Section A-Research paper

SCREENING THE ANTIMICROBIAL PROPERTIES OF *Benincasa hispida* (Thunb.) Cogn. LEAVES

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Abstract: The purpose of this study was to determine the antibacterial properties and antibiotic potentials of Benincasa hispida Linn leaves. The study aims to evaluate the antimicrobial activities and determine the inhibition zones for extracts against some bacterial strains and fungi. The antimicrobial activity was investigated in the present study using an ethanolic leaf. The ethnomedicinal plant (an ethnomedicinal herb) was evaluated for its potential to act as an antimicrobial agent against medically significant bacterial and fungus strains. Extracts were tested using agar disc diffusion. The antibacterial and antifungal activities of Benincasa Hispida extracts were evaluated against Gram-positive Staphylococcus aureus and Bacillus Cereus strains, two gram-negative strains, Escherichia Coli & Pseudomonas aeruginosa strains, and two fungal strains Aspergillus Niger & Candida Albicans strains. The zone of inhibitory activity was compared with that of Amphotericin, a standard antifungal medication and Gentamycin, an antibiotic standard. The results indicated that the Bacillus cereus growth was inhibited and that no antifungal effect was seen. The plants were analysed for phytochemicals. Benincasa hispida's microbial activity was due to secondary metabolites. This plant may be used to find bioactive compounds, which could lead to new pharmaceutical research.

Keywords: antibacterial, ethnomedicine, antifungal, phytochemical.

1. Introduction:

Microorganisms that cause infection, such as bacteria and mold, are a persistent problem and continue to increase. Different microorganisms can cause different diseases. The type of infection determines the severity and its causes, many possible treatments exist. Antifungal drugs are the most common treatment for fungal infections are caused by bacteria and viruses that cause pathogenic bacteria. Parasites are responsible for some diseases. Antiparasitic drugs are available on the market today as a treatment. Concomitant illnesses can exacerbate infections and make them lethal. Antimicrobial resistance, host toxicity and other unwanted side effects have made it necessary to find new antimicrobial molecules. To treat infections caused by pathogenic microorganisms, the research is currently focused on natural compounds that can show antimicrobial resistance. Existing in vitro techniques can be used to test a compound's antimicrobial and antifungal properties. Diffusion, thin-layer Chromatography (TLC), Bioautography and dilution are the most commonly used methods. Methods are divided further by testing technique and time-kill test. The cytofluorometric flow test (FCM) as well as the adenosine Triphosphate bioluminescence (ATP) test are other 1 1 1, 1 1 1, 1 2, 2 3, 5 20 22, 24 methods. Despite their time-consuming nature and high cost, they are less commonly used due to the need for more equipment. Results are available quickly (1,2). Fungal infections cause mycoses every year. They can be systemic,

subcutaneous or cutaneous. According to studies, dermatophytes, yeasts and non-dermatophyte mycoses were the most common agents. The study showed that Trichophyton (especially Trichophyton rubrum, 73%) and T. Interdigital & Mentagrophytes complex (16%) were the most common cause of superficial fungus infection. Candida and Cryptococcus are also pathogens that can cause serious fungal infections. They are often the cause of nosocomial disease, and mortality rates reach up to 40% (3). Other factors, such as organ transplantation or nonfungal infections, can exacerbate pathogenic diseases. Patients with HIV or cancer treated by antiretroviral drugs or cytokine inhibitors are at a high risk of developing invasive fungal infections or have a higher death rate from invasive fungus infection (5,6). Antifungal agents can treat fungal-caused illnesses. These antifungal drugs are classified as azoles (echinocandins), polyenes (pyrimidine analogues), and allylamines. Antifungal drugs are chosen for a specific disease based on its type, the pathogens involved and the target. Antifungal agents primarily target fungal ergosterol, glucan, membranes, or nucleic acid synthesis. Depending on the drug class, it is possible to target and inhibit different pathogens. Many strategies have been developed to improve the treatment of IFIs, due to their alarming increase in incidence worldwide, which can result in 1.7 million deaths per year. Amphotericin (AMB) is the first-line polyene against this problem. The gram-positive bacteria Streptomyces Nodusus produces it. It works by disrupting fungi membranes. Combinatorial therapy can be used to maximize the effectiveness of treatment. This reduces the likelihood of developing resistance to monotherapy and also allows for the reduction of dosages of drugs with significant side effects, such as polyenes. Antimicrobials include antibacterial, antibiotics, and anti-parasitic medications. Before antibiotics, infections were the main cause of death and morbidity in humans. Even though many antifungal drugs are available, the number of invasive fungus infections continues to increase. Infections caused by bacteria, viruses and parasites are also on the rise, posing serious risks to public health. This is because of the rise in antimicrobial resistance (AMR). AMR infections can cause serious illnesses and prolonged hospitalization. The biggest problem is the high toxicity of drug-drug interactions and their unwanted side effects in patients with immunodeficiency. AMB is the first-line antifungal drug and can cause nephrotoxicity. Hepatotoxicity can be caused by antifungal drugs such as voriconazole and azole-fluconazole. Patients with liver disease or liver failure should use these medications with caution. Scientists are trying to create more effective drugs by finding new antifungal compounds. The number of diseases and deaths due to microbial infections and fungi is rising worldwide. To develop new antimicrobials, scientists still heavily rely on natural compounds derived from eukaryotes and prokaryotic bacteria, plants, animals, and microorganisms. Researchers are currently focusing on secondary metabolites, newly synthesized molecules, and plant and microbial isolates. Various methods are currently used to determine new natural compounds' antifungal or antimicrobial properties. These health benefits have been threatened over the last few decades as many antibiotics are less effective in treating certain diseases. This is not only because many produces toxic reactions but also because drug-resistant bacteria have emerged. Newer drugs that are less resistant to resistance should be investigated. Natural drugs are important in the treatment and prevention of many human diseases. Traditional medicine is a primary system of healthcare in many developing countries. Herbs have been extensively used in traditional medicine, and their therapeutic potential is well-documented. Natural products accounted for 61% of all new drugs between 1981 and 2001. They were very successful in areas such as cancer and infectious diseases. Recent trends show, however, that the rate of discovery of novel active chemical entities has declined. [5] Natural products from higher plants may be used to create novel antimicrobial agents. Many researchers have studied the effects of plant extracts against bacteria in various parts of the globe. In India, much work has been conducted on ethnomedicinal plant species. [9] Plants are rich in secondary metabolites

such as flavonoids and tannins. Since ancient times, men have used herbal medicines. Traditional medicine practitioners have used Indigenous plants to treat many diseases. The antimicrobial properties in medicinal plants have been reported more and more from around the globe. 80% of people worldwide use plant extracts or their active components as folk medicine. [13] Drugs are used to control harmful microorganisms, but this results in multiple drug-resistant strains of bacteria. This has led to alarming clinical conditions in the treatment of infections. Resistance to antibiotics by microorganisms is increasing. Bacteria can acquire and transmit resistance to synthetic drugs used as therapeutic agents. [14] To broaden the spectrum of natural antibacterial agents, ethanolic leaf extracts of Benincasahispida (Thunb.) Cogn. Leaves showed no antifungal activities. Since their introduction, antibiotics have been one of the most effective weapons against bacterial infections. They also improve human health. These health benefits have been threatened over 1 1 4 6 7 8 12 13 the last few decades as many antibiotics commonly used to treat certain diseases have become less effective. This is not only due to toxic reactions but also because drug-resistant bacteria have emerged. To find newer drugs that are less resistant, it is important to research them. Natural drugs play an important role in treating and preventing human diseases. Traditional medicine is a primary system of healthcare in many developing countries. Herbs have been extensively used in traditional medicine, and their therapeutic potential is well-documented. Natural products accounted for 61% of all new drugs between 1981 and 2001. They were very successful in areas such as cancer and infectious diseases. Recent trends show, however, that the rate of discovery of novel active chemical entities has declined. Natural products from higher plant species could provide new antimicrobials with novel mechanisms. Many researchers have studied the effects of plant extracts against bacteria in various parts of the world. In India, much work has been conducted on ethnomedicinal plant species. [9] Plants are rich in secondary metabolites such as flavonoids and tannins. For centuries, men have used herbal medicines. Traditional medicine practitioners have documented the therapeutic efficacy of indigenous plants for various ailments. The antimicrobial properties in medicinal plants have been reported more and more from around the globe. According to the World Health Organization, 80% of people around the globe use plant extracts and their active components in folk medicine. [13] Drugs are used to control harmful microorganisms, but this results in multiple drug-resistant strains of bacteria. This has led to alarming clinical conditions in the treatment of infections. Resistance to antibiotics by microorganisms is increasing. Bacteria can acquire and transmit resistance to synthetic drugs used as therapeutic agents. In an attempt to broaden the spectrum of natural antibacterial agents, the ethanolic leaf extract of Benincasahispida (Thunb.) Cogn. Leaves showed no antifungal activities.

2. Materials and Methods:

2.1 Plant Material (*Benincasa hispida*):

Benincasa Hispida leaves were collected from the local market and the cultivation area. The Botanical Survey of India in Shibpur, Howrah and West Bengal has authenticated the plant material by taxonomically identifying it.

2.2 Preparation of Ethanolic Extract:

Fig 1: Preparation of coarse Powder from *Benincasa hispida leaves*

1. The plant material was gently cleaned in tap water to remove dirt. They were then shadedried in the lab at room temperature (24+- 2degC for 3-4 weeks).
2. The dried plant material was pulverized using a machine grinder, followed by sieving to get a coarse powder.
3. After 72 hours, petroleum ether (60-8o) was treated with coarse powder to remove fatty substances.
4. The extract was treated for 36 hours with 95 per cent ethanol using the Soxhlet apparatus.
5. Filter paper No. 42 filters the crude extract solution. Then, the crude extract solution is filtered using Whatman No.42 and then concentrated in vacuum under for reduced pressure and then dried in hot air oven and store the extract at 4˚C.
6. The extract is then used to further the study.

Fig 2: Extraction Procedure by using soxhelet apparatus

2.3 Test Microorganisms and Growth Media:

The microorganisms like Bacterial strains Staphylococcus aureus ATCC 11632, Bacillus cereusv MTCC 430, Escherichia coli, Pseudomonas aeruginosa15442 and fungal strains Aspergillus Niger and Candida albicans were procured from ATCC (American type culture collection) (HiMedia, India) and MTCC (Microbial type culture collection) CSIR, Institute of Microbial Technology, Chandigarh India chosen based on their clinical and pharmacological importance. (Microbial type cell collection) from were used for evaluating antibacterial and antifungal activity. The bacterial strains were grown in Nutrient agar plates and incubated at

37°C for 24 hours whereas the fungi were grown in Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) media, respectively, at 28°C. The stock cultures were maintained at 4°C.

2.4 In vitro antimicrobial Activity:

2.4.1 Agar well diffusion assay:

In vitro antibacterial and antifungal activities were examined for ethanolic extracts. Antibacterial and antifungal activities of plant part extract against four pathogenic bacteria (two Gram-positive and two-gram negative bacteria) and two pathogenic fungi were investigated by the agar disk diffusion method based on the zone of inhibition and the standard antibiotic Gentamicin and Amphotericin B were used. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by syringe filter and stored at 4°C. The ethanolic plant extracts *Benincasa hispida* of different concentrations (100mg/ml, 200mg/ml) were screened for antibacterial activity against the *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* and each 100µl bacterial indicator strain ($1x10^8$ CFU/ml) was seeded on Mueller Hinton agar (MHA) with cotton swab and wells were made using a sterile cork borer (6mm) and added 100µl of extract was added on each of different concentration along with standard antibiotic Gentamicin incubated for 24 hours 37°C and as well as for antifungal activity for *Candida albicans* and *Aspergillus niger* , these fungal strains was seeded with 100µl ($1.0X10^7$cfu/ml) on SDA and PDA plates with 100µl of extract on each wells . Amphotericin B antibiotic as a standard was used and DMSO as a negative control and incubated at 28°C for 48-72 hours. The experiment was carried out in triplicate and results were interpreted based on the zone of inhibition in each well (mm in diameter).

3. Results:

3.1 Result on In vitro antifungal activity of ethanolic leaf extract against candida albicans:

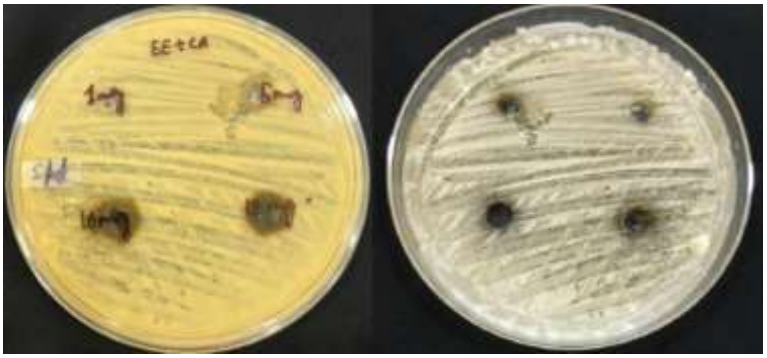


Fig 3: Ethanolic extract 1mg/ml, 5mg/ml, 10mg/ml and 18mg/ml *Candida albicans* ATCC 10231 against using agar well diffusion incubated for 48 hours

Fig 4: Ethanolic extract 1mg/ml, 5mg/ml and 10mg/ml against *Candida albicans* ATCC10231 usingagar well diffusionincubated for48hours

Fig 5: Amphotercin B concentration 1mg/ml,250µg/ml,500µg/ml against candida albicans ATCC10231 using agar well diffusion incubated for 48hrs**.**

3.2 In vitro antifungal activity of ethanolic leaf extract against Aspergillus niger:

Fig 6: Ethanolic extract 1mg/ml, 5mg/ml, 10mg/ml and 18mg/ml against Aspergillus niger ATCC 16888 by agar well diffusion incubated for 3 days

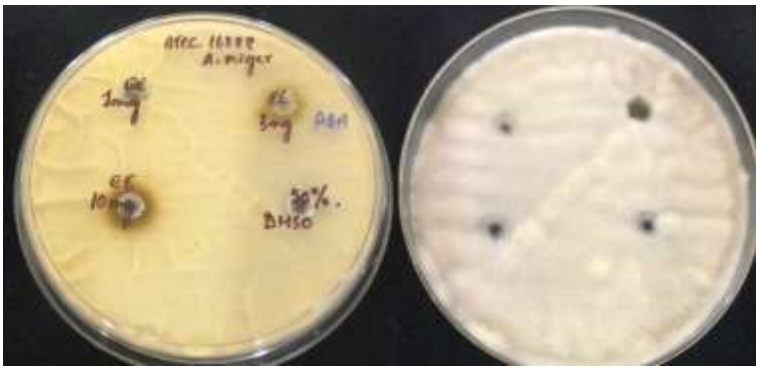


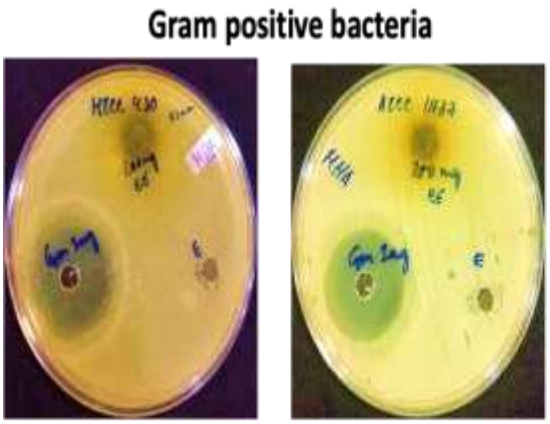
Fig 7: Ethanolic extract 1mg/ml, 5mg/ml, 10mg/ml and DMSO against Aspergillus niger ATCC 16888 by agar well diffusion incubated for 3 days

3.3 Antibacterial activity of ethanolic leaf extract against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus:*

By using the disk diffusion method, antibacterial activity was assessed against Escherichia coli (ATCC 25922), Pseudomonas aeruginosa MTCC 24,53 and Bacillus Aureus (ATCC 1162) by ethanolic extracts of plants (100 or 200 mg/ml). Interpretation of the zone of inhibition (in diameter). Use Gentamicin (one milligram per ml) as a positive test. Negative control using a solvent.

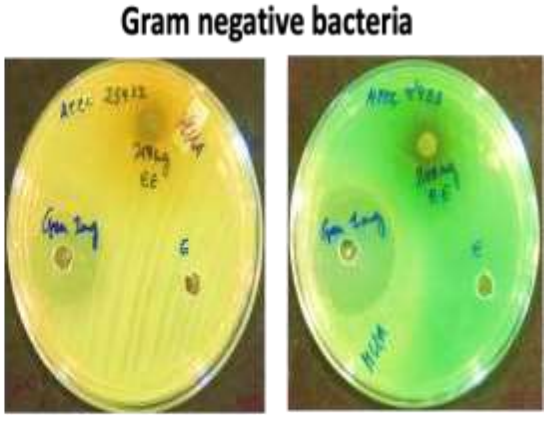
Table 1: inhibition zone in diameter

	Inhibition zone in diameter (mm)			
Ethanolic extract concentration	**Escherichia coli** **ATCC 25922**	**Pseudomonas aeruginosa** **MTCC 2453**	**Staphylococcus aureus** **ATCC 11632**	**Bacillus cereus** **MTCC 430**
100 mg/ml	_	_	_	**8±0.3**
200 mg/ml	_	_	_	**12±0.3**



Bacillus cereus MTCC 430 | *Staphylococcus aureus* ATCC 11632

Fig 8



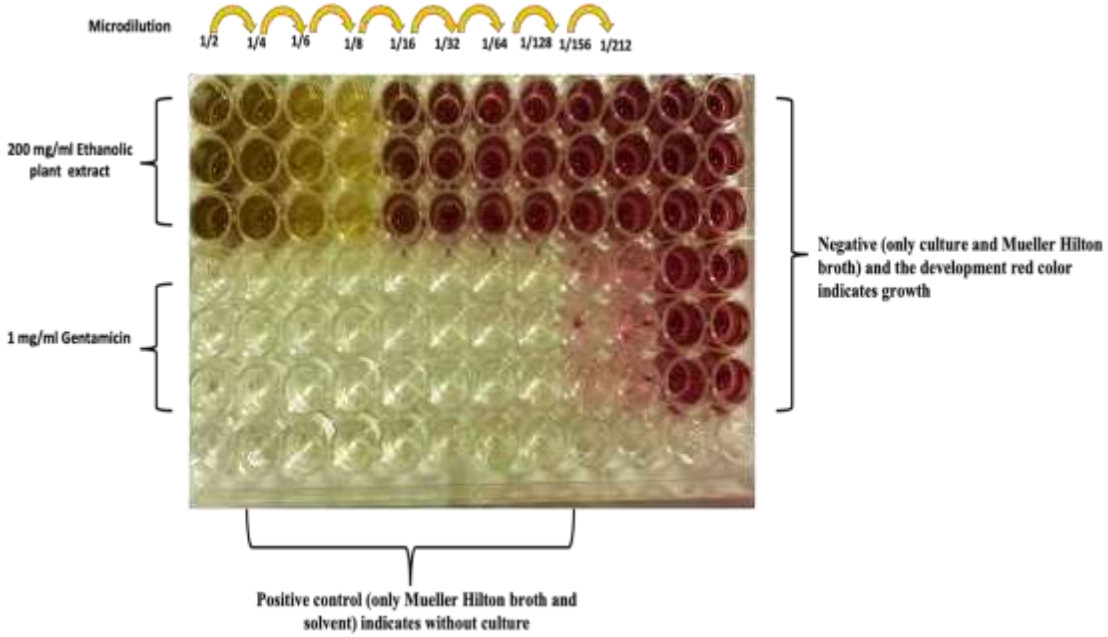
Escherichia coli ATCC 25922 | *Pseudomonas aeruginosa* MTCC 2453

Fig 9

3.4 Minimum Inhibition concentration (MIC) assay for ethanolic leaf extract against Bacillus cereus:

Minimum inhibition concentration of Ethanolic leaf extract (stock concentration 200mg/ml)

Fig 10: determination using TTC colorimetric method by 96-well microplate



against *Bacillus cereus* MTCC 430 which has antibacterial activity was determined by 96 microplates well microdilution method using *w/v* 2,3,5-triphenyltetrazolium chloride (TTC) colorimetric measurement at OD 540nm wavelength.
The result of the MIC determination of the Ethanolic leaf extract against *Bacillus cereus* showed in 25mg/ml was observed.

4. Discussion:
When tested using standard fungal strains, Candida albicans ATCC 10231 and Aspergillus ATCC 16888 ATCC 16888, the ethanolic leaf extraction from Benincasa Hispida did not show any significant antifungal activity. Amphotericin B served as a control positive in the experiment. DMSO is used to dissolve ethanolic extracts of plants. The agar-well method was used to test the antibacterial activity against Escherichia Coli and other bacteria such as Staphylococcus aureus, Pseudomonas Aeruginosa, Bacillus Cereus and Staphylococcus aureus. Gentamicin served as a control positive & DMSO was used as a solvent.

Bacillus cereus MTCC 430 showed antibacterial activity against 100mg/ml & 200mg/ml conc. with an average zone diameter of 8±0.3 & 12±0.3mm respectively. No significant antibacterial activities were observed against other bacterial strains used for this purpose. MIC values for the extract against Bacillus cereus MTCC 430 was performed using 96 microplates well microdilution method using w/v 2,3,5-triphenyltetrazolium chloride (TTC) colometric measurement at OD 540nm wavelength. The result of the MIC determination of the Ethanolic plant extract against Bacillus cereus showed in 25mg/ml was observed. From the above observations it is clear that the plant extract shows antibacterial activity against

specific bacterial strain though not very significant compared to positive control Gentamicin. This finding may be considered for taking further studies on the extract regarding its antimicrobial activities.

5. Conclusion:

From the above discussion, it may be concluded that the subject leaf extract of Benincasa hispida shows antibacterial activity against Bacillus cereus, a prominent gram-positive microbe. This is also evident from the MIC value of the extract shown after performing the test as per standard protocol. The findings were also compared with positive control using standard antibiotic Gentamycin. The extract did not show better activities compared to the antibiotics, still can be a substitute being natural product. Further research on the extract could make it a prominent lead compound showing antimicrobial property.

6. References:

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