PHYTO CHEMICAL INVESTIGATION AND EVALUATION OF ANALGESIC ACTIVITY OF PHYSALIS MINIMA


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ABSTRACT

Physalis minima (solanaceae) is an annual herbaceous plant commonly found in tropical countries like India, Africa, Afghanistan, Srilanka and is an Ayurvedic plant with huge medicinal importance. The ethanol and ethyl acetate extracts of Physalis minima plant were screened for phytochemical investigation and analgesic activity using hot plate method in albino wistar rats of either sex. The various doses used are 200mg/kg & 400mg/kg body weight of alcohol & ethyl acetate extract of Physalis minima showed comparable analgesic activity in hot plate method. The significant and nearlyequal activity was observed in ethyl acetate and alcoholic extract.

KEYWORDS: Physalis minima, Analgesic activity, Hot plate method, Phytochemical Investigation.

INTRODUCTION

Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals[1]. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases[2].

Pain is an unpleasant sensation no doubt, but on the whole it is usually beneficial to man (or animal). It is mainly a protective mechanism for the body, occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus[3].

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it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia), or persistent long after the precipitating injury has healed (e.g. phantom limb pain). It can also occur as a consequence of brain or nerve injury (e.g. following a stroke or herpes infection). With many pathological conditions, tissue injury is the immediate cause of the pain, and this results in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation[4]. Analgesics are the drugs which relieve pain without causing loss of consciousness. Non steroidal anti inflammatory (NSAIDS) drugs make up one of the largest groups of drugs used for pain[5].

*Physalis minima* belongs to the family solanaceae, which is commonly known as wild cape gooseberry in English.kupanti, budda and budamma in telugu. It is a small delicate, erect, annual, pubescent herb. Leaves are ovate to cordate, pubescent, delicate, exstipulate acuminate having reticulate palmate venation. Flowers are pedicellate, hermaphrodite, complete, solitary, fruits are berry. The plant is reported as diuretic, laxative, useful in inflammations, supplement for vit.c, enlargement of spleen and abdominal troubles[6].

**MATERIALS AND METHODS**

**PLANT MATERIAL**

The whole plant of *Physalis minima* was collected from surroundings of Nirmala College of Pharmacy, Atmakuru, Mangalagiri, Guntur (Dt), Andhra Pradesh and the same were authentified by Assistant Professor Dr. S. M. Khasim, Department of Botany, Acharya Nagarjuna University, Guntur A.P., voucher specimen were deposited at Department of Pharmacognosy for further reference.

**EXTRACTION PROCEDURE**

The shade dried plant material were reduced to moderately coarse powder and extracted successively with ethyl acetate and alcohol using soxhlet apparatus after defating[7].

**PRELIMINARY CHEMICAL TESTS**

The dried plant materials of the Physalis minima were powdered and subjected to the following systematic chemical test in order to identify the nature of chemical constituents present(Kokate C.K.1994)
1. ALKALOIDS
   a. MAYER’S TEST
   The substance is treated with Mayer’s reagent. A white or pale yellow colored precipitate is formed.

   b. DRAGENDORFF’S TEST
   The substance is treated with Dragendorff’s reagent, orange or red precipitate is formed.

   c. HAGER’S TEST
   The substance is treated with Hager’s reagent, a yellow crystalline precipitate is formed.

   d. WAGNER’S TEST
   The substance is treated with Wagner’s reagent; a reddish brown precipitate is formed.

2. CARBOHYDRATES
   a. MOLISCH’S TEST: Treating the substance with α-Naphthol and concentrated H2SO4 which gives purple colour.

   b. FEHLING’S TEST: To the solution add equal quantity of Fehling’s solution A&B. After heating, brick red precipitate is obtained.

   c. BENEDICT’S TEST: The substance is treated with Benedict’s reagent and heated in water bath. It gives reddish brown precipitate.

   d. BARFOED’S TEST: 1ml of extract is heated with 1ml of Barfoed’s reagent, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min.) may also cause reduction, owing to partial hydrolysis to monosaccharide’s.

3. GLYCOSIDES
   General test: Test A: Extract 200mg of drug with 5ml of dilute sulphuric acid by warming on a water bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1ml of Fehling’s solution A and B until it becomes alkaline (test with pH paper) and heat on a water bath for 2 minutes. Note the quantity of red precipitate formed and compare with that of formed in Test B.

   Test B: Extract 200mg of the drug using 5ml of water instead of sulphuric acid. After boiling add equal amount of water as used for sodium hydroxide in the above test. Add 0.1ml
Fehling’s A and B until alkaline (test with pH paper) and heat on water bath for 2 minutes. Note the quantity of red precipitate formed. Compare the quantity of precipitate formed in Test B with that of formed in Test A. If the precipitate in Test A is greater than in Test B then Glycoside may be present. Since Test B represents the amount of free reducing sugar already present in the crude drug, whereas Test A represents free reducing sugar plus those related on acid hydrolysis of any glycoside in the crude drug.

4. TANNINS

a) FERRIC CHLORIDE TEST
Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

b) PHENAZONE TEST
Add about 0.5 gm of sodium acid phosphate to 5ml of extract warm it and filter. To the filtrate add 2% phenazone solution, bulky precipitate is formed, which is often colored.

5. STEROIDS AND TRITERPENOIDS

a) LIBERMANN-BURCHARD TEST
Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoids.

b) SALKOWSKI TEST
Treat the extract with few drops of concentrated sulphuric acid red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

c) SULFUR POWDER TEST
Add small amount of sulfur powder to the extract, it sinks at the bottom.

6. SAPONINS

FOAM TEST
Dilute 1ml of alcohol and aqueous extracts separately with distilled water to 20 ml and shake in a graduated cylinder for 15 min. the formation of foam indicates the presence of saponins.
7. **FLAVONES**

a) The powder is treated with sodium hydroxide; formation of yellow color indicates the presence of flavones.

b) The powder is treated with concentrated sulphuric acid, formation of yellow or orange indicates flavones.

8. **PROTEINS**

**MILLONS TEST**

To the substance add few drops of Millons reagent, reddish brown color shows the presence of proteins.

**NINHYDRIN TEST**

To the substance add few drops of Ninhydrin reagent purple color shows the presence of proteins.

**XANTHOPROTEIN TEST**

Warm small quantity of substance in water then treat with concentrated with nitric acid, add enough ammonia to make alkali, first a yellow color obtained then on addition of ammonia the color changes to oranges.

**Experimental animals:** Wistar albino rats weighing between 150-200gm were obtained from westbengal. The animals were maintained on the suitable nutritional and environmental conditions throughout the experiment as per the rules and regulations of the institutional animal ethical committee.

Experimental protocols for the pharmacological studies were reviewed and approved by the institutional animal ethical committee 1629/PO/a/12/CPCSEA.

**Analgesic activity of Ethyl Acetate and Alcoholic Extract of *Physalis minima.***

**Analgesic activity by hot plate method**[9-13]: Albino wistar rats of either sex weighing between (150-200gms) were divided into groups of six animals in group. the basal reaction time by observing hind paw licking or jump response (which ever appears first) in animals when placed on hotplate maintained at constant temperature (55° c) was taken.

GROUP-1  Control

GROUP-2  Standard

GROUP-3  Ethanolic extract of *Physalis minima* (200mg)
GROUP-4  Ethanol extract of *Physalis minima* (400mg)
GROUP-5  Ethyl acetate extract of *Physalis minima* (200mg)
GROUP-6  Ethyl acetate extract of *Physalis minima* (400mg)

RESULTS

PRELIMINARY PHYTOCHEMICAL SCREENING OF EXTRACTS OF *Physalis minima* LINN

The extracted plant material is subjected to the following preliminary Phytochemical screening

Table:1.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Chemical groups</th>
<th>Alcoholic extract</th>
<th>Ethyl acetate extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>6</td>
<td>Proteins</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>7</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Flavones</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table :2.

Analgesic Activity Screening of Extracts of *Physalis Minima* Linn

<table>
<thead>
<tr>
<th>ANIMALS</th>
<th>30MIN</th>
<th>60MIN</th>
<th>90MIN</th>
<th>120MIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>1.0 ±0.20</td>
<td>1.0 ±0.26</td>
<td>1.1 ±0.30</td>
<td>2.16 ±0.38</td>
</tr>
<tr>
<td>STANDARD</td>
<td>6.5 ±3.25</td>
<td>9.13 ±5.32</td>
<td>8.2 ±6.19</td>
<td>8.4 ±5.51</td>
</tr>
<tr>
<td>ALC HOL(T1)</td>
<td>2.2 ±0.30</td>
<td>4.45 ±0.58</td>
<td>4.65 ±0.58</td>
<td>4.85 ±0.68</td>
</tr>
<tr>
<td>ALC HOL(T2)</td>
<td>5.21 ±1.42</td>
<td>5.47 ±2.42</td>
<td>6.66 ±3.95</td>
<td>5.4 ±2.46</td>
</tr>
<tr>
<td>ALCOHOLIC EXTRACT (T3)</td>
<td>2.68 ±0.34</td>
<td>4.13 ±0.36</td>
<td>4.85 ±0.66</td>
<td>5.53 ±2.47</td>
</tr>
<tr>
<td>ETHYL ACETATE EXTRACT (T4)</td>
<td>4.01 ±0.20</td>
<td>5.88 ±2.18</td>
<td>6.76 ±3.96</td>
<td>5.95 ±2.19</td>
</tr>
</tbody>
</table>
DISCUSSION
The various extracts at a dose of 200 mg/kg and 400 mg/kg body weight showed comparable analgesic activity in hot plate method (Table 1). Ethyl acetate extract showed a higher analgesic activity (6.7 sec. P<0.05 at 90 min) followed by alcoholic extract (6.6 sec. at 90min p<0.05) as compared to control at both the doses. The significant and nearly equal activity was observed in ethyl acetate and alcoholic extract.

CONCLUSION
The various extracts of whole plant of Physalis minima at a dose of 200mg/kg and 400mg/kg body weight were investigated for analgesic activity. The ethyl acetate and ethanol extracts showed significant analgesic activity (p<0.05) from 1 hour onwards as compared to standard drug tramadol. The significant analgesic and activity may be due to the presence of flavones. Flavones are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception [7-14].

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REFERENCES


