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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT & ANTIMICROBIAL ACTIVITYOF AQUEOUS EXTRACT OF BENINCASA HISPIDA FRUIT

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ABSTRACT

The present study was carried out to evaluate the phytochemical constituents, antioxidant activity and antimicrobial activity of the fruit extract of Benincasa hispida. The fresh fruits were collected from a local vegetable market and authenticated in the Department of Pharmacognosy. The fruits were cleaned, dried and powdered for further extraction. The powdered material was subjected to successive solvent extraction using petroleum ether, methanol, ethyl acetate and distilled water to obtain different extracts. The obtained extracts were stored under refrigerated conditions for further analysis. Preliminary phytochemical screening was performed to identify the presence of various bioactive constituents such as alkaloids, flavonoids, phenols, proteins, steroids and quinines using standard qualitative tests. The antioxidant activity of the extract was evaluated using different in-vitromethods such as. Total antioxidant capacity by phosphomolybdate method Superoxide radical scavenging activity (SOD method) DPPH radical scavenging assay. These methods were used to determine the free radical scavenging potential of the plant extract. The antimicrobial activity of the extract was assessed using the cup plate (agar diffusion) method against selected microorganisms to determine the zone of inhibition and antimicrobial potential. Thus, the study involved extraction, phytochemical screening, antioxidant assays and antimicrobial evaluation to determine the therapeutic potential of Benincasa hispida fruit extract.

KEYWORDS: Preliminary phytochemical screening was performed to identify the presence

of various.

INTRODUCTION

The continuous formation of free radicals in human's body can be controlled naturally by different beneficial compounds known as antioxidants. Oxidative stress can be caused in result of free radicals formation.[1] Aging and different chronic diseases including diabetes, cancer and cardiovascular diseases could be caused by oxidative stress. Free radicals are stabilized or deactivated by antioxidants before they attack cells. Antioxidants are important factor to maintain optimal cellular and human body health. Furthermore, fortification of food formulation by adding antioxidant compounds lead to prevention of oxidative reactions which adversely affect food quality attributes. Several epidemiologic studies revealed that consumption of foods containing high amount of antioxidant compound lowering the risk of human disease occurrence.[2] Oxidative stress depicts the existence of products called free radicals and Reactive Oxygen Species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation in vivo and in vitro and create oxidative stress which results in numerous diseases and disorders[3,4] such as cancer[5], cardiovascular disease[7], neural disorders[6], Alzheimer's disease[8], mild cognitive impairment, Parkinsons disease, alcohol induced liver disease, ulcerative colitis[10], ageing, atherosclerosis[11] Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries.[12] Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system.[13] Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. The family of free radicals generated from the oxygen is called ROS which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O2-) and hydroxyl radicals (OH), as well as non-free radicals (H2O2) and singlet oxygen.[14] In the body, free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides, etc.[15] When

oxygen traps single electron, it becomes unstable and thus very reactive, since it generates harmful chain reactions against many biological molecules. The extreme toxicity of oxygen is related to its high capability of generating free radicals and in turn destroying many major biological molecules. They can attack on lipids and proteins and destroy membranes. ROS can damage DNA and lead to mutation and chromosomal damage. Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids.[16] ROS can attack various substrates in body and contribute to development of chronic diseases. For example, oxidatively modified LDL has been hypothesized to be a causative agent in the development of cardiovascular diseases. Exogenous chemicals and endogenous metabolic processes in human body produce free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death. Superoxide anion radicals increase under stress conditions such as heavy exercise, certain drugs, infection and various disease states. During normal metabolic processes, human body generates more than 2 Kg of O2 - per year. Cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as Super Oxide Dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury.[17] Molecules such as vitamin C and E inhibit lipid peroxidation in cell. When the mechanism of antioxidant protection becomes unbalanced in human body, antioxidant supplement may be used to help reduce oxidative damage. Several degradation reactions, which may occur on heating or during long term storage, deteriorate fats and oils and the lipid constituents of foods. Oxidation reactions and the decomposition of oxidation products are the main processes which result in decreased nutritional value and sensory quality.[18] Research has implicated oxidative and free-radical- mediated reactions in degenerative processes related to ageing and diseases such as cancer, coronary heart disease and neurodegenerative disorders such as Alzheimer's disease. The prevention or retardation of these oxidation processes is essential for the food producer and almost everyone involved in the entire food chain from “farm to fork”. Various methodologies may be employed to inhibit oxidization, including prevention of oxygen access, use of lower temperature, inactivation of enzymes catalyzing oxidation, reduction of oxygen pressure and the use of suitable packaging.[19] Another common method of protection against oxidation is to use specific additives which inhibit or retard oxidation. These oxidation inhibitors are generally known as antioxidants.[20] The common agricultural products are rice, wheat, jute, tea, cotton, sugarcane, flower, vegetables, fishes and seed development, livestock, horticulture are main agricultural sectors. Microbial contamination is

one of the most alarming causes of agricultural production.

According to the Food and Agriculture Organization (FAO), pests and diseases are responsible for about 25% of crop loss.[21] To solve this issue, new methods are needed to detect diseases and pests early such as novel sensors that detect plant odours, and spectroscopy and bio photonics that are able to diagnostic plant health and metabolism.[22] Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. It has been reported that more than 400,000 species of tropical flowering plant showed medicinal properties and the usages of medicinal flowering plant make the folk medicine cheaper the other medicine.[23] The methanolic extracts of B. hispida showed excellent protection against histamine-induced bronchospasm probably through an antihistamine activity that is H1 receptor- antagonism.[24] The methanol extract of winter melon fruit showed significant inhibition against fungus namely Candida albicans[25] also methanolic and petroleum ether extracts showed significant inhibition of carrageenan-induced paw edema, histamine induced paw edema and cotton pellet induced granuloma in a rat model.[26,27,28] The potentially of the different organic extract of B. hispida will be assayed to find strong antimicrobial activities. If they found B. hispida would be a potential source that may help to develop natural antimicrobial agents for the control of plant pathogens. Benincasa hispida (Synonym: Benincasa cerifera)[29], which commonly called (winter melon, ash gourd, ash guard, winter gourd, white pumpkin and wax gourd. white gourd, tallow gourd, gourd melon and Chinese watermelon) belongs to the Cucurbitaceae family, it was probably native in Japan and Java, cultivated more or less throughout India and in warm countries. It is a popular vegetable crop, especially among Asian communities both for nutritional and medicinal purposes.[30,31] It was preferred as a cooked vegetable, boiled alone, boiled with meat, or included in a variety of dishes. Also, it was used raw like sliced cucumbers.[32] However, the plant was used medicinally in various complains such as gastrointestinal problems, respiratory disease, heart diseases, diabetes mellitus and urinary diseases.[33] Fruits were traditionally used as a laxative, diuretic, tonic, aphrodisiac, cardiotonic, urinary calculi, blood disease, insanity, epilepsy, schizophrenia and other psychologic disorders, jaundice, dyspepsia, fever, and menstrual disorders.[34,35,36] The major constituents of Benincasa hispida fruits are volatile oils, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, ß-sitosterin and uronicacid.[30,37,38,39,40,41] The pharmacological studies revealed that the plant exerted many pharmacological activities, including central nervous effects (anxiolytic, muscle relaxant, antidepressant, in the treatment

of Alzheimer's disease and to minimize opiates withdrawal signs), antioxidant, anti- in flammatory, analgesic, antiasthmatic, diuretic, nephroprotective, antidiabetic, hypolipidemic and antimicrobial effects. The present review aimed to highlight the chemical constituents and pharmacological effects of Benincasa hispida.

AIM

The Principle aim of the study is to evaluate the phytochemical analysis, antioxidant activity and the anti-microbial activity of 'Benincasa hispida' fruit extract without any side effects and also to ensure a new approach in the treatment of disease.

PRIMARY OBJECTIVE

To evaluate the anti-microbial activity of the "Benincasa hispida" fruit extract.

SECONDARY OBJECTIVE

- To analyze the preliminary phytochemical screening on Benincasa hispida fruit.
- To evaluate the antioxidant activity study of Benincasa hispida fruit extract.

PLAN OF WORK

Collection and authentication of Benincasa hispida fruit

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Procurement of Benincasa hispida Fruit

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Preparation of fruit Extract Using Methanol

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Synthesis of medicinal extract

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Standardization & Preliminary Phytochemical Analysis of fruit extract

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Pharmacological Evaluation

- Evaluation of antioxidant activity of super oxide dismutase, total antioxidant activity and DPPH scavenging activity(1,1- diphenyl-2- picrylhydrazyl).
- Evaluation of antimicrobial activity of cup plate method.

MATERIAL AND METHOD

The fresh fruit of B. hispida (Thunb.) Cogn. Fruit was collected in the month of September 2025 from the local vegetable market of Kangeyam, Tiruppur. The Authentication of the plant was done in the department of Pharmacognosy, Excel College of Pharmacy, Komarapalayam, Namakkal, India. The fruit was dried, seeds were separated and powdered, and passed to 60 mesh. The powder of fruit (500 g) was first defatted with petroleum ether (500 ml x 6; yield was 1% w/w). The defatted powder was then dried and extracted with methanol (500 ml x 6; yield was 20% w/w) and thereafter with ethyl acetate (500 ml x 4; yield was 3% w/w) successively. The remaining powder was dried and extracted with distilled water to give aqueous extract (500 ml x 6; yield was 5% w/w). The dried fractions were stored in a refrigerator at 4 °C throughout the study.

The cleaned plant materials were then sun-dried to completely remove moisture, powdered with a mechanical grinder, and stored.

Benincasa hispida (B.hispida) also known as Kundur fruit, Chalk umra, wax gourd, winter gourd, ash gourd, winter melon, white gourd, tallow gourd, Chinese preserving melon, ash pumpkin, and (alu) puhul, a creeper grown for its very big size fruit, is eaten as a green mature vegetable or greens. There is a fine hairs fuzzy coating outer side of the young fruit and has solid thick white flesh of sweet in tastes. The mature fruit sheds its hairs and forms a waxy white coating, giving the name of “wax gourd.” The gourd wax coating increases the storage facilities of it. It can grow of a length up to 80 cm and also have broad leaves and yellow flowers. The taste is rather bland. B. hispida is a native of South and Southeast Asia. However, it is commonly grown all over Asia, including Japan, Burma, Ceylon, Sri Lanka, Java, and Australia.

Traditional Uses and Ethnopharmacology

In India, B. hispida is used as a winter season vegetable for a wide variety of diseases. Its medicinal properties have been also recognized in the Ayurvedic system of medicine, spiritual traditions of India and Yoga. In Vietnam, its soup (cooked with pork short ribs) is traditionally used by in the treatment of epilepsy, cough, lung disease, hiccups, asthma, internal bleeding, and urinary retention. In India, a fruit compote called Petha Cubes is made from the pulp of the fruit, which is recommended for vegetarians.

The fruit is also used in peptic ulcer, and it is also used in diabetes mellitus, urinary infection, haemorrhages from internal organs, insanity, epilepsy, and other nervous disorders in

Ayurveda.

The fruit is sweet and traditionally used as a cooling, styptic, antiperiodic, laxative, diuretic, tonic, aphrodisiac, and cardiotonic, and also in jaundice, dyspepsia, urinary calculi, blood disease (e.g., haemorrhages from internal organs), insanity, epilepsy, asthma, diabetes, vitiated conditions of pitta, fever, menstrual disorders, and balancing the body heat.

Preparation of Benincasa hispida fruit extract

The fruits were sliced in to two halves and the peel was carefully removed with a manual peeler and the seeds were selectively collected and removed manually then the pulp obtained was homogenized in a warring commercial blender and dried in the shade at room temperature. Samples were ground in a Coffee grinder and dried under hot-air oven then stored in airtight container. 2g each of the fruit sample were separately extracted using petroleum ether (60-80) and methanol sequentially using Soxhlet's apparatus for about 3 hours each using 30 ml of the corresponding solvent. The extracts were then dried and dissolved in 10 ml petroleum ether/methanol (HPLC Grade, Merck). It was then filtered through 0.20 mm membrane filter. The extract was used for this analysis.

STANDARDIZATION AND PHYTOCHEMICAL ANALYSIS OF Benincasa

hispida

Determination of pH Value

The Benincasa hispida was weighed to about 5g and immersed in 100 ml of water in a beaker. The beaker left behind for 24 hours in room temperature. pH of supernatant of the formulation was determined using a calibrated digital pH Meter.

PREPARATION OF THE EXTRACT

Preparation of extracts of Benincasa hispida by hot continuous percolation method

About 500 gm of dried powdered fruits of Benincasa hispida T. was properly packed in Whatmann filter paper (grade no.1) and kept in thimble and the Soxhlet Apparatus was set up. The extraction of powder was done with different solvents with solvents of increasing polarities like petroleum ether (60- 80º C), chloroform, and methanol. Here temperature maintenance is based on the solvents used for extraction. The solvents were removed under reduced pressure using rotary evaporator and stored in desiccators. This fluid is taken for analysis.

Test For Calcium:- 2ml of the above prepared extract is taken in a clean test tube. To this add

2ml of 4% ammonium oxalate solution A white precipitate Indicates the presence of Calcium.

Test For Chloride: The extract is treated with silver nitrate solution, A white precipitate is formed indicates the presence of Chloride.

Test For Lead :	The extract is treated with potassium iodide.	A yellow
precipitate	indicates presence of	lead.

Test For Ferrous Iron: The extract is treated with concentrated Nitric acid and ammonium thiocyanate solution. Blood red color indicates the presence of Ferrous Iron.

Test For Carbonate: The substance is treated with concentrated hydrochloride, Brisk effervescence indicates the presence of Carbonate.

Test For Tannic Acid: The extract is treated with ferric chloride. Blue black precipitate indicates presence Tannic acid.

Test For Sulphate: 2ml of the extract is added to 5% Barium chloride solution. A white precipitate indicates the Presence of sulphate.

Test For Zinc: The extract is treated with Potassium Ferro cyanide. White precipitate indicates presence of Zinc.

Preliminary phytochemical screening

The presence of phytochemicals like alkaloids, flavonoids, saponins, tannins, terpenoids, polyphenols, cardiac glycosides, anthraquinones and carbohydrates in the leaves of Benincasa hispida extract was evaluated using standard testing methods.

Test for carbohydrates: 1 mL of Molisch’s reagent was added to 2 mL of extracts. Then, few drops of concentrated sulphuric acid were added. The presence of reddish colour was indicated the presence of carbohydrate.

Test for Alkaloid: 6 mL of aqueous extract was added, stirred with 3 mL 1% of HCl on steam bath, and Mayer’s reagent was added. Turbidity of the resulting precipitate was taken as an evidence the presence of alkaloid. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

Test for Saponins : 3 mL of aqueous extract was dissolved in 5 mL of distilled water. Then,

the mixture was shaken vigorously. The formation of stable persistent froth was showed the presence of saponins.

Test for flavonoids (Alkaline reagent test) : 2 mL of the aqueous extract were treated with few drops of sodium hydroxide solution. Then the formation of intense yellow colour was indicated for the presence of flavonoids.

Test for Terpenoids: 3 mL of the aqueous extract was dissolved in 2 mL of chloroform. Then, 2 mL of concentrated sulphuric acid was added, a reddish brown colour in inter phase was indicated the presence of terpenoids.

Test for polyphenols (phenolic compounds): 1 mL of the aqueous extract and 2 mL of distilled water was mixed. Then, it was treated with10% ferric chloride and the formation of blue colour was indicated for the presence of phenols or polyphenols.

Test for Quinines: To the 1 ml of extract added 1ml of 1% of sodium hydroxide and mixed well. Appearance of blue green or red indicates presence of quinines.

Test for Protein: Few drops concentrated nitric acid was added to 1 ml of the extract. Yellow colour indicates the presence of proteins.

Test for Steroids: 1 ml of extract mixed with 1 ml of chloroform and concentrated H2SO4 sidewise. A red colour presence at the lower chloroform layer indicates presence of steroids.

INVITRO ANTIOXIDANT STUDIES

Total Antioxidant Assay by Phosphomolybdate Method Principle

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphor molybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH.

Procedure

0.1 mL of sample (100 lg) solution is combined with 1 mL of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95o C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 695 nm against blank in

UV spectrophotometer. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it is incubated under same conditions as rest of the sample. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid with methanol.

Superoxide radical scavenging activity (SOD) principle

Although superoxide anion is a weak oxidant, it ultimately produces powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress.

Procedure

Superoxide dismutase (SOD) activity was determined by the inhibition in photo reduction of nitro blue tetrazolium by the SOD enzyme. To the 0.1ml of sample added 1ml of the reaction mixture I (1ml of 50mM PBS, 0.075ml of 20mM L Methionine, 0.04ml of 10mM hydroxyl amine hydrochloride, and 0.1ml of 50mMEDTA) and incubate the sample at 300C for 5minutes. After incubation added 50µM riboflavin and the sample was allowed to expose under 200W fluorescent light. 1ml of the reaction mixture II (1% Sulphanilamide in 5% phosphoric acid) was added and the measurement was read at 543nm under spectrophotometer.

$$\% \text{ Inhibition of nitrite formation} = 1\text{-AS/AC X } 100$$

DPPH scavenging activity Principle

The molecule 1, 1-diphenyl-2-picrylhydrazyl (a,a-diphenyl-b picryl hydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored.

Procedure

Free radical scavenging activity of the extracts was determined by DPPH assay. DPPH solution (0.004%, w/v) was prepared in methanol. Stock solution (1 mg/ml) standard ascorbic acid (0.05 g/ml) were prepared using methanol. 200, 400, 600, 800, 1000µl of the sample solution and 1ml of DPPH solution was added along with 0.4 ml of 50mM Tris HCL buffer, tube was incubated in dark for 30 minutes and the reading was measured at 517nm using spectrophotometer (LT 291 Labtronics microprocessor). Methanol was used as a blank, and the mg/g of the DPPH was calculated by using the ascorbic acid as a standard. The percentage of the DPPH radical scavenging is calculated using the equation as given below.

% inhibition of DPPH radical = ([Abr- Aar])/ Abr * 100 37

Where, Abr is the absorbance before reaction and Aar is the absorbance after reaction has taken place

MICROBIOLOGICAL ASSAY

Anti -bacterial Activity of Benincasa hispida fruit exrtract

Antibacterial activity of the extract was identified by using well diffusion method against the bacteria. Mueller hinton agar (39gm of media was dissolved in 1000ml of distiller water and sterilized under autoclave at 1210C for 15 minutes) was prepared, and poured to petriplate for solidification, after solidification, 70µl of the bacterial culture (E.coli, K.pneumoniae, S.typhi, S.aureus) was swabbed using cotton swab and well were made with cork borer followed by the sample was added (50µl). Antibiotic disc (Cefazolin-CZ30mcg) was placed as a positive control and dimethyl sulfoxide was used as negative control. Then the plate was incubated at 370C for 24 hrs. After incubation anti-bacterial activity of the sample was measured based on the zone of inhibition in mm.[76]

6. RESULTS

ORGANOLEPTIC EVALUATION

The aqueous extract of Benincasa hispida dried fruit was subjected to evaluation of organoleptic characters and the results obtained were illustrated.

S.NO	PARAMETERS	OBSERVATION
1	Description	Finepowder
2	Colour	Light yellow
3	Odour	Aromatic
4	Taste	Sweet

PHYSICOCHEMICALEVALUATION

The aqueous extract of Benincasa hispida was subjected to Physicochemical evaluation of and the results obtained were illustrated in Table no: 6

Table 6: Physicochemical evaluation on Benincasa hispida fruit extract.

S.NO	PROPERTIES	OBSERVATION
1	pH	6.0

QUALITATIVE ANALYSIS

Table 7: Minerals evaluation on Benincasa hispida fruits extract Phytochemical Analysis of the Plant Extracts.

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1	TESTFOR CALCIUM 2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% ammonium oxalate solution	A white precipitate of calcium oxalate is formed	Indicates the Presence of Calcium
2	TESTFOR CHLORIDE The extract is treated with silver nitrate Solution	A white curdy precipitate is formed	Indicates the Presence of Chloride
3	TESTFORLEAD The extract is treated with potassium iodide	No yellow precipitate is formed	Absence of Lead
4	TESTFORFERROUS IRON The extract is treated with conc. Nitric acid and ammonium thiocyanate Solution	Dark blue precipitate is formed	Indicates the presence of Ferrous Iron
5	TESTFORCARBONATE The substance is treated with concentrated Hcl.	Brisk effervescence is formed due to CO2	Presence of Carbonate
6	TESTFORTANNICACID The extract is treated with ferric chloride.	No Blue black Precipitate is formed	Absence of Tannic acid
7	TESTFORSULPHATE The extract is treated with barium chloride	white precipitate is formed	Presence of Sulphate
8	TEST FOR FERRIC IRON The extract is acidified with Glacial acetic acid and potassium ferrocyanide.	Blood red color formed	Presence of Ferric iron
9	TEST FOR ZINC The extract is treated with potassium ferrocyanide	white precipitate is formed	Presence of Zinc

The qualitative analyses of bioactive compounds found in Benincasa hispida fruit extracts are as follows. The presence and absence of useful bioactive substances such as alkaloids,

tannins, flavonoid, terpenoids, Cardiac glycosides, polyphenols, and other pharmacologically active compounds in the extracts were revealed by a confirmatory test involving colour changes.

Benincasa hispida fruit was extracted in this study, and the presence of phytochemicals such as alkaloids, tannins, saponins, flavonoid, terpenoids, Cardiac glycosides, polyphenols, and others was determined. Based on the findings, naturally occurring phytochemicals in the fruit extract were used as a reducing and capping agent. Figure 4 depicts the results of a qualitative phytochemical analysis of the Benincasa hispida fruit extract, which revealed the presence of secondary metabolites such as terpenoids, Phenols, saponins, steroids and tannins, among others. As a result, the phytochemicals in extract are capable of reducing ion by donating electrons, capping, and stabilizing the prepared test drug.

Table 8: Phytochemical analysis for the aqueous extract of Benincasa hispida fruit extract.

Phytochemical constituents	Observation
Alkaloids	-
Terpenoids	+
Phenols	+
Sugar	-
Saponins	+
Flavanoids	-
Quinines	-
Proteins	+
Steroids	+

+ indicates presence of phytoconstituent

- indicates absence of phytoconstituent

Fig.4: Qualitative Phytochemical Analysis of the Benincasa hispida extract Invitro.

ANTIOXIDANT STUDIES

Total Antioxidant Assay by Phosphomolybdate Method

A phosphomolybdenum assay was used to determine the quantitative antioxidant capacity of biosynthesized extract; this technique measures the reduction of Mo (VI) to Mo (V) by antioxidant compounds. The formation of a green phosphate/Mo (V) complex, with maximum absorption at 695 nm, indicates reducing activity. In the current study, biosynthesized extract demonstrated 321mg/g of phosphomolybdenum reduction activity, while the corresponding absorbance value for standard Ascorbic acid ranged from 640 mg/g. It should be noted that electron transfer occurs at different redox potentials in the ferrozine and phosphomolybdenum assays, and the reducing activity in each case is determined by the structure of the antioxidant under investigation.

Table 9: Antioxidant activity of biosynthesized Benincasa hispida by Phosphomolybdate Method.

Sample	Total antioxidant(mg/g)
Ascorbic acid	640
Sample	321

Superoxide radical scavenging activity

Oxidation is life, but in addition to so many other necessary processes of life, during normal oxygen metabolism, various free radicals and superoxide are continuously produced. The presence of a high level of this superoxide radical is known to be harmful to cellular components, contributing to tissue damage and a variety of diseases. The scavenging activity of Biosynthesized Benincasa hispida leaf extract for super oxide radicals shows 86.2 % inhibition was comparable to that of ascorbic acid.

Table 10: Antioxidant activity of biosynthesized Benincasa hispida by Superoxide radical scavenging method.

Sampleused	Absorbance
Control	0.261
Sample	0.128

DPPH scavenging activity

A DPPH scavenging activity was used to determine the quantitative antioxidant capacity of biosynthesized extract. The DPPH radical has been widely used to assess a compound's

ability to act as a free radical scavenger or hydrogen donor, and thus its antioxidant efficacy. A drastic colour change from purple to yellow made it obvious. Biosynthesized Benincasa hispida fruit extract were found to have(327mg/g) antioxidant activity and standard ascorbic acid (625mg/g).The lowest value indicates greater antioxidant activity. Over positive standards, this value was significantly higher. The presence of phenolic components could explain the leaf extract's significant DPPH radical scavenging capacity. The presence of tannins and flavonoids in the extract also contributed to the extract's DPPH radical scavenging activity. Furthermore, alkaloids are typically the most abundant component found in the leaf and are expected to contribute hydrogen- donating ability.

Table 11: Antioxidant activity of biosynthesized Benincasa hispida by DPPH Method.

Sample	Total antioxidant (mg/g)
Ascorbic acid	625
Sample	327

MICROBIOLOGICAL ASSAY

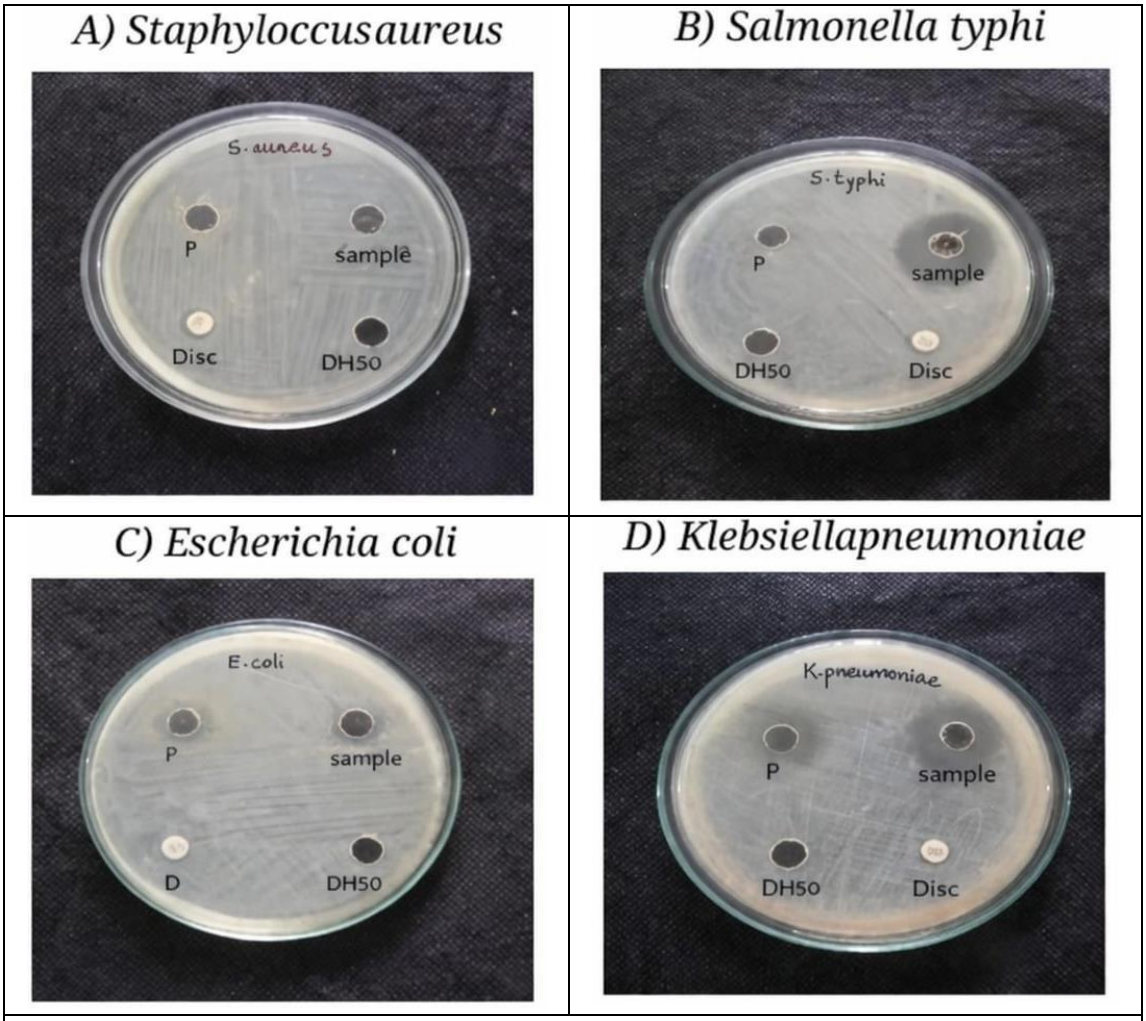
Anti-bacterial Activity of Benincasa hispida fruit extract

The antibacterial properties of Benincasa hispida fruit extract were tested against Gram-positive S.typhi, S.aureus and Gram negative E. coli, K.pneumoniae. Using the agar well diffusion method, Benincasa hispida fruit extract demonstrated significant antibacterial activity against three bacterial strains in the agar well diffusion method. From the above-mentioned experiment it can be found that the Benincasa hispida fruit extract are effective in killing/inhibiting a range of bacterial growth. The ZOI of around 5.33± 0.33was observed for the Gram-positive bacteria strain S.typhi and in the case of the Gram- negative bacteria strain E.coli and K.pneumoniae, the detected ZOI was 3.33±0.33mm and 5.33± 0.33 respectively. There are some reports on the mechanism underlying antimicrobial activity. Benincasa hispida fruit extract has a significant zone of inhibition on K.pneumoniae and S.typhi in his catse. Proposed mechanism is that the Benincasa hispida fruit extract will attach to the negatively charged bacterial cell wall and rupture it, causing protein denaturation and cell death.

Table 5: Zoneof Inhibition in Microorganism.

Samples used	Zone of inhibition in mm			
	Staphyloccus aureus	Salmonella typhi	Escherichia Coli	Klebsiella pneumoniae

Benincasa hispida fruit Extract	Nil	Nil	2.33± 0.33	3.67± 0.33
Sample	Nil	5.33± 0.33	3.33± 0.33	5.33± 0.33
DMSO	Nil	Nil	Nil	Nil
Disc	1.33± 0.33	Nil	Nil	Nil



Bacterial cultures showing the inhibition zones around wells loaded with 50 µg of Benincasa hispida fruit extract and (B)Salmonella typhi representing inhibition zone formed with 5.33± 0.33nm size of Benincasahispidafruit extract, (C) Escherichia Coli representing inhibition zone formed with 3.33± 0.33nm size of Benincasa hispida fruit extract, (D) Klebsiella pneumonia represent ingihibition zone formed with 5.33±0.33nm size of Benincasa hispida fruit extract.

Fig. 6: Zone of Inhibition in Microorganism.

CONCLUSION

From the present investigation, it can be concluded that the fruit extract of Benincasa hispida contains several important phytochemical constituents that contribute to its biological activity. The extract exhibited significant antioxidant activity through different free radical scavenging assays, indicating its ability to reduce oxidative stress.

The antimicrobial studies also demonstrated that the extract possesses noticeable antibacterial activity, suggesting its potential role in controlling microbial infections. These findings support the traditional medicinal use of Benincasa hispida and indicate that it may serve as a natural source of antioxidant and antimicrobial agents.

Further studies involving isolation of active constituents, toxicity evaluation and clinical investigations are recommended to explore its therapeutic applications in the treatment of various diseases.

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