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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LAGENARIA *SICERARIA* (MOL) STANDAL

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ABSTRACT
The antimicrobial effect of leaf extracts of *Lageneria siceraria* (Molina) Standl was evaluated on bacterial strains like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. The acetone, methanol, alcohol and distilled water leaf extracts exhibit significant activity against all test organisms. The petroleum ether showed least activity against test bacterial strains. The *in vitro* antimicrobial activity of the extract was compared with standard antibiotic Cephlexin. The most susceptible gram positive bacterium is *Bascillus subtilis* and gram negative bacterium is *Pseudomonas aeruginosa*.
Whereas, the *Candida albicans* does not show any significant activity**.** The MIC values of the extracts were also determined which ranged from 10 to 50mg/ml. The plant contains tannins, saponins, flavonoids, terepenoids, alkaloids, steroids and cardiac glycosides. The present study suggests that the leaf extract of *Lageneria siceraria* can be used in treating diseases caused by the test organisms.

Key Words: *Lagenaria Siceraria, Antimicrobial Properties and Phytochemical Screening*

INTRODUCTION
It is estimated that there are about 250000 to 500000 plants species widely used in ethnomedicine on earth and relatively small percentage i.e. 1 to 10% of it is used as food (Moerman, 1996). Clinical problems during the treatment of infectious diseases are usually caused by micro-organisms and they can be controlled by antibiotics but now a day's micro-organisms develop resistance to many antibiotics due to indiscriminate use of commercial antimicrobial drugs (Davis, 1994). Therefore phytochemists are engaged in search of new antimicrobial plant substances from medicinal plants (Karaman *et al*., 2007).
The medicinal efficacy of these plants depends on the bioactive phytochemical constituents which involve in physiological action in human body **(**Akinomoladun *et al.,* 2007). The most important bio active constituents are alkaloids, essential oils, flayonoids, tannins, terepenoids, saponin (Edeoga *et al*., 2005).
Lagenaria siceraria is a vigorous annual herb. The stem is prostrate or climbing, angular, ribbed thick, brittle, softly hairy. Leaves are simple with long petiole up to 30 mm long thick, hallow, densely hairy with two small lateral glands located at the leaf base. Leaf lamina is broad; five lobed, cordate, pubescent and softly hairy and the tendrils are branched.
Flowers are pedicellate, solitary axillary, unisexual, monoecious. Petals are five, creamy or white coloured opening in the evening. Fruits are large, fleshy, densely hairy variable cylindrical or flask shaped or globose, constricted at the middle, indehiscent and green but at maturity turn to yellow. The pulp is pale brown, dry fruit has a thick hard hollow structure. Seeds are many, embedded in the spongy pulp, compressed with two flat facial ridges.

Medicinal Uses
Lagenaria siceraria of family Cucurbitaceae known as bottle gourd, Doodhi and Lauki, is a common fruit vegetable used throughout India. Fruits are traditionally used as a nutritive agent having cardio protective, cardio tonic, diuretic and aphrodisiac properties. It is also used as an antidote to certain poison (Deore, *et.al*., 2009; Kirtikar and Basu; 2001; Nadkarni ,1992; Rehman, 2003 and Shirwaikar, 1995). Other uses include the treatment of cough, asthma, jaundice, kidney stone and measles (Jain and Sharma, 1967) also

in Africa *Lagenaria siceraria* used as edible and medicinal because the fruit contain moderate amounts of Vitamins C, B complex and proteins. Its high water content is reputed for cooling and lubricating effect (Morimoto *et a*l., 2005). In addition to medicinal uses it has appreciable antioxidant activity (Erasto and Mbwambo, 2009).

MATERIALS AND METHODS

Plant Material

The root, stem and leaves of *Lagenaria siceraria* were collected during November 2010 from various places of Parbhani district and identified using standard floras Naik (1979) and Naik, *et al*., (1998). The collected plant material was shade dried, powdered and stored in airtight container.

Preparation of Extracts

Powder obtained was subjected to successive soxhlet extraction with increasing order of polarity i.e. acetone (56 to 60^0c), alcohol (60-70^0c), petroleum ether (60-80c) methanol (65.5-65.5c) and water (Daniel, 1991).

Test Microorganisms

Escherichia coli MTCC (3260), *Staphylococcus aureus* MTCC (1073), *Bacillus subtilis* MTCC (1091), *Pseudomonas aeruginosa* MTCC (708), *Candida albicans* MTCC (3971), were maintained on Muller Hinton agar and potato dextrose agar slant for bacteria and fungi respectively and stored at 6^0c until used. The slants were incubated at 37^oC for 24 hours and inoculums were prepared by Mac Farland turbidity standards (Stainer *et al*., 1986).

Antimicrobial Assay

Antimicrobial activity of was determined using agar well diffusion method using Nutrient agar for bacteria and potato dextrose agar for fungi. Plant extract was dissolved in DMSO (Dimethyl Sulphoxside) at concentration of 2mg /ml Cephalexin 20mg/ml was used as standard. Each plate was inoculated with 20mg/ml microbial suspension having a concentration of 10^8 cells/ml 0.1ml extract was added to each well. The bacterial plates were incubated at 37^0C for 24 hours and those containing fungi were incubated at 25^0C for seven days. The antimicrobial activity was observed as inhibition zone which was compared with standard.

Table 1: Antimicrobial activity of leaf extract of *Lagenaria siceraria*

S. No.	Organisms	Zone of inhibition(mm)					
		Plant extract					Control
		ALL	DTL	ACL	MTL	PTL	
1	*Escherichia coli*	6.1	**9.1**	4.1	**11.0**	8.1	10.1
2	*Pseudomonas aeruginosa*	7.0	**8.1**	**9.1**	**8.1**	7.5	5.1
3	*Staphylococcus aureus*	**10**	6.1	6.1	**7.1**	4.0	5.0
4	*Bacillus subtilis*	**9.1**	5.0	**10.1**	**9.**0	1.0	8.1
5	*Candida albicans*	**7.1**	2.1	4.1	5.0	3.1	10.2

ALL- Alcohol leaf extract, DTL- Distilled water leaf extract, ACL- Acetone leaf extract, MTL- methanol leaf extract, PTL- Petroleum ether leaf extract

The MIC was also determined by broth dilution method. The cultures were diluted in nutrient agar broth at a density adjusted to turbidity of 0.5 Mac Farland standards. Equal volume of each extracts and nutrient broth were mixed in test tubes 0.1ml standard inoculums was added to each tube. The lowest concentration of the extract that effects visible bacterial growth and compared with standard regarded as MIC (Table 3) Perez *et al*., (1996) and Stainer, *et al*., (1986).

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RESULTS

Antimicrobial assay was performed by agar well diffusion method against four bacterial species and one fungal species. The maximum antimicrobial activity was exhibited by alcohol extract against *Staphylococcus aureus*. The distilled water extract exhibited considerable activity against *Escherichia coli* and *Pseudomonas aeruginosa*.The acetone leaf extracts exhibit good activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. The methanol extract is active against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.* The distilled water extract is active against Escherichia *coli*, *Pseudomonas aeruginosa*. Whereas Petroleum ether extract is less effective against all test strains and the Candida *albicans* is resistant to all the extracts as comparable to antibiotic Cephalexin (Table no. 1).

Phytochemical Analysis

Phytochemical analysis of aqueous plant extract revealed that the presence of tannin, saponins, flavanoids, terpenoids, alkaloids, steroids and cardic glycoloids (Table no.2).

Table 2: Preliminary phytochemical screening of *Lagenaria siceraria*

S. No.	Plant part	Tannin	Pholobo tannins	Saponins	Flavonoid	Terpenoids	Cardiac glycosides	Alkaloid	Steroid
1	Root	-	-	+	+	+	+	+	+
2	Stem	-	-	+	+	+	+	+	+
3	Leaf	+	-	+	+	+	+	+	+

+: present --: absent

Table 3: Minimum inhibitory concentration (mg/ml)

S. No.	Organisms	Minimal inhibitory zone				
		ALL	DTL	ACL	MTL	PTL
1	*Escherichia coli*	50	20	50	10	40
2	*Pseudomonas aeruginosa*	50	20	10	40	10
3	*Staphylococcus auerus*	10	40	50	30	50
4	*Bacillus subtilis*	30	50	10	10	50
5	*Candida albicans*	50	50	50	40	40

DISCUSSION

The antimicrobial effect of *Lagenaria siceraria* has a broad spectrum activity in alcohol, acetone and methenolic extracts because there is maximum inhibition of the growth of gram negative bacteria such as *Escherichia coli,* and *Bacillus subtilis.* Similar findings have also been reported by Tomori *et al*., (2007) on antibacterial activities of *br Lagenaria eviflora* in Nigeria. However, the solvent and distilled water extracts does not exhibit any significant activity against *Candida albicans.*

The preliminary phytochemical screening revealed that plant extracts shows the presence of alkaloids, steroids, flavonoids, terpenoids, saponins which may exhibit antimicrobial activity. Flavonoids are the major group of phenolic compounds reported for their antiviral and antimicrobial properties Barnard *et al.,* (1993) and Afolayan and Mayer (1997).

Conclusion

This vividly underscores the potentials of herbal remedies which have been reported to produce beneficial responses (Morimoto *et al*., 2005) In actual findings it is observed that some of these antibacterial phytochemicals exhibited antibacterial activity against *Escherichia coli, and Bacillus subtilis* Comparative analysis of this study reveals that the bacterial inhibition elicited by the plant extract was higher than that of antibiotic Cephalexin.

This study clearly indicates that the leaf extracts of *Lagenaria siceraria* possess potent antimicrobial activity. The present investigation proves that the use of *Lagenaria siceraria* by rustics for the treatment diseases caused by the test organisms.

It is not yet clear that what type of mechanism of action of extract occurred during antibacterial activity. It is therefore difficult to speculate what could be the factor responsible for this differential response (Tomori *et al*., 2007). It should therefore be essential to follow-up this type of investigation to isolate and elucidate the active antibacterial principles from plants for their better economic and therapeutic utilization.

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