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# Synthesis of Silver Nanoparticles and Phytochemical Analysis of *Benincasa hispida* Peel Extract

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## Abstract

*Benincasa hispida* is a member of family cucurbitaceae it has nutritional and medicinal properties. *Benincasa hispida* is considered as one of the sacred fruit in India. This fruit found to have wide applications. Current research was carried out on the evaluation of *in vitro* activities and silver nanoparticle synthesis of *Benincasa hispida* peel extract. The phytoconstituents of *Benincasa hispida* fruit peel includes alkaloids, steroids, resin and vitamins. *Benincasa hispida* fruit peel gives red florescence at 365nm (under long UV) in Benzene, Diethyl ether, Chloroform, Ethanol and acetone. Proximate analysis which includes ash value, moisture content, extractive values, total solid content and crude fiber content of fruit peel powder was determined. The pharmacological activities such as anti-arthritis, thrombolytic, anti-oxidant, anti-diabetic showed high economic value of fruit peel. The extract was found to have antifungal activity against *Trichophyton* spp., fungi responsible for athlete's foot infection. Herbal soap was prepared using *Benincasa hispida* peel extract found to be effective for the treatment of athlete's foot infection. Green synthesis of silver nanoparticles using peel extract of *Benincasa hispida* is a rapid and ecofriendly method. The characterization of silver nanoparticles, was done using FTIR, SEM, XRD, and UV-Visible spectrophotometer. The silver nanoparticles found to have maximum absorbance at 350nm and the size of nanoparticles found to be in the range 28-38nm with irregular shape. Antifungal activity against *Trichophyton* sp. (Causing Athletes foot infection) showed by silver nanoparticles. The H<sub>2</sub>O<sub>2</sub> detecting ability of silver nanoparticles was found.

## Keywords

*Benincasa hispida* peel, fluorescence analysis, H<sub>2</sub>O<sub>2</sub> detection, pharmacological activities, phytochemicals, proximate analysis, Silver nanoparticles.

\*\*\*\*\*

## 1. INTRODUCTION:

*Benincasa hispida* (synonym: *Benincasa cerifera*) [1] which commonly called as (winter melon, ash gourd,

winter gourd, white pumpkin and wax gourd. In ayurveda (Sanskrit) *Benincasa hispida* called as Kooshmanda. It is a popular vegetable crop, especially among Asian communities both for nutritional and medicinal purposes [2, 3]. Kooshmanda is widely used in India as vegetable and for making petha and candy [4]. It is specially valued as brain tonic in the treatment of mental disorders, and is an effective antidote for mercury and alcohol poisoning [5]. *Sarangdhara Samhitaa* describes its use for the treatment of haemorrhage (especially ulceration of lungs) and pulmonary consumption. Its juice is considered as specific in the first stage of consumption, while the juice from cortical portion is used in the treatment of diabetes [6]. The plant was used medicinally in various complaints such as gastrointestinal problems, respiratory diseases, heart diseases, diabetes mellitus and urinary diseases [7]. Fruits were traditionally used as a laxative, diuretic, tonic, cardiogenic, urinary calculi, blood disease, insanity, epilepsy, schizophrenia and other psychologic disorders jaundice and menstrual disorders [8]. The fruit has been valued as a nutritious vegetable as it provides a good source for natural sugars, amino acids, organic acids, mineral elements and vitamins.

Nanotechnology is an important enabling technology which has wide variety of potential applications in the biomedical, agricultural, optical and electronic fields [9]. Silver nanoparticles nowadays in the center of attraction of modern researchers because of their diversity and unique properties such as high biocompatibility [10], enhanced opto-electronic properties [11], low toxicity [12] and improved antimicrobial activity [13]. Silver nanoparticles exhibit new or improved properties depending on their size, morphology and distribution. The "Green" environment friendly processes in chemical technologies are becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental concerns [14]. Plant parts such as fruit, leaf, bark, seed, and stem extracts are being effectively used in green synthesis. Recently biosynthetic methods are used to synthesize nanoparticles by employing naturally occurring reducing, capping, and stabilizing agents such as polysaccharides, plants, bacteria, fungi [15]. Silver is the one of the most commercialized nano-material with five hundred tons of silver nanoparticles production per year [16] and is estimated to increase in few years. Biological methods are ecofriendly method used to synthesis of silver nanoparticles without used of any toxic, expensive and harsh chemical substances. Plant

extracts may acts as reducing agents and stabilizing agents in the synthesis of nanoparticles [17]. Including its profound role in field of high sensitivity biomolecular detection, catalysis, biosensors; it is been acknowledged to have strong inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory, and anti-angiogenesis activities [18, 19].

## 2. MATERIALS AND METHODS:

### 2.1 Collection of sample and solvent extraction:

The fresh fruits of *Benincasa hispida* were collected in month of January, 2019 from local vegetable market of Pune, Maharashtra.

#### 2.1.1 Extraction of *Benincasa hispida* peel:

The fruit peel was allowed to dry naturally i.e. under shade drying. After completion of drying process peel of fruit was ground in grinder [20].

##### 2.1.1 a) Cold extraction method:

Peel powder (*Benincasa hispida*) was mixed with the solvent (Methanol) in 1:10 proportion. Mixture was kept for 24hrs. It was filtered through whatman filter paper no.1. Filtrate was collected, and evaporation of the solvent was done. Extract was stored at 4°C until use.

##### 2.1.1 b) Hot extraction method:

*B. hispida* fruit peel extract was obtained using methanol solvent. Extraction was done using Soxhlet apparatus and temperature was adjusted to 50°C.

### 2.2 Determination of phytochemicals from fruit peel extract:

The qualitative tests were performed for detection of phytochemicals of *Benincasa hispida* peel extract [21].

#### 2.2.1 Qualitative test of Phytochemicals

##### i) Test for Alkaloids:

The extract was dissolved in dilute HCL and used for test. Mayer's and Wagner's test were done for the alkaloid content.

##### a) Mayer's test:

Few ml of extract was treated with of Mayer's reagent (1.36 gm of mercuric chloric and 5 gm of Potassium iodide in 100 ml water) by side of test tube were added. Then White or creamy precipitation was considered as a positive test.

##### b) Wagner's test:

Few ml of extract was treated with Wagner's reagent (1.27gm iodine, 2gm potassium iodide and 100 ml distilled water) by side of tube. Reddish brown precipitation was observed.

##### ii) Test for Flavonoids:

Few drops of dilute NaOH solution was added to 1ml of extract. Intense yellow colour solution becomes

colourless after addition of few drops of dilute HCl which indicates positive test.

**iii) Test for Phenol:**

The extract was dissolved in water and then treated with 10% FeCl<sub>3</sub> and then dark green colour formation indicates presence of phenol.

**iv) Test for Terpenoids:**

5 ml of extract was dissolved in 2ml of CHCl<sub>3</sub> and 1ml of concentrated H<sub>2</sub>SO<sub>4</sub> then reddish brown color interface was observed.

**v) Test for Steroids:**

To 1 ml extract, added 10 ml Chloroform and equal volume of concentrated H<sub>2</sub>SO<sub>4</sub> by sides. Upper layered turned into red and H<sub>2</sub>SO<sub>4</sub> layer showed yellow colour with green fluorescence.

**vi) Test for Resins:**

Extract was treated with a 3-4 ml of CuSO<sub>4</sub> solution after proper mixing for 1-2 min and green precipitation proves resins.

**vii) Test for Tannins:**

Extract was dissolved in 100ml distilled water and it was filtered and to the 1% FeCl<sub>3</sub>, then appearance of green, purple, blue or black colour indicates positive test.

**viii) Test for Quinones:**

Extract was treated with KOH solution and then blue colour was observed.

**ix) Test for Carboxylic acid:**

Extract was mixed with sodium bicarbonate solution and checked for effervescence.

**x) Test for Oxylates:**

4 ml extract was mixed with 2 ml of Acetic acid, 1 drop of FeCl<sub>3</sub> and 2 ml concentrated H<sub>2</sub>SO<sub>4</sub> brown ring indicated positive test for oxylates.

**xi) Test for Carbohydrates:**

Hot Fehling's solution was added to 4ml of extract and red brick colored precipitation indicates carbohydrate content.

**xii) Test for proteins:**

Ninhydrin reagent was added to the extract then mixture was boiled and blue colored was observed.

**2.2.2 Thin layer chromatography of phytochemicals:**

Thin layer chromatography (TLC) was carried out using methanol extract of the *B.hispida* peel. The phytoconstituents were qualitatively determined [22].

**2.3 Proximate Analysis:**

The parameters determined for proximate analysis includes ash value, moisture content, extractive value, total solid content and crude fiber content of the fruit peel powder [23].

**2.3.1 Determination of Ash values:**

**2.3.1. a) Total ash:**

2 gm of the fruit peel powder was weighed in a tarred silica dish and it was incinerated at a temperature not exceeding 450°C until free from carbon. The sample was cooled and weighed. The residue was collected on ash less filter paper was incinerated and then filtrate was evaporated to dryness and ignited at a temperature not exceeding 450°C. The

percentage of ash was calculated with reference to the air dried powder of fruit peel.

### 2.3.1. b) Acid-insoluble ash:

The ash obtained described as total ash was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on an ash-less filter paper and washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried powder of fruit peel.

### 2.3.1. c) Water soluble ash:

Ash was dissolved in 25ml water and boiled for 5 minutes. Then filtered and collected water insoluble matter was washed with hot water and dried at below 450°C for 15 minutes. Weight of water soluble ash was determined.

### 2.3.2 Moisture Content and total solid content:

Powdered peel of *Benincasa hispida* (W2, 2g) was placed in a weighed petridish (W1). The petridish was kept in a hot air oven at 60°C till constant weight (W3). The sample was placed in a desiccator after it had achieved constant weight and then weighed to determine the moisture content and total solids using the following formula:

$$\text{Moisture (\%)} = \frac{(W1+W2)-W3}{W2} \times 100$$

$$\text{Crude fiber content} = \frac{\text{Loss in weight on ignition (W2-W1)-(W3-W1)}}{\text{Weight of sample}} \times 100.$$

## 2.4. Fluorescence Analysis:

Dried peel powder of 0.5 gm was taken into clean and dried test tubes. To each tubes 5ml of different organic solvents like distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, acetic acid, sulphuric acid, nitric acid, hydrochloric acid, 5% FeCl<sub>3</sub>, 5% iodine, picric acid, 1N NaOH and 1N NaOH+ Methanol were added separately. Then all the tubes were shaken and they were allowed to stand about 20 - 25 minutes. These solution were observed under the different wavelength i.e., visible light and UV light (254nm and 365nm) for their characteristic color reaction and compared with a standard color chart and colors were recorded [24].

## 2.5. Determination of antifungal activity:

### 2.5.1. Isolation and Identification of fungi causing athletes foot infection:

The sample was collected from patient having athletes foot infection with patient's consent. Sample was collected using sterile cotton swab and placed on sterile potato dextrose agar. The culture plate was incubated at room temperature (25°C). Incubated plates were inspected for fungal growth

Where, W1 = Weight of petridish, W2 = Weight of sample, W3 = Weight of dried sample + weight of petridish

**Total solid content was determined by the following formula:**

$$\text{Total solids (\%)} = 100 - \text{Moisture (\%)}$$

### 2.3.3 Crude fiber content:

For the removal of fat, 2gm of peel powder was treated with petroleum ether. Petroleum ether was removed and powder was dried. Dried powder was boiled in 200 ml of conc. sulphuric acid for 30 minutes with bumping chips. It was filtered and washed with boiling water. It was boiled with 200 ml of sodium hydroxide for 30 mins. It was again filtered and wash with sulphuric acid, 350 ml portions of water and lastly 25 ml alcohol. Residue was removed and transferred to ashing dish (Prewighed dish W1). For drying two hours at 130±2°C. It was cooled in desiccators and weighed (W2). It was ignited for 30 minutes, at 600°C. It was cooled in a dessicator and reweighed (W3).

on daily basis. Identification was done based on microscopic and macroscopic characteristic of fungi.

### 2.5.2. Determination of antifungal activity of *Benincasa hispida* peel extract:

The fungal (*Trichophyton* spp.) culture was incubated for 48 hrs at room temperature on potato dextrose agar plate. Fungal spore suspension was prepared and adjusted absorbance to 0.2 at 600 nm. The 0.1ml suspension of test organism was spread on sterile PDA plate. Using a sterile cork borer (8mm diameter), wells was made on each plate. 50 µl of extract was added to well. Plates were kept at 4°C for 1-2 hrs for pre diffusion. Then plates were incubated at RT for 48 hrs. The zone of inhibition was observed. For control, methanol solvent was used.

### 2.5.3 Formulation using *Benincasa hispida* peel extract and testing its *in vitro* antifungal activity against *Trichophyton* spp.:

Herbal soap was prepared using basic soap ingredients and *B. hispida* peel extract. 66gm of sodium hydroxide was dissolved in 20ml of water and allowed to cool at 35-45°C. 50ml of mixture of coconut oil and almond oil were heated in water bath at 55°C. Both aqueous and oil phases were mixed

together and 10gm of *Benincasa hispida* peel extract was added. The mixture was gently stirred to form viscous gel and kept for 2days [25].

#### 2.5.3.1 Antifungal assay of herbal soap:

In potato dextrose medium containing tubes, the herbal soap and the basic soap (control) were incorporated. As a growth control, the tube containing potato dextrose broth, seeded with test organism was used. The tubes were inoculated with a *Trichophyton* fungal suspension and incubated at room temperature for 3- 5 days [26].

#### 2.6. In vitro anti-arthritic activity of *Benincasa hispida* peel extract:

Inhibition of protein denaturation was used for evaluation of in vitro anti –arthritic activity using diclofenac sodium as standard. 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of test solution (100 µg/ml of synthesized compound) was used for the preparation of 0.5ml test solution. For the preparation of test control solution (0.5ml), 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml distilled water was used. Standard solution (0.5ml) consists of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of diclofenac sodium. pH was adjusted at 6.3 using 1N HCl for all solutions. The samples were incubated at 37°C for 20 minutes and then temperature was increased to at 57° C for 3minutes. 2.5 ml of phosphate buffer was added after cooling previous solution. Using UV-Visible spectrophotometer the absorbance was measured at 416nm. The control represents 100% protein denaturation.

The percentage inhibition of protein denaturation could be calculated as:

$$\% \text{ of Inhibition} = [100 - (\text{Absorbance of test solution} - \text{Absorbance of product control})] \times 100$$

The control represents 100% protein denaturation. The results were compared with diclofenac sodium [27].

#### 2.7. In-vitro thrombolytic test of *Benincasa hispida* peel extract:

The dried peel powder was dissolved in dimethyl sulfoxide to get 100 µg/ml concentrations. 5 ml of venous blood were distributed in five different pre-weighed sterile micro centrifuge tubes (1 ml/tube) and incubated at 37°C for 45 min. The serum was completely removed after clot formation. To determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone). 100 µl of the extract was added separately into pre-weighed clot micro centrifuge tube. For positive control, 100 µl of streptokinase (SK) was used while 100 µl of distilled water was used for negative control. All the tubes were then incubated at 37°C for

90 min and observed for lysis of clot. Released fluid was removed after incubation and weights of the tubes were determined. After clot disruption, the difference in weight was calculated and then expressed percentage of clotlysis [28].

**% of clot lysis = (weight of released clot / clot weight) × 100.**

### 2.8. Antioxidant activity of *Benincasa hispida* peel extract:

The antioxidant activity *in vitro* of the methanolic extract of *Benincasa hispida* peel was carried out according to methods: free radical scavenging DPPH and Ferric reducing antioxidant power, FRAP assay [29].

#### 2.8. a) Free radical scavenging DPPH assay:

To explore the use of herbal extracts as antioxidants, the free radical scavenging activity was measured. For 0.3mM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) was prepared into methanol. 1ml of methanol and 1ml extract was added into tubes and 2ml of DPPH solution was mixed. The tubes were placed in the dark at room temperature for 30 minutes after stirred by vortex. The absorbance was measured at 517 nm. Tube containing 2ml of the DPPH methanol solution and 1ml of methanol was used for negative control.

Ascorbic acid was used as synthetic antioxidants of reference. A percentage of discoloration of the DPPH in solution of methanol was estimated by the capacity of the antioxidant to scavenge the free radical.

The percentage of antioxidant activity was determined according to the following equation:

**% Antioxidant activity = (Abs control - Abs sample / Abs control) × 100**

#### 2.8. b) Ferric reducing antioxidant power, FRAP assay:

In FRAP assay the reduction of Fe<sup>3+</sup> TPTZ (2, 4, 6-Tripyridyl-S-Triazine) complex (colorless complex) to Fe<sup>2+</sup>-tripyridyltriazine (blue coloured complex) formed by the action of electron donating antioxidants at low pH. The preparation of FRAP reagent was done by mixing 300 mM acetate buffer, 10 ml TPTZ in 40mM HCl and 20 mM FeCl<sub>3</sub> .6H<sub>2</sub>O (in the proportion 10:1:1 ) at 37 °C. 5µl of the peel extract was added to freshly prepared FRAP reagent & ferric tripyridyl triazine ( Fe<sup>3+</sup> TPTZ) complex was reduced to ferrous ( Fe<sup>2+</sup>) form was given intense blue color complex For reagent blank , 3.995 ml FRAP reagent and 5 µl distilled water were added and

incubated for 30 minutes at 37° C . The absorbance was measured at 593 nm.

### 2.9. *In vitro* anti- diabetic activity *Benincasa hispida* peel extract:

Anti-diabetic activity was determined by using assay based on inhibition of glucose and amylase activity.

#### 2.9.1 Inhibition assay for amylase activity:

Four concentrations of plant extract were prepared by dissolving in double distilled water. These were 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. A total of 500 µl of plant extract and 500 µl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (0.5mg/ml) were incubated for 10 minutes at 25°C. After pre-incubation, 500 µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at 5s intervals. This reaction mixture was then incubated for 10 minutes at 25°C. 1ml of DNSA colour reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10 ml distilled water following which absorbance was measured at 540nm [30].

$$\% \text{ inhibition} = \frac{(A_{540} \text{ CONTROL} - A_{540} \text{ EXTRACT})}{A_{540} \text{ CONTROL}} \times 100$$

#### 2.9.2. Glucose diffusion inhibitory study:

A dialysis membrane ( Dialysis membrane -70 approximately capacity 2.41ml/cm) containing prepared *Benincasa hispida* peel extract and glucose solution (0.2 Mm in 015 M Sodium chloride ) was kept in beaker which contains 40 ml of 0.15 M sodium chloride and 10 ml distilled water. The beakers were placed at room temperature on orbital shaker and glucose movement into external solution was observed. The protocol was carried out in triplicates [30].

### 2.10. Synthesis and characterization of silver nanoparticles using *Benincasa hispida* peel extract:

1mM solution of AgNO<sub>3</sub> (0.017gm) silver nitrate was dissolved in 100ml deionized (DI) water and then added 10 ml soxhlet methanol extract of *Benincasa hispida* peel. The reacting solution was incubated in darkness and gradual change in color of solution was observed. After 2 days incubation the intense brown coloured colloidal mixture was centrifuged at 20,000 rpm for 30min. After centrifugation pellet formed at the bottom of centrifuge tube were carefully re-dispersed in small amount of DI water. The colloidal particles dried to get powder of silver nanoparticles.

Using UV-Visible spectrophotometer, the absorption spectra of the solution was studied. Chemical composition of synthesized silver nanoparticles was studied by using FTIR spectrometer. The size of synthesized silver nanoparticles was determined by X-ray diffraction spectroscopy. The morphological features of synthesized silver nanoparticles were studied by SEM (scanning electron microscopy) [31].

### 2.10.1. Colorimetric sensing H<sub>2</sub>O<sub>2</sub> detection using *Benincasa hispida* peel extract:

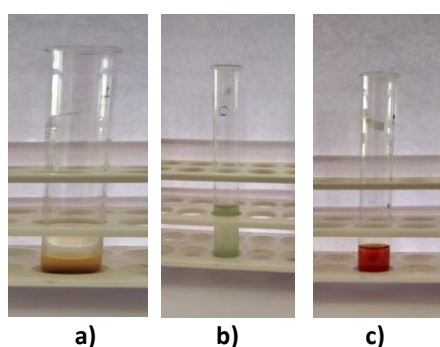
1 ml of aqueous solution of H<sub>2</sub>O<sub>2</sub> (10 mM) was added to solution of silver nanoparticles. The colour of

nanoparticles changed from brown to colorless instantly after addition of H<sub>2</sub>O<sub>2</sub> solution. The stock solution of H<sub>2</sub>O<sub>2</sub> (10mM) was diluted in required amount of de-ionized water to prepare 5 different concentration solution of H<sub>2</sub>O<sub>2</sub> (i.e.10<sup>-1</sup> to 10<sup>-4</sup>) for assessing the minimum detectable concentration of H<sub>2</sub>O<sub>2</sub> [32].

## 3. RESULTS AND DISCUSSION:

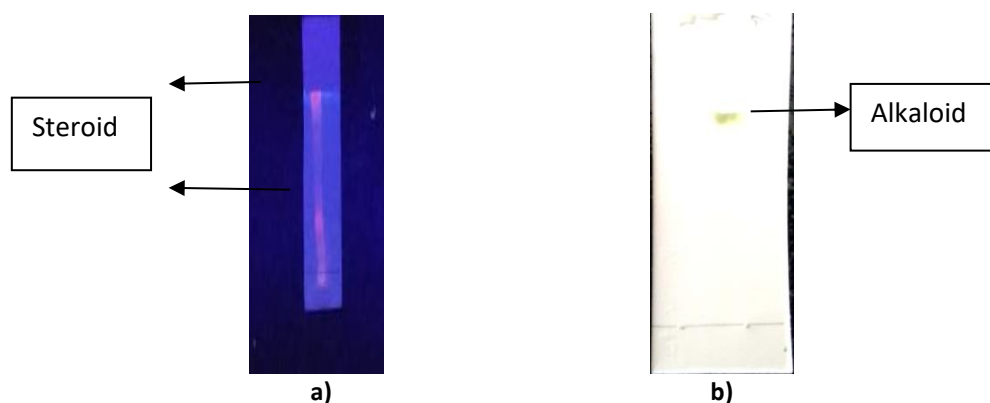
**3.1** Fruit peel of *B. hispida* was dried and powdered. Methanol solvent was used for the extraction of phytochemicals.

**3.2.1 Phytochemical screening:** The *Benincasa hispida* peels methanol extract found to contain alkaloids, steroids and resins as shown in Fig.1.



**Fig. 1.** Qualitative test for phytochemicals of *Benincasa hispida* peel extract. a) Test of Alkaloids b) Test of Resins c) Test of Steroids.

**3.2.2 TLC of *Benincasa hispida* peel extract.:** TLC was carried out for the determination of bioactive compounds in *Benincasa hispida* peels methanol extract.



**Fig. 2 TLC of phytochemicals: a) TLC of steroid:** TLC of steroids in Methanol extract of *Benincasa hispida* peel using (chloroform: Ethanol: Water) solvent system and UV light used as developer. **b) TLC of alkaloids:** TLC of alkaloids in methanol extract of *Benincasa hispida* peel using (Ethyl acetate: Chloroform: Water) solvent system and Mayer's reagent used as developer.

*Benincasa hispida* peels methanol extract found to contain steroids with 0.8 Rf value and 0.4 Rf value and alkaloids with 0.9 Rf value.

**3.3 Proximate Analysis of *Benincasa hispida* peel extract:** Ash value is important factor in determining the authenticity as well as purity of *Benincasa hispida* peel powder. The total ash value, acid insoluble ash,



water soluble ash was found to be 47%, 38.29% and 23.40% respectively. (Table no.2).

**Table 1: Proximate Analysis of *Benincasa hispida* peel extract**

Experimental studies	Observation for powdered peel of <i>Benincasa hispida</i>
Total ash	47%
Acid –insoluble ash	38.29%
Water soluble ash	23.40%
Moisture content	11%
Total solid content	89%
Crude fiber content	20%

**3.4 Fluorescence Analysis of *Benincasa hispida* peel extract:** The organic solvents such as diethyl ether, benzene, chloroform, acetone & ethanol showed red fluorescence under long UV light (365nm).

**Table 2: Fluorescence Analysis of *Benincasa hispida* peel extract**

Solvent	visible	Short UV 254nm	Long UV 365nm
Hexane	Green	Green	Green
Methanol	Green	Green	Green
Ethyl acetate	Green	Green	Green
Sulphuric acid	Black	Black	Black
Benzene	Green	Green	Red fluorescence
1N NaOH	Greenish brown	Greenish brown	Greenish brown
Diethyl ether	Green	Green	Red fluorescence
Nitric acid	Green	Green	Green
Chloroform	Green	Green	Red fluorescence
HCL	Black	Black	Black
Acetic acid	Green	Yellow	Brown
Water	Light green	Light green	Light green
Picric acid	Yellowish green	Yellow	Yellow
Ethanol	Green	Green	Red fluorescence
Iodine	Green	Green	Light green
5% FeCl <sub>3</sub>	Dark green	Brown	Brown
Acetone	Green	Green	Red fluorescence
1N NaOH+ Methanol	Green	Green	Brown



**Fig. 3: The fluorescence analysis was observed under long UV (365nm).**

### 3.5 Determination of antifungal activity:

**3.5.1 Isolation and identification of fungi causing athletes foot:** Patients foot skin was used for the isolation of fungi causing athletes foot infection. White growth of fungi was observed on potato

dextrose agar after 48 hours of incubation. Lactophenol cotton blue staining of fungi was done for the identification of fungi. Both macro and micro conidia were observed which showed resembles with *Trichophyton* spp.



Fig. 4: Cotton Blue staining of athlete's foot infection causative agent *Trichophyton* spp.

**3.5.2 Antifungal activity of *Benincasa hispida* peel extract:** The extract of *Benincasa hispida* peel showed activity against fungal strain (*Trichophyton* spp). The activity of methanol extract of *Benincasa*

*hispida* peel showed significant antifungal activity by using well diffusion method. Zone of inhibition found to be 9mm.



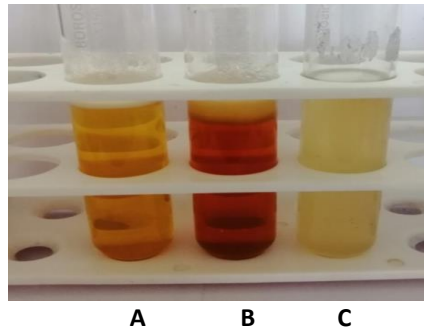
Fig. 5: Antifungal activity using *Benincasa hispida* peel extract against *Trichophyton* spp by well diffusion method.

**3.5.3. Antifungal assay of herbal soap of *Benincasa hispida* peel extract:** Antifungal activity of the basic soap and herbal soap was compared for its activity against *Trichophyton* spp. Extensive growth was observed in potato dextrose broth inoculated with

*Trichophyton* and tube containing basic soap plus inoculum of *Trichophyton*. No Fungal growth was observed in potato dextrose broth containing herbal soap and *Trichophyton* inoculum.



Fig.6 Formulated herbal soap of *B.hispida* peel extract.



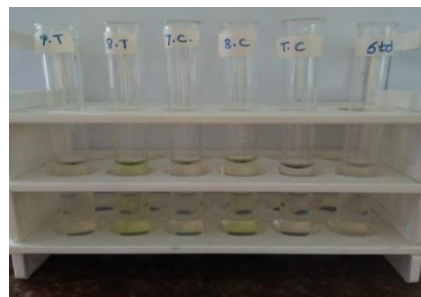
**Fig.7: Antifungal assay of formulated soap against *Trichophyton* spp.** a) Tube containing extract soap without fungus b) Tube containing extract soap containing growth of fungus c) Tube containing growth of *Trichophyton* in potato dextrose broth.

**3.6. *In vitro* anti-arthritis activity of *Benincasa hispida* peel extract:** *Benincasa hispida* peel hot and cold extracts of methanol and diclofenac sodium were tested for anti-arthritis activity and found significant percentage inhibition in protein denaturation.

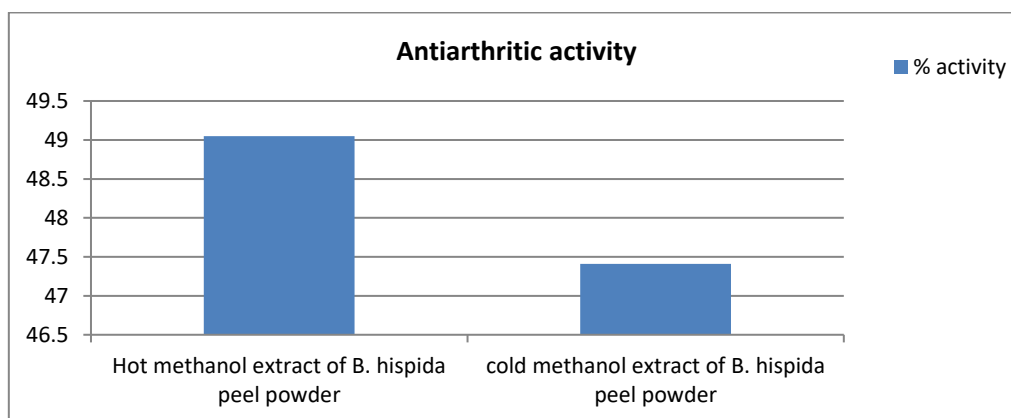
*In vitro* protein denaturation test, the *Benincasa hispida* have shown significant activity and its effect was compared with the standard drug diclofenac sodium. The maximum percentage inhibition of protein denaturation was observed as 66% methanol extract and 55.5% for Soxhlet methanol extract.

**Table 3: *In vitro* anti-arthritis activity of *Benincasa hispida* peel extract:**

Extract	% of inhibition $\pm$ SD
<i>Benincasa hispida</i> peel cold extract methanol	66 $\pm$ 0.288
<i>Benincasa hispida</i> peel hot extract methanol	55.5 $\pm$ 0.2013



**Fig.8 *In vitro* anti-arthritis activity of *Benincasa hispida* peel extract.**



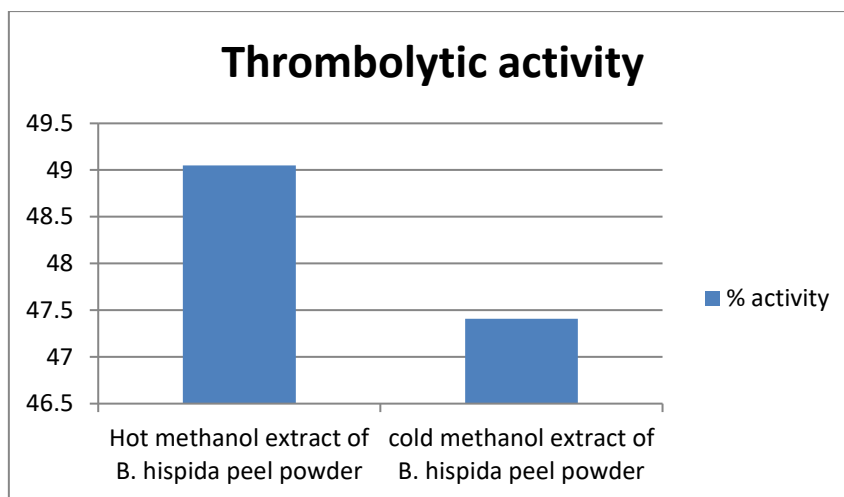
**Fig. 9 *In vitro* anti-arthritis activity of *Benincasa hispida* peel extract.**

**3.7. In vitro anti-thrombolytic activity of *Benincasa hispida* peel extract:** *Benincasa hispida* peel hot and cold extracts of methanol showed significant thrombolytic activity with lysis of clot. In *in-vitro* thrombolytic test, *Benincasa hispida* possesses

significant thrombolytic activity. So that, we may assume that these extracts can be considered as a potential source of natural anti-arthritis as well as thrombolytic agent

**Table 4: In vitro anti-thrombolytic activity of *Benincasa hispida* peel extract:**

Extract	Wt. Of empty tube ( A ) gm	Wt. of with clot ( B ) gm	Wt. of clot C=B-A	Wt. of clot after lysis (D)gm	Wt. of lysis release clot (B-D)gm	% of clot lysis
Hot methanol extract of <i>Benincasa hispida</i> peel powder	1.04	2.100	1.06	1.58	0.52	49.05 ± 0.13
Cold methanol extract of <i>Benincasa hispida</i> peel powder	1.03	2.190	1.16	1.64	0.55	47.41± 0.11



**Fig .10 Thrombolytic activity of *Benincasa hispida* peel extract.**

**3.8. Antioxidant activity of *Benincasa hispida* peel extract:** FRAP and DPPH assays were used for the evaluation of antioxidant activity of *Benincasa hispida* peel extract.

58.9% antioxidant activity by free radical scavenging DPPH method. The cold methanol extract of *Benincasa hispida* peel gives 74.3% antioxidant activity by free radical scavenging DPPH method.

**3.8.a) Free radical scavenging DPPH:** The hot methanol extract of *Benincasa hispida* peel gives

**Table 5: Free radical scavenging DPPH of *Benincasa hispida* peel extract**

Extract	Absorbance at 517nm	% antioxidant activity
Hot methanol extract of <i>Benincasa hispida</i> peel powder	0.16	58.9%
Cold methanol extract of <i>Benincasa hispida</i> peel powder	0.10	74.3%
Standard	0.39	100%

**3.8. b) Free reducing antioxidant power (FRAP) of *Benincasa hispida* peel extract:** The FRAP value for hot methanol extract of *Benincasa hispida* peel gives 0.60mg/ml. The FRAP value for cold methanol extract of *Benincasa hispida* peel gives 0.21mg/ml.

**Table 6: Free reducing antioxidant power (FRAP) of *Benincasa hispida* peel extract:**

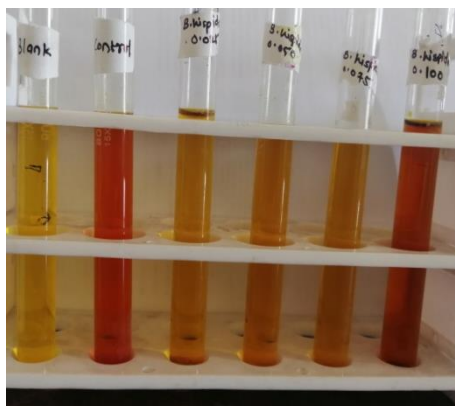
Extract	Absorbance at 593nm	TEAC (mg/gdw)
Hot methanol extract of <i>Benincasa hispida</i> peel powder	0.60	0.60 ± 0.01
Cold methanol extract of <i>Benincasa hispida</i> peel powder	0.21	0.21± 0.015

TEAC (Trolox equivalents antioxidant capacity) (mg, Trolox equivalents/gdw).

**3.9 In vitro anti- diabetic activity of *Benincasa hispida* peel extract:**

**3.9.1 Inhibition assay for  $\alpha$ - amylase activity of *Benincasa hispida* peel extract:** % Inhibition of  $\alpha$ -amylase enzyme brought about by methanolic extract of varying concentrations of *Benincasa hispida* peel as compared to a control. In context of

the discussion, it would be interesting to investigate the causative components mechanism for clot lysis by these Peel extracts and for protein denaturation activity. *Benincasa hispida* peel showed the maximum inhibition of  $\alpha$ -amylase activity in various concentrations (table no.10).



**Fig.11 Inhibition assay for  $\alpha$ - amylase activity of *Benincasa hispida* peel extract.**

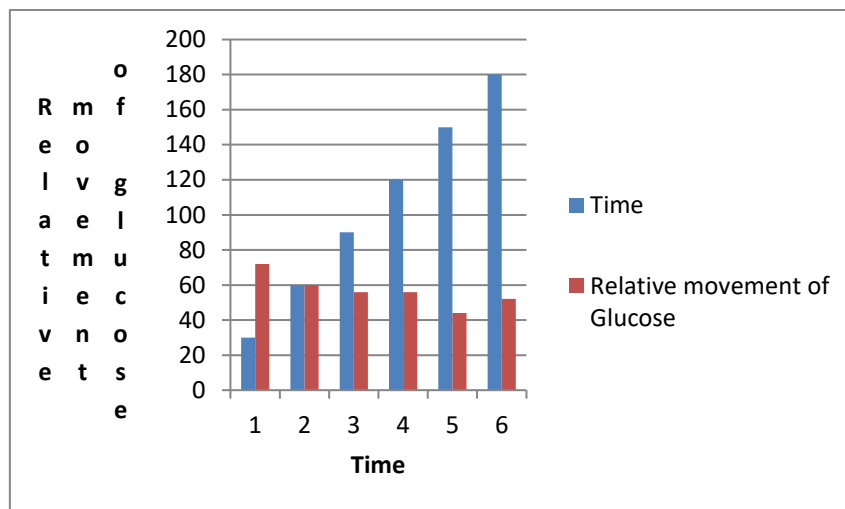
**Table 7: Inhibition assay for  $\alpha$ - amylase activity of *Benincasa hispida* peel extract:**

Concentration (gm/ml)	Absorbance 540nm	<i>Benincasa hispida</i> peel %
0.025	0.15	71% ± 0.15
0.050	0.22	65% ± 0.15
0.075	0.25	63% ± 0.23
0.100	0.51	25% ± 0.15

**3.9.2 Glucose diffusion inhibitory study of *Benincasa hispida* peel extract:**

**Table 8: Glucose diffusion inhibitory study of *Benincasa hispida* peel extract:**

Sr no.	Time ( min)	Control ( Glucose +Buffer)	Relative movement of glucose %
1	30	0.25	72
2	60	0.25	60
3	90	0.25	56
4	120	0.25	56
5	150	0.25	44
6	180	0.25	52



**Fig.12** Glucose inhibitory study of *Benincasa hispida* peel extract.

### 3.10. Synthesis and Characterization of silver nanoparticles using *Benincasa hispida* peel extract:

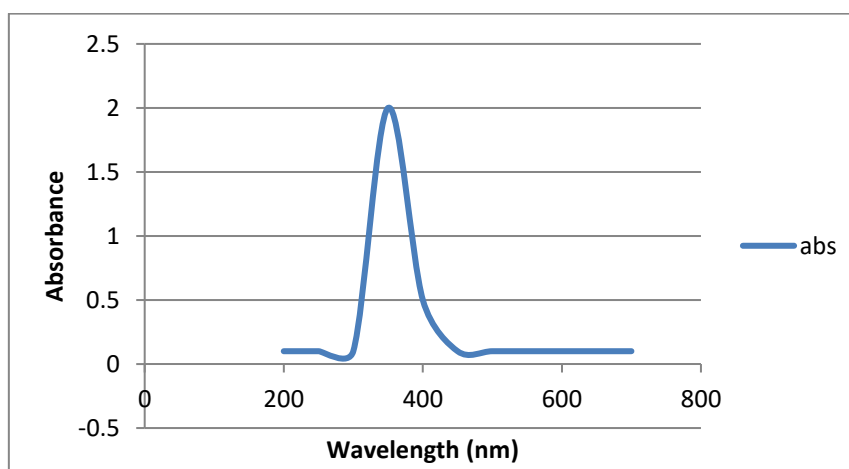
*Benincasa hispida* peel extract was tested for its potential to serve as reducing agent.



**Fig.13** Synthesis of silver nanoparticles.

#### 3.10.1 UV-Spectrum analysis of synthesized silver nanoparticles:

The UV-vis spectra of silver nanoparticles synthesized by *Benincasa hispida* peel are shown in figure. In the UV-vis absorption spectrum, a distinct peak observed at 350 nm.



**Fig.14** UV-Spectrum analysis of synthesized silver nanoparticles.

**3.10.1. Fourier transforms infrared spectroscopy (FTIR) of synthesized silver nanoparticles:** FTIR spectroscopy of synthesized silver nanoparticles shown in fig.7. FTIR is an important tool for the identification of functional groups. The bands

observed at  $2917.06\text{ cm}^{-1}$ ,  $1378.47\text{ cm}^{-1}$  and  $1031.85\text{ cm}^{-1}$  were assigned to C-H stretch, N=O bend and C-N stretch vibration of the alkanes and aliphatic amines, respectively [32].

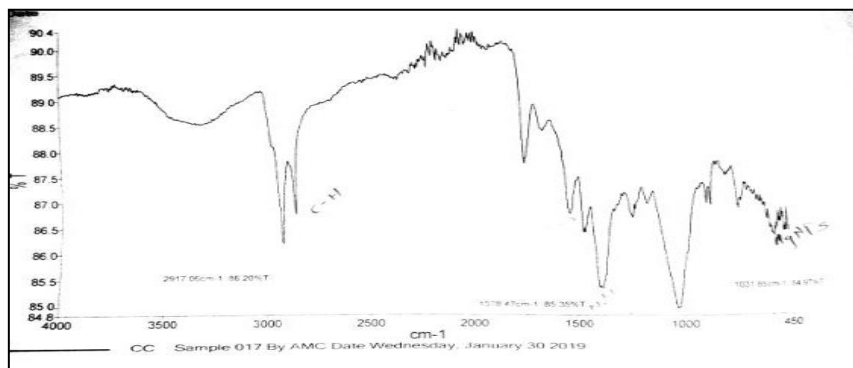


Fig.15 FTIR of synthesized silver nanoparticles.

**3.10.2. XRD analysis of synthesized silver nanoparticles:** Silver nanoparticles was confirmed by the analysis of XRD pattern as shown in Fig.17. The three distinct diffraction peaks at 2 theta values of  $27.56^\circ$ ,  $31.89^\circ$  and  $45.85^\circ$  can be indexed to the

(1148.62), (1623.69) and (728.97) reflection planes of face centred cubic structure of silver. Biosynthesized silver nanoparticles found to have size between 28 to 38 nm.

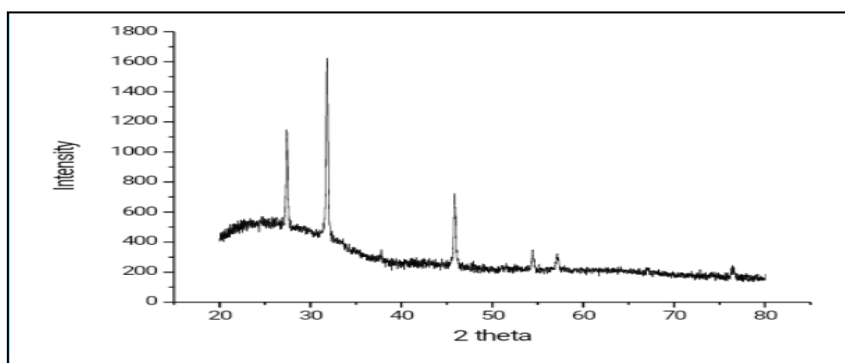


Fig.16 XRD curve of synthesized silver nanoparticles.

**3.10.3 SEM analysis of synthesized silver nanoparticles:** SEM analysis of colloidal silver nanoparticles is shown in Fig.16. The synthesized

silver nanoparticles were found to be in irregular shape.

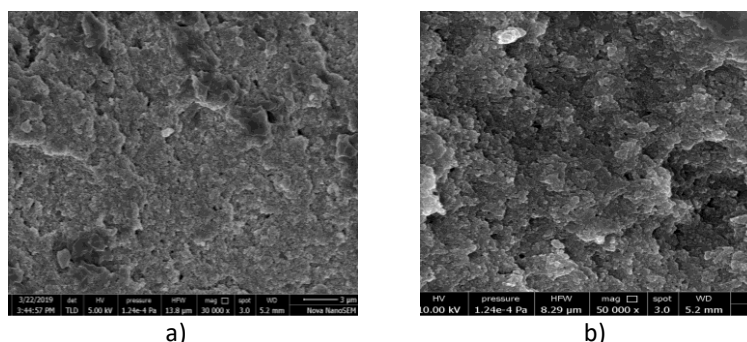


Fig.17 a) & b) SEM images of synthesized silver nanoparticle by using *B. hispida* peel extract.

### 3.10.4 Colorimetric sensing H<sub>2</sub>O<sub>2</sub> detection using *Benincasa hispida* peel extract:

The colorimetric detection was performed using various concentration of H<sub>2</sub>O<sub>2</sub> and the result indicated that the minimum detectable concentration of H<sub>2</sub>O<sub>2</sub> that may be visually by this process was 1mM.

#### 4. CONCLUSION:

The methanol extract of *Benincasa hispida* peel contained steroids, resins, alkaloids and this extract exhibited effective antifungal, antioxidant, arthritic, thrombolytic activities. Soap formulation of methanol extract of *Benincasa hispida* peel gives effective antifungal activity. The present study concluded that the plant *Benincasa hispida* peel can be excellent source of silver nanoparticles. For the conformation of silver nanoparticles color change was observed. Characterization of synthesized silver nanoparticles was done by using UV-Visible spectrophotometer, SEM, XRD and FTIR spectroscopy.

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