PRELIMINARY PHYTOCHEMICAL SCREENING AND TO EVALUATE THE ANTHELMINTIC ACTIVITY OF HYDRO-ALCOHOLIC AND PETROLEUM ETHER EXTRACT OF NYCTANTHES ARBOUR-TRISTIS ROOT. (FAMILY-NYCTAGINACEAE)

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INTRODUCTION

Nyctanthes arbor-tristis one of the well known and most useful medicinal plants. It is commonly called—Night jasmine[English], due to fact that its flowers emit a very strong and pleasant fragrance during the whole night. The flowers start falling after midnight and by the daybreak, the plant appears dull. The generic name—Nyctanthes has been coined from two Greek words Nykhta (night) and Anthos (flowers) The specific name arbor-tristis means as it loses its brightness during the daytime. NAT plant has been screened for anti-malarial anti-histaminic activity, anti-arthritis activity, local anaesthetic, antihypnotic, analgesic, anti- uker, anti-pyretic, anti-depressant, anti- leishmaniasis, anti-cancer, anti-larvicial, anti-allergic, anti-viral, Immunomodulatory, anti-helminthic, antioxidant, anti-diuret. Therapeutic properties of medicinal plants have been used traditionally to treat human diseases. Growing populations of developing countries use plant-derived medicines to be a normal part of primary health care. Hundreds to thousands of diverse secondary metabolites with different biological activities were found recorded in higher plants. In recent years, multiple drug resistance (MDR) human pathogenic microorganisms have developed due to the indiscriminate use of synthetic antimicrobial drugs commonly used in the treatment of infectious diseases [1, 2].

Introduction to helminthiasis

Helminthiasis or worm infection is one of the most prevalent diseases. Many worms are parasitic in humans and cause a serious complication. It is estimated that one-fourth of the world population may be infected by worms. In Helminthiasis this organism multiply outside of the definitive host and have the unique ability to evade host immune defences, for reasons that are not fully understood. Helminthiasis tends to be chronic, possibly lasting an entire lifetime of the host. Infected host humans are divided into two categories or phyla.

- **Platy helminthes (flatworms):**
  - In which a. Cestodes (tapeworms), b. Trematode (flukes) is included.
  - Nematodes (roundworms):
    - In which a. Roundworm, b. Hoo1oworm, c. Pinworm, d. Whipworm is included [3, 4].

MATERIALS AND METHODS

Plant material and collection

The root of Nyctanthes arbor-tristis have been collected from a local area of Guwahati (dist. kamrup) the root was dried at room temperature (30-40 °C). The plant was authenticated by Dr. P. P. Baruah, professor and head, Department of Botany, Gauhati University, Assam, India.

Worms Collection

Indian earthworm Phereitima posthuma (Annelida) were collected from the waterlogged areas of soils Indian earthworms are identified at Girijanandachowdhury institute of pharmaceutical science (Azara, Guwahati).

Chemicals and reagents

Petroleum Ether, Methanol, Dragendorff reagent, Mayer’s reagent, Wagner’s reagent, Benedict’s reagent, sulphuric acid, lead acetate, Molsch’s reagent, Fehling solution A and B, sodium citrate, copper sulphate, ferric chloride, sodium hydroxide, glacial acetic acid, benzene, chlo roform, ammonium nitrate, potassium nitrate, gelatine, Albendazole suspension [Zentel, GSK Pharmaceuticals Ltd. Bangalore], and CMC [Rankem Lab.] were used during the experimental protocol. All the chemicals used are a laboratory and analytical grade.

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Preparation of the plant extract

The root of night flowering jasmine was collected and washed with tap water, remove all the soil and dirt's and shade dried. Shade-dried roots were grinded and formed powdered, then it passed through sieve number 60 and then the material was extracted with non-polar to polar solvents. At first, the plant material was defatted with petroleum ether then the extraction is carried out in a hydro-alcoholic solvent that is methanol and distilled water in a Soxhlet apparatus by continuous heat extraction. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50 °C[5].

Phytochemical screening

Test for alkaloids

A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and observed for the formation of a reddish-brown precipitate.

Test for tannins

Two ml of the extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for saponins

One ml aliquot of floral extract was combined with 5 ml distilled water at 60 °C, shaken for 2 min, as saponins are known to possess frothing activity, the volume of froth produced in this experiment was observed and recorded every 10 min for a period of 30 min.

Tests for glycosides

To 2 ml of the extract, added 3 ml of CHCl3 and 10% ammonia solution. Formation of pink colour indicates the presence of glycosides.

Test for carbohydrates (Molisch’s test)

Few drops of Molisch’s reagent were added to 2 ml of the methanolic extract. This was followed by addition of 2 ml of conc. H2SO4 down the side of the test tube. The mixture was then allowed to stand for 2-3 min without shaking. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Test for alkaloids: A fraction of extract was treated with 3-5 drops of Wagner’s reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and observed for the formation of a reddish-brown precipitate.

Test for flavonoids

The extract was dissolved in diluted NaOH and HCl. A yellow solution that turns colourless indicates the presence of flavonoids.

Test for proteins

One ml of methanolic extract was mixed with 2 ml of Millon’s reagent. Appearance of a white precipitate which turned red upon gentle heating confirms the presence of protein.

Test for phenolic compounds

To 1 ml of the extract, few drops of 0.5% ferric chloride solution were added. Formation of bluish black colour indicated the presence of phenolic compounds [6-8].

Anthelmintic activity

For the anthelmintic activity of Nyctanthes arbortristis, Indian adult earthworms (phere Verma posthuma) in 6 cm in length and 0.1-0.1-2 cm in width were used. The earthworms were divided into ten groups of six earthworms in each group. The extract was dissolved in DMSO in different concentrations and the final volume was adjusted to 10 ml; the extract and standard drugs were freshly prepared before starting the experiments. The extract of different concentration and standard solution were poured in different Petri dishes. All the earthworms were washed into normal saline solution before they are released into Petri dishes. Observation was made for the time taken to paralyze (paralysis was said to occur when earthworms didn’t revive in normal saline) and death (death was concluded when earthworms lost their motility and followed with their body colors fading away).

RESULTS

The study shows the phytochemical screening, anthelmintic activity of the hydro-alcoholic and petroleum ether extract of the plant Nyctanthes arbortristis. The yield % of the extraction of the hydro-alcoholic was 4.65 % and petroleum ether 4.88%.

Phytochemical screening

Table number 2 showed the result of Phytochemical screening of both extract hydro-alcoholic and petroleum ether. Where the (+) positive mean present and (-) negative mean absent.

Anthelmintic activity

Table number 2 showed the anthelmintic activity of the plant extract. The hydro-alcoholic and petroleum ether extract of the Nyctanthes arbortristis root having anthelmintic activity against earthworms (phere Verma posthuma).

<table>
<thead>
<tr>
<th>Chemical test</th>
<th>Pet. ether</th>
<th>Hydro alcoholic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Proteins and amino acid</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

(+ve) Presence, (-ve) Absence

Table 2: Antimicrobial activity of root hydro-alcoholic and petroleum ether extract Nyctanthes arbortristis observed against the earthworms

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/ml)</th>
<th>Time for paralysis(min)</th>
<th>Time for death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Standard(Albendazole)</td>
<td>50</td>
<td>14.43</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6.62</td>
<td>9.51</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>50</td>
<td>16.09</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12.2</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>8.5</td>
<td>13.9</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>50</td>
<td>10.09</td>
<td>15.35</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.14</td>
<td>12.24</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5.53</td>
<td>10.05</td>
</tr>
</tbody>
</table>
DISCUSSION

Phytochemical analysis

The Phytochemical test of Nyctanthes arbortristis root showed the presence of various phytoconstituents. Hydro-alcoholic extract and petroleum ether having Carbohydrates, Flavanoids, Tannin, Phenol, Glycosides, Saponins and Alkaloids, Saponins (table 1).

Anthelmintic activity

In the present study, it was observed that all the extracts of Nyctanthes arbor tristis root have exhibited a positive response to the certain degree of anthelmintic activity. Extracts exhibited more potent activity at higher concentration (200 mg/ml) against Pheretimaposthuma (earthworm). Evaluation of anthelmintic activity was compared with reference standard Albendazole as shown in  table 2 and plot the graph for comparison of different extract (fig. 1)

CONCLUSION

The study has shown that petroleum ether and methanolic extract of Nyctanthesarbor tristis root have significantly determined anthelmintic activity. But the methanolic extract of Nyctanthesarbortristis leaves shown most significant anthelmintic activity as compared to petroleum ether extract. Further studies can be done to identify the possible phyto constituents responsible for the anthelmintic action. In conclusion the traditional use of plant

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declare none

REFERENCES