QUALITATIVE PHYTOCHEMICAL SCREENING & ACUTE TOXICITY STUDY AND LD_{50} DETERMINATION OF DATURA METEL.

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ABSTRACT

To perform the qualitative phytochemical screening and acute toxicity of Datura metel L. Root. Method: The root is subjected to various extractions using ethanol and distilled water. Extraction process used was percolation method. With the extract phytochemical screening was done. R_f value was also calculated there after acute toxicity test was performed. After phytochemical screening different types of compounds namely alkaloids, triterpinoids, fats and fixed oils, flavonoids, carbohydrates are found in aqueous extract and flavonoids are found in ethanolic extract. After performing the acute toxicity with 2000mg/kg, 2640mg/kg, 3280mg/kg, 3920mg/kg, 5000 mg/kg. The animal dies at 3920mg/kg & 5000 mg/kg dose. This study helps us to get the information about the phytochemical present and their TLC value in the root of the plant and gives the LD_{50} of the extract.

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INTRODUCTION

Medicinal herbs constitute the corner stone of traditional medicinal practice worldwide. These herbs are relatively cheap and easily available. These medicinal plants represent a great deal of untapped reservoir of drugs and the diversity of their component molecule makes a valuable source of novel lead compounds\[4\]. Traditional medicine in many areas of the world relies on the use of a wide variety of plant species. Phytotherapy still plays an important role in the management of diseases, mainly among populations with very low income.\[2\]

*Datura metel* (D. metel) is a widespread annual plant from the Solanaceae family. It is one of the widely well known folklore medicinal herb. It is a wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH3) and include the anti-cholinergic drugs atropine, and scopolamine. From ancient civilization it was traditionally used for religious visionary purposes throughout the world and used by witchcraft in medieval Europe. The plant finds application in the treatment of diarrhoea and skin diseases. It is used in the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation, skin-ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and Treacheries.\[3\]


DESCRIPTION OF THE PLANT:

**Synonym**

*D. fastuosa* Linn.

**Family**

*Solanaceae*.

**Habitat**

Throughout India, particularly in waste place.

**English**

Thornapple, Downy *Datura*, Common thorn apple, jimson weed, devil's trumpet, devil's weed, tolguacha, Jamestown weed, stinkweed, locoweed, datura, pricklyburr, devil's cucumber, hell's bells, moonflower, amaduudu (Luganda), gathumba (Kikuyu), muana (Kiswahili), ngwata (Kamba), silulu (Kitosh)

**Ayurvedic**

Dhattuura, Dhuurtta, Dhastura, Unmatta, Shivapiiya, Harapiiya, Hema, Haatta, Dhsstuura, Dhsstuuraka, Kanaka, Maaatula. Also equated with Raaj-dhatuura. (white var.)

**Unani**

Dhaturaa.

**Siddha/Tamil**

Oomatthai, Karuvoomatthai.

**Action**

Various plant parts are used in headache, hemiplegia, epilepsy, delirium, convulsions, cramps, rigid thigh muscles, rheumatism. Leaf-antitumour, antirheumatic. Leaf and corolla-anti-inflammatory.\[4\]

TRADITIONAL USE

*Datura* has a wide range of traditional applications, including the treatment of epilepsy, hysteria, insanity, heart diseases, fever with catarrh, diarrhea and skin diseases. Crushed leaves are used to relieve pain. In China, the plant is used in the treatment of asthma. In Vietnam, the dried flowers and leaves are cut into small chips and used in antiasthmatic cigarettes. About 3 to 5g of the flower extract can be used as an anesthetic through oral consumption that produces general anesthesia within 5 minutes, which lasted for about 5 to 6 h. The flower of the *D. metel* is used in the treatment of pain, chronic bronchitis and asthma (Kam and Liew 2002; Kol, 1999). In Bangladesh, leaves are used for scabies, eczema and allergy (Chowdhury et al., 1996). Dried whole plant powder is used to smoke to cure excessive or abnormal breathing, applied around the eyes to enlarge pupils. Application or drinking of leaf juice relieves pain and swelling. Leaf juice is mixed with a little opium and applied to the affected area to reduce swelling of gums or base of ears. Leaf juice is mixed with lime and turmeric and applied to the breasts to reduce breast pain (Rahmatullah et al., 2010). The flowers of *Datura metel* have been used in traditional Bangladeshi medicine for the treatment of asthma, convulsions, pain, and rheumatism for centuries.\[8\]

EXTRACTION FROM ROOT OF SAMPLE

This was carried out according to the method of percolation. The dried, crushed root and solvent is taken into two conical flask containing distilled water and ethanol in 1:25 ratio (root : solvent) in both the cases respectively. Each was allowed to stand for two weeks with constant shaking at regular interval at room temperature. The percolates were then filtered and the solvents (ethanol and water) were evaporated to obtain ethanolic and aqueous extracts of the latex. Then the extract is stored in a refrigerator at 4°C until needed for experiment.

PHYTOCHEMICAL SCREENING

The preliminary phytochemical evaluation of ethanolic and aqueous extract of *Datura metel* L. roorevealed the presence of flavonoids, carbohydrates, steroids, carbohydrate and glycosides. The result of phytochemical analysis were significant in aqueous extract. This observation clearly indicate that most of bioactive compounds of are *Datura metel* soluble in water than ethanol. The result of phytochemical analysis were shown in Table 1.
Table 1: Phytochemical analysis of aqueous and ethanolic root extracts of *Datura metel* L.

<table>
<thead>
<tr>
<th>PHYTOCHEMICAL CONSTITUENTS</th>
<th>AQUEOUS EXTRACT</th>
<th>ETHANOLIC EXTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tanins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids and triterpenoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fats and fixed oil</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mucilage</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

“+” ve indicates presence of compounds
“-” ve indicates absence of compounds

TLC ANALYSIS OF ROOT EXTRACT

The $R_f$ value (Retardation factor) is calculated for identifying the spots i.e. in Qualitative analysis. $R_f$ value is the ratio of distance travelled by the solute to the distance travelled by the solvent front.

$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$

The $R_f$ value ranges from 0 to 1. But ideal values are from 0.3 to 0.8. $R_f$ value is specific and constant for every compound in a particular combination of stationary and mobile phase. When the $R_f$ value of a sample and reference compound is same, the compound is identified by its standard. When the $R_f$ value differs, the compound may be different from its reference standard. [6]

RE RESULT OF TLC ANALYSIS

Table 2: Phytochemical analysis of aqueous and ethanolic and aqueous Datura extract by TLC ($R_f$ value determination)

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Drugs</th>
<th>Solvent system</th>
<th>$R_f$ value of aq extract</th>
<th>$R_f$ value of ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td>Alkaloidal drug</td>
<td>Acetone : water : concentrated ammonia (9:0.7:0.3)</td>
<td>No run but 2 spots are separated</td>
<td>0.28</td>
</tr>
<tr>
<td>ii)</td>
<td>Ethyl acetate : methanol : water (10:0.1:35:1)</td>
<td>0.64</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>iii)</td>
<td>Toluene : chloroform : ethanol (2.85 : 5.7: 1.45)</td>
<td>0.4</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>iv)</td>
<td>Chloroform : methanol (5 : 5)</td>
<td>0.862</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>i)</td>
<td>Flavonoid drug</td>
<td>Eth acetate : formic acid : acetic acid: water (10:1:1:2.7)</td>
<td>0.58</td>
<td>0.6</td>
</tr>
<tr>
<td>ii)</td>
<td>Chloroform : acetone : formic acid (7.5 : 1.6 : 0.9)</td>
<td>0.78</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>i)</td>
<td>Cardiac glycoside</td>
<td>Ethyl acetate : methanol: water (10:1:35:0.1)</td>
<td>0.66</td>
<td>0.5</td>
</tr>
<tr>
<td>ii)</td>
<td>Eth acetate : methanol :ethanol: water (8.1:1:0.4:0.8)</td>
<td>0.8</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>iii)</td>
<td>Chloroform : methanol: water (6.4:0.5:0.0.1)</td>
<td>0.636</td>
<td>0.545</td>
<td></td>
</tr>
</tbody>
</table>
ACUTE TOXICITY STUDY

Acute toxicity describes the adverse effects of a substance that result either from a single exposure \[3^{[6]}\] or from multiple exposures in a short space of time (usually less than 24 hours).

To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance. \[7\]

It is widely considered unethical to use humans as test subjects for acute (or chronic) toxicity research. However, some information can be gained from investigating accidental human exposures (e.g., factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, in vitro testing methods and inference from data on similar substances. \[13^{[2]}\]

Solution reagents and chemicals:

Freshly prepared solutions and analytical grade chemicals were used in all the experiments. Datura root extract were calculated 2000mg/kg for animal and made aqueous suspension of 0.6% Na-CMC solution of latex and Datura and kept in freeze until experiment. \[8\]

Animals:

For the purpose of the study institutional ethical permission is taken as reference no.BCPSR/IAEC/2013/002. Inbred Wistar mice of both sexes with average weight of 30 g were used as test animals. The animals, which were obtained from the laboratory animal facility of the Bengal College of Pharmaceutical Sciences and Research, Durgapur-713212, were housed in stainless steel cages at room temperature of 28–32 degree Centigrade and under a light period of 16–18 h daily. They were fed on standard commercial feed. \[9\]

METHODOLOGY:

Animals should be fasted prior to dosing (e.g., with the mice, food but not water should be withheld overnight; with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period of time, it may be necessary to provide the animals with food and water depending on the length of the period.

One animal each was given Datura root extract of 2000mg/kg into i.p route and observed for 48 hrs and given the food and diet regularly according to time. At the end of each of the experiments, the sign of toxicity, the number of mice that were dead or alive and the time of death were recorded. \[10\]

Table 3: Observation table for acute toxicity test of preliminary limit test for animals:

<table>
<thead>
<tr>
<th>Sl No</th>
<th>No Of Animals</th>
<th>Weight Of Animal</th>
<th>Dose Administered</th>
<th>Route Of Administration</th>
<th>Sign of toxicity or death</th>
<th>Death of Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>30.02 gm</td>
<td>2000mg/kg</td>
<td>i.p</td>
<td>Appetite, locomotion reduced for one day. Not died</td>
<td>Not died and was live and animal become normal. Died after 24 hour.</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>33 gm</td>
<td>2640mg/kg</td>
<td>i.p</td>
<td>1 animal died</td>
<td>Died after 12 hour. 5 animal died. Died within 1 hour. Within 15 minutes.</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>34 gm</td>
<td>3280mg/kg</td>
<td>i.p</td>
<td>Movement reduced. 1 animal died. 5 animal died. Died within 1 hour. Within 15 minutes.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>32.05 gm</td>
<td>3920mg/kg</td>
<td>i.p</td>
<td>6 animal died.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>34.45 gm</td>
<td>5000mg/kg</td>
<td>i.p</td>
<td>6 animal died.</td>
<td></td>
</tr>
</tbody>
</table>

\[10\]
RESULTS AND DISCUSSION

Thus after performing the phytochemical study of \textit{Datura metel} root we found the presence of Alkaloids, fats and fixed oils, carbohydrates, glycosides, steroids and tri terpinoids in aqueous extract and flavonoids in ethanolic extract. This is presented in table no 1. So, the compound present in \textit{Datura metel} root is more soluble in water than ethanol. From the table no 2 the TLC value with different solvents are represented for different phytochemical present. After performing the acute toxicity with 2000mg/kg, 2640mg/kg, 3280mg/kg, 3920mg/kg, 5000 mg/kg. The animal dies at 3920 and 5000 mg/kg dose. It is presented in table 3.

After performing the acute toxicity test we can conclude that the animal dies at the 3290 mg/kg dose, which is a lethal dose of the drug for the animal. The LD\(_{50}\) of the drug is 2640mg/kg. It is represented in graph 1.

FUTURE PROSPECT

Novelty of the work and future application includes the fact that natural products are more safe for use as compared to that of the synthetic compounds. As these products are natural, potential for market penetration in India as well as abroad is high, subject to approval by relevant regulatory authorities. Moreover India being a developing country consists of a major portion of population who are below the poverty level, i.e. the BPL people. Many of them die of simple and treatable diseases without any medication because in today’s world the cost of medicines are rising high. Hence, if we can establish and proof the fact of the presence of immense medicinal values in a waste-land plant like \textit{Datura metel} and utilize it to an optimum limit, then we can hope of bringing this drugs in an affordable limit of the poverty struck people and give them the required medication, because while doing this project it has been observed that the plant Dhatura (Datura metel) possess immense medicinal values and it would be advantageous to treat various kinds of diseases at the same time using its different plant parts. With the above result we will go for further study for different invivo study.

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REFERENCES


