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Research Article

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Assessment of antimicrobial efficacy of *Citrullus lanatus* methanolic seed extract

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ABSTRACT

The present study was carried out to investigate the antibacterial and antifungal potential of Citrullus lanatus seeds. Determination of antimicrobial activity of methanol extract of Citrullus lanatus seed was carried out against 10 bacterial species (Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Salmonella typhi, Enterococcus faecalis, Vibrio cholerae, Shigella dysenteriae, Proteus mirabilis, Pseudomonas aeruginosa) and 5 fungal species (Aspergillus flavus, Aspergillus niger, Penicillium notatum, Trichophyton mentagrophytes, Candida albicans). Inhibition zones formed by the extract were compared with the standards: Streptomycin and Amphotericin B. The methanolic seed extract at a concentration of 1000 µg/ml was effective against bacteria: Vibrio cholerae (7 mm), Proteus mirabilis (7 mm), Shigella dysenteriae (7 mm), Staphylococcus aureus (6 mm), Escherichia coli (6 mm), Enterococcus faecalis (6 mm), B. subtilis (6 mm), Salmonella typhi (5 mm) and fungi: Aspergillus niger (13 mm), Aspergillus flavus (12 mm), Penicillium notatum (11 mm), Candida albicans (9 mm). The results of Citrullus lanatus seed extract demonstrated antimicrobial activity against the organisms tested. Hence, this extract can be used to discover novel bioactive natural products that may lead to the development of new antimicrobial drug.

Keywords: Medicinal plant; Water melon; Disc diffusion; Inhibition zone; Phytocompounds

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INTRODUCTION

Medicinal plants constitute an important natural wealth and they provide primary health care services to more than 80% of the world population. People living in rural areas prefer using traditional medicines for the treatment of various diseases and disorders [1]. Therapeutic value of theses medicinal plants depend on the presence of one or more constituents possessing certain physiological and pharmacological activity. Thousands of species are reported to have medicinal value in texts and literature, and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times [2].

Antibiotics are one of the most important weapons in fighting bacterial, fungal and viral infections, and have greatly benefited the health-related quality of human life [3]. However, past three decades have seen a dramatic increase in microbial resistance to these antimicrobial agents [4]. Recently, multiple drug resistance has developed simultaneously due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of various infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reaction [5]. Such situation stimulates the development of new anti-microbial agents in order to treat infectious diseases in an effective manner [6]**.** At this juncture, drugs derived from natural sources play a significant role in prevention and treatment of several infectious diseases.

Citrullus lanatus (Family – Cucurbitaceae) is commonly called as the watermelon. The Ayurvedic Pharmacopoeia of India indicated the seeds to possess a high lipase activity comparable to that of wheat germ, in addition to high

lipoxygenase, urease and trypsin-inhibitory activities. Aqueous extract of the seeds also exhibit amylase inhibitory activity [7]. It also reported to have antioxidant [8], α-glucosidase inhibitory activity [9], analgesic and anti-inflammatory activity [10], anti-ulcerative activity [11] and antimicrobial activity [12]. The fruits have been proved to contain laxative [13], antioxidant [14] and anti-hyperlipidemic property [15].

In this study, we evaluated the antimicrobial activity of methanolic extract of C*itrullus lanatus* seed against few common pathogenic microorganisms by using agar disc diffusion method.

EXPERIMENTAL SECTION

2.1 Plant Material

Citrullus lanatus seeds were collected from Vellore, Tamilnadu. The plant was authenticated by Prof. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai and a voucher specimen was deposited in the herbarium at PARC with the reference number: PARC/2012/1195. The seeds were air-dried and made into a coarse powder using a blender and stored in air-tight containers for further use.

2.2 Extraction

The air-dried, coarsely-powdered *C. lanatus* seeds were subjected to hot soxhlet extraction with methanol for 24 h. The liquid extract was filtered and solvent was partially removed using rotary evaporator under reduced pressure. The remaining solvent was completely removed by evaporation in water bath. The residual extract, *C. lanatus* methanolic seed (CLS) extract was used for further assays.

2.3 Microorganisms

Microbial cultures were obtained from Royal Bioresearch Centre, Velachery, Chennai. The following 10 bacterial cultures: *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Salmonella typhi, Enterococcus faecalis, Vibrio cholerae, Shigella dysenteriae, Proteus mirabilis, Pseudomonas aeruginosa* and 5 fungal cultures: *Aspergillus flavus, Aspergillus niger, Penicillium notatum, Trichophyton mentagrophytes, Candida albicans* were used for the study.

2.4 Preparation of inoculum

Stock cultures were maintained at 4°C on nutrient agar slants. Active cultures for the experiment were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth and were incubated for 24 h at 37ºC.

2.5 Antibacterial activity

Antibacterial activity of CLS extract was determined by disc diffusion method [16]. The Mueller Hinton Agar medium (MHA) was weighed as 3.8 g and dissolved in 100 ml of distilled water and to this 1 g of agar was added. After sterilization, the media was poured into sterile petriplates and were allowed to solidify for 30 min. After solidification, the inoculums were spread on solid plates with sterile swab moistened with the bacterial suspension. Various concentration of the CLS extract was prepared (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and62.5 µg/ml) using 20 µl of 1% DMSO. A negative control: 20 µl of 1% DMSO and a positive control: 10 µg/ml of standard antibiotic streptomycin was added on the respective discs and placed on MHA plates. All the dishes were incubated at 37°C for 24 h. Then, the bacterial growth was determined by measuring the diameter of zone of inhibition formed.

2.6 Antifungal activity

Antifungal activity of CLS extract was determined by disc diffusion method [16]. The strains were cultured on Potato Dextrose Broth. Potato Dextrose Agar (PDA) medium was prepared by weighing 3.9 g of PDA and 1 g of agar dissolved in 100 ml distilled water. PDA medium was then poured into sterile petriplates and were allowed to solidify. The inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Different concentration of CLS extract was prepared (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml) with 20 µl of 1% DMSO. 20 µl of 1% DMSO and10 µg/ml of standard antibiotic amphotericin B were used as negative and positive controls respectively. All the dishes were incubated at room temperature for 24 h. Diameter of the inhibition zones formed around the discs was measured.

RESULTS AND DISCUSSION

Medicinal plants represent a rich source of antimicrobial agents. Antibacterial and antifungal properties of medicinal plants are being increasingly reported from different parts of the world. World Health Organization encourages the use of plant extract or their active constituents that are used as folk medicine in traditional therapies

[3]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal, and anti-inflammatory properties of plants [17]. Some of these observations have helped in identifying the active principle responsible for such activities and in developing drugs for the therapeutic use in human beings. Therefore, this study has been carried out to evaluate the antimicrobial activity of methanolic extract obtained from *Citrullus lanatus* seed against few bacterial and fungal strains. The results were compared with standard drugs.

The present study revealed CLS extract with remarkable bacterial and antifungal activity. Antibacterial activity exerted by CLS extract is summarized (Table 1). For each organism, the zone of inhibition formed was different depending on the concentration used. Large zones of inhibition were formed at a concentration 1000 µg/ml for *V. cholerae* (7 mm), *P. mirabilis* (7 mm), *S. dysenteriae* (7 mm), *S. aureus* (6 mm), *E. coli* (6 mm), *E. faecalis* (6 mm) and *S. typhi* (5 mm). *B. subtilis* (6 mm) were vulnerable even at low concentration of the extract, 62.5 µg/ml. CLS extract was however not effective in controlling *K. pneumoniae* and *P. aeruginosa.* Streptomycin, on the other hand in mild concentration was more effective than CLS as evidenced by much larger zones. This might be probably due to the fact that streptomycin is a pure compound while the extract being crude in nature.

The activity of the plant against both gram-positive and gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds, general metabolic toxins or pharmacological active metabolites like furostanol and spirostanol saponins [18], flavonoid glycosides [19], phytosterols and some amides [20] in the extract. CLS was efficient against almost all the tested bacterial strains except *K. pneumoniae* and *P. aeruginosa*. High activity was observed against *V. cholerae*, *P. mirabilis*, *S. dysentriae*, *S. aureus*, *E. coli*, *E. faecalis*, *S. typhi* and *B. subtilis.* This confirms that CLS can be utilized as a source of new antimicrobial compound against the aforementioned bacteria.

Table 1: Antibacterial activity of *Citrullus lanatus* seed methanol extract

S. No.	Microorganisms	Zone of Inhibition (mm)						
		Concentration (µg/ml)					1% DMSO	Streptomycin 10 µg/ml
		1000	500	250	125	62.5		
1	*S. aureus*	6	6	-	-	-	-	17
2	*K. pneumoniae*	-	-	-	-	-	-	15
3	*B. subtilis*	6	6	6	6	6	-	15
4	*E. coli*	6	6	-	-	-	-	9
5	*S. typhi*	5	5	-	-	-	-	16
6	*E. faecalis*	6	6	5	-	-	-	19
7	*S. dysenteriae*	7	-	-	-	-	-	10
8	*P. mirabilis*	7	7	-	-	-	-	11
9	*P. aeruginosa*	-	-	-	-	-	-	-
10	*V. cholerae*	7	7	-	-	-	-	18

Antifungal activity exhibited by CLS extract is tabulated (Table 2). CLS was very much effective against *A. niger* (13 mm) followed by *A. flavus* (12 mm), *P. notatum* (11 mm) and *C. albicans* (9 mm) at 1000 µg/ml concentration. However the extract did not prove useful against *T. mentagrophytes.* Surprisingly, the standard amphotericin B used in the study was ineffective against all the fungal strains, except with *C. albicans* showing 8 mm inhibitory zone. Hence, CLS extract even in its crude form is found to be more valuable as an antifungal agent than the antibiotic itself. The antifungal activity of CLS may be attributed to various phytochemicals detectable in the extract like saponins. The action mechanisms of saponins lie in damaging the membrane and causing leakage of cellular materials, ultimately leading to cell death [21]. CLS extract possessed strong antifungal activity and can be used as a better antifungal drug. Hence, the results of present investigation clearly indicate CLS property to inhibit the growth of microorganisms and thereby prevent infectious diseases.

Table 2: Antifungal activity of *Citrullus lanatus* seed methanol extract

S. No.	Microorganisms	Zone of Inhibition (mm)						
		Concentration (µg/ml)					1% DMSO	Amphotericin B 10 µg/ml
		1000	500	250	125	62.5		
1	*A. flavus*	12	10	-	-	-	-	-
2	*A. niger*	13	10	-	-	-	-	-
3	*P. notatum*	11	9	6	-	-	-	-
4	*T. mentagrophytes*	-	-	-	-	-	-	-
5	*C. albicans*	9	8	8	8	8	-	8

CONCLUSION

The present antimicrobial investigation of the methanolic extract of *C. lanatus* seed showed that the extract was effective against the tested microorganisms when compared to the available standard drugs. However, the extract had better antifungal activity than antibacterial. The study thus ascertains the value of this plant used as a traditional medicine, which could be of considerable interest to develop new herbal drugs. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antimicrobial activity.

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REFERENCES

[1] JN Ramalivhan; CL Obi; A Samie; BC Iweriebor; P Uaboi-Egbenni, JE Idiaghe; MN Momba. *Afr. J. Biotech.*, **2014**, 13(4), 616-625.
[2] J Parekh; D Jadeja; S Chanda. *Turk. J. Biol.*, **2005**, 29, 203-210.
[3] B Pankaj; Nariya; NR Bhalodia; VJ Shukla; RN Acharya. *Ayu.*, **2012**, 32(4), 585-588.
[4] I Chopra; J Hodgson; B Metcaif; G Poste. *JAMA.*, **1996**, 275, 401-403.
[5] J Davis. *Science*, **1994**, 264, 375-382.
[6] SJT Lachumy; Z Zuraini; S Sasidharan. *Res. J. Pharma. Biol. Chem. Sci.*, **2010**, 1(4), 391-398.
[7] CP Khare. Indian medicinal plants: an illustrated dictionary, Springer International, New Delhi, **2007**.
[8] J Sathya; FG Shoba. *Asian J. Plant Sci. Res.*, **2014**, 4(5), 35-40.
[9] J Sathya; M Parimala; FG Shoba. *J. Pharmacog. Phytochem.*, **2014**, 3(5), 12-14.
[10] P Madhavi; M Rao; K Vakati; H Rahman; MC Eswaraiah. *Int. Res. J. Pharm. App Sci.*, **2012**, 2(4), 104-108.
[11] A Bhardwaj; R Kumar; V Dabas; N Alam. *Int. J. Pharm. Pharma. Sci.*, **2012**, 4(5), 135-139.
[12] LEA Hassan. *J. Med. Plants Res.*, **2011**, 5(8), 1338-1344.
[13] S Sharma. *Pharmacol. Online*, **2011**, 2, 790-797.
[14] NS Gill. *Am. J. Pharm.*, **2011**, 30(3), 429-34.
[15] A Poduri; DL Rateri; SK Saha; S Saha; A Daugherty. *J. Nutr. Biochem.*, **2013**, 24(5), 882–886.
[16] AW Bauer; WM Kirby; JC Sherris; M Turck. *Am. J. Clin. Pathol.*, **1966**, 45(4), 493–496.
[17] B Mahesh; S Satish. *World J. Agric. Sci.*, **2008**, 4(S), 839-843.
[18] I Kostova; D Dinchev. *Phytochem. Rev.*, **2005**, 4(2-3), 111-137.
[19] NA Saleh; AA Ahmed; MF Abdalla. *Phytochem.*, **1982,** 21(8), 1995-2000.
[20] YX Xu; HS Chen; HQ Liang; ZB Gu; WY Lui; WN Leung; TJ Li. *Planta Med.*, **2000**, 66(6), 545-550.
[21] V Mshvildadze; A Favel; F Delmas; R Elias; R Faure; Q Decanosidze; E Kemertelidze; G Balansard. *Pharmazie.*, **2000,** 55(4), 325-326.