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Antibacterial activity of Cucurbita maxima Duchesne ex. Lam. (fam. Cucurbitaceae)

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Cucurbitaceae family is also known as squash family. In India, it is represented by 84 species out of a total of 750 species of the world. Plants of this family are mostly used as vegetables, a few yield delicious summer fruits and a few are medicinal. In fact members of Cucurbitaceae form a source of a variety of compounds like alkaloids, flovonoids, terpenoids, glycosides tannins and saponins. In view of the medicinal importance of this family, the present investigation was undertaken. *Cucurbita maxima* is a giant pumpkin and locally called as 'kaddu'. The present paper describes the findings of the antibacterial activity of this plant. The present study was conducted in the year 2016-17. The plant material was collected locally from Barwani and identified by the experts. The plants material was air-dried in shade for 60 days and powdered in a wearing blender using a mixer. The powder was stored at 4^0C for further analysis. The powdered material was extracted with aqueous and ethanol solvents using Soxhlet apparatus. Extraction with each solvent was carried out for 10 h. Both the solvent extracts were dissolved in 2 ml of ethanol and distilled water separately and solubility was tested. Sterile paper discs (Whatman No. 1) were dipped in the solvent extracts and aseptically placed on seeded plates. All the plates were incubated at 37^0C FOR 24 h. The organisms used were *Bacillus subtilis* (MTCC-441), *Escherichia coli* (MTCC-1687), *Pseudomonas areogenosa* (MTCC-1934), and *Staphylococcus aureus* (18 mm against 16 mm in aqueous extract) while all the rest three organisms selected for this study showed more inhibition zone in aqueous extract i.e. *E. coli* with 15 mm, *Bacillus subtilis* with 20 mm, and *Pseudomonas areogenosa* with 14 mm respectively. Thus from the present study it is inferred that *Cucurbita maxima* plant extracts of various solvents posses potential of folk medicinal properties. Further research work is in progress.

Keywords: *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas areogenosa* and *Staphylococcus aureus*.

Introduction:

Earlier, the antimicrobial properties of several compounds responsible for the colour of plant tissues have been clearly demonstrated. Anthocyanin pigments are present in almost all higher plants bring predominant in flower and fruits. Although these pigments are better known for their food coloring capabilities, but they are also inhibitory to certain bacteria. Other Phenolic compounds have been demonstrated to exhibit antimicrobial activites. Tannins and tannic acid are present in bark rinds and other structural tissues of plants and are known to possess antimicrobial activities (Trivadi, 2004). Naturally occurring antimicrobial compounds are present in plant leaves, stems, bark, roots, flowers and fruits. Information on the antimicrobial activity of plant substances particularly spices and herbs have been available to us for centuries (Beuchats, 1994). Compounds which are largely responsible for their antimicrobial activites include many simple and complex derivatives of phenols. They are volatile at room temperature and higher concentrations of these compounds is necessary for inhibiting growth or various metabolic activities in micro-organisms and their concentration often exceeds to those normally used in food (Trivedi, 2004). The antimicrobial effect of red and white wines should be proportional to the flavonoid, tannin content (Singleton and Esau, 1969). Extracts of blueberries, apples, strawberries red wines, grape juice, apple juice and tea were studied for their antiviral activities by Knowalchuk and Speirs (1976 a, b; 1978 a, b).

Material method

Sampling:

Fresh samples of the selected plants were collected in May, 2016. The entire plants of *Cucurbita maxima* were collected from the agricultural land of Nandgoan village while for another selected plant *Momordica dioica*, the samples were collected from the hilly area of Pati village both the places belonging to Barwani district of Madhya Pradesh, India.

Identification of Plant material:

The taxonomic identities of both *Cucurbita maxima* and *Momordica dioica* species were confirmed by Dr. S. K. Mahajan, Formerly Professor of Botany, Govt .P. G .College, Khargone (M.P.).

Preparation of plant material:

The entire plants of *Cucurbita maxima* and *Momordica dioica* were collected and washed under running tap water thoroughly and shade dried for 15 days. The shade dried materials of these plants were then powdered separately with the help of electric grinder. Afterwards the powdered samples were wrapped in papers and packed in air tight containers in order to avoid moisture and contamination.

Extraction process:

The extraction of dried plant materials of both the selected plants *Cucurbita maxima* and *Momordica dioica* was carried out separately by soxhlation method. A quantity of 15 gm of powdered sample was extracted in soxhlet apparatus with two solvents water and ethanol for 24h and 36h respectively. The temperature of water was kept at 70^0C ±5 and that of ethanol at 50^0C ±5.

Antimicrobial susceptibility test

The antimicrobial activity of the selected alcoholic ethanolic and aqueous extract was determined by disk diffusion method (Bauer *et al* 1966).Liquid Nutrient Agar media were used in antibiotic sensitivity testing. Various petriplates were sterilized by autoclaving at 121^0C for 15 minutes at 15 Psi pressure and was then used for sensitivity tests under septic condition in the laminar air flow (LAF) prepared NA (nutrient medium) medium was poured into each petriplates to obtain a uniform depth of 3mm and was allowed to solidify. After solidification, the entire agar surface was streaked with cotton with *Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Psuedomonas oreuginosa* strains. After swabbing was done between each Whatmann's filter paper no.1 was cut into small disk of 6 mm diameter by using punching machine and were autoclaved. The autoclaved disc was then dipped into for different concentration of each aqueous and ethanolic extracts of both the plants. The discs were transferred to the petriplates using flame sterilized forceps. Each disc was gently pressed to the agar inoculums surface; the plates were then incubated in an incubator at 37^0C$^{\pm}$ for 24 hour. After incubation period the zone of inhibition was measured by using diameter measurement scale.

Result:

The aqueous extract of *Momordica dioica* showed different zones of inhibition against *Staphylococcus aureus* (14 mm) *Pseudomonas aeruginosa* (15 mm) *Escherichia coli* (14 mm) and *Bacillus subtilis* (16 mm) (Table-1 Plate 1 and Fig. 1). When the ethanolic extract of *Momordica dioica* was studied, then it also showed different zones of inhibition against *Staphylococcus aureus* (16 mm) *Pseudomonas aeruginosa* (15 mm) *Escherichia coli* (15 mm) and *Bacillus subtilis* (16 mm) **(**Table-1).

Table 1: Observation of antimicrobial susceptibility of *Cucurbita maxima* (in aqueous/ethanolic) against bacteria

S. N.	Bacteria	*C. maxima (aq.)*		*C. maxima (eth.)*	
		Inhibition	Zone size (mm)	Inhibition	Zone size (mm)
1	*S. aureus*	+ve	16	+ve	18
2	*B. subtelis*	+ve	20	+ve	15
3	*E. coli*	+ve	15	+ve	13
4	*P. aeruginosa*	+ve	14	+ve	12

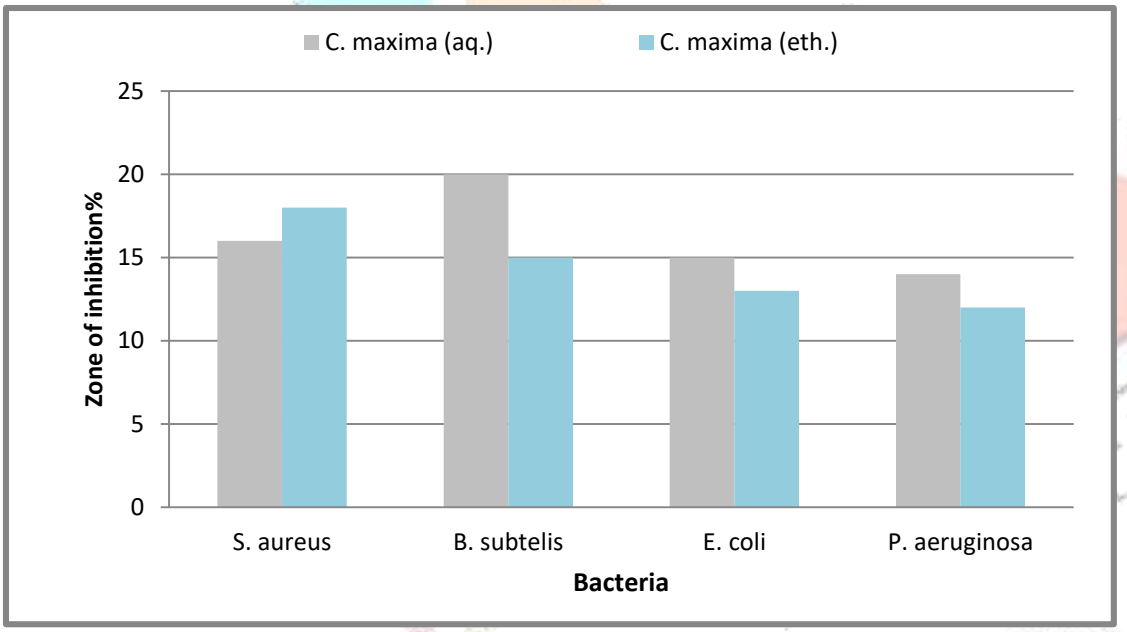
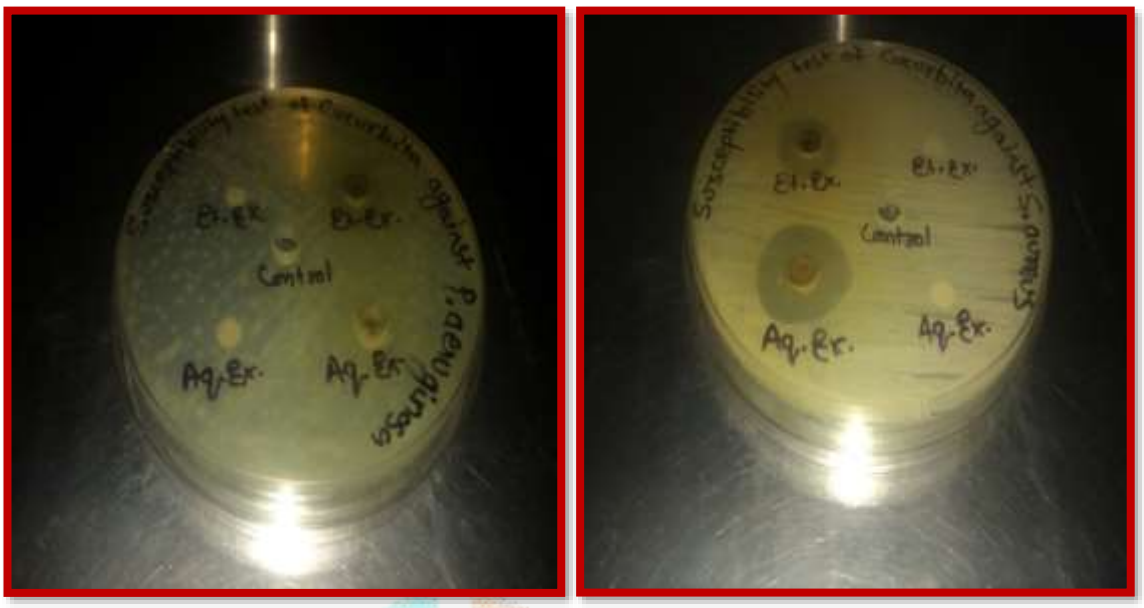


Fig. 1: Antimicrobial susceptibility test of *C. maxima* aqueous extract and ethanolic extract

PLATE- 1



i. Susceptibility of *Cucurbita maxima* in ethanolic and aqueous extracts against *P. aeruginosa* and *S. aureus*

ii. Susceptibility of *Cucurbita maxima* in ethanolic and aqueous extract against *E.coli* and *B.subtilis*

Conclusion:

The study revealed that the ethanolic extract showed more inhibition zone in case of *Staphylococus aureus* (18 mm against 16 mm in aqueous extract) while all the rest three organisms selected for this study showed more inhibition zone in aqueous extract i.e. *E. coli* with 15 mm, *Bacillus subtilis* with 20 mm, and *Pseudomonas areugenosa* with 14 mm inhibition zone.

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