and a mixture of oleic, linoleic, linolenic and stearic acids (Han, 1998). Leaves contain tartaric acid and citric acids, calcium oxalate, flavones (acacetin and 7,4'-diOMe apigenin), glycoflavones (4'-OMe vitexin, 4'-OMe iso-vitexin and 3',4'-diOMe orientin), flavonols (3',4'-diOMe quercetin) and phenolic acids such as p-hydroxybenzoic, vanillic and syringic acids (Danie, 2006). The methanolic extract of *Oxalis corniculata* (MEOC) has been proven experimentally to possess antioxidant activity in in-vitro methods (Yalla Reddy *et al.* 2010). The presence of flavonoids and related polyphenols may be responsible for the antioxidant and anti-inflammatory activity (Archana R Juvekar *et al.*, 2010). Sumei *et al.* (2006) have reported the aqueous extract of whole plant can eliminate the evil wetness, urethritis, neurasthenic, injuries from falls, skin ulcer, foot ringworm, eczema, scald, ringworm on feet. Leaf decoction is used in treating cough, dysentery and as an astringent. The potential of the plant can be simply observed from overall result of zone of inhibition and the presence of phytochemicals that they may play significant role as the antibacterial.

Conclusion

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. Some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002) has led to the search of new antimicrobial agents in particular from medicinal plants. This study is substantial step and further in-depth research is required to isolate the bioactive compounds of this species as well as further studies on its bio efficiency against human pathogens. Also there is

need to study the mechanism of action of and the toxicity level of the plant.

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