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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIBACTERIAL ACTIVITY OF CUCURBITA MAXIMA SEED EXTRACT

K. Ravishankar*, GVN. Kiranmayi, GV. Appa Reddy, VVL. Sowjanya, V. Baba Sainadh, VG. Lakshmi Durga, V. Siva Prasad, PV. Swaminaidu and T. Prasad

Sri Sai Aditya Institute of Pharmaceutical Sciences and Research, ADB Road, Surampalem, East Godavari District, Andhra Pradesh, India.

ABSTRACT

The objective of present work is to study medicinally active substances present in ethanolic -extract obtained from seeds of *Cucurbita maxima*. Preliminary Phytochemical screening of the extracts revealed the presence of Carbohydrates, Steroids, Proteins and amino acids. The presence of these bioactive constituents is associated with the antimicrobial activity of the plant. The seed extract of *Cucurbita maxima* solvented by ethanol, showed the spectrum of inhibition on *Staphylococcus aureus, Bacillus subtilis, Staphylococcus werneri, Pseudomonas putida, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Escherichia coli* by Cylinder plate method. The observations revealed significant zone of inhibition and supports to antibacterial activity.

Keywords: Cucurbita maxima, Phytochemical, Antibacterial activity.

INTRODUCTION

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components1. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world[2-5]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine[6]. In the present study, medicinal plant *Cucurbita maxima* belonging to the family **Cucurbitaceae** was selected to assess the antibacterial activity.

Cucurbita maxima seeds have many health benefits, as they are a good source of protein, zinc, and other vitamins, and they are even said to lower cholesterol[7]. *Cucurbita maxima* has been claimed to combat benign prostatic hyperplasia[8] . *Cucurbita maxima* seed oil contains essential fatty acids that help

maintain healthy blood vessels, nerves and tissues[9] . The medicinal properties of *Cucurbita maxim* include anti-diabetic, antioxidant, anti-carcinogenic, and anti-inflammatory[10]. The present study is aimed to demonstrate and determine the antibacterial effect of *Cucurbita maxima* seeds on various strains of bacteria.

MATERIALS AND METHODS

Plant material

Fresh plant seeds were collected from Aditya gardens situated in surampalem village, East Godavari district, Andhra Pradesh , and the plant was identified by Botanist Dr.T.U. Ragharam. The seeds obtained from the plant was dried in shade and then pulverized into powder.

Preparation of crude ethanol extract

The seed powder was repeatedly macerated with 95% ethanol in a percolator. The combined filtrate was evaporated to dryness under reduced pressure at 40–50°C. The resulting crude ethanol extract was then stored at 10–15°C.

Test Organism Used

The various organisms like *Staphylococcus aureus ATCCBAA 1026, Bacillus subtilis ATCC 11774, Staphylococcus werneri ATCC 27836, Pseudomonas putida ATCC 700007, Pseudomonas aeruginosa ATCC 10662, Proteus mirabilis ATCC 14153 , Escherichia coli ATCC 10536 , Kleibsella pneumonia ATCC 33495* are procured from Microbes Speciality Lab Danavaipeta , Rajahmundry , East Godavari District 533103 , Andhra Pradesh , India .

Antimicrobial Agent

The reference standard **Amikacin** was procured from Pradeep Organics and chemicals Pvt. Ltd, Hyderabad

Phytochemical screening

The powdered seeds were evaluated for qualitative determination of major phytoconstituents *i.e.* Alkaloids , Carbohydrates ,Glycosides, Phenolic compounds , tannins , Saponins, Steroids, Flavonoids, proteins and aminoacids ; which were further confirmed by thin layer chromatography.

Qualitative screening

Alkaloid detection was carried out by extracting 1 g powdered sample with 5 ml methanol and 5 ml of 2N HCl; and then treating the filtrate with Mayer's and Wagner's reagents. The samples were scored positive on the basis of Reddish brown or cream precipitation. Flavonoids were tested by heating 1 g powdered sample with 10 ml ethyl acetate over a steam bath (40–50°C) for 5 min; filtrate was treated with 1 ml dilute ammonia. A yellow coloration demonstrated positive test for Flavonoids. Saponins content was determined by boiling 1 g powdered sample in 10 ml distilled water for 15 min and after cooling, the extract was shaken vigorously to observe froth formation. Cardiac glycosides were identified by Borntrager's test**.** Ammonical layer turning to pink was indicative of cardenolides/cardiac glycosides[11]

Thin layer chromatography (TLC)

TLC plates were prepared by using silica gel G for TLC, were left overnight for air drying. These plates were activated by hot air oven at 100ºc for 1hr.Cold alcoholic extract was plotted on TLC plates[12]. The plates were dried and developed in suitable solvents for rapid screening. Pure ethyl acetate, 50% chloroform/ methanol, 1:1 ethyl acetate/methanol. The plates were run in the above solvent systems and dried at room temperature. Derivatisation of TLC plates was done by spraying 10% H_2SO_4 in methanol.

Different bands were observed and corresponding Rf values are determined. R_f value of each spot was calculated as

R_f = Distance travelled by the solute / Distance travelled by the solvent.

Antibacterial Assay

Seed extracts of *Cucurbita maxima* was evaluated for antibacterial activity against several gram positive and gram negative organisms.

The antibacterial activity of ethanolic seed extract was performed using Agar cup-plate method. 20ml of sterile nutrient agar medium was poured into sterile Petri-dishes and allowed to solidify. The Petri dishes were incubated at 37^o C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile top agar (4 ml) contained 1 ml culture. Bores were made on the medium using sterile borer. Dried ethanolic extract of seeds of *Cucurbita maxima* was

dissolved in water to obtained different concentration (100 , 200 mg/ml) and sterilized by filtration through a Whattman filter paper no. 1, and 0.05 ml of the different concentrations of extract were added to the respective bores. 0.05ml of Amikacin at a concentration of (25 µg/ml) was taken as standard reference. All the plates were kept in a refrigerator at 2 to 8 ^{o} c for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37ºc for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening

Phytochemical screening of the extracts of *Cucurbita maxima* revealed the presence of Carbohydrates, Steroids, and proteins and amino acids (table1).

Table 1: Preliminary Phytochemical analysis of *Cucurbita maxima seed* extract

Components	*Cucurbita maxima* {ethanolic seed extract}
Alkaloids	-
Carbohydrates	+
Glycosides	-
Phenolic compounds and tannins	-
Proteins and amino acids	-
Saponins	+
Steroids	-
Flavonoids	+

Thin layer chromatography

The presence of phytoconstituents was further confirmed by thin layer chromatography and their R_f values have been presented as 0.7 (Figure 1). The components were best resolved in screening system using pure ethyl acetate, 50% chloroform/ methanol, 1:1 ethyl acetate/methanol.

Pink colour indicates presence of Proteins and Aminoacids on ninhydrin spray

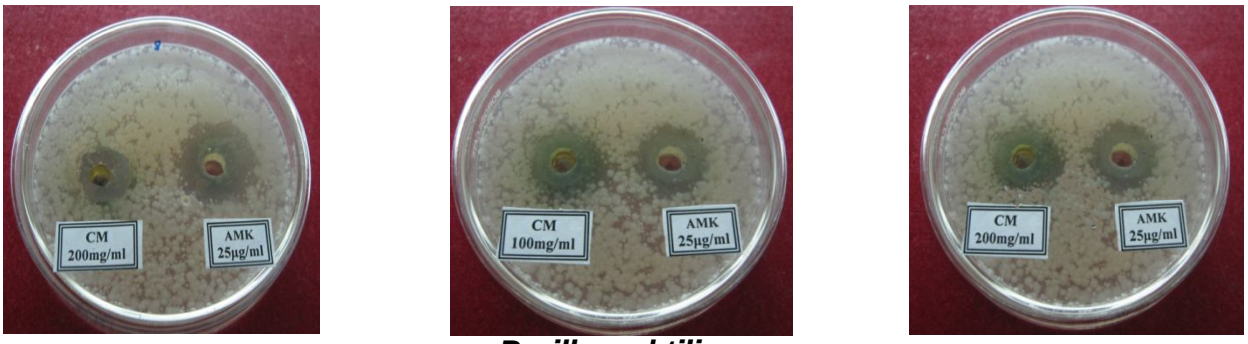
Fig. 1: TLC of *Cucurbita maxima*

Antibacterial Activity

The seed extract of *Cucurbita maxima* was studied for antibacterial activity employing standard cylinder method. Microbes used were *Staphylococcus aureus, Bacillus subtilis, Staphylococcus werneri, Pseudomonas putida, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia* and *Escherichia coli.* The zone of inhibition recorded for various organisms was determined ,*Klebsiella pneumonia* (20mm) ,*Proteus mirabilis* (19mm), *Staphylococcus aureus* (16mm), *Pseudomonas putida* (16mm), *Pseudomonas* aeruginosa (16mm), *Escherichia coli* (16mm) , *Staphylococcus werneri* (15mm) , *Bacillus subtilis (14mm).* Activity of ethanolic extract of the plant seed was compared with standard Amikacin (25µg). Cucurbita maxima seed extract exhibited good antimicrobial activity were tabulated along with figures (table 2; figure 2 ,3,4) .

Table: 2 Antibacterial activity of *Cucurbita maxima* seed extract

Microorganism	Zone of inhibition(mm) 100mg/ml	200mg/ml	Amikacin 25µg/ml
Gram positive			
Staphylococcus aureus	11 ± 1.02	16 ± 2.36	19 ± 1.39
Bacillus subtilis	9 ± 2.09	14 ± 3.96	18 ± 1.52
Staphylococcus werneri	9 ± 3.68	15 ± 2.87	19 ± 1.23
Gram negative			
Pseudomonas putida	14 ± 5.20	16 ± 2.87	14 ± 2.31
Pseudomonas aeruginosa	13 ± 4.02	16 ± 2.39	13 ± 3.21
Proteus mirabilis	18 ± 3.06	19 ± 1.26	22 ± 5.63
Klebsiella pneumonia	12 ± 2.80	20 ± 1.29	15 ± 1.28
Escherichia coli	12 ± 2.29	16 ± 1.68	14 ± 1.36



Proteus mirabilis ***Bacillus subtilis*** ***Staphylococcus werneri***

Fig. 2: Zone Of Inhibition of *Cucurbita maxima*

Pseudomonas aeruginosa **Standard Amikacin**

Fig. 3: Inhibition Zone Of *Cucurbita maxima* seed Extract *Against* Gram Positive Organisms

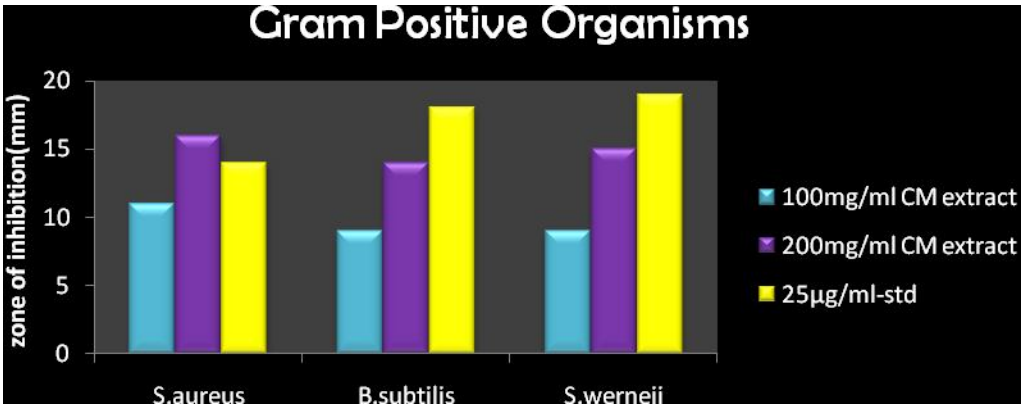
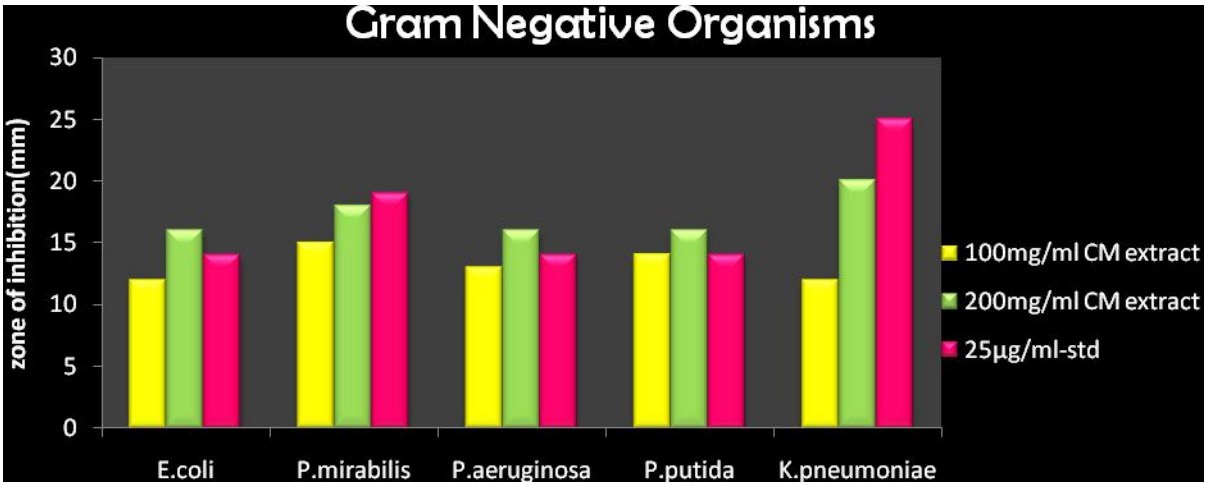


Fig. 4: Inhibition Zone Of *Cucurbita maxima* seed Extract against Gram negative Organisms



CONCLUSION

The scientific paper establishes that Cucurbita maxima seed extract has good significant antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Staphylococcus werneri, Pseudomonas putida, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia and Escherichia coli .The antibacterial activity of seed extract with different concentrations 100 and 200mg/ml was very well compared with standard reference drug Amikacin 25 µg/ml . From this study it can be concluded that Cucurbita maxima seed possessed antibacterial activity. We believe that the present investigation supports the antibacterial properties of Cucurbita maxima seed extract. The study explains the rationale use of the plant in treating various infections.

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