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Antimicrobial effect of *Lagenaria siceraria* (Mol.) Standley, against certain bacteria and fungal strains

Y. P. Nagaraja*, K. N. Geetha and M. S. Vinay

Department of Biotechnology, Nagarjuna College of Engineering and Technology, Mudugurki, Venkatagirikote post, Devanahalli Taluk, Bangalore - 562 110 (Karnataka), INDIA
*Corresponding author. E-mail: Drnagaraja_yp@rediffmail.com

Abstract: This study was performed to evaluate the antibacterial and antifungal potency of *Lagenaria siceraria* (Mol.) Standley, commonly known as bottle gourd belonging to the family Cucurbitaceae. Petroleum Ether, Chloroform, Methanol, Absolute alcohol and Water showed a majority of the compound including Steroids, Alkaloids, Tannins, Flavonoids, Lactones and Carbohydrates. All the five extracts were prepared by using soxhlet apparatus and the extracts showed moderate to potent antimicrobial activity against the bacterial strains: *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus* and antifungal strains: such as *Aspergillus flavus*, *Aspergillus oryzae* and *Trichoderma harzianum*.

Keywords: *Lagenaria siceraria*, Antibacterial, Antifungal, Phytochemicals, Leaf extracts

INTRODUCTION

The use of plant extracts and phytochemicals both with known antimicrobial properties can be of great significance in therapeutic treatments (Shapoval *et al.*, 1994). Many plants are used as medicines because of their antimicrobial nature, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances (Geethalakshmi *et al.*, 2010). The demand on plant based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, easily biodegradable producing minimum environmental hazards, no side effects and easily available at affordable price. India is the largest producer of medicinal herbs and traditional practitioners of this country use more than 6000 medicinal plants in primary health care (Shariff *et al.*, 2006).

Lagenaria siceraria (Mohl.) Standley fruit (Syn. *L. vulgaris* Ser., *Cucurbita legenaria*, Linn., *L. leucantha* Rusbey, belongs to the family Cucurbitaceae), commonly known as Bottle gourd is distributed in Africa, Asia and America. The first and foremost important use of bottle gourd was as a water carrier. Medicinally it is used as a purgative, an antidote for certain poisons and a cure for coughs (Uphof, 1959). An infusion of the seeds has been used to cure chills and for headaches, and juice from the leaves was taken against jaundice and to cure baldness (Moerman, 1998 and Deshponde *et al.*, 2008). The present study was undertaken to evaluate the potentiality of aqueous and organic solvent extracts of *L. siceraria* against some medically important bacterial and fungal strains.

MATERIALS AND METHODS

Test plant material : The fresh plant material of the *L. siceraria* was collected from Hoskote forest ranges near Bangalore in Karnataka, India during February 2009.

Test organisms : To find out the antimicrobial activity of various extracts of *L. siceraria*, the different strains of pathogenic bacteria used are *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC29212, *Klebsiella pneumonia* ATCC31488, *Salmonella typhi* ATCC6539, *Staphylococcus aureus* ATCC25923 and antifungal strains includes *Aspergillus flavus* ATCC32611, *Aspergillus oryzae* ATCC76080, *Trichoderma harzianum* ATCC20476.

Extraction and phytochemical analysis: The surface of the leaves were washed in distilled water to remove the surface microflora and are then shade dried, pulverized by a mechanical grinder and passed through a 40 µm mesh sieve to get the fine powder and stored in an airtight container. The dried powder (50g) was extracted by soxhlet extraction method using different solvents namely, Petroleum Ether, Chloroform, Methanol, Absolute alcohol and Water sequentially. The solvents from various extracts were then concentrated in a rotavapour at reduced pressure below 40^{0}C and the extract thus obtained was stored in airtight bottles at 4^{0}C for further experiments.

Qualitative tests for phytochemical analysis: The phytochemicals in the plants were investigated by performing standard qualitative tests for the presence of

Table 1. Preliminary phytochemical screening of *Lagenaria siceraria*.

Sl.No.	Tests	Occurrence
1.	Steroids	+
2.	Alkaloids	+
3.	Tannins	-
4.	Flavonoids	+
5.	Lactones	+
6.	Carbohydrates	+

alkaloids, flavanoids, saponins, tannins, terpenoids, steroids, lactones and carbohydrates were evaluated according to the methods described by Edeoga *et al.* (2005).

Antimicrobial activity assay

Disc diffusion test: The *in vitro* antimicrobial activity of the sample solution was done by disc diffusion method (Bauer *et al.*, 1966). Plates were prepared by pouring 20 ml of sterile nutrient agar media (Hi media) into sterile petridishes and were inoculated with a loopful broth culture of each organism. Sterile whatmann filter paper discs (6 mm diameter) impregnated with 20 ml quantity of dimethyl sulfoxide solution of various extracts was air dried and placed on the agar plates. The plates were incubated at 37^0C for 24h. control studied with Chloramphenicol and Nystatin 30mg were used as standards for bacteria and fungi, the solvent DMSO control was concurrently (Umadevi *et al.*, 2003). The growth inhibition of each microbial strain was calculated as the percentage of inhibition of radial growth relative to the control. The plates were used in triplicate for each treatment. The relative growth inhibition of treatment compared to control was calculated by percentage, using the following formula:

$$\text{Inhibition \%} = 1 - \frac{\text{Radial growth of treatment (mm)}}{\text{Radial growth of control (mm)}} \times 100$$

Minimal inhibitory concentration (MIC) assay: Minimum Inhibitory Concentration assay was performed by dilution method as described by National Committee for Clinical Laboratory Standards (NCCLS, 1993) using Muller Hinton agar medium (Hi-media). All the test extracts including standard was taken in discs. The discs were dipped in extracts having the concentrations of 0.0625, 0.125, 0.25 and 0.5mg/10^3. The plates were incubated at 37^0C for 24h. The assay was repeated twice and the antimicrobial potency of different microbial strains was determined by observing the inhibitory zone of microbial strains. The lowest concentration showing no inhibitory zone was considered as the MIC.

RESULTS

Extraction yield and phytochemical estimation: The results of phytochemical analysis of petroleum ether, chloroform, methanol, absolute alcohol and water extract has revealed the positive results for alkaloids, steroids, flavonoids, lactones and carbohydrates and negative results for tannins. The phytochemical analysis of water and ethanol extract has shown the presence of saponins and terpenoids respectively (Table 1).

The results of antibacterial activity of *L. siceraria* extract *in vitro* are given in Tables 2 and 3.It was observed that, the plant is possessing more activity against *Escherichia coli* 14.2±3.77, 13.4±3.13, 14.4±2.88, 9.8±1.48, *Enterococcus faecalis* 13.6±3.21, 12.4±2.88, 17.0±4.00, 8.0±1.41, and *Staphylococcus aureus* 14.6±2.61, 13.0±2.00, 13.8±3.03, 6.6±2.07 in petroleum ether, chloroform, methanol and water extracts respectively, whereas in case of *Klebsiella pneumonia* 11.4±1.34, 04.6±6.39, 11.8±1.79, 7.0±2.24 and *Salmonella typhi*15.2±4.60, 04.8±6.72, 11.6±2.19, 5.6±3.21, the growth of inhibition was less.

In the case of antifungal activity, the growth of inhibition was comparatively more with respect to *Aspergillus flavus* 13.2±2.28, 13.4±2.19, 10.8±1.10, 5.0±3.81, *Aspergillus oryzae* 12.8±3.35, 13.0±1.00, 10.4±0.89, 3.6±3.36, but the growth of inhibition was less in case of *Trichoderma harzianum* 00.0±0.00, 14.2±2.28, 11.2±1.79, 4.2±3.03.

Thus the different solvent extracts have showed different results with different microorganisms. The petroleum ether extracts has showed MIC at 0.25 x 10^3 mg/ml in bacterial strains whereas in fungi MIC was shown positive for *A. oryzae*. The chloroform extract in bacteria

Table 2. Antimicrobial activity of various extracts of *L. siceraria* on different microbes.

	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Microorganisms	----------(mm)----------			
Bacterial Strains				
Escherichia coli	14.2±3.77	13.4±3.13	14.4±2.88	9.8±1.48
Enterococcus faecalis	13.6±3.21	12.4±2.88	17.0±4.00	8.0±1.41
Klebsiella pneumonia	11.4±1.34	04.6±6.39	11.8±1.79	7.0±2.24
Salmonella typhi	15.2±4.60	04.8±6.72	11.6±2.19	5.6±3.21
Staphylococcus aureus	14.6±2.61	13.0±2.00	13.8±3.03	6.6±2.07
Fungal Strains				
Aspergillus flavus	13.2±2.28	13.4±2.19	10.8±1.10	5.0±3.81
Aspergillus oryzae	12.8±3.35	13.0±1.00	10.4±0.89	3.6±3.36
Trichoderma harzianum	00.0±0.00	14.2±2.28	11.2±1.79	4.2±3.03

The values are mean ± S. D. of zone of inhibition

Table 3. Antimicrobial activity of various extracts of L. siceraria on different microbes.

Microorganisms	Petroleum ether extract	Chloroform extract	Alcohol extract	Water extract
	----------(mg)----------			
Bacterial Strains				
Escherichia coli	0.43	0.66	0.25	0.25
Enterococcus faecalis	0.66	0.25	0.66	0.25
Klebsiella pneumonia	0.25	0.8	0.43	0.25
Salmonella typhi	0.43	0.8	0.25	0.25
Staphylococcus aureus	0.25	0.43	0.43	0.25
Fungal Strains				
Aspergillus flavus	0.43	0.66	0.11	0.8
Aspergillus oryzae	0.11	0.11	0.66	0.8
Trichoderma harzianum	0.8	0.11	0.11	0.8

The values are mean ± S. D. of MIC.

showed MIC in *E. coli*, *E. faecalis* and *S. aureus* at 0.25 x 10^3 mg/ml and in case of fungi, the extract was able to show the MIC at 0.11 x 10^3 mg/ml and 0.25 x 10^3 mg/ml. The methanol extract has showed MIC in 0.25 x 10^3 mg/ml and 0.43 x 10^3 mg/ml in bacterial strains and 0.11 x 10^3 mg/ml and 0.66 x 10^3 mg/ml in the case of fungi (Table 3).

DISCUSSION

The results obtained in this study showed the presence of antimicrobial activity of *L. siceraria*, in the preliminary screening of antimicrobial activity using disc diffusion method. The antibacterial activity of *L. siceraria* water extract was lower than the organic solvent extracts however the results are concurrent with the earlier report done by Tolulope (2007) in *Hibiscus sabdariffa* against *S. aureus, E. coli and K. pneumoniae.* Ghosh *et al.* (2008) have also used similar bacterial sources in their investigation. It includes, *Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhi* and *Staphylococcus aureus* and got more zone of inhibition in the methanol extract. However, in the present study also it is revealed that the extracts of petroleum ether, chloroform and methanol have showed more zone of inhibition than the water extract and it is also suggesting that, the inhibition is more in *E. coli, E. faecalis* and *S. aureus* but it was comparatively less in case of *K. pneumonia* and *S. typhi.*

The antifungal property of the plant species has been done by using three different strains amongst *A. flavus* which showed more zone of inhibition than the other two strains. This is due to the chemical property of the extract which inhibited the growth of the fungi. Similar types of results were also observed by Cichewicz and Thorpe (1996). Many plants have been used to screen the antimicrobial property because of their antimicrobial traits, which are due to chemical compounds sythesised in their secondary metabolism of the plant such as alkaloids (O. T. A. Plants, 1985 and Omulokoli *et al.*, 1997); flavonoids (Batista *et al.*, 1994). Incase of petroleum ether extract, the MIC was found to be more at 0.25 x 10^3 mg/ml. Even in case of fungi *A. oryzae* showed MIC at 0.11 x 10^3 mg/ml and *A. flavus* at 0.25 x 10^3 mg/ml. The results are similar to the report mentioned by the earlier workers like Geethalakshmi *et al.*, (2010). It was concluded that, the most susceptible bacterial was *E. faecalis* while most resistant was *S. typhi* and in case of fungi the most susceptible fungal strain was *A. flavus* while most resistant was *T. harzianum.*

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