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Research Article

Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant

M.P. Raghavendra, S. Satish and K. A. Raveesha[‡]
Agriculture Microbiology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, 570 006, India.

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Abstract

Oxalis corniculata Linn. (Family; Oxalidaceae) was tested for antibacterial activity against three important pathovars of *Xanthomonas* and fourteen human pathogenic bacteria. Powdered leaf material was extracted with different solvents viz., petroleum ether, benzene, chloroform, methanol and ethanol using Soxhlet apparatus. All the solvent extracts were evaporated to dryness using rotary flash evaporator. Dry residue was dissolved in respective solvents (1:10 w/v) and tested for antibacterial activity. Among five solvents tested, methanol and ethanol extracts showed significant antibacterial activity when compared with K-cycline and Bact-805 for plant pathogens, Gentamicin and Streptomycin for human pathogens. Phytochemical analysis of the leaf material revealed that the antibacterial activity of the plant material is because of the presence of phenolic compounds.

Introduction

Inspite of use of all available means of plant protection, about 1/3rd of the yearly harvested food commodities of the world is destroyed by pests and the loss due to this is expected to be nearly 6000 crores annualy (Bhan and Misra, 1998). Quick and effective management of plant diseases in agricultural commodities is generally achieved by the use of synthetic pesticides (Mathur et al. 1999). However, the indiscriminate application of these chemical pesticides has caused health hazards in animals and humans due to their residual toxicity. Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops and are reported to have developed resistance to kanamycin, ampicillin, penicillin and streptomycin (Verma et al. 1989). There are several reports of antibiotic resistance of human pathogens to available antibiotics (Mitsuyama et al. 1987; Gutmann et al. 1988; Mathias et al. 2000; Ganguly et al. 2001 and Martino et al. 2002). Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human and plant pathogens and hence in the present investigation, an acid herb commonly called 'Indian sorrel' (*Oxalis corniculata*

[‡]For correspondence: raveesha@sancharnet.in

Linn.) was tested for its efficacy to inhibit pathovars of *Xanthomonas* and some human pathogens. Comparative efficacy of the plant extract with some synthetic antibiotics was also tested. *Oxalis corniculata* Linn. is a small procumbent herb, with stems rooting and pubescent with appressed hairs, leaves palmately 3-foliolate. This plant is well known for its medicinal value as a good appetiser and as a remover of kapha, vata and piles. It is also known to cure dysentery, diarrhea and skin diseases (Kirtikar and Basu, 1975).

Materials and methods

Collection of plant material

Healthy, disease free, mature leaves of *Oxalis corniculata* collected from Mysore, Mysore district, Karnataka (India) were used for the preparation of aqueous and different solvent extracts. A voucher specimen of the plant has been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore.

Preparation of Solvent extracts

Thoroughly washed mature leaves were shade dried and then powdered with the help of a blender. 25 g of the powder was filled in the thimble and extracted successively with petroleum ether, benzene, chloroform, methanol and ethanol using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. All the extracts were subjected to antibacterial activity assay and phytochemical analysis.

Phytochemical analysis

Phytochemical analysis of all the evaporated solvent extracts was conducted following the procedure of Indian Pharmacopoeia (1985). By this analysis, the presence of several phytochemicals listed in table 3 was tested.

Test pathogens

Three pathovars of *Xanthomonas* viz., *Xanthomonas axonopodis* pv. *malvacearum* (X.a.m.) causative agent of angular leaf spot of cotton, *X. a.* pv. *phaseoli* (X.a.p.) the causal organism of common blight of bean and *X. campestris* pv. *vesicatoria* (X.c.v.) the causal organism of bacterial spot of tomato were obtained from DANIDA laboratory, DOS in Applied Botany, University of Mysore, Mysore. Human pathogens were collected from different hospitals around Mysore, Karnataka.

Antibacterial activity assay

Antibacterial activity of solvent extracts was determined by cup diffusion method on nutrient agar medium (Anon, 1996). Solvents used for extraction served as control. Plates inoculated with test pathogens were incubated for 24h. and zone of inhibition if any around the wells was measured in mm. The procedure was repeated for recommended dose of K-cycline and Bact 805 and other synthetic antibiotic discs viz., Gentamicin and Streptomycin.

Results

Antibacterial activity of solvent extracts

Antibacterial activity assay of all the five solvent extracts revealed that methanol and ethanol extracts showed significant activity against both phytopathogenic and human pathogenic bacteria. Other solvent extracts viz., petroleum ether, benzene and chloroform did not show significant zone of inhibition when compared with control. Methanol extract showed highly significant activity when compared with K-cycline and Bact-805 against plant pathogenic bacteria (table 1). In case of human pathogenic bacteria methanol extract showed moderately significant antibacterial activity when compared with Streptomycin (table 2). Among plant pathogens X.a.p. was highly sensitive. Among human pathogens *S. flexneri*, *Salmonella paratyphi* B, *Streptococcus faecalis* and *S. aureus* were highly sensitive to methanol and ethanol extracts.

Table 1: Antibacterial activity of different solvent extracts of *Oxalis corniculata* on phytopathogenic bacteria (zone of inhibition measured in mm)

Extracts	X. a. pv. *m*	*X. a.* pv. *p.*	*X. c.* pv. *v.*
Petroleum ether	0.00	0.00	0.00
Benzene	0.00	0.00	0.00
Chloroform	0.00	0.00	0.00
Methanol	21.00±0.11	27.00 ± 0.12	15.66 ± 0.12
Ethanol	15.00 ± 0.11	17.00 ± 0.12	14.66 ± 0.11
K-cycline	22.66 ± 5.0	16.00 ± 0.00	14.66 ± 0.32
Bact-805	20.66 ± 0.37	18.00 ± 0.37	15.66 ± 0.35

Average of four replicates

X. a. pv. *m.*: *Xanthomonas axonopodis* pv. *malvacearum.*

X. a. pv. p.: *Xanthomonas axonopodis* pv. *phaseoli.*

X. c. pv. v.: *Xanthomonas campestris* pv. *vesicatoria.*

Phytochemical analysis

Phytochemical analysis of all the solvent extracts revealed the presence of carbohydrates and glycosides, phytosterols, phenolic compounds/tannins, flavonoids, proteins and aminoacids and volatile oils in both methanol and ethanol extracts (table 3). Further phytochemical analysis of methanol extract (Harborne, 1992) revealed that the antibacterial activity of the methanol and ethanol extract is due to the presence of phenolic compounds.

Discussion

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been a continuing search for new and more potent antibiotics (Heisig, 2001). According to World Health Report on infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium.

Table 2. Antibacterial efficacy of different solvent extracts of *Oxalis corniculata* against human pathogenic bacteria (Zone of inhibition measured in mm)

Sl. No.	**Organisms**	**Petroleum ether**	**Benzene**	**Chloroform**	**Methanol**	**Ethanol**	**Gentamicin**	**Streptomycin**
1.	*Proteus mirabilis*	5.75 ± 0.14	0.00	5.87 ± 0.12	8.9 ± 0.02	8.13 ± 0.09	9.25 ± 0.15	0.00
2.	*Citrobacter sp.*	5.87 ± 0.12	5.50 ± 0.00	5.87 ± 0.12	10.05 ± 0.05	7.85 ± 0.11	15.75 ± 0.15	13.62 ± 0.10
3.	*Klebsiella sp.*	5.50 ± 0.00	0.00	0.00	8.87 ± 0.12	7.00 ± 0.00	15.62 ± 0.10	11.75 ± 0.15
4.	*E. coli*	0.00	0.00	0.00	1.00 ± 0.00	8.10 ± 0.05	14.62 ± 0.10	10.62 ± 0.10
5.	*S. aureus*	6.75 ± 0.14	7.42 ± 0.07	0.00	16.87 ± 0.12	13.37 ± 0.12	22.37 ± 0.10	22.75 ± 0.15
6.	*S. faecalis*	8.75 ± 0.14	7.12 ± 0.12	7.61 ± 0.06	19.37 ± 0.12	15.75 ± 0.14	15.75 ± 0.15	0.00
7.	*Pseudomonas aeruginosa*	0.00	6.75 ± 0.14	0.00	14.25 ± 0.14	9.25 ± 0.14	14.62 ± 0.10	7.5 ± 0.00
8.	*Salmonella paratyphi A*	0.00	0.00	0.00	14.81 ± 0.11	13.67 ± 0.00	18.37 ± 0.10	16.37 ± 0.10
9.	*S. p. B.*	0.00	0.00	0.00	15.67 ± 0.00	13.75 ± 0.16	21.62 ± 0.10	18.72 ± 0.15
10.	*S typhi*	0.00	0.00	0.00	12.75 ± 0.14	12.25 ± 0.14	20.62 ± 0.10	0.00
11.	*Salmonella typhimurium*	0.00	0.00	0.00	12.25 ± 0.14	9.87 ± 0.12	16.62 ± 0.10	12.00 ± 0.17
12.	*Shigella boydii*	0.00	0.00	0.00	13.87 ± 0.12	13.25 ± 0.14	21.75 ± 0.10	0.00
13.	*Shigella flexneri*	0.00	0.00	0.00	16.87 ± 0.12	16.00 ± 0.20	14.40 ± 0.10	9.25 ± 0.15
14.	*Shigella sonnei*	0.00	0.00	0.00	13.75 ± 0.14	6.75 ± 0.14	18.75 ± 0.15	0.00

Results of four trials

Hence, the last decade witnessed an increase in the investigations on plants as a source of human disease management (Aiyelagabe et al. 2000; Prashanth et al. 2001; Mouniswamy et al. 2002; Woldemichael et al. 2003 and Baek et al. 2004) and not many reports are available on the exploitation of plants for the management of plant diseases (Satish et al. 1999; Bisignano et al. 2000). This is mainly due to lack of information on the screening/evaluation of diverse plants for their antibacterial potential. Therefore, in the present investigation *Oxalis corniculata* an important weed was evaluated for its antibacterial potential for the first time against important pathovars of phytopathogenic *Xanthomonas pathovars*, which are known to cause many diseases on important crops like tomato, cotton, French bean, paddy, pepper etc., and also against fourteen human pathogenic bacteria. The present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human and plant systems. The phytochemical analysis revealed that the active principle responsible for the antibacterial activity is a phenolic compound.

Table 3: Phytochemical analysis of *Oxalis corniculata*

Test for	**Petroleum ether**	**Benzene**	**Chloroform**	**Methanol**	**Ethanol**
Alkaloids	- -	- -	- -	- -	- -
Carbohydrates and glycosides	- -	- -	- -	++	++
Phytosterols	- -	- -	- -	++	++
Fixed oils and fats	- -	- -	- -	- -	- -
Phenolic compounds/tannins	- -	- -	- -	++	++
Saponins	- -	- -	- -	- -	- -
Flavonoids	- -	- -	++	++	++
Protiens and Aminoacids	- -	- -	- -	++	++
Gums and mucilage	- -	- -	- -	- -	- -
Volatile oils	- -	- -	- -	++	++

- -: Absent ++: Present

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