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COMPARATIVE ANALYSIS OF ANTIMICROBIAL POTENTIAL OF PEEL AND MESOCARP OF *LAGENARIA SICERARIA* FRUIT EXTRACTS IN VARIOUS SOLVENTS AGAINST CLINICALLY IMPORTANT PATHOGENS

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Abstract

The fruit of *Lagenaria siceraria* is commonly used as a vegetable. The aim of the present work was to compare the antimicrobial potential of extracts of its peel and mesocarp in various solvents against clinically important standard microbial strains including *Pseudomonas aeruginosa* ATCC9027, *Escherichia coli* ATCC8739, *Bacillus subtilis* ATCC6633, *Staphylococcus aureus* ATCC6538 and *Candida albicans* ATCC10231. In general, mesocarp was more potent than peel. *P. aeruginosa* was most susceptible, and methanolic extracts of both peel and mesocarp showed higher activity than antibiotic Amoxil. Ethyl acetate fractions of both the fruit parts and *n*-butanolic fraction of mesocarp showed highly significant activity against *P. aeruginosa*. Hexane fractions of peel and mesocarp and ethyl acetate fraction of mesocarp showed higher activity against *C. albicans* than Amoxil. In conclusion, the fruit of *L. siceraria* possesses exploitable antimicrobial activity, and further phytochemical investigation may yield good candidates for new anti-infection drugs.

Keywords: *Lagenaria siceraria*, peel, mesocarp, antimicrobial potential

Introduction

Modern antibiotics that have provided strong defense against pathogenic infections ever since their first discovery in 1928, have limitations of their own. They not only have side effects and are expensive, they also lose efficacy because of resistance that the pathogens soon develop against them. Drug resistance is, in fact, a great global challenge.[1] According to WHO (World Health Organization), antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.[2] The situation necessitates extensive efforts to explore new affordable, safer and more effective antimicrobial remedies. Natural products constitute a tested alternative, and the recent years have witnessed abundant studies on plants with the aim to discover new anti-infection therapeutic agents.[3-8]

Lagenaria siceraria (family *Cucurbitaceae*) is a plant with many folkloric medicinal uses. The plant is locally known as Lauki/Kaddu/Ghia in Pakistan, and its fruit is a popular vegetable, which is fleshy with numerous seeds. Therapeutic properties associated with the fruit include diuretic, cardio-tonic, cardio-protective, antihyperlipidemic and aphrodisiac properties. It is also used for the treatment of jaundice, measles, cough, kidney stone and asthma.[6] The antimicrobial activity of the leaves, seeds and fruit flesh of Ethiopian cultivar of *Lagenaria siceraria* has been evaluated and found to show good activity against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*, but not against clinical isolates of *Staphylococcus aureus* and *Escherichia coli.*[9] In another study, chloroform, acetone, methanolic and hexane extracts of peel of *L. siceraria* fruit were evaluated against a number of microbes.[8] The leaf extracts of the plant in different solvents were found to show moderate to potent antimicrobial activity against some clinically important bacterial and fungal strains.[6,7]

The objective of the present work was to explore and compare the antimicrobial properties of methanolic extracts peel and mesocarp of the fruit and their fractions in different solvents against a number of clinically important standard microbial strains. This is the first ever study of this type on the fruit of *Lagenaria siceraria*.

Materials and Methods

Chemicals

Solvents used for extraction and fractionation were of HPLC grade. Mueller-Hinton and nutrient agars were purchased from Sigma-Aldrich and dimethyl sulfoxide (DMSO) from RDH.

Collection and preparation of plant material

The fresh fruit of *Lagenaria siceraria* was collected from the farms of Pattoki, District Kasur, Punjab (Pakistan). Fresh fruit of *L. siceraria* was cut manually to separate epicarp (peel) and mesocarp. A weighed amount of each part was ground to obtain a paste like material.[10] The ground material of each part was soaked in methanol for 15 days for extraction. The extract obtained on filtration was concentrated on a rotary evaporator (Buchi R-210). The dried methanolic extract was suspended in distilled water and extracted into solvents with increasing polarity. Consequently, hexane, ethyl acetate, *n*-butanolic and aqueous fractions were obtained.

Test organisms

To explore antimicrobial potential of extracts and fractions of *L. siceraria* fruit peel and mesocarp, four clinically important bacteria and one fungus were used. The Gram-negative bacteria were *Pseudomonas aeruginosa* ATCC9027 and *Escherichia coli* ATCC8739, while Gram-positive bacteria included *Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC6538. The fungal strain was *Candida albicans* ATCC10231.

Study of antimicrobial activity

Antimicrobial activities of methanolic extracts of peel and mesocarp of *Lagenaria siceraria* and their fractions in different solvents were determined in terms of zones of inhibition (ZOI) and minimum inhibitory concentrations (MIC).

Determination of zones of inhibition (ZOI)

Ability of plant samples to inhibit growth of test microorganisms was determined by agar well diffusion method.[11,12] Antibacterial and antifungal susceptibility test was performed by using Mueller-Hinton agar and potato dextrose agar. Each plant sample was prepared in 100% DMSO (dimethyl sulfoxide) with concentration of 40 mg/mL. The Mueller-Hinton agar and potato dextrose agar were melted and cooled to 48-50 °C. Standardized inoculum ($1.5x10^8$ CFU/mL), 0.5 McFarland (1.17% $BaCl_2.2H_2O$ + 1% H_2SO_4) of each microorganism was separately added to molten agar. The mixture was poured into a Petri plate and allowed to solidify. Wells were made in each plate with the help of

sterilized cork borer of 8.5 mm diameter. Fruit samples were poured into wells labelled accordingly. The plates were incubated for 24 h at 37 °C and 28 °C for bacteria and fungus, respectively. Diameters of the inhibition zones were recorded in mm. DMSO was used as a negative control, while Cefixime and Amoxil were used as positive control. Each experiment was repeated thrice and the average values were calculated for antibacterial and antifungal activity.

Determination of MIC values

The lowest concentration of a drug that is sufficient to inhibit the growth of a microorganism is called minimum inhibitory concentration or MIC. It provides us a measure of how effective a drug is. MICs of methanolic extracts of peel and mesocarp of *Lagenaria siceraria* fruit and their fractions were determined by agar dilution method according to a reported procedure.[13] Various dilutions of each fruit sample were prepared in MHA, which were into separate Petri plates and allowed to solidify. Microbial emulsions in sterile saline were then spotted onto the agar. A control plate contained MHA alone. After incubation for 24 h, the plates were observed to note the minimum concentration that was sufficient to inhibit the growth of a microorganism.

Data analysis

All determinations of zones of inhibition and MICs were carried out at least three times. Statistical analysis was performed using Microsoft Excel 2010, and the results were expressed as mean ± SEM.

Results

Zones of inhibition (ZOI)

Antimicrobial assay for methanolic extracts of peel and mesocarp of *Lagenaria siceraria* and their fractions were conducted by agar well diffusion method against four standard bacterial strains and one standard fungal strain. Zones of inhibition were measured in mm and results are displayed in Tables 1-3. Methanolic extracts of both peel and mesocarp showed highest activity against *P. aeruginosa* showing ZOI of 16.50±0.70 mm and 25.00±0.00 mm, respectively. Activity of peel was comparable to that of Amoxil (ZOI, 17.25±1.76 mm) while mesocarp was more potent. The extracts of peel and mesocarp were active against all bacterial strains with significant zone of inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus, Bacillus subtilis*. Ethyl acetate fraction of peel exhibited maximum antimicrobial activity against *P. aeruginosa* with ZOI of 21.00±0.00 mm. The aqueous extract of peel exhibited significant activity against *P. aeruginosa* (ZOI, 18.00±0.00 mm) and *B. subtilis* (ZOI, 16.50±0.70 mm), whereas aqueous extract of mesocarp exhibited significant activity against *B. subtilis* (ZOI, 16.00±0.00 mm). The *n*-butanolic extract of peel exhibited good activity against *P. aeruginosa* (ZOI, 16.75±0.35 mm) and *S. aureus*(ZOI, 15.50±0.70 mm), whereas *n*-butanolic fraction of mesocarp exhibited excellent activity against *P. aeruginosa* (ZOI, 21.00±0.00 mm) which was higher than that of standard antibiotic drug Amoxil. The hexane fractions of peel and mesocarp exhibited notable activity against *S. aureus*(ZOI, 17.00±0.00 mm) and *P. aeruginosa* (ZOI, 17.75±035 mm), respectively. All the plant samples, excluding *n*-butanolic fraction of mesocarp, exhibited good activity against the fungus *Candida albicans*, which was comparable to that of antibiotics Cefixime and Amoxil. Hexane fraction of peel and ethyl acetate fraction of mesocarp exhibited highest antifungal activity against *Candida albicans* with ZOI of 20.0 and 22.0 mm, respectively. They were more potent than Amoxil (ZOI, 19.6 mm).

Minimum inhibitory concentrations (MICs)

Minimum inhibitory concentrations of methanolic extracts of *Lagenaria siceraria* fruit peel and mesocarp and their fractions were determined and results are exhibited in Tables 4 and 5. MIC values ranged from 0.6 to 1.6 mg/mL. MICs of methanolic extracts of peel was 1.0 mg/mL against all microorganisms except *S. aureus* against which it was 1.4 mg/mL. MICs of mesocarp against all microbes were 1.0 mg/mL. Ethyl acetate fraction of peel was lethal to *P. aeruginosa* at MIC of 0.6 mg/mL. MIC of ethyl acetate and hexane fractions of mesocarp was 0.6 mg/mL against *B. subtilis*. Aqueous fraction of mesocarp showed MIC as 0.6 mg/mL against *P. aeruginosa.* Remarkably, *P. aeruginosa* was most susceptible among all the test microorganisms.

Discussion

Lagenaria siceraria is a fruit vegetable with numerous therapeutic properties. It is cultivated on large scale and is, therefore, easily available to common people. Antimicrobial remedies based on the plant can provide cost effective and safer

alternatives to the current antibiotics. The methanolic extract of mesocarp exhibited higher activity against all the test bacteria than that of peel, but lower activity against the fungus. The methanolic extracts of both epicarp and mesocarp of the fruit were especially effective against *P. aeruginosa*. The study can provide foundation for more specialized investigation exploring efficacy of the fruit of *Lagenaria siceraria* against infections caused by this microorganism such as those of skin, ear and eye. Since *P. aeruginosa* is known to particularly affect people with weak immune system, vegetable based medication should be especially beneficial for such patients. Mesocarp was more potent than epicarp against most of the test organisms. Ethyl acetate fraction of epicarp and *n*-butanolic fraction of mesocarp exhibited notably higher effectiveness than Amoxil against *P. aeruginosa*. In the study, Gram-negative bacteria proved to be more susceptible than Gram-positive bacteria, and *Pseudomonas aeruginosa* was most vulnerable. It is hypothesized that a paste of the fruit of *L. siceraria* should be effective against skin infections caused by *P. aeruginosa*.

While possibility of synergistic effect cannot be ruled out, phytochemical investigation may result in the isolation of individual compounds with exploitable antimicrobial potential.

Conclusion

This study revealed that peel and mesocarp of *Lagenaria siceraria* fruit possessed antimicrobial potential of varying degree against different microorganisms. The demonstration of wide-ranging antibacterial activity by *L. siceraria* fruit peel and mesocarp has great significance and suggest that consumption or application of this fruit may provide defense against different pathogens. While isolation of individual bioactive phytochemicals has its own importance in drug discovery, the synergistic effect of different natural products should not be ruled out.

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Table 1: Antimicrobial activity of methanolic extract of peel and methanolic extracts of mesocarp of *Lagenaria siceraria* fruit relative to two standard antibiotics in terms of zones of inhibition (mm) against common microorganisms (concentration of each of the drugs and fruit samples was 40 mg/mL).

Microorganisms	Antibiotic drugs		Fruit extracts	
	Cefixime	Amoxil	Methanolic	
			Peel	Mesocarp
Bacterial strains				
Pseudomonas aeruginosa(-)	31.00±0.00	17.25±1.76	16.50±0.70	25.00±0.00
Escherichia coli (-)	40.25±1.76	37.00±0.71	15.00±1.41	15.25±1.76
Bacillus subtilis(+)	38.00±0.00	43.25±1.60	15.25±0.35	17.00±0.00
Staphylococcus aureus (+)	38.00±0.00	43.25±1.60	13.00±0.00	18.00±0.00
Fungal strain				
Candida albicans	32.25±1.76	19.60±0.56	16.00±0.00	15.00±0.00

(+) Gram-positive; (-) Gram-negative

Table 2: Antimicrobial activity of different fractions of methanolic extract of *Lagenaria siceraria* peel in terms of zones of inhibition (mm) against common microorganisms (concentration of drugs and fruit samples was 40 mg/mL).

Microorganisms	Fractions in different solvents			
	Hexane	Ethyl acetate	n-Butanolic	Aqueous
Bacterial Strains				
P. aeruginosa	16.50±0.70	21.00±0.00	16.75±0.35	18.00±0.00
E. coli	14.00±0.00	13.50±0.70	12.75±0.35	14.00±0.00
B. subtilis	13.00±0.00	16.00±0.00	15.00±0.00	16.50±0.70
S. aureus	17.00±0.00	14.00±0.00	15.50±0.70	13.75±0.35
Fungal Strain				
C. albicans	20.00±0.00	16.00±0.00	-------	14.00±0.00

Table 3: Antimicrobial activity of different fractions of methanolic extract of *Lagenaria siceraria* mesocarp in terms of zones of inhibition (mm) against common microorganisms (concentration of drugs and fruit samples was 40 mg/mL).

Microorganisms	Fractions in different solvents			
	Hexane	Ethyl acetate	n-Butanolic	Aqueous
Bacterial Strains				
P. aeruginosa	17.75±0.35	18.00±0.00	21.00±0.00	14.00±0.00
E. coli	16.00±0.00	15.00±0.00	16.00±0.00	15.50±1.41
B. subtilis	15.00±0.00	17.75±0.35	-------	16.00±0.00
S. aureus	13.75±0.35	15.00±1.41	14.75±0.35	13.75±0.35
Fungal Strain				
C. albicans	20.00±0.00	22.00±0.00	-------	10.30±0.42

Table 4: MICs (mg/mL) of methanolic extract of *Lagenaria siceraria* peel and its fractions against different microorganisms.

Microorganism	Methanolic	Hexane	Ethyl acetate	n-Butanolic	Aqueous
Bacterial Strains					
P. aeruginosa	1.0	1.6	0.6	1.4	1.4
E. coli	1.0	1.6	1.6	1.6	1.6
B. subtilis	1.0	1.6	1.0	1.0	1.4
S. aureus	1.4	1.0	1.6	1.4	1.6
Fungal Strain					
C. albicans	1.0	0.6	1.0	2.0	1.6

Table 5: MICs (mg/mL) of methanolic extract of *Lagenaria siceraria* mesocarp and its fractions against different microorganisms.

Microorganism	Methanolic	Hexane	Ethyl acetate	n-Butanolic	Aqueous
Bacterial Strains					
P. aeruginosa	1.0	1.0	1.0	1.0	0.6
E. coli	1.0	1.0	1.0	1.6	1.0
B. subtilis	1.0	0.6	0.6	1.0	1.0
S. aureus	1.0	1.0	1.0	1.4	1.0
Fungal Strain					
C. albicans	1.0	0.6	0.6	1.0	0.6