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ORIGINAL ARTICLE

Qualitative Analysis of Metabolites and Bioactivity Determination of Different Solvent Extracts from Seeds and Leaves of *Benincasa hispida a*gainst Selected Microbes

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ABSTRACT
Benincasa hispida belongs to the family Cucurbitaceae and is used as traditional remedy of various illnesses since time immemorial. Our study was carried out to test the presence of various phytochemicals and to determine the antimicrobial activity of the seed and leaf extracts of Benincasa hispida. It showed the presence of various metabolites such as carbohydrate, reducing sugar, protein,amino acid, saponin, alkaloid, flavonoid, steroid, terpenoid, phenol, glycoside and tannin in different solvents. Antimicrobial activity was tested by agar well diffusion method against Staphylococcus aureus, Eschericia coli, Pseudomonas aeruginosa, Proteus mirabilis, Bacillus cereus and Aspergillus niger. The Ethanolic and Methanolic seed extracts of Benincasa hispida were found to be most effective against the tested microbes whereas Chloroform and Aqueous extracts were less effective. The maximum zone of inhibition was found in the Ethanolic seed extract against Bacillus cereus (20.66 ±0.057). Similarly the Ethanolic and Methanolic leaf extracts of Benincasa hispida were also very effective against all the baceterial and fungal strains tested for whereas Chloroform and Aqueous extracts were totally ineffective. The maximum zone of inhibition was found in the Methanolic leaf extract against Aspergillus niger (16.66 ± 0.115). Ampicillin and Cefotaxine were used as positive control and DMSO was used as negative control. The presence of wide range of phytochemicals and effective antimicrobial activity of the seed and leaf extract suggest that the plant can be effectively used for synthesis of new therapeutic agent. The present study provides scope for further research on the plant to isolate and identify the bioactive compounds.
***Keywords**: Phytochemical analysis, Antimicrobial activity, Benincasa hispida*

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INTRODUCTION

Plants are used as medicine in different countries for treating various diseases and are a source of many potent and powerful drugs [1]. According to WHO, a plant can be considered as medicinal if it contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs[2]. Due to the growing resistance of the microbes against the available antibiotics it has become very important to search for newer sources of antibiotics. Plant based antimicrobials are however cheaper and safer alternative sources of antimicrobials [3,4,5]. In view of this fact the present study was carried out to evaluate the phytochemicals and to determine bioactivity of different solvent extracts from seeds and leaves of *Benincasa hispida* against some selected microbes. *Benincasa hispida* belongs to the family cucurbitacae. It is considered to be the best vegetable fruit and medicine. It is used in various ailments like epilepsy, bleeding and insanity [6]. It is also reported to be useful in nervous disorder, acidity and ulcer [7 &8]. The plant is also used as anti diarrheal and for treating inflammation and pain [9& 10].

MATERIAL AND METHODS

Collection of Plant Sample

Fresh fruits and leaves of *Benincasa hispida* were collected from a local vegetable field of Dibrugarh, Assam. The seeds were separated from the fruit and the leaves along with the seeds were washed

properly and shade dried. Later on these were crushed into powder and the later were stored in air tight containers.

Extraction of bioactive compounds

Samples were macerated using different solvents (250 ml) such as Water, Methanol, Ethanol, Chloroform and Petroleum ether for about 48 hours and filtered through Whatman No 1 filter paper. The filtrate were concentrated by heating on a hot plate at about 30º 40ºC until a semidried, sticky mass was formed. The later were preserved at 4^0 C until use.

Phytochemical Analysis

The crude plant extracts were used for phytochemical analysis to identify the presence of primary as well as secondary metabolites such as carbohydrates, proteins, alkaloids, cholesterols, flavonoids, saponins, terpenoids, glycosides, tannins, phenols, following standard methods [11,12 &13].

Micro-organisms and standard antibiotics:

The antimicrobial activity was carried out against five bacterial strains and one fungal strain obtained from Microbial Type Culture Collection and Gene Bank (IMTECH, Chandigarh, India).The microbes used for antimicrobial study were *Staphylococcus aureus* (MTCC 87), *Escherichia coli* (MTCC 10312), *Pseudomonas aeruginosa* (MTCC-3542), *Proteus mirabilis* (MTCC-3310), *Bacillus cereus* (MTCC 1305) and *Aspergillus niger* (MTCC-9652). The bacterial strains were maintained on Nutrient Agar and fungi on Potato Dextrose Agar. The microbes were sub-cultured at an interval of one month. Standard antibiotics such as Ampicillin and Cefotaxime were used as positive control.

Screening for bioactivity

Agar well diffusion method was followed to test the antimicrobial activity of the crude extract [14]. About 25 ml of media was poured in sterile Petri dishes. The plates were allowed to solidify at room temperature. 0.1 ml of standardized inoculum was uniformly spread onto the surface of sterile agar plates. After drying, well of 5 mm diameter were made on the agar plates with the help of sterilized cork borer. The crude extract was dissolved in DMSO and about 100 µl of each extract were poured in the respective well and the plates were incubated for 24 hours at 37°C. The experiment was performed in triplicate Diameter of the zone of inhibition was measured in mm and expressed as Mean ± Standard Deviation. Ampicillin and Cefotaxime (60µg/ml) were used as positive control and DMSO was used as negative control.

RESULT AND DISCUSSION

Phytochemical analysis of seed of *Benincasa hispida* showed the presence of various phytochemicals (Table I and II). Carbohydrate was found to be present in all the solvent extracts of seed except ethanolic extract. Protein and alkaloids were present in ethanolic, methanolic and aqueous extract. Saponin was found only in aqueous extract, steroid in ethanolic extract and flavonoid in chloroform extract. Reducing sugar, phenol, tannin and tepenoid were absent in all the extracts. Glycoside was present in ethanolic and methanolic extract. In leaf extract- Carbohydrate, reducing sugar, protein and alkaloid were present in ethanolic, methanolic and aqueous extract but absent in chloroform and aqueous extract. Steroids were present in ethanolic and aqueous extract. Terpenoid present in methanolic and chloroform extract. Flavanoids were found only in chloroform and phenol in ethanolic extract. Glycosides were present in ethanolic and aqueous extract.

Table I: Qualitative phytochemical analysis of seed extract of *Benincasa hispida*

Sl. No.	Phytocompounds	Ethanolic Extract	Methanolic Extract	Chloroform Extract	Aqueous Extract
1	Carbohydrate	-	+	+	+
2	Reducing sugar	-	-	-	-
3	Saponin	-	-	-	+
4	Glycoside	+	+	-	-
5	Protein	+	+	-	+
6	Phenol	-	-	-	-
7	Steroid	+	-	-	-
8	Tannin	-	-	-	-
9	Terpenoid	-	-	-	-
10	Alkaloid	+	+	-	+
11	Flavanoid	-	-	+	-

Table II: Qualitative phytochemical analysis of leaf extract of *Benincasa hispida*

Sl. No.	Phytocompounds	Ethanolic Extract	Methanolic Extract	Chloroform Extract	Aqueous Extract
1	Carbohydrate	+	+	-	+
2	Reducing sugar	+	+	-	+
3	Saponin	-	-	+	+
4	Glycoside	+	-	-	+
5	Protein	+	+	-	+
6	Phenol	+	-	-	-
7	Steroid	+	-	-	+
8	Tannin	+	+	+	+
9	Terpenoid	-	+	+	-
10	Alkaloid	+	+	-	+
11	Flavanoid	-	-	+	-

'+' indicates the presence of chemical constituent '-' indicates the absence of chemical constituent.

The antimicrobial activity of the seed and leaf extract was tested against five bacterial strains and one fungal strain and the results are shown in table III and IV respectively. The Ethanolic and Methanolic seed extracts of *Benincasa hispida* were found to be most effective against the test the microbes whereas Chloroform and Aqueous extracts were less effective. The maximum zone of inhibition was found in the Ethanolic seed extract against *Bacillus cereus* (20.66 ±0.057). Similarly the Ethanolic and Methanolic leaf extracts of *Benincasa hispida* were also very effective against all the baceterial and fungal strains tested for. Chloroform and Aqueous extracts were totally ineffective. The maximum zone of inhibition was found in the Methanolic extract against *Aspergillus niger*(16.66 ± 0.115) [Fig I and Fig II].

Table III: Antimicrobial activity of seed extracts of *Benincasa hispida*

	Zone of inhibition in mm (Mean SD)						
Name of test organism	Ethanolic Extract	Methanolic Extract	Chloroform Extract	Aqueous Extract	Ampicillin (60µg/ml)	Cefotaxime (60 µg/ml)	Negative control
Bacillus cereus	20.66± 0.057	12.66±0.251	13±0.100	-	25±0.383	24± 0.231	-
Staphylococcus aureus	12.33±0.057	11±0.100	-	-	17±0.058	11±0.058	-
Escherichia coli	16±0.100	19±0.300	18.66±0.152	7.66±0.057	-	21±0.100	-
Pseudomonas aeruginosa	14.33±0.057	15±0.200	12±0.100	-	20±0.141	13±0.071	-
Proteus mirabilis	11±0.100	13 ±0.100	-	-	26±0.435	28±0.289	-
Aspergillus niger	13.33±0.152	14±0.100	-	6 ±0.100	-	28±0.354	-

Table IV: Antimicrobial activity of leaf extracts of *Benincasa hispida*

	Zone of inhibition in mm (Mean SD)						
Name of test organism	**Ethanolic Extract**	**Methanolic Extract**	**Chloroform Extract**	**Aqueous Extract**	**Ampicillin (60 g/ml)**	**Cefotaxime (60 g/ml)**	**Negative control**
Bacillus cereus	15±0.200	12.66±0.208	-	-	25±0.383	24± 0.231	-
Staphylococcus aureus	11.33±0.152	11.33±0.057	-	-	17±0.058	11±0.058	-
Escherichia coli	14.66±0.152	14.66±0.208	-	-	-	21±0.100	-
Pseudomonas aeruginosa	11.33±0.115	14±0.100	-	-	20±0.141	13±0.071	-
Proteus mirabilis	11.66±0.100	16±0.100	-	-	26±0.435	28±0.289	-
Aspergillus niger	10.33±0.057	16.66±0.115	-	-	-	28±0.354	-

Ampicillin and Cefotaxine were used as positive control and DMSO was used as negative control. Some extracts were inactive against some microbes. But it may not necessarily signify the absence of the bioactive active compounds. The compounds may not be completely dissolved in the respective solutions or may be present in lesser amount to show bioactivity. Phytochemicals are known to endow the plant with medicinal properties. Thus these phytochemicals which are found to be present in different leaf and seed extracts may be considered to be responsible for their antimicrobial activity. *E.coli* and *Aspergillus niger* were found to show no zone of inhibition against the standard antibiotic Ampicillin suggesting that these organisms have undergone resistance against the said antibiotic. But our plant extract were found to be effective. Thus our present also study provides a scientific basis and justifies the use of this plant in ethnomedicinal system for treating various diseases since time immemorial. From the present study it can be concluded that the leaf and seed extract of *Benincasa hispida* contains various phytochemicals and it also exhibits significant antimicrobial activity against gram positive and gram negative bacteria as well as against the fungal strain tested for. The presence of various phytochemicals and significant antimicrobial activity thus justifies it use as medicine for treating different diseases. From this study it can be suggested that *Benincasa hispida* can be a potential source for discovery of new drugs. Further research to isolate and identify the bioactive compounds and toxicological study will be useful in new drug discovery from the plant

Fig I: Graphical representation of antimicrobial activity of seed extracts of *Benincasa hispida*

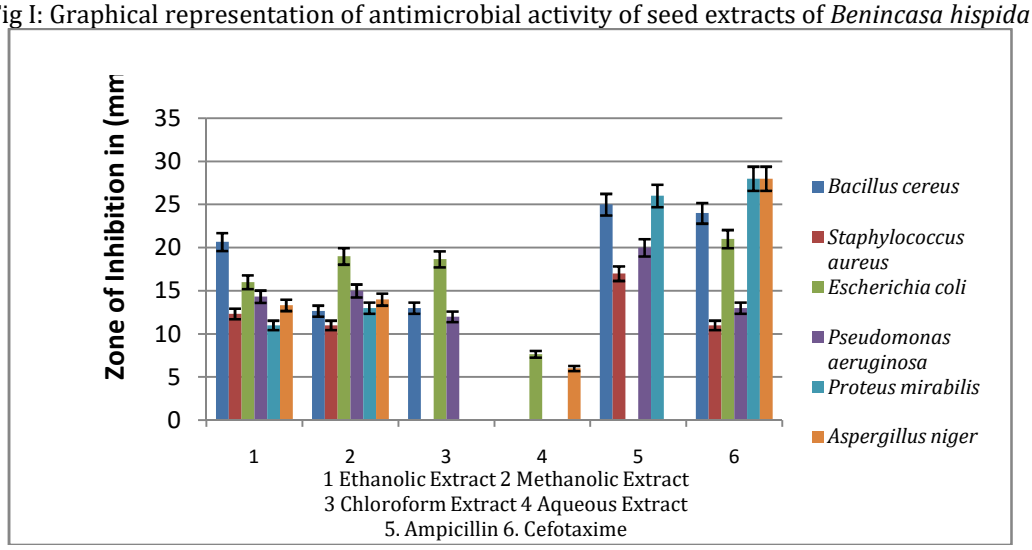
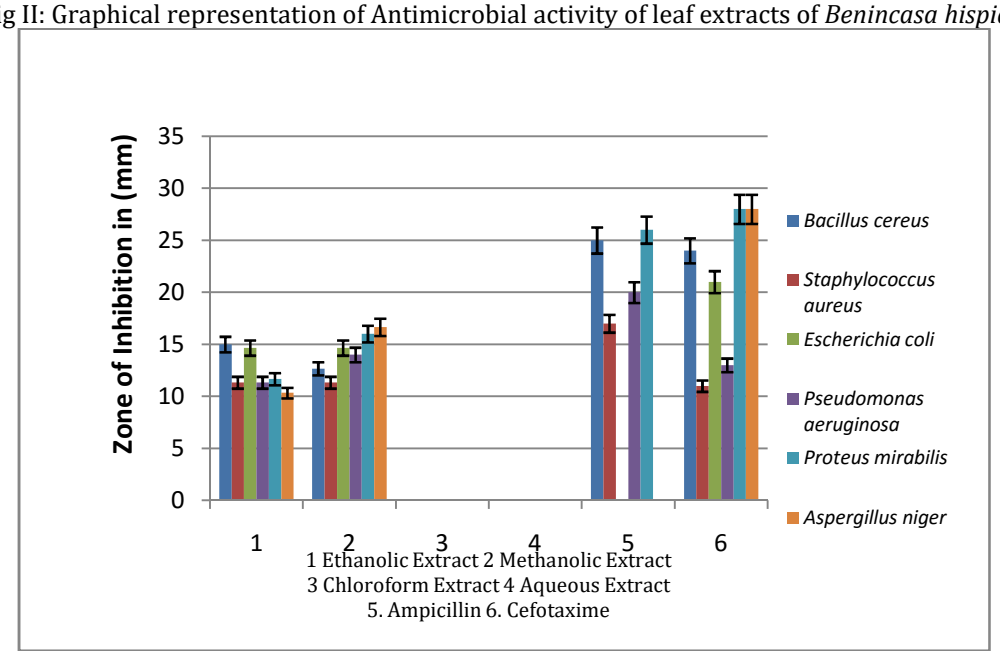


Fig II: Graphical representation of Antimicrobial activity of leaf extracts of *Benincasa hispida*



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REFERENCES

1. Mahesh B, Satish S. (2008). Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. *World J Agric Sci,* **4 (S)**: 839-843.
2. Junaid S A, Olabode A O, Onwuliri F C, Okworiu A E J, Agina S E. (2006). The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *Afri J Biotechnol* , **5 (22)**: 2315-2321.
3. Pretorius C J, Watt E. (2001) Purification and identification of active components of *Carpobrotus edulis L. J Ethnopharm* ,**76**: 87-91.
4. Sharif M D M, Banik G R.(2006) Status and Utilization of Medicinal Plants in Rangamati of Bangladesh. *Res J Agric Biol Sci* ,**2(6)**: 268-273.
5. Doughari J H**,** El-mahmood A M, Manzara S. (2007).Studies on the antibacterial activity of root extracts of *Carica papaya* L. *Afri J Microbiol*, Res. 037- 041.Available online (http://www.academicjournals.org/ajmr).
6. Ghosh K, Baghel M S.(2011). A pharmacognostical & physiolochemical study of *Benincasa hispida* with Ayurvedic review *IJRAP*, 2(6):1664-1668.
7. Sharma L K. (1984). Food Medicines. Practical Nature Cure. Nature cure publication house. Puddukkottai .India p 169.
8. Warier PK. (1994). Indian Medicinal Plants .Orient Longman Limited, India, p 261.
9. Gill N S, Dhiman K, Bajwa J, Sharma P, Sood S. (2010). Evaluation of Free radical scavenging, Anti inflammatory and Analgesic potential of *Benincasa hispida* seed extract. *Int J Pharm,* **6**:625-657.
10. Manish R A, Sunita J M. (2008). Gastroprotective effect of *Benincasa hispida* fruit extract. *Int J Pharm,* **40**:271-275.
11. Sadasivam S, Manickam A (1996). *Biochemical Methods for Agricultural Sciences*, New Age International (P) Limited, New Delhi, India .
12. Tyler V. (1994). Phytomedicines in Western Europe: their potential impact on herbal medicine in the United States *Herbalgram*, **30**, 24-30.
13. Harborne J B. (1973). Phytochemical methods. Chapman and Hall. London.
14. Ahmad I, Beg A Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human Pathogens. *J Ethnopharmacol,* **74**: 113-123.