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Isolation & Purification Of Benincasa Hispida Leaves For Anti-Inflammatory Activity

1.Dr. Shoheb Shakil Shaikh[1*] 2.Nazmin Nisar Shaikh 3. Moin Akil Shaikh 5.Sarah Nihal Shaikh 4.

1. Associate Professor, Siddhi's Institute of Pharmacy, Thane 421401.

2. Research Scholar, Pravara Rural College of Pharmacy, Loni.

3. Research Scholar, Vaman Rao Ithape Institute of Pharmacy, Sangamner.

4. Vidyaniketan Institute of Pharmacy, Bota Sangamner.

Abstract:

From the thousands of years, the use of *Benincasa hispida* fruit from Cucurbitaceae family is for medicinal purpose. It is very popular vegetable for nutritional purposes. The fruit of *Benincasa hispida i*s mostly use for various type of medicinal problem as like respiratory system, gastrointestinal problem, heart diseases, urinary function and diabetes. The plant shows many pharmacological activities as central nervous effect, anti-oxidant, anti-inflammatory, analgesic, anti-asthmatic, diuretic, nephron protective, antidiabetic, hypolipidemic and antidepressant etc. This plant mainly in Asian communities is mostly use as food because of its nutrition and medicine value. There are lot of study on the fruit part of this plant so that's why now this research article present the Pharmacognostical account as well as physicochemical evaluation, which shows the effect in the treatment of disease by using the leaf part of this plant. Leaf of this plant is also having the various medicinal use. So, the *Benincasa hispida* leaves is phytochemically evaluated using the ethanol extract of leaves to show which secondary metabolite are present and to check the anti-inflammatory activity. The result shows presence of alkaloids, tannins, phenol and flavonoids. So, the leaves of *Benincasa hispida* responsible for different pharmacological activity.

Keywords: *Benincasa hispida*, Physicochemical, Alkaloids, Tannin, Flavonoids, Phenol, Anti-inflammatory activity.

Introduction:

Nature is the best source of the medicine. There are many sources as plant, animal and minerals which are obtained from the nature and this are helpful for treating various disease. Medicinal plants are presently in demand and their acceptance is increasing progressively[1]. From this growing medicinal plant use the *Benincasa hispida* is the most popular plant use for the medicinal purpose and pharmacological activity[2]. Plants are reservoir of potentially useful chemical compound which serve as drug. So, the herbal medicine is promising choice over modern synthetic drugs. They show very less side effect[3]. The fruit of *Benincasa hispida* is mainly contain flavonoids, terpenoids, carbohydrates, alkaloids and phenol etc. So, it is useful in the various disease treatment as like bleeding disorder, epilepsy, insanity and nervous disorder[4]. A lot of work has been done on the various parts of the *Benincasa hispida* but there is no any record on the Pharmacognstic parameter of leaves. Also, the physicochemical and phytochemical evaluation helps to show which secondary metabolites are present in leaves and due to presence of this secondary metabolite such as alkaloids, flavonoids, tannins and phenol of leaves of *Benincasa hispida* is helpful in showing various pharmacological activities.

Material and methods Collection of plant material

The leaves of *Benincasa hispida* plant (Family- Cucurbitaceae) was collected from the local area of Loni (BK) Ahmednagar,

Maharashtra, India. The plant was authenticated at the herbarium of Department of Botany and Research Centre, Padmashri

Vikhe Patil College of Arts, Science and Commerce, Loni, Pravaranagar-413 713. With the Ref No: PVPC/Bot/2020-21/IID15.

Chemicals and Reagents

Various type of chemical and reagents were used to carry out the research work.

Preparation of Benincasa *hispida* leaves extract

The leaves of *Benincasa hispida* was collected. Then dried it and this dried leaves then pulverized in grinder to make coarse powder which used for the extraction.

- Method: Soxhlet extraction
- Solvent: Ethanol.

Taking the 100 gm of powder drug of *Benincasa hispida* leaf and extracted with 100ml ethanol at 70 c for 48 hrs continuously. After the 48 hrs of extraction, round bottom flask was cooled at room temperature and the extract were filter and collected. Evaporated by using rotary evaporator. Then dried this extract and store at 4°c until further use.

Figure No 1: Extraction Process of *Benincasa hispida* Leaves.

Pharmacognstic study :

The plant materials were studied for different parameter as given below:

- Macroscopic feature
- Microscopic examination

Macroscopic feature of *Benincasa hispida* leaves:

Various type of organoleptic and morphological characters of *B. hispida* leaves are as colour, shape, size, taste, odour and fracture etc were studied[5].

Microscopic feature of *Benincasa hispida* leaves:

The green leaves are used for microscopy of the *Benincasa hispida*. Taking the thin section of leaf that is transvers section which take by blade. It was then stained by the phloroglucinol and hydrochloric acid. Before treating this, it was clear by the clearing agent i.e. chloral hydrate which will remove the dirt and the green part of leaves. The glycerine should used to prevent the sample drying. Different reagent was use for the different structural components. The stains should be just under the coverslip and not anywhere else for the purpose of exact focusing. Different cell component are studied as per the standard methods [6,7]. So the different layers of cells and identifying characters were observed and then photomicrography was done. **Powder microscopy**

The dried powder of leaves was observed under the microscope. By using different staining reagent for the detection of lignified components like stomata, trichome etc were detect under the microscope[8].

Physico-chemical standards

Physico-chemical standard of leaves sample were carried out using aqueous and other organic solvent like methanol and ethanol[8].

Priliminary qualitative phytochemical screening for extracts

This preliminary phytochemical screening of leaves was carried out using aqueous and the organic solvent. Various type of phytochemical test was carried out that are Molisch 'test, Benedict's test, Fehling's test, Barford's test, Selwinoff's test, Dragendroff's test, Hager' test, Mayer's test, tannic acid test, Wagner's test, Sulphuric acid test, Lead acetate test, Bromine test, Salkowaski test etc are the test taken for presence carbohydrate, reducing sugar, monosaccharides, hexose sugar, alkaloids, flavonoids, tannins and steroid using standard procedure[8].

Pharmacological activity Anti-inflammatory activity[9]:

Dose selection- Dose were selected according to previous paper, It was found that the dose increasing up to 2000mg/kg body weight shown no toxicity or mortality in experimental animals. The LD50 of ethanolic extract is 100mg/kg B.W.

Carrageenan-induced rat paw edema

The carrageenan -induced rat paw edema assay was carried out according to N.S. Gill K. Dhiman et al 2010. Wister rats has separated into 5 groups each of consisting 6 animals.

Group 1: (Disease Control): Carrageenan (1%) was given in the planter surface of rat.

Group 2: (Standard): Diclofenac sodium (12.5mg/kg, p.o)

Group 3: (EEBH 100mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (100mg/kg, p.o)

Group 4: (EEBH 200mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (200mg/kg, p.o) **Group 5:** (EEBH 300mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (300mg/kg, p.o)

Edema was induced on the left hind paw of the rats by subplantar injection of 0.1ml of a solution of 1% (w/v) carrageenan in 0.9% NaCl(w/v). The paw volume was measured at intervals of 60, 120, 180 min by the mercury displacement method using a plethysmograph after administration of extract/drug orally. The percentage inhibition of paw edema in drug treated group was compared with the carrageenan control group and calculated. Increase in the linear diameter of left hind paw were taken as an indication of paw edema. After this edema formation the extract should be given to that rat group by above procedure. So after the given time intervals the reading was calculate.

The percentage inhibition of the inflammation was calculated from the formula: 13%inhibition=[(VT-VO) control-(VT-VO) treated groups] / (VT-VO) control*100, where Vo represents paw volume of the rat before administration of carrageenan, VT represents paw volume of the rat after administration of carrageenan at different time intervals.

Statistical Analysis

The statistical analysis were done for the results obtained from the anxiolytic activity of different doses of ethanolic and aqueous extract .Results of the experiments and observations will be expressed as Mean ± Standard Error of Mean (SEM). The significance differences between groups will be determined using one-way analysis of variance (ANNOVA) followed by at least one of the following Dunnet's tests: . * P< 0.05, **P< 0.01, ns-not significant when compared to vehicle treated group.

Result and discussion Pharmacognostical study Macroscopic study of leaves

- Color – Green
- Odour - Characteristics
- Taste - Bitter
- Size – 9.8 to 14.1 cm in length.

Fig No 2: *Benincasa hispida* leaf.

The different pharmacognostical parameters were studied such as Color, Odor, Taste, Size, Shape and the results were obtained as Green, Characteristics, Bitter, 9.8cm long respectively.

Microscopic study of leaves

Epidermal cell- The upper epidermal cells are polygonal and the lower epidermal cell are irregular.

Stomata- There are three types of stomata present in the leaf that are anomocytic, tetracytic and anisocytic.

Stomatal index- 29.03-40.0(32.39±5.11)

Trichomes- glandular and covering trichomes are present.

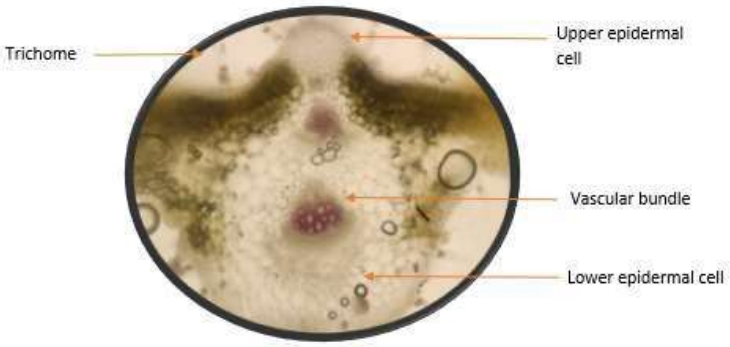

Fig No 3: T.S of *Benincasa hispida* leaf.

Powder Study

In powder characteristic of *Benincasa hispida* leaves appear the pinkish colour to the vascular bundles, then there are presence of stomata and the shape of trichomes. Also, the brownish colour shows the polygonal epidermal cells.

Fig(4)c-Stomata

Fig(5)a-Trichomes

Fig (6)b-Epidermal layer

Physico-chemical standards

There is various parameter study should done in the physico-chemical standard that are LOD, total ash, acid insoluble ash, water soluble ash and extractive values were determined as mention in table 1.

Parameter	Value obtained on dry weight basis (% w/w)
Loss on drying	0.2%
Total ash	30%
Water soluble ash	30%
Acid insoluble ash	20%
Water soluble extractive	16%
Alcohol soluble extractive	32%

Table No 1: Physico-Chemical Standard of *Benincasa Hispida* Leaves.

Preliminary phytochemical screening

Preliminary phytochemical screening of methanolic extract *Benincasa hispida* leaves shows the presence of various phytochemicals which are in table no 2. The methanolic extract shows the presence of alkaloids, phenol, glycoside, tannin and flavonoid etc. By this phytochemical study it concludes the presence of compound in the leaf extract which exerted medicinal uses.

Types of phytoconstituent	Methanol extract
Alkaloids	+
Carbohydrates	-
Tannins	+
Flavonoids	+
Proteins	-
Phenols	+
Glycosides	+
Steroids	-
Saponins	-

Table No 2: Preliminary Phytochemical Screening. (+)- Present, (-)- Absent

Anti-inflammatory activity:

Carrageenan-induced rat paw edema:

Effect of Benincasa hispida leaves extract on carrageenan induced rat paw edema.

Group	Paw size(mm)at different time intervals(min)after carrageenan inducted paw edema.			
	0min	60min	120min	180min
Control	3.77±0.12	7.25±0.08	7.15±0.09	7.03±0.13
DICLO (12.5mg/kg)	3.80±0.32	7.27±0.28*	6.12±0.13**	5.30±0.13***
EEBH (100mg/kg)	3.81±0.21	7.24±0.27	6.92±0.16	6.10±0.10*
EEBH (200mg/kg)	3.78±0.31	7.26±0.26	6.63±0.17*	5.70±0.09**
EEBH (300mg/kg)	3.79±0.41	7.27±0.11*	6.45±0.14**	5.42±28***

Table 3: Anti-inflammatory activity by Carrageenan induced rat paw edema.

All values are mean ±SEM (n=6); *p< 0.05 when compared to control. Ethanolic extract of Benincasa hispida leaves respectively.

(DICLO- Diclofenac, EEBH- Ethanolic extract of Benincasa hispida).

(EEBH 100mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (100mg/kg, p.o)

(EEBH 200mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (200mg/kg, p.o)

(EEBH 300mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (300mg/kg, p.o).

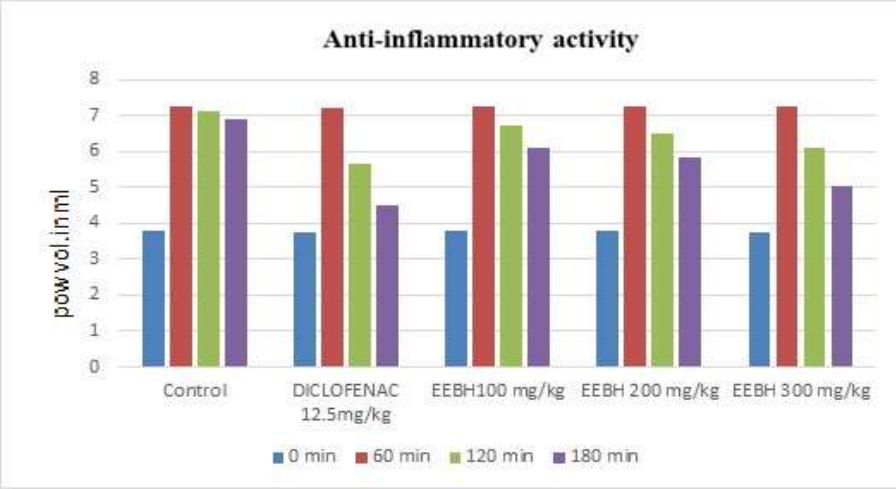

Figure: Effect of Benincasa hispida leaves ethanolic extract on carrageenan induced rat paw edema.

(EEBH 100mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (100mg/kg, p.o)
(EEBH 200mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (200mg/kg, p.o) (EEBH 300mg/kg, p.o): Ethanolic extract of *Benincasa hispida* (300mg/kg, p.o).

Conclusion

The leaves of this Benincasa *hispida* evaluated using the methanolic extract which shows the presence of alkaloids, tannins and flavonoids in maximum quantity. Until in very less quantity the glycosides are present. The above study concludes that the *Benincasa hispida* leaves shows the significant anti-inflammatory activity on carrageenan induced rat paw edema model. The ethanolic extract at 300mg /kg dose shows the significant anti-inflammatory activity as compare to standard.

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Conflict of interest: Authors have declared that no conflict of interest exists.

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