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Assessment of Antibacterial and Phytochemical analysis of *Lagenaria vulgaris* Ser. against Respiratory Tract Pathogens

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ABSTRACT

Microbial drug resistance has fascinated a lot attention in recent years. In present study, the antibacterial activity of various extracts of *Lagenaria vulgaris* was studied against five bacterial pathogens i.e. *Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442. Shade dried seeds were crushed and extracted in petroleum ether (PET), acetone (ACE), methanol (MeOH) and water (H_2O) by using Soxhlet apparatus. The agar well diffusion method was adopted to examine antibacterial activity of extracts against the susceptible organisms. Erythromycin was used as positive control to determine the sensitivity of the strains. Phytochemical analysis was done for plant extract. Results showed that seed extracts of *L. vulgaris* exhibited moderate antibacterial activities. The maximum inhibition by acetone extract was found against *H. influenzae* (16 mm), *S. pneumoniae* (14 mm) and *S. pyogenes* (14 mm) respectively. Least activity was found against *S. aureus* (12 mm) followed by MeOH, H_2O and PET. The phytochemical screening for ACE extract of *L. vulgaris* has shown that plant contains flavonoids, glycosides, alkaloids, steroids, terpene, saponins and tannins. The investigation supports a good response to the use of *L. vulgaris* in herbal medicine and as a base for the development of new drugs and phytomedicine.

Keywords: Antibacterial activity, agar well diffusion method, *Lagenaria vulgaris*, Phytochemical screening.

INTRODUCTION

Herbs are staging a comeback all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other, were used for medicinal purposes.

Present day infectious diseases pose serious problems to health and they are the main cause of morbidity and mortality worldwide (Beaglehole *et al*. 2004). The uses of herbs in treatment of animal and human

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diseases have long been established. Most plant extracts have been shown to possess antimicrobial agents active against microorganisms *in vitro*. These plants contain medicinal properties which make them potent to cure or prevent diseases.

Lagenaria vulgaris Ser. is commonly known as "Unnab". It is a member of gourd bearing vines belongs to the cucurbitaceae family. The young fruit is used as vegetables, the large, strong, hard-shelled, and buoyant fruits have long been used as containers for water and food, musical instruments. Traditionally, the vegetable is claimed to possess antipyretic activity, cardioprotective, cardiotonic, aphrodisiac, diuretic, nutritive properties (Kirtikar, 2001).Hence, this study is an attempt to explore the antibacterial potential of *L. vulgaris* seed extracts against respiratory tract pathogens.

MATERIAL AND METHODS

Plant Material - Plant was collected from Baramadpur Village, Kadipur, Sultanpur, Uttar Pradesh and authenticated at Botanical Survey of India, Northern Regional Center, Dehradun. Collected seed material was dried under shade at room temperature and crushed to small pieces by using pestle and motar.

Preparation of Extract - Plant extracts were prepared by immersing 200 g of powdered plant material in 600 ml of four different solvents i.e. petroleum ether (PET), acetone (ACE), methanol (MeOH) and water (H_2O), loaded in Soxhlet assembly and extracted for 72 h through successive method (Ahmed *et al*., 1998). Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30°C. Residues were stored at 4°C until further use. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/ml for agar well diffusion method.

Test Microorganisms - The five bacterial and one fungal strains causing respiratory infections used in this study were *Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442. Bacterial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of Inoculums - Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37°C.

Antibacterial testing - The antibacterial activity of different extracts was determined by agar well-diffusion method (Ahmed *et al*., 1998). 0.1 ml of 12-16 h incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium no. 173 (Hi media Pvt. Ltd., Mumbai, India) and poured in pre-sterilized petri plates. A cork borer (6 mm diameter) used to punch wells in solidified medium and filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic erythromycin (positive control). The plates were incubated at 37°C for 24 h in BOD incubator and the diameter of the zone of inhibition was measured in millimetre. Each sample was assayed in triplicate and the mean values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest

millimetre (mm) as observed from the clear zones surrounding the wells.

Phytochemical screening - Major phytocompound, in the crude extracts of *T. cordifolia* were subjected to phytochemical analysis to determine the presence of bioactive components by using standard qualitative methods (Trease and Evans, 1996).

Test for alkaloids - Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids.

Test for flavonoids - On addition of conc. HCl in methanolic extract of material, a red colour appeared which indicated the presence of flavonoids.

Test for glycosides - Plant extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with $Ba(OH)_2$. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. H_2SO_4 and after hydrolysis the presence of sugars was determined with help of Fehling's solution.

Test for Steroids - The extract mixed with 3 ml $CHCl_3$ and 2 ml conc. H_2SO_4 was poured from side of test tube and colour of the ring at junction of two layers was noted. A red colour showed the presence of steroids.

Test for Saponins - Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Tannins - Extract was added in 1% ferric chloride and observed the colour. Bluish black colour appeared which disappeared on addition of dilute H_2SO_4 follow a yellow brown precipitate indicates the presence of tannins.

RESULTS AND DISCUSSION

The data characterizing the antibacterial activity of crude extract of *L. vulgaris* seeds are presented in Table 1. The study showed that the crude extract of *L. vulgaris* was found active and exhibited moderate antibacterial activities against test bacterial organisms. The maximum inhibition by ACE extract was found against *H. influenzae* (16 mm), *S. pneumoniae* (14 mm) and *S. pyogenes* (14 mm) respectively. The minimum activity was found against *S. aureus* (12 mm) followed by MeOH, H_2O and PET.

Table 1: The inhibition zones diameters of various extracts of *Lagenaria vulgaris* (seeds)

Microorganisms	*Diameters of the inhibition zone (mm)				Positive Control
	PET	ACE	MeOH	H_2O	(Erythromycin)
H. influenzae	-	16	10	-	21
P. aeruginosa	-	13	11	11	17
S. aureus	-	12	11	-	31
S. pneumoniae	9	14	11	12	20
S. pyogenes	-	14	14	13	26

** Values are mean of three replicates; Cork borer diameter: 6 mm;*

The phytochemical screening of *L. vulgaris* extracts has shown that plant contains flavonoids, glycosides, alkaloids, steroids, terpenes, saponins and tannins which are very important constituent when looking for pharmacologically active phytochemicals in the plant. In some findings, *L. vulgaris* was reported with major phytoconstituents i.e. saponins, fibres, flavanoids and triterpenoid cucurbitacins B, D, G, H (Evans, 1996; Sonja, 2000), fucosterol, campesterol, flavones C-glycosides (Shirwaikar and Sreenivasan, 1996) present in its various parts. The pharmaceutical cough syrups and drugs use it

as principle ingredient. It is also used in treatment of pain, ulcer, fever, pectoral cough, asthma and other bronchial disorders (Anonymous, 1962; Khare, 2004).

On the basis of results, it is concluded that seeds *L. vulgaris* has good antimicrobial potential against selected respiratory tract pathogens. This study supports the traditional use of *L. vulgaris* and indicated that it contains some major bioactive compounds inhibiting the growth of microorganisms there by proving very effective source of derived drugs.

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