Anti-bacterial Evaluation and Minimum Inhibitory Concentration Analysis of Oxalis corniculata and Ocimum santum against Bacterial Pathogens

Article *in* Biotechnology(Faisalabad) · April 2010

DOI: 10.3923/biotech.2010.533.536 · Source: DOAJ

3 authors, including:

Md Shahedur Rahman
Nanjing University of Science and Technology
52 PUBLICATIONS **973** CITATIONS

SEE PROFILE

Mohammad Abu Hena Mostofa Jamal
Islamic University (Bangladesh)
81 PUBLICATIONS **884** CITATIONS

SEE PROFILE

Biotechnology, 2010
ISSN 1682-296X

Anti-bacterial Evaluation and Minimum Inhibitory Concentration Analysis of *Oxalis corniculata* and *Ocimum santum* against Bacterial Pathogens

Md. Shahedur Rahman, Md. Mahboob Hossain Khan and Md. Abu Hena Mostofa Jamal
Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh

Abstract: In this present study, it is tried to find out the antimicrobial effect and Minimum Inhibitory Concentration (MIC) of *Oxalis corniculata* leaf and *Ocimum sanctum* leaf extract against clinical isolates from urine, stool and sputum and their successive comparison with commercially, available antibiotic discs. Powdered leaves were prepared and used for extraction with various solvents, viz., methanol, ethanol and chloroform in case of *Oxalis corniculata* and ethanol, methanol, n-hexane and petroleum spirit in case of *Ocimum santum*. All the solvent extracts were evaporated to dryness in air. Using the disc diffusion method, the bacterial growth were inhibited. Among the solvent extracts tested, methanol extract of *Oxalis corniculata* leaf showed higher antibacterial activity compared to Erythromycin and Nalidixic acid against *Staphylococci* sp. whereas the methanol extract of *Ocimum sanctum* leaf showed higher antibacterial activity compared to CIP-5 against *Staphylococcus aureus*. The best MIC values were recorded to be 256 μg mL^{-1} against *Salmonella typhi* for ethanol extract of *Oxalis corniculata* leaf and 128 μg mL^{-1} against *Staphylococcus aureus* for the methanol extract of *Ocimum sanctum* leaf.

Key words: Medicinal plants, antibacterial activity, *Oxalis corniculata, Ocimum santum*, MIC

INTRODUCTION

The use of higher plants and their extracts to treat infections is an age old practice in traditional medicine. Traditional medical practice has been known for centuries in many parts of the world (Onyeagba *et al*., 2004). About 80% of the world's population depends wholly or partially on traditional medicine for its primary health care needs (Kunwar and Adhikari, 2005). Bangladesh possesses a rich flora of Medicinal plants. Out of 5000 species of phanerogams and pteridophytes growing in this country more than a thousand are regarded as having medicinal properties (Batugal *et al*., 2004). More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh. The use of herbs is the most ancient approach to healing known. The herbal medicines may be in form of powders, liquids, or mixtures, which may be raw or boiled, ointments, liniments and incisions (Akpata, 1979). Roots, barks and leaves of various plants are employed in ethnomedicine. Plant extracts are given singly or as concoctions for various ailments. Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants (El-Shouny and Magaam, 2009; Misra *et al*., 1992; Habtemarian *et al*., 1993) and quite a number of chemical compounds of plant origin have been shown to possess antimicrobial activities (Prabuseenivasan *et al*., 2006; De Billerbeck *et al*., 2001; Corthout *et al*., 1992). In diseases of microbial origin, the plants function as a result of antimicrobial activity against the causative agents (Sofowora, 1993).

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 (Raghavendra *et al*., 2006). *Ocimum sanctum* L. (Labiatae) is a strongly scented small annual herb, up to 18 inches tall and grows into a low bush and is commonly known as holy basil, Tulsi' or Tulasi (Mahmood *et al*., 2008). It is being used as a tonic for the treatment of nervous disorders, stress related headaches, migraines and allergies (Bargava and Singh, 1981). Due to peculiar essence of *O. sanctum* oil, used to clear the mind and relieve the intellectual fatigue, while giving clarity and mental strength. The oil is also administered for asthma, bronchitis, sinus infections, constipation, nausea, vomiting and cramp (Gupta *et al*., 2002).

In this present study it is so tried to find out the antimicrobial effect and Minimum Inhibitory Concentration (MIC) of *Oxalis corniculata* leaf and

Corresponding Author: Md. Shahedur Rahman, Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh

Ocimum sanctum leaf extract against clinical isolates from urine, stool and sputum and their successive comparison with commercially available antibiotic discs.

MATERIALS AND METHODS

This research was conducted from July, 2007 to December 2007 at Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh. The bacterial strains used in this study were *Pseudomonas aeruginosa, Staphylococci* sp., *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Shigella* sp., *Enterobacteria* sp., *Escherichia coli* which were isolated from clinical specimens like stool, urine and sputum collected from infected patients by standard methods. The plants were taken from local garden in Kushtia district. Then leaves were collected, washed and cut into small pieces and dried in the shade, away from the sun (this will avoid the denaturing of the enzyme and prevent excessive dehydration). Once the leaves were dried, it was grounded into finely divided powder. *Ocimum sanctum* leaf was dissolved in selected solvents like n-hexane, ethanol, petroleum spirit and methanol to produce their respective extracts for their antibacterial activity. Similarly, for *Oxalis corniculata* leaf, chloroform, ethanol and methanol extracts were used as solvents. Twenty gram of the ground samples were soaked in 100 mL of different solvents in conical flasks sealed with foil. The flasks were placed in the shaker for 24 h for *Oxalis corniculata* leaf and 36 h for *Ocimum sanctum* leaf so that the oil in the leaf powder extracts out into the solvent. After shaking, the debris of the leaves was removed by filtration. The solutions were filtered twice to ensure no leaf particle gets retained in the crude extract. Into this, a pinch of charcoal was added to absorb the color of the extract. The crude extract was filtered again to remove the undissolved charcoal. The filters were finally transferred into the beaker and left in the air for drying till the solvents evaporates leaving a concentrated crude extract. All extracts were stored at 4°C when not in use. Sterile filter paper disks of 5 mm diameter were impregnated with different crude extracts and dried in a hot air oven at 60°C for 5 min.

Agar plates were inoculated with 0.1 mL broth culture of test organisms and spread with an L-shaped glass rod. Then the commercially available antibiotic discs were placed on the inoculated Petri dishes by using sterile forcep in the laminar Airflow cabinet. Discs prepared from the extract from the leaves were also placed in the same way. Then the plates were left for incubation at 37°C for 48 h. The Minimum Inhibitory Concentration (MIC) of all the solvents were also determined by serially diluting the crude extract from 512 μg mL^{-1} to 2 μg mL^{-1} with distil water (Reiner, 1982).

RESULTS AND DISCUSSION

The extract of *Oxalis corniculata* is prepared by dissolving their powders into different solvents like methanol, ethanol and chloroform. From Table 1 it has been seen that methanol extract of *Oxalis corniculata* leaf showed the effective zone formation against all the micro-organisms, while chloroform shows the least amount of zone formation. When a comparative study was made, it was seen that the crude extract of the methanol showed a high activity than E-15, TS-25 and NA-30 against *Staphylococci* sp. The chloroform extract of *Oxalis* leaf also showed more activity then the commercial discs, i.e., E-15, NA-30 and TS-25 against the *Staphylococci* sp. The ethanol extracts showed less antibacterial activity when compared to the commercial discs. Table 1 also mentioned that methanol extract of *Ocimum* leaf showed a good response when compared with the other extracts. It was noticed that the *Staphylococcus aureus* showed higher sensitivity to

Table 1: Antibacterial activity of *Oxalis corniculata* and *Ocimum sanctum* leaf extracts on pathogenic bacteria (zone of inhibition in cm) and their comparison with commercial antibiotics

	Zone of inhibition (cm)											
	Commercial discs					Crude extracts of *Oxalis* leaf			Crude extract of *Ocimum* leaf			
Samples	E-15	NA-30	TS-25	CIP-5	CX-5	1	2	3	4	5	6	7
Escherichia coli	-	1.4	2.3	2.5	1.0	1.15	0.7	-	-	-	-	1.0
Pseudomonas aeruginosa	-	0.5	-	-	-	-	-	-	-	-	-	-
Staphylococci sp.	0.70	1.1	-	2.5	3.2	0.85	1.6	1.4	0.9	0.8	-	0.7
Salmonella typhi	1.4	1.6	-	3.2	2.7	1.0	-	-	-	-	-	-
Klebsiella pneumonia	2.0	1.0	1.9	-	1.5	-	0.95	-	-	-	-	-
Staphylococcus aureus	3.8	0	2.9	1.5	3.9	0.65	1.5	-	1.7	-	-	0.8
Shigella sp.	2	2.25	3.1	1.5	2.8	-	-	1.0	1.1	-	-	0.85
Enterobacteria sp.	1.5	-	3.3	0.9	-	-	1.4	-	0.67	0.9	-	-

E: Erythromycin, NA: Nalidixic acid, 1: Ethanol Extract of *Oxalis* leaf, 2: Methanol extract of *Oxalis*, 3: Chloroform Extract of *Oxalis*, 4: Methanol extract of *Ocimum* leaf, 5: N-Hexane extract of *Ocimum* leaf, 6: Petroleum spirit extract of *Ocimum* leaf, 7: Ethanol extract of *Ocimum* leaf, -: No inhibition zone

Table 2: Determination of Minimum Inhibition Concentration (MIC) of *Oxalis corniculata* and *Ocimum sanctum* leaf in different solvents (μg mL^{-1})

	Determination of Minimum Inhibition Concentration (MIC)						
	Oxalis corniculata leaf extract			*Ocimum sanctum* leaf extract			
Test of sample	Methanol	Ethanol	Chloroform	Ethanol	Methanol	N-hexane	Petroleum spirit
Escherichia coli	Crude	Crude	-	Crude	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Staphylococci sp.	512	Crude	512	Crude	Crude	Crude	-
Salmonella typhi	Crude	256	-	-	-	-	-
Klebsiella pneumonia	Crude	-	-	-	-	-	-
Shigella sp.	-	-	Crude	Crude	128	-	-
Enterobacteria sp.	Crude	-	-	-	Crude	Crude	-

methanol extract of *Ocimum* leaf than CIP-5 and NA-30. These finding agrees with that of other researchers (Joshi *et al*., 2009; Unni *et al*., 2009; Mahmood *et al*., 2008; Cock, 2008; Raghavendra *et al*., 2006; Taranalli *et al*., 2004; Burt, 2004).

Due to variable diffusability in agar medium, the antibacterial property may not demonstrate as ZOI commensurate to its efficacy. Therefore Minimum Inhibitory Concentration (MIC) value has also been computed in this study. MIC is the lowest concentration of antibacterial substance required to produce a sterile culture (Cheesbrough, 1987).

From Table 2 it is observed that in case of *Oxalis corniculata* leaf extract the lowest MIC value in observed at 256 μg mL^{-1} of the ethanol extract against *Salmonella typhi*. Where *Ocimum sanctum* leaf extract lowest MIC value was observed at 128 μg mL^{-1} concentration of methanol extract against *Shigella* sp. In general, it can be stated as that the methanol (Kathiriya *et al*., 2010) and chloroform extract of *Oxalis* and ethanol extract of *Ocimum* leaves showed higher antibacterial activity then the commercial discs. Thus, these plants can be used as efficiently for curing common diseases like diarrhea, cough, fever, etc.

These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. In conclusion, the results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases (Bylka *et al*., 2004; Kilani, 2006; Joshi *et al*., 2009).

CONCLUSION

The best MIC values were recorded to be 256 μg mL^{-1} against *Salmonella typhi* for ethanol extract of *Oxalis corniculata* leaf and 128 μg mL^{-1} against *Staphylococcus aureus* for the methanol extract of *Ocimum sanctum* leaf.

REFERENCES

Akpata, L., 1979. Practice of Herbalism in Nigeria. University of Ife Press, Nigeria.

Bargava, K.P. and N. Singh, 1981. Effect of *Ocimum sanctum* on noise induced changes in neutrophil functions. Ind. J. Med. Res., 73: 143-151.

Batugal, P.A., J. Kanniah, S.Y. Lee and J.T. Oliver, 2004. Medicinal plants research in Asia. Volume 1, The Framework and Project Workplans. International Plant Genetic Resources Institute, Serdang, Selangor DE, Malaysia.

Burt, S.A., 2004. Essential oils: Their antibacterial properties and potential applications in foods: A review. Int. J. Food Microbiol., 94: 223-253.

Bylka, W., M. Szaufer, J. Matlawska and O. Goslinska, 2004. Antimicrobial activity of isocytisoside and extracts of Aquilegia vulgaris. Lett. Applied Microbiol., 39: 93-97.

Cheesbrough, M., 1987. Medicinal Laboratory Manual for Tropical Countries Volume 1. 2nd Edn., Elsevier Publisher, Philadelphia, pp: 524.

Cock, E.I., 2008. Antimicrobial activity of *Aloe barbadensis* Miller leaf gel components. Internet J. Microbiol.,

Corthout, J., L. Pieters, M. Claeys, D.V. Berghe and A. Vlietinck, 1992. Antiviral caffeoylester from *Spondias mornbin*. Phytochemistry, 31: 1979-1981.

De Billerbeck, V.G., C.G. Roques, J.M. Bessiere, J.L. Fonvieille and R. Dargent, 2001. Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. Can. J. Microbiol., 47: 9-17.

El-Shouny, W.A. and S. Magaam, 2009. Sensitivity of multidrug resistant *Pseudomonas aeruginosa* isolated from surgical wound-infections to essential oils and plant extracts. World J. Med. Sci., 4: 104-111.

Gupta, S.K., J. Prakash and S. Srivastava, 2002. Validation of traditional claim of Tulsi, *Ocimun sanctun* Linn. as a medicinal plant. Ind. J. Exp. Biol., 40: 765-773.

Habtemarian, S., A.I. Gray and P.G. Waterman, 1993. A new antibacterial sesquiterpene from *Premma oligotrichia*. J. Nat. Prod., 56: 140-143.

Joshi, B., S. Lekhak and A. Sharma., 2009. Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. Kathmandu Univ. J. Sci. Eng. Technol., 5: 143-150.

Kathiriya, A.K., K. Das, M. Joshipura and N. Mandal, 2010. *Oxalis corniculata* Linn. the plant of Indian subtropics. Herbal Tech. Ind.,

Kilani, A.M., 2006. Antibacterial assessment of whole stem bark of Vitex doniana against some enterobactriceae. Afr. J. Biotechnol., 5: 958-959.

Kunwar, R.M. and N. Adhikari, 2005. Ethnomedicine of dolpa district Nepal: The plants their vernacular names and uses. Lyonia, 8: 43-49.

Mahmood, K., U. Yaqoob and R. Bajwa, 2008. Antibacterial activity of essential oil of *Ocimum sanctum* L. Mycopath, 6: 63-65.

Misra, T.N., R.S. Sigh, N.S. Pandey, C. Prasal and B.P. Singh, 1992. Antifungal essential oil and a long chain alcohol from *Achyranthis aspera*. Phytochemistry, 31: 1811-1812.

Onyeagba, R.A., O.C. Ugbogu, C.U. Okeke and O. Iroakasi, 2004. Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). Afr. J. Biol., 3: 552-554.

Prabuseenivasan, S., M. Jayakumar and S. Ignacimuthu, 2006. *In vitro* antibacterial activity of some plant essential oils. BMC Complem. Alternative Med., 6: 39-39.

Raghavendra, M.P., S. Satish and A. Raveesha, 2006. Phytochemical analysis and antibacterial activity of *Oxalis corniculata*, an known medicinal plant. My Sci., 1: 72-78.

Reiner, R., 1982. Antibiotica: An Introduction. La Roche and Co. Ltd., Switzerland, pp: 23.

Sofowora, A., 1993. Introduction to Medicinal Plants and Traditional Medicine. John Wiley and Sons, New York, pp: 97-145.

Taranalli, D., S.V. Tipare, S. Kumar and S.S. Torgal, 2004. Wound healing activity of *Oxalis corniculata* whole plant extract in rats. Ind. J. Pharm. Sci., 66: 444-446.

Unni, B.G., A. Borah, S.B. Wann, H.R. Singh, B. Devi and M. Bhattacharjee, 2009. Phytochemical and antibacterial study of traditional medicinal plants of North East India on *Escherichia coli*. Asian J. Exp. Sci., 23: 103-108.