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Antimicrobial activity of *Cucurbita maxima* flowers (Pumpkin)

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Abstract
The present study was conducted to investigate the antimicrobial activities of Cucurbita maxima medicinal plant. The compound isolated from ethyl acetate fractions of C. maxima flowers extract has a significant antibacterial activity against bacteria and fungi. For bacterial strains such as S. typhi, E. coli, E. faecalis and B. cereus and two fungal strains such as C. lunata and C.albicans were tested by using disc diffusion method. The anti-bacterial activity of the compound isolated from ethyl acetate fraction is almost comparable with the standard Chloramphenicol and the anti-fungal activity of the compound isolated from ethyl acetate fraction is almost comparable with the standard Fluconazole. The drugs from Cucurbita maxima flowers destroy the growth of bacteria and fungi. It has also the ability to prevent or treat bacterial and fungal infections. The present research aims to compile medicinal values of Cucurbita maxima generated through the research activity using modern scientific approaches and innovative scientific tools.

Keywords: Cucurbita maxima flowers, Antibacterial activity, Antifungal activity, Diffusion method, Chloramphenicol, Fluconazole etc.

Introduction
"Let food be your medicine and medicine be your food" is an incentive Hippocrates launched more than 2400 years ago (Hakim, 1988) [6], gaining more and more followers nowadays, as we become more aware about the benefits of a healthy living. Medicinal plants are those ones with medicinal properties, i.e. those ones that can be directly or indirectly used for medical purposes; the background principle of this approach is that these plants contain certain biologically active substances that influence the metabolic processes of humans. Considering it's proved health effects, Cucurbita maxima Duch. (pumpkin) can also be included among the other medicinal plants. Pumpkin is known to possess pharmacological activities like antitumor (Hartwell, 1967; Saha *et al*., 2011) [7, 12] Nutritional and health protective value of pumpkin draws considerable attention of food scientists in recent years (Fokou *et al*., 2004) [5]. Pumpkin belongs to the family Cucurbitaceae which is an angiosperm, genus Cucurbita with different varieties (Alfawaz, 2004) [1].
Pumpkin is gourd-like squash belongs to genus Cucurbita and the family Cucurbitaceae. It normally belongs to the species Cucurbita pepo, Cucurbitamixta, Cucurbita maxima, and Cucurbita moschata and its native is North America. They naturally have a thick, orange or yellow shell. Pumpkins are broadly grown for commercial use, and are used both in food and recreation. In India, it is a most consuming vegetables. Pumpkins are considered to be a fruit and it contains 90 percent water. Pumpkins have antioxidant beta-carotene, which help to improve the immune function and can reduce the risk of diseases like heart disease and cancer. In Australia 'pumpkin' is generally called as winter squash. The term pumpkin derived from the Greek word pepon that means large melon". Pumpkins are a squash like fruit that range in between 9-18 lbs (4-8 kg) to 75 lbs (34 kg). Pumpkin stems are rigid, spiky and angular than squash stems, which are generally softer, more rounded where joined to the fruit. Pumpkins are generally orange or yellow, some pumpkin fruits are dark green, pale green, orange-yellow, white and gray. It is monoecious plant and it has both male and female flowers on the same plant. Pepita is the term used in Spanish to mean for the pumpkin seed. The seeds are characteristically flat and asymmetrically oval, and light green in color [8, 4, 11].
Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves.

Therefore, it is useful to investigate the potential of local plants against these disabling diseases (Amaral *et al*., 2006; Koko *et al*., 2008) [2, 9]. The plant has been used traditionally in many countries suchas China, India, Yugoslavia, Brazil and America as antidiabetic, antitumor, antihypertensive, antiinflammatory, immunomodulatory and anti-bacterial agents (Saha *et al*., 2011; Caili *et al*., 2006) [13, 14, 3].

Vegetables are edible plants or parts of plants which can be eaten cooked or raw without any serious processing. They nourish the body and also serve as good sources of vitamins and minerals to the body. Vegetables contain essential food substances which include carbohydrates, proteins, minerals, oil and vitamins. These vegetables are important commodities for poor households because their prices are relatively affordable compared with other food items (Okon and James, 2014) [10]. The aim of this study was to elucidate the antibacterial and anti-fungal activity of Cucurbita maxima using the compound isolated from ethyl acetate fraction.

Materials and Method

Extraction and fractionation

Fresh flowers (1 kg) of Cucurbita maxima were collected at O. Koothur village, Ariyalur district, during the month of August and identified by Dr. John Britto, Director, Rabinat Herbarium and Center for Molecular Systematics, St. Joseph's College (Campus), Tiruchirappalli, Tamilnadu. India. The flowers were extracted with 90% ethanol (5x500 ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80 ^{0}C) (6x250 ml), Peroxide free diethyl ether (4x250 ml) and ethyl acetate (8x250 ml). Petroleum ether fraction and diethyl ether fraction did not yield any isolable material. The compound isolated from ethyl acetate fraction was taken for screening anti-microbial activities.

Antimicrobial Procedure

Screening of Antibacterial Activity

Bacteria tested

Four bacterial strains such as S. typhi, E. coli, E. faecalis and B. cereus were used throughout this investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of inoculums

Stock cultures were maintained at 4 ºC on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) that was incubated without agitation for 24 hrs at 37 ºC. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to $2.0x10^6$ colony forming units (CFU/ml).

Antibacterial susceptibility test

The disc diffusion method was used to screen the antibacterial activity. In-vitro antibacterial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The test samples of concentration 10 mg/ml, 20 mg/ml, 30 mg/ml, 40mg/ml were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37 ºC for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Standard antibiotic Chloramphenicol of concentration 1mg/ml was used as positive control.

Screening of Antifungal Activity

Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 105 CFU/ml.

Determination of antifungal activity

The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with the test sample. Standard antibiotic (Fluconazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37 ºC for 72 h. The diameters of zone of inhibition observed were measured.

Results and Discussion

In the present study, isolated ethyl acetate fraction of Cucurbita maxima flowers exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in Table I that the sample possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 10 mg/ml as 5 mm, 5 mm, 0 mm and 0 mm, for 20 mg/ml as 12 mm, 13 mm, 11 mm and 13 mm, for 30 mg/ml showing 20 mm, 16 mm, 18 mm and 23 mm and for 40 mg/ml as 29 mm, 25 mm, 28 mm and 30 mm, against S. typhi, E. coli, E. faecalis and B. cereus respectively when compared with standard drug Chloramphenicol showing 17 mm, 18 mm, 23 mm and 21 mm zone of inhibition respectively. Then it is evident from the data presented in Table II that the sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 10 mg/ml as 0 mm and 0 mm, for 20 mg/ml as 16 mm and 13 mm, for 30 mg/ml as 19 mm and 20 mm and for 40 mg/ml as 22 mm and 30 mm against C. lunata, and C.albicans respectively when compared with standard drug Fluconazole showing 21 mm and 19 mm of inhibition respectively. The above result shows that the activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers shows significant antibacterial and antifungal activities.

Table 1: Anti-bacterial activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers in different strains

S. No.	Name Of Organisms	Zone of inhibition(mm)				
		Standard (Chloramphenicol)	Sample Concentration (mg/ml)			
			10	20	30	40
1.	S. typhi	17	5	12	20	29
2.	E. coli	18	5	13	16	25
3.	E. faecalis	23	0	11	18	28
4.	B. cereus	21	0	13	23	30

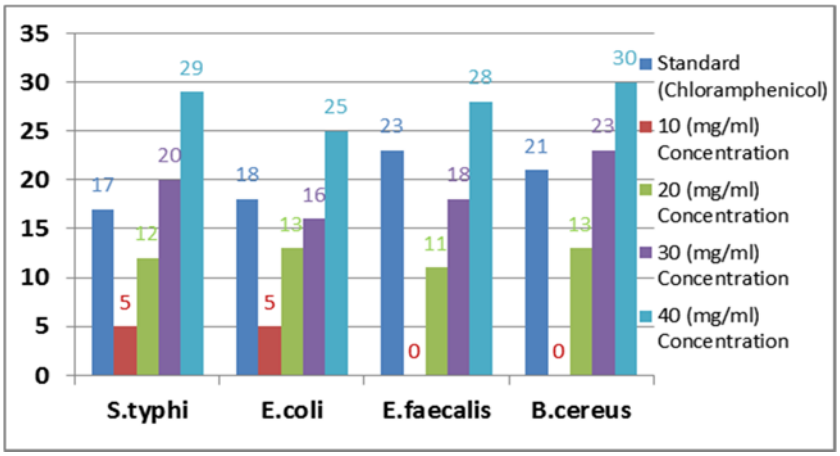

Fig 1: Graphical representation of anti-bacterial activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers. (Standard: Chloramphenicol, concentration 1 mg/ml)

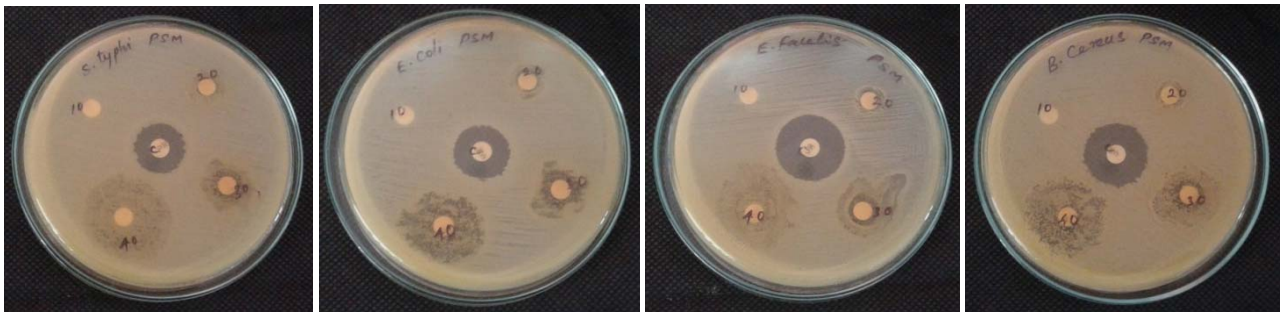

Fig 2: Inhibition of anti-bacterial growth of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers by disc diffusion method.

Table 2: Anti-fungal activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers in different strains

S. No.	Name Of Organisms	Zone of inhibition(mm)				
		Standard (Fluconazole)	Sample Concentration (mg/ml)			
			10	20	30	40
1.	C. lunata	21	0	16	19	22
2.	C. albicans	19	0	13	20	30

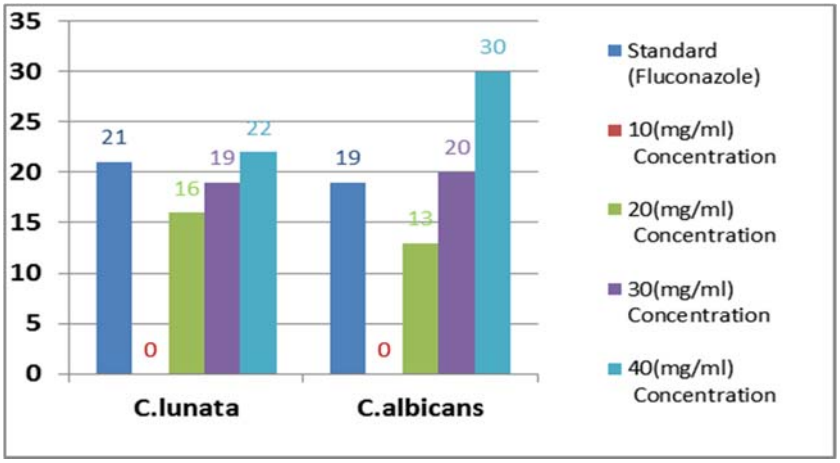

Fig 3: Graphical representations of anti-fungal activity of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers. (Standard: Fluconazole, concentration 1 mg/ml)

C. lunata **C. albicans**

Fig 4: Inhibition of anti-fungal growth of the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers by disc diffusion method.

Conclusion

Based on the result of the above study on the Cucurbita maxima we conclude that the compound isolated from ethyl acetate fraction of Cucurbita maxima flowers shows superior antibacterial and antifungal activity against the following microorganisms such as S. typhi, E. coli, E. faecalis, B. cereus and C. lunata, C. albicans. Also it justifies the claimed uses of flower parts of the Cucurbita maxima in the traditional system of medicine to treat various infectious disease caused by the microbes. Antimicrobial activities are aggravated by increasing the quantity of this compound, which can be used as an alternative for antibiotics. Therefore, it is necessary to characterization their active compounds and should be investigated for better understanding of its safety, efficacy and properties.

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