ANTHELMINTIC SCREENING OF *BENINCASA HISPIDA* (THUNB.) FRUIT AGAINST *PERITIMA POSTHUMA*

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**ABSTRACT**

In this study fruit of *Benincasa hispida* (Thunb.) Cogn. (Cucurbitaceae) have been investigated for anthelmintic properties against *Pheritima posthuma* (Indian earthworms). The pulverized fruit powder of *Benincasa hispida* was subjected to successive extraction in a Soxhlet apparatus using benzene, chloroform, alcohol and water. Three dilutions with concentration of 1.0 mg/ml, 5.0 mg/ml and 10.0 mg/ml of all extracts; standard Piperazine citrate (10 mg/ml) and Control (Normal saline) were screened to record the time taken to paralyse (P) and kill (D) the worms. The worms which were treated with aqueous extract has shown the significant effect causing paralysis and death of worms at concentration of 100 mg/ml as compared to the standard and control.

**KEYWORDS:** *Benincasa hispida* Fruit, Anthelmintic activity, *Pheritima posthuma*.

**INTRODUCTION**

*Benincasa hispida* (Thunb.) Cogn. belongs to Cucurbitaceae family.\(^1\) It is called as Winter melon, Ash gourd, Wax gourd, Kondol in English, Kusmandah in Sanskrita, Petha in Hindi, Kohala in Marathi language.\(^2, 3\) The plant is possibly indigenous to Malaysia, now obtained all over the tropics. It is cultivated in India, Burma, Ceylon, and on the hills up to 4,000’. A large annual or biennial trailing gourd climbing by means of tendrils; Fruits broadly cylindric 30-80cm long, hairy throughout, eventually covered with a waxy bloom; Fruit contains numerous white colored embedded seeds.\(^2, 4, 5\)

The fruits are sweet, cooling, styptic, laxative, diuretic, tonic, aphrodisiac and antiperiodic. They are useful in asthma, cough, diabetes, haemoptysis (haemophysis), heamorrhages from internal organs, epilepsy, fever and vitiated conditions of pitta. The seeds are sweet, cooling and anthelmintic, and are useful in dry cough, fever, urethrorrhea, syphilis, hyperdipsia and vitiated conditions of pitta. According to an old Korean medical encyclopedia, the “Donguibogam”, the *Benincasa hispida* is effective against diabetes, dropsy, diseases related
to liver, leucorrhea, and good for the detoxication of minerals, the removal of fever, and to strengthen the function of bladder and small and large intestines.\[^2,6\]

It is also utilized to make candy termed ‘Petha’ in North India and Pakistan. In South Indian cooking, it is used to prepare curries. It is used to amplify appetite; the fresh juice of *Benincasa hispida* is used to treat kidney stones in Ayurvedic medicines. The seeds boiled in cow milk are administered to raise “sperm count” and to advance “sperm locomotion”. The soup made by cooking fruit pulp with pork short ribs helps for increasing the milk in lactating women.\[^7\] It is used as anthelmintic, insanity (psychosis) and other nervous diseases, and as a bitter tonic, in India and China. Fruits are suggested in Ayurvedic remedies for the treatment of peptic ulcers. The seed of winter melon are utilized as “vermifuge”.\[^1\] Seeds have the main role of diuretics and hence utilized for treating edema of liver and beriberi. Seed extract assist in mucus secretion and has expectorant action, prevents gastric ulcer, histamine inhibitory effects and anti-tumor effects.\[^6,8\]

*Benincasahispida* has shown the presence of four triterpenes and two sterols together with a flavonoid C-glycoside, acylated glucose, and a benzyl glycoside.\[^8\] Seeds principally contain saponins, urea, citrulline, linoleic acid, oleic acid and fatty acids and triterpenoids known as isomultiflorenol, proteins such as trigonelline, foffearin, and osmotin, steroids such as β-sitosterol and stigmast-5-ene-3-beta-ol, alkaloids such as 5-methylcytosine, and triterpenoids such as cucurbicitin B.\[^6\] The fruit also contains amino acids, mucins, mineral salts, starch (about 32%), vitamins B and C, fixed oil (about 44%), cucurbitine, acid resin, myosin, and sugar (about 4%). Phytochemical study show two triterpenes, alunsenol and mutiflorenol, having mast cell stabilizing action in rats.\[^9\] Fruit extract of *Benincasa hispida* has gastroprotective effect\[^10\], and has showed protection against acetylcholine and histamine induced bronchospasm in guinea pigs\[^11\]. The fruit also contains water-soluble and hemicellulosic polysaccharides\[^12\]. From sarcocarp a protease has been purified by two steps of chromatography and identified that protease in a cucumisin like serine protease.\[^13\] Different extracts of *Benincasa hispida* has shown anti-angiogenic effect\[^5\], anti-ulcerogenic effect\[^8\], hypoglycemic effect\[^14\], anxiolytic-like effect\[^15\], antidepressant activity\[^16\], nootropic activity\[^17\], prevent morphine withdrawal in mice\[^18\], fruit rind is anti-inflammatory\[^19\].

In helminthiasis any organ of the body is infected by worms like pinworm, roundworm or tapeworm. Naturally, occurrence of helminths is more in the gastrointestinal tract as compared to the liver and other parts of the body.\[^20\] The approximation found by WHO is that “a shocking two billion people harbour parasitic worm infection. Helminthiasis is widespread globally (1/3 of
world community) and it is more ordinary in developing countries like India where the personal
and environmental cleanliness is inferior mainly in regions with water having the residence of
freshwater snails, that generally transmit the parasite. In human body, GIT is the habitat of many
helminths. Helminthiasis is hardly ever fatal, but is a major cause of ill health universally.[21]

Even though the helminthiasis is usually restricted to tropical expanses, create an immense
danger to health and add to the dominance of “malnutrition, anaemia, eosinaphilia and
pneumonia”.[22]

There is a principal difficulty in treating helminthiasis as the gastro-intestinal helminths
develop resistance to presently obtainable synthetic medicines for the disease,[23], for this reason
there is rising demand in the direction of natural anthelmintic medicines. The present study is an
attempt to screen the anthelmintic potential of *Benincasahispida* fruits.

**MATERIALS AND METHODS**

**Plant collection and authentication:** The fruits of *Benincasahispida* Thunb. were collected from
local area of Nandurbar, Maharashtra and authenticated by Dr. S. K. Tayde, Dept. of Botany,
Art’s, Science and Commerce College, Shahada, Dist-Nandurbar, Maharashtra, India. The
voucher specimen (SHD/2011/02) has been preserved in Dept. of Pharmacognosy and
Phytochemistry, College of Pharmacy, Shahada for future reference.

**Preparation of extracts:** The dried pieces of fruits were subjected to size reduction to get coarse
powder, and then passed through sieve no. 40 to get uniform powder. The pulverized fruit
powder (250 gm) was subjected to successive extraction in a Soxhlet apparatus using benzene,
chloroform, alcohol & water. The extracts are concentrated in a rotary flash evaporator and then
evaporated to dryness.

**Worm selection and Authentication:** Healthy adult earthworms, *Pheritimaposthuma* (Annelida,
Megescoleidiae) was selected due to its anatomical and physiological resemblance with the
intestinal roundworm parasites of human beings,[24-26], and also because of their easy availability.
Earthworms were collected from the water logged areas from the local area of Nandurbar,
Maharashtra, India. All worms are washed with normal saline, and kept in beakers containing
normal saline. The worms are authenticated by Dr. A. S. Patil, Director & Head, P.G. Dept. of
Zoology, P. S. G. V. Mandal’s Art’s, Science and Commerce College, Shahada, Dist-Nandurbar,
Maharashtra, India. The voucher specimen (SHD/Zoo/2011/2) has been preserved for future
reference.
Materials: Extracts used: Chloroform, benzene, alcohol and aqueous extracts of Benincasahispida fruits; Standard: Piperazine Citrate (GSK Ltd., Mumbai); Nutrient Agar Plate (Petri dish 9 cm²) and Digital Stop watch.

Sample preparation and Dose selection: All the extracts are taken to prepare suspensions of different concentration (10, 50 and 100 mg/ml) by using 0.2% v/v of Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration, as given below:

- 100 mg of extract was mixed in 10 ml 0.2% v/v of Tween 80 to get 10 mg/ml
- 500 mg of extract was mixed in 10 ml 0.2% v/v of Tween 80 to get 50 mg/ml
- 1000 mg of extract was mixed in 10 ml 0.2% v/v of Tween 80 to get 100 mg/ml
- 100 mg of Piperazine Citrate was mixed in 10 ml distilled water to get 10 mg/ml (Standard)

Preparation of Groups: Following 14 groups of just about identical size earthworms, having 3 worms individually in each group were taken.

a) Alcoholic extract of Benincasahispida 10 mg/ml  
b) Alcoholic extract of Benincasahispida 50 mg/ml  
c) Alcoholic extract of Benincasahispida 100 mg/ml  
d) Aqueous extract of Benincasahispida 10 mg/ml  
e) Aqueous extract of Benincasahispida 50 mg/ml  
f) Aqueous extract of Benincasahispida 100 mg/ml  
g) Chloroform extract of Benincasahispida 10 mg/ml  
h) Chloroform extract of Benincasahispida 50 mg/ml  
i) Chloroform extract of Benincasahispida 100 mg/ml  
j) Benzene extract of Benincasahispida 10 mg/ml  
k) Benzene extract of Benincasahispida 50 mg/ml  
l) Benzene extract of Benincasahispida 100 mg/ml  
m) Piperazine Citrate 10 mg/ml (Standard)  
n) Normal saline (Control)

Method: The anthelmintic evaluation was performed as stated by the method of Ajaiyeoba et al. with slight alterations. Pheretimaposthuma were released in 9 cm petridishes containing 25 ml extracts of 3 different concentrations separately (10, 50 and 100 mg/ml in 0.2% v/v Tween 80). This was done in triplicate for all the groups in all extracts of Benincasa hispida fruit. Observations were noted for time taken to paralyze all worms (Mean time for paralysis; P, in minutes) when no movement of any sort was observed, except when the worms were shaken.
vigorously and the time of death of worm (D, in minutes) were recorded after ascertaining that worms neither moved (completely vanished their motility) when shaken vigorously nor when dipped in warm water (50°C) followed with fading away of their body colors. Piperazine Citrate (10 mg/ml) was included as reference compound while normal saline as control for all groups.\[27\]

The data obtained in results were expressed as Mean ± SEM. Significant intergroup difference in each worm was analysed and one-way analysis of variance (ANOVA) was carried out.\[28\]

RESULTS

The Time of Paralysis (P) found to be 56.25, 47.90 & 38.93 min. for Benzene extract; 54.58, 46.81 and 37.88 min. for Chloroform extract; 45.89, 32.10 and 26.60 min. for Ethanol extract; 45.21, 33.51 and 27.41 min. for aqueous extract, at conc. of 10, 50 and 100 mg/ml respectively when compared to standard i.e. Piperazine citrate (5.55 min.) at the conc. of 10 mg/ml and to normal saline as control.

While the Time of Death (D) found to be 104.84, 93.02 and 78.08 min. for Benzene extract; 96.32, 81.38 and 68.73 min. for Chloroform extract; 60.28, 48.28 and 39.33 min. for Ethanol extract; 61.62, 49.05 and 38.87 min. for aqueous extract, at conc. of 10, 50 and 100 mg/ml respectively when compared to standard i.e. Piperazine citrate (11.27 min.) at the conc. of 10 mg/ml and to normal saline as control.

The results obtained indicate that ethanol and aqueous extracts of *B.hispida* fruit has shown significant anthelmintic activity at conc. of 100 mg/ml against *Pheritimaposthuma*.

The results of anthelmintic activity are shown in Table 1. The graphical representations of results were shown in Figure 1 and Figure 2.
Table 1: Anthelmintic activity of *Benincasahispida* fruit extracts on *Perithimaposthuma* (Indian Earthworm)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Groups (n=3) of Extract</th>
<th>Conc. (in mg/ml)</th>
<th><em>Perithimaposthuma</em> (Indian Earthworm)</th>
<th>Time of Paralysis (P) &amp; Death (D) in min. (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>1</td>
<td>Benzene</td>
<td>10</td>
<td>56.25±0.1049</td>
<td>104.84±0.2107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>47.90±0.3724</td>
<td>93.02±0.4081</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>38.93±0.3658</td>
<td>78.08±0.2831</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>10</td>
<td>54.58±0.3378</td>
<td>96.32±0.1274</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>46.81±0.3099</td>
<td>81.38±0.1014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>37.88±0.1889</td>
<td>68.73±0.3691</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>10</td>
<td>45.89±0.4473</td>
<td>60.28±0.1146</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>32.10±0.0549</td>
<td>48.28±0.1012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>26.60±0.3132*</td>
<td>39.33±0.1170</td>
</tr>
<tr>
<td>4</td>
<td>Aqueous</td>
<td>10</td>
<td>45.21±0.1572</td>
<td>61.62±0.2111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>33.51±0.5167</td>
<td>49.05±0.2446</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>27.41±0.3564*</td>
<td>38.87±0.1894</td>
</tr>
<tr>
<td>5</td>
<td>Control (Normal Saline)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>Standard (Piperazine Citrate)</td>
<td>10</td>
<td>5.55 ± 0.2226</td>
<td>11.27 ± 0.1524</td>
</tr>
</tbody>
</table>

Significant difference from Positive Control (Std.) by One Way ANOVA (n = 3) *p*<0.01
BEN = Benzene Extract, CL = Chloroform Extract, ALC = Alcoholic Extract, AQ = Aqueous Extract, STD = Standard (Piperazine Citrate)

DISCUSSION:

Security is fundamental standards in acquisition of normal arrangements and natural drugs for restorative organizations, and isolating some bit of huge worth control. These measures give accommodating particular bearing to checking success of normal drugs inside pharmacovigilance.
structures. Thriving seeing of home created meds is looked in, out & all around with that of different cures right now tried in setting of WHO International Drug Monitoring Program. While there are administrative and social separations in accessibility & utilization of unmistakable sorts of courses of action, they are all also as crucial from pharmacovigilance point of view. Rules were made with perspective that, inside current pharmacovigilance structures, seeing of flourishing of courses of action ought to be upgraded and extended in ways that will permit gainful checking of natural pharmaceuticals.

CONCLUSION

Helminthiasis is growing concern specifically in developing countries like India and some under developed countries. Available medicament as per the reports of WHO showing resistance and hence its need of hour that alternative options must be develop. The fruits of Benincasahispida Cogn (Cucurbitaceae) are used traditionally as anthelmintic, in insanity, other nervous diseases, asthma, cough, diabetes, haemoptysis, heamorrhages from internal organs, peptic ulcers, epilepsy, fever and vitiated conditions of pitta. Present study was having prime objective to evaluate anthelmintic potential and our findings confirmed the anthelmintic potential of Benincasahispida fruits against Indian earthworms; and hence can also be used for treatment of various types of worm’s infestation. To bring this material as treatment option in main treatment regimen need further investigation.

ACKNOWLEDGEMENT

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