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Extraction, isolation and antimicrobial studies on fruit extract of *Benincasa hispida* (thunb) cong

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ABSTRACT

The *Benincasa hispida* fruit was extracted with solvent like methanol and water. The extracts were studied for both antibacterial and antifungal activity. The extracts did not show any antibacterial activity but it has remarkable antifungal activity against *Aspergillus niger* and *Candida albicans*. The methanolic extract was subjected to column chromatography, chloroform and ether fraction (40:60) produced crystals. The isolated crystals (Inositol) were confirmed through characterization such as IR, ^{1}H NMR and chemical test.

KEYWORDS

Benincasa hispida;
Antibacterial activity;
Antifungal activityl;
Carbohydrate;
Inositol.

INTRODUCTION

Benincasa hispida (Thump) cogn belong to Family: Cucurbitaceae, it is available throughout India. The fruit juice is used in folklore medicine and also recommended by ayurvedic text for respiratory diseases (cough and asthma), Gastro intestinal problems (dyspepsia, burning sensation, peptic ulcer, piles and constipation), Nervous disorders (insanity and epilepsy), heart disease, cataract, syphilis, sexual dysfuntion, vermifuge, diabetes, urinary disease (difficulty in urination and bladder stones), excessive thirst and antidote for alcohol and mercury poisoning[1-3]. In this study *Benincasa hispida* was extracted with methanol and water, the extracts were tested for antibacterial (*Bacillus cereus, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis* and *Salmonella typhi*) and antifungal activity (*Aspergillus niger* and *Candida albicans*) using cylinder plate method. The methanolic extract of *Benincasa hispida* was subjected to column chromatography in that one crystal was eluted in chloroform and ether. IR, ^{1}H NMR and chemical test confirmed the eluted crystal (inositol).

MATERIALS AND METHODS

The well-matured fruits of *Benincasa hispida* were brought from the local market in the month of July and August. The fruit extracts were prepared by maceration process. Fruits of *Benincasa hispida* were washed with water, peal and seeds were removed and the pulp was subjected to the process of maceration. The mashed pulp 1kg of each in two different glass containers was taken and extracted the pulps with

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methanol and water for one week with occasional shaking. On the eighth day the contents were filtered using a muslin cloth and the filtrates were distilled under reduced pressure. The percentage yield of extracts was calculated and the values are 3.8 and 3.5 respectively. The extracts were subjected to different chemical test[4] and antimicrobial screening such as antibacterial (*Bacillus cereus, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis* and *Salmonella typhi*) and antifungal activity (*Aspergillus niger* and *Candida albicans*) using cylinder plate method at the concentration of 100µg/ml. Amoxycillin (10µg/ml), Cefaclor (30µg/ml) and Ketoconazole (100µg/ml) were used as standard.

The methanolic extract was subjected to column chromatography. A suitable column of 2.5cm in diameter and 60cm in length was selected and thoroughly washed with water, dried and rinsed with acetone and then dried completely. Little pure cotton was placed in to the column with the help of a big glass rod up to the neck to avoid the leakage of smaller particles of the adsorbent. A piece of filter of suitable size was placed over the cotton. The column was packed with silica gel 100-200 mesh up to 1/3 of its length by pouring the silica gel into the petroleum ether solvent. The adsorbent was carefully poured through the funnel to prevent the formation of air bubbles in the column. The sides of the column were tapped slowly in order to facilitate the packing of the adsorbent. The prepared column was thoroughly washed with petroleum ether and the liquid level was always kept above the surface of the column to prevent the cracking of the column. A round filter paper of suitable size was placed above the packed column. About 2 gm of the concentrated methanolic extract was weighed and mixed with suitable quantity of silica gel (100-200mesh) to ensure the free flow of the extract along with the adsorbent. It was packed in the column through the funnel then petroleum ether was added through the column and kept aside without disturbance for overnight to settle the extract. The column was eluted with different organic solvents in the order of increasing polarity; they are light petroleum, cyclohexane, carbon tetrachloride, toluene, benzene, chloroform, diethyl ether and ethyl acetate. The crystals were eluted in the fraction of chloroform and ether (50:50, 40:60, 30:70, 20:80 and 10:90). The eluted crystals were examined for spectral studies such as I.R and ^{1}H NMR for the identification of chemical structure[5]. The eluted crystals were also confirmed through chemical tests.

TABLE 1: Antifungal activity of extracts

S.no	Test	Zone of inhibition(mm)	
		A.N	C.A
1	Methanolic extract 100µg/ml	24	22
2	Water extract 100µg/ml	20	21
3	Ketoconazole 100µg/ml	23	25

Antimicrobial activity

The antibacterial activity[6] of the extracts were tested against gram (+) bacteria (*Bacillus cereus* NCCS 2106, *Staphylococcus aureus* NCCS 2079 and *Staphylococcus epidermis*) and gram (-) bacteria (*Escherichia coli* NCCS2065, *Pseudomonas aeruginosa* NCCS2200 and *Salmonella typhi*) using nutrient agar medium and fungi (*Aspergillus niger* NCCS 1196 and *Candida albicans* NCCS 3471) using sabourand dextrose agar medium.

Paper disc diffusion method

The sterilized (autoclaved at 120^{0}C for 30min), liquefied medium (40-50°C) was inoculated (1ml/100ml of medium) with the suspension of the microrganism (matched to McFarland barium sulphate standard) and poured into the petridish to give a depth of 3-4mm. The paper discs impregnated with the extracts (100µg/ml and 250µg/ml for antibacterial & 100 for antifungal activity using dimethyl sulphoxide as solvent) were placed on the solidified medium. The plates were refrigerated (pre-incubated) for two hours at 4°C and then incubated at 37°C for 24h and 48h for antibacterial and antifungal activity respectively at the end of which the zone of inhibition was observed (TABLE 1). Amoxycillin (10µg/disc), cefaclor (30µg/disc) and Ketoconazole (100 µg/disc) were used as standards.

RESULTS AND DISCUSSION

Benincasa hispida fruit was extracted with methanol and water. The chemical test of methanolic and water extract shown that it contains carbohydrate, flavanoids, steroids, triterpinoids, tannins and proteins. Methanolic extract was subjected to column chromatography with

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different organic solvents, in this chloroform and ether fraction (50:50, 40:60, 30:70, 20:80 and 10:90) produced pure crystals. The eluted crystals were confirmed as carbohydrate through molisch test and conformed to U.V, I.R, and ^{1}H NMR for identification of chemical structure and also confirmed with authentic sample. The eluted crystal shown m.p -225-226ºC, UV λ_{max} (H_2O): 280nm, IR absorption at 3568 and 780, ^{1}H NMR ($CDCl_3$) δ: at 3.36(m, 6H; CH) and 2.09(m, 6H; -OH). The spectral data showed that the isolated crystal was carbohydrate (Inositol[7]). The methanolic and water extract of *Benincasa hispida* does not shown any antibacterial activity against the tested organisms at the concentration of 100µg/ml and 250µg/ml. Both the extracts showed good antifungal activity against *Aspergillus niger* and *Candida albicans* at the concentration of 100 µg/ml. In this methanolic extract showed potent antifungal activity against *Aspergillus niger* comparable to that of standard (ketoconazole).

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