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ANTIMICROBIAL POTENTIAL AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF *CUCURBITA MAXIMA* SEEDS

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ABSTRACT

In the present study, aqueous extract of *Cucurbita maxima* seeds was evaluated for its antibacterial activity against five different bacterial strains viz. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcous faecalis* and *Pseudomonas aeruginosa*. The susceptibility of bacterial strains against the extract was determined using disc diffusion method. All of the bacterial strains were found to be significantly susceptible against the extract except *P. aeruginosa,* which was least susceptible showing Zone of Inhibition (ZOI) of 4 mm. However, maximum antibacterial activity of *C. maxima* seed extract was observed against *E. coli* and *E. faecalis* with ZOI of 7 mm. Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponins, proteins, carbohydrates and glycosides in the extract. The data suggests that *C. maxima* seed extract could be developed as a promising bactericidal agent for curing several bacterial infections.

KEYWORDS: ***Cucurbita maxima seeds, antimicrobial, phytochemical and bactericidal agent***

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INTRODUCTION

The most valuable nature's gift to mankind is a plethora of different varieties of herbs and plants. Each part of these plants has different aroma, colour and medicinal properties on the basis of which they have been used since ancient times as food, flavouring agent, dying agent and also as medicine to treat a number of ailments. It is well known that this variation in colour, aroma and medicinal properties of plants is due to different chemical constituents and elements present in them. It becomes important to mention here that the presence of different phytochemicals and phytoelements in different plants depends upon their vegetation. Contemporary research has revealed the potential of several herbs and plants as exemplary sources of drugs[1]. Medicinal plants besides being efficient therapeutic agents are also a great source of different chemical constituents which could be developed as potential drugs. These phytoconstituents could serve as novel leads and clues for modern drug designing[2]. Correlation between phytoconstituents and bioactivity of medicinal plants is therefore desirable in order to synthesize specific bioactive compounds for treating specific diseases[3]. Furthermore, the active components of herbal remedies have the added advantage of being combined with many other components that appear to be inactive. However, these complementary components make the plant safer and provide better efficacy due to synergy in comparison to its isolated and pure active components[4]. Recently, increased resistance of human pathogenic microorganisms against existing antibiotics has become a global concern[5]. Thus, screening of medicinal plant extracts and their products for antimicrobial activity is the need of the hour as it has been reported that higher plants represent a potential source of novel antibiotic prototypes[6]. Pumpkin originated from South America, are known as 'kaddu' in Hindi. It belongs to the family Cucurbitaceae. They are classified into different varieties viz. *C. maxima, Cucurbita pepo*, *Cucurbita moschata* and *Cucurbita mixta* according to their texture and shape of stem. The pumpkin flesh with bright orange colour indicates that the pumpkin are rich in carotenoids, having great nutritional and health protective promoting value and hence used as vegetable[7]. Even the pumpkin seeds can be dried and eaten raw or baked, which have valuable nutrient oils, proteins and vitamins. Pumpkin seeds have enjoyed a long history in traditional medicine for use as tenifuges, due to their ability to remove intestinal parasites from the body. Curcurbitin is a substance correlated with antiparasitic activity due to its reported L-tryptophan content[8,9]. The seeds have been suggested as a treatment for depression[10], while eating pumpkin seeds can help preventing the most common types of kidney stones[11,12] by both reducing the levels of substances that promote stone formation in the urine and increasing the levels of substances that inhibit stone formation. Hence, the seeds of the medicinal plant, *C. maxima* were selected for the present study and their aqueous extract was prepared for evaluating their antimicrobial efficacy against different bacterial strains. The results were also correlated with the presence of several phytochemicals using standard procedures.

MATERIALS AND METHODS

(i) *Chemicals*

All the chemicals used in phytochemical testing were of analytical grade and purchased from Merck (India) Ltd. Luria Bertani broth (Himedia), Luria Bertani Agar (Himedia) and sterile discs as well as ciprofloxacin discs (Himedia) were used in antibiotic sensitivity testing.

(ii) *Plant material*

The seeds of *C. maxima* plant were procured from the local market of Allahabad, UP, India and authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, UP, India. A voucher specimen has been submitted to the University herbarium. The seeds were washed well with water and dried in shade. The shade dried seeds were powdered and extracted with hot distilled water. The extract obtained was filtered, concentrated and lyophilized to get a powder. The dry powder so obtained was stored at -40^{o}C for further use.

(iii) *Bacterial strains, stocks and growth in vitro*

Bacterial strains of *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis* were clinical isolates obtained from the Department of Biotechnology, All India Institute of Medical Sciences, New Delhi, India and the microbiologist of the department confirmed the identity based on microscopic examination, Gram's character and biochemical test profile. Bacterial stocks were maintained and stored as 1 ml aliquots at –80^{o}C in Luria Bertani (LB) broth for the above bacterial strains supplemented with 0.1% Tween 80. Bacterial stocks were revived from –80ºC and grown in Luria Bertani (LB) broth containing 0.1% Tween 80. All cultures were grown at 37ºC in a shaker incubator (190-220 rpm) overnight.[13]

(iv) *Determination of Zone of Inhibition (ZOI)*

The freshly prepared inoculum of *S. aureus*, *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *E. faecalis* were swabbed all over the surface of the LB Agar plates using sterile cotton swab. Three sterile discs of 6 mm diameter were placed on the medium with the help of disc dispenser and were numbered properly. The aqueous seed extract solution was prepared of 100 µg/µl concentration and 10, 20 and 30 µl of this solution were poured on the discs with the help of sterilized micropipette to load 1, 2 and 3 mg/disc. Discs were left for some time till the extract diffused in them. The effects were compared with that of the standard antibiotic, ciprofloxacin loaded sterile disc at a concentration of 30 mcg/disc. Finally, samples were incubated with closed lids at 37°C for 24 hours and ZOI of each disc was measured in terms of the diameter of inhibition zone using ruler[13].

(v) *Preliminary phytochemical testing*

Phytochemical screening of the seeds of the selected medicinal plant, *C. maxima* was carried out using standard methods for identification of various phytochemical constituents[14,15]. Preliminary phytochemical testing of the extract was carried out as follows

Table 1
Phytochemical testing of C. maxima seed extract (CMSE).

Phytoconstituents		Tests	Observation
Tannins	***(Braymer's test)***	2ml extract + 2ml H_2O + 3 drops $FeCl_3$ (5%)	Formation of green colour
Flavonoids		1ml extract + 1ml $Pb(OAc)_4$ (10%)	Appearance of yellow colour
Terpenoids		2ml extract + 2ml $(CH_3CO)_2O$ + 3 drops conc. H_2SO_4	Deep red colouration
Saponins	***(Foam test)***	5ml extract + 5ml H_2O + heat	Froth appears
	(Emulsion test)	5ml extract + Olive oil (few drops)	Emulsion forms
Steroids	***(Salkowski's test)***	2ml extract + 2ml $CHCl_3$ + 2ml conc. H_2SO_4	Formation of reddish brown ring at the junction
Phlobatannins	***(Precipitate test)***	2ml extract + 2ml HCl (1%) + heat	Red precipitate
Carbohydrates	***(Molisch's test)***	2ml extract + 10ml H_2O + 2 drops Ethanolic α-naphthol (20%) + 2ml conc.H_2SO_4	Reddish violet ring at the junction
	(Fehling's test)	2ml extract + 1ml of Fehling's solution A and B + heat	Reddish colour precipitate
Glycosides	***(Molisch's test)***	2ml extract + 10ml H_2O + 2 drops Ethanolic α-naphthol (20%) + 2ml conc.H_2SO_4	Reddish violet ring at the junction
	(Salkowski's test)	2ml extract + 2ml $CHCl_3$ + 2ml conc. H_2SO_4	Formation of reddish brown ring at junction
	(Liebermann's test)	2ml extract + 2ml $CHCl_3$ + 2ml CH_3COOH	Violet to blue to green colouration
Coumarins		2ml extract + 3ml NaOH (10%)	Yellow colouration
Alkaloids	***(Hager's test)***	2ml extract + few drops of Hager's reagent	Yellow precipitate
	(Wagner's test)	2ml extract + 2 drops HCl (1.5%) + 3 drops Wagner's reagent	Brown precipitate
	(Mayer's test)	2ml ethanolic extract + 3 drops Mayer's regent	Reddish brown ring at junction white precipitate
Proteins	***(Xanthoproteic test)***	1ml extract + 1ml conc. H_2SO_4	White precipitate
	(Biuret test)	1ml extract + 5-6 drops w/v NaOH + 2 drops $CuSO_4$ (30%w/v)	Violet red precipitate
Emodins		2ml extract + 2ml NH_4OH + 3ml Benzene	Red colouration
Anthraquinones	***(Borntrager's test)***	3ml extract + 3ml Benzene + 5ml NH_3 (10%)	Pink, violet or red colouration
Anthocyanins		2ml extract + 2ml HCl (2N) + NH_3	Pinkish red to bluish violet colouration
Leucoanthocyanins		5ml extract + 5ml Isoamyl alcohol	Organic layer turns into red

RESULTS

Table 2 illustrates the antibacterial activity of *C. maxima* seed extract (CMSE) assessed against five different bacterial strains viz. *E. coli*, *S. aureus*, *K. pneumoniae*, *E. faecalis* and *P. aeruginosa* by disc diffusion method. Results reveal that the seeds of *C. maxima* were found to be active against all the five tested bacterial strains. Extent of effects of the extract was evaluated by measuring the Zone of Inhibition (ZOI). The method of ZOI was opted for the present study to get a more precise picture of effectiveness of different concentrations against various strains and hence results can be compared well. The results were also compared with the standard antibiotic 'Ciprofloxacin' of 30 mcg/disc concentration.

Table 2
Antibacterial activity of CMSE and standard,Ciprofloxacin.

Plant/standard used	Diameter of ZOI (Zone of Inhibition) in mm				
	E. coli	*S. aureus*	*K. pneumoniae*	*E. faecalis*	*P. aeruginosa*
CMSE (1 mg/disc)	7	5	5	5	4
CMSE (2 mg/disc)	6	5	5	6	5
CMSE (3 mg/disc)	6	6	6	7	5
Ciprofloxacin (30 mcg/disc)	20	20	19	15	25

All the three concentrations viz. 1, 2 and 3 mg/disc of CMSE exhibited significant activity against all the five bacterial strains. However, the highest activity at minimum concentration of 1 mg/disc was observed in case of *E. coli* with ZOI of 7 mm. Whereas, the same ZOI of 7 mm was observed in case of *E. faecalis* at maximum concentration of 3 mg/disc. Moreover, all the concentrations of 1, 2 and 3 mg/disc were least effective against *P. aeruginosa* having ZOI 4, 5 and 5 mm, respectively. Though, against which the standard drug, ciprofloxacin was most effective with ZOI of 25 mm against the same strain of *P. aeruginosa.* It is important to note that the CMSE was noted very effective against *S. aureus* and *K. pneumoniae* even at

maximum concentration. Table 3 represents the results of phytochemical screening of CMSE for different phytoconstituents using standard procedures. Results reveal the presence of tannins, saponins, alkaloids, carbohydrates, proteins and glycosides.

Table 3
Preliminary phytochemical analysis of CMSE.

Phytoconstituents tested	Results observed
Tannins	++
Saponins	++
Carbohydrates	++
Glycosides	++
Proteins	+
Alkaloids	++
Flavonoids	-
Terpenoids	-
Steroids	-
Phlobatannins	-
Coumarins	-
Anthraquinones	-
Anthocyanins	-
Leucoanthocyanins	-
Emodins	-

DISCUSSION

All the bacterial strains showed ZOI in a concentration dependent manner i.e. ZOI increases with increase in concentration of the extract except *E. coli*. It is interesting to note that *E. coli* showed highest ZOI of 7 mm at lowest concentration of 1 mg/disc. Whereas, *E. faecalis* showed the same ZOI of 7 mm at the highest concentration of 3 mg/disc of the extract. Though, these inhibitions of 7 mm against *E. coli and E. faecalis* are significant yet lower than the standard drug, ciprofloxacin. Moreover, in case of standard drug ciprofloxacin the highest ZOI was observed against *P. aeruginosa* and least ZOI was observed against *E. faecalis.* Whereas, CMSE showed highest ZOI against *E. coli* and *E. faecalis* and least ZOI against *P. aeruginosa.* Phytochemicals elicit chemotherapeutic or chemoprophylactic properties against an array of infectious enteric diseases. Furthermore, presence of specific phytoconstituents viz. tannins, saponins, alkaloids and their glycosides in *Cucurbita maxima* seed extract may thus be responsible for its antibacterial activities. Several mechanisms of antimicrobial action of phytochemicals have been suggested by different researchers, acknowledging that phytochemicals may act either by inhibiting microbial growth or by inducing cellular membrane perturbations or by interference with certain microbial metabolic processes, as well as modulation of signal transduction or gene expression pathways[16-18]. As tannins have been reported to exhibit antimicrobial potential which could be either due to the inhibition of enzyme activity by complexation with substrates of bacteria or due to the direct action of tannins on the microorganism metabolism, through the inhibition of oxidative phosphorylation or by decreasing the availability of essential ions to the bacterial metabolism by complexation of tannins with metabolic ions[19]. Moreover, tannic acid has been reported to show inhibitory activity against several bacterial strains. The inhibitory effect of tannic acid on the growth of bacteria may be caused by its strong iron binding capacity[20]. Moreover, saponins and their glycosides occurring widely in plants have been reported to possess antimicrobial property against different bacteria and the results were also compared favourably with the standard antibiotic, penicillin[21]. Saponins might act by altering the permeability of cell walls and hence exert toxicity on all organized tissues. They exert some antibacterial activity once combined with cell membranes to elicit changes in cell morphology leading to cell lysis[22]. Alkaloids are heterocyclic nitrogen compounds characterized by different antimicrobial activities. These compounds were found to show antibacterial activity in the alkaloid rich fractions of leaf, pod and flower of *Prosopis juliflora*. Piperidine alkaloids were found to be present in all the active fractions as shown by Data Analysis in Real Time-Mass Spectrometry (DART-MS). Presence of two groups of alkaloids showed one group with indolizidine ring in the centre of the molecule and the second group without indolizidine ring. Antibacterial activity of the first group of alkaloids had been reported by Ahmed et al. 1978[23], however Singh et al., 2011[24] showed that other group of alkaloids also had the potential to inhibit bacterial growth. Alkaloids have been found to accumulate in cells driven by the membrane potential. They are also excellent DNA intercalators active on several microorganisms including bacteria with a target on RNA polymerase, gyrase and topoisomerase IV as well as on nucleic acid[25,26]. Alkaloids have been acknowledged as drug scaffolds in modern antibacterial chemotherapy[27]. In terms of mechanism of action, it has been proposed that alkaloids inhibit the activity of bacterial cell growth enzymes. In addition to direct antibacterial and antibiotic-enhancing activities of alkaloids, they have also been reported to act as antibiotic prophylaxis in order to inhibit bacterial virulence[28]. Thus, evaluation of antibacterial activity of CMSE extract confirms that it could be developed as a novel chemotherapeutic as well as chemoprophylaxis agent of high value.

CONCLUSION

The aqueous extract of *C. maxima* seeds plays a vital role not only in curing the bacterial infections but also in preventing these infections. Hence, it could be explored

not only for the development of novel chemotherapeutic agent but also as novel chemoprophylactic agent, especially being alkaloid rich, against various bacterial infections. Since, the need of the hour is to develop efficient antibacterial agents to fight against the prevailing multi-drug resistant bacteria therefore, this discovery would be of great help in bringing out the products for commercialization with high chemoprophylactic value.

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