Toxicity of Datura alba leaf extract to aphids and ants

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Citation

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Abstract

Datura alba, is a medicinal plant that belongs to the family of solonaceae. In the Indian subcontinent, indigenous medical practitioners use extracts of this plant to treat various inflammatory diseases. Ants and Aphids were chosen as an experimental model as they were common insecticides for local farmers in Sri Lanka. Therefore, the objective of this study was to analyse the inorganic and organic contents in the leaf of Datura alba and to investigate the acute toxicity at varying concentrations on aphids and black ants. We determined the calcium, magnesium and phosphorous in the ionic state quantitatively and carried out screening tests and solvent extraction using chloroform and petroleum ether to find out the presence of organic groups such as alkaloids, flavanoids, saponins and cardiac-glycosides. The concentration of Ca2+, Mg2+, Fe3+ and PO43− were found to be (3.17 ± 0.02) × 104, (3.37 ± 0.01) × 104, (1.38 ± 0.004) × 104 and (4.20 ± 0.03) × 104 ppm respectively. The screening tests confirmed the presence of alkaloids and steroids or terpenoids and the absence of saponins, cardiac glycosides and flavanoids. Increasing concentrations of the plant extracts (2500-15000 ppm) were added to ants (n = 10) and aphids (n = 10) in an experimental chamber. After 10 minutes of exposure, the numbers of live insects were counted. The results showed the EC50 value was 12000 ppm for aphids and 11600 ppm for ants. Percentage mortality increased from 20-60% with increasing concentrations. Our results indicated that extract of Datura alba leaves at higher concentrations was more toxic it can be used as an insecticide against aphids and black ants.

INTRODUCTION

Medicinal plants have emerged as some of the most widely studied plants and significant interest has been shown in their chemistry because of their potential application in medicine [1,2,3]. Many of these medicinal plants contain chemical constituents that could cause harmful effects to human if taken in large quantities. Alkaloids occurring in a large amount make these plants poisonous [4]. Large quantity of oxalic acid, a proto plasmic poison in the form of oxalates of calcium, sodium and potassium also produces poisoning [5,6,7].

Some of these plants are poisonous to insects and pests [8,9,10]. There is a necessity to find cheap insecticides for the diverse needs of agriculture, destruction of house holds pests and prevention of vectors of diseases such as malaria in developing countries such as Sri Lanka and India. Plant based insecticides [11,12] are preferred to chemical insecticide as they have little or no negative effect on the agricultural environment [13]. At present, there is not much knowledge of these plant based insecticides in the literature. The evidence suggests that plants containing a high percentage of rotenone [14], powdered young leaves and twigs of Javanica (Blume) and nepalenis (Benn) are used to kill mosquito Larvae [15].

In a quest to identify a plant based insecticide in this study we investigate the toxic effects of Datura alba, an indigenous medical plant used in ancient Indian medical system. Datura alba, which is under the family of solanaceae, is one of the most useful medicinal plant used in treating asthma, muscle spasm, whooping cough, skin ulcers etc [16]. This plant grows in warmer parts of the world particularly in South and Southeast Asia including India and Sri Lanka. This annual herb is bushy, smooth, fetid, 0.5 to 1.2 m in height also attaining 6 feet or more in rich soils. The leaves are 18 cm long and the flowers are white in colour (Figure 1). Various parts of the plant (leaves, seeds, roots and fruits) are used for different purposes in medicine [17].

Figure 1

Figure 1:

The aim of this study was to determine the inorganic contents quantitatively and organic contents qualitatively in the leaves of Datura alba and test its toxic activity in common agricultural insects such as aphids and black ants.

MATERIALS AND METHODS

INORGANIC ANALYSIS

(A) MOISTURE CONTENT

The wet weight of the fresh Datura alba leaves was recorded before placing them in a hot air oven at 1000° C for an hour for the complete evaporation of water. The sample was taken out, cooled and weighed to obtain the dry weight. This process was repeated until a constant weight was obtained carried out in triplicate.

(B) TOTAL ASH CONTENT

Oven dried leaf sample was weighed in a porcelain crucible and ignited in a muffle furnace for several hours until a constant weight was obtained.

(C) ORGANIC MATTER

The difference between the weight of the oven dried sample and the ash was taken as the weight of the organic matter.

(D) CALCIUM CONTENT

10.0 ml of test solution and 2 ml of KOH were added to a titration flask and stirred for 5 minutes. 0.5 g of Patton reader's indicator and masking agent (2 ml) were added and the resulting solution was titrated against standard EDTA solution.

(E) TOTAL AMOUNT OF CALCIUM AND MAGNESIUM CONTENT

10.0 ml of test solution was taken and 2 ml of buffer solution (pH = 10), 2 ml of masking reagent were added and resulting solution was titrated against standard EDTA solution using Erichrome black T as an indicator.

(F) PHOSPHOROUS CONTENT

Standard phosphorous solution (1000 ppm) was prepared. Different concentrations of solutions were made by using series of dilutions (0-1000 ppm). A standard curve was obtained by plotting absorbance versus concentrations.

Test solution (5.0 ml) was pippetted out and molybdate vanadate composite reagent (5.0 ml) was added to it and the resulting solution was mixed well and allowed to stand for 10 minutes. The intensity of the yellow colour developed was measured.

(G) IRON CONTENT

Standard iron solution (1000 ppm) was prepared. Different concentrations of solutions were made by using series of dilutions (0-30 ppm). A standard curve was obtained by plotting absorbance versus concentrations.

Hydroxylamine hydrochloride (10%, 2 ml), distilled water (1 ml) and 1, 10 ortho phenanthraline reagent (2 ml) were added to the test solution and the intensity of the red colour developed was measured.

QUALITATIVE ORGANIC ANALYSIS

(A) SCREENING FOR SAPONINS

A small amount of the plant material was taken in a test tube and water was added. Then the plant material was shaken vigorously. The tube was observed over a period of one hour to find out whether there was any froth formation that indicates the presence of saponins.

(B) SCREENING FOR CARDIAC-GLYCOSIDES

Fifty grams of powdered plant leaf material was refluxed with 100 ml of ethanol in a round bottomed flask in a steam bath. After an hour of refluxing the flask was cooled to room temperature. Then this mixture was filtered and washed with fresh rectified spirit (50 ml) the combined filtrate and washings were used for the screening tests.

Test solution (0.2 ml) was placed as a concentrated spot on the centre of a piece of filter paper and circular chromatography was performed with $CHCl_3$ as the developer. The filter paper was air dried with kedde reagent

and dried again.

(C) SCREENING FOR FLAVANOIDS

Test solution (15 ml) was evaporated to dryness. The residue was defatted with petroleum ether, dissolved in rectified spirit (2 ml) and the solution was divided in to two equal parts in test tubes. To one portion concentrated HCl (0.5 ml) and Mg turnings were added, cooled and shaken with butanol. The colour of the solution was compared with that in the second test tube.

(D) SCREENING FOR ALKALOIDS

The test solution (70 ml) was evaporated to dryness and 10 ml of HCl (2 N) was added and heated in a steam bath for 5 minutes with stirring. This solution was then filtered. The filtrate was divided in to 4 equal portions in separate test tubes.

(1). A few drops of Mayer's reagent were added to one of the test tubes.

(2). A few drops of Wagner's reagent were added to the solution in the second test tube.

(3). The remaining two fractions were combined, basified with concentrated NH_3 and the solution was extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried over anhydrous $MgSO_4$ and concentrated. The solution was subjected to TLC with $CHCl_3$:MeOH = 9:1 as the developing solvent. Then the plates were sprayed with dragondroff reagent.

(E) SCREENING FOR STEROIDS

Test solution (15 ml) was evaporated to dryness. The residue was stirred with petroleum ether (10 ml) and the organic layer was discarded. The residue was dissolved in $CHCl_3$ (10 ml) and divided in to 3 equal portions in separate test tubes.

(1). One of the test tubes was used as reference.

(2).The second test tube was held at an angle of 45° and concentrated H_2SO_4 (2 ml) was allowed to run along the side of the tube and observed for any changes.

(3). Acetic anhydride (AC_2O) (3 drops) was added to the remaining portion and mixed. Then concentrated H_2SO_4 (1 drop) was added to this solution and mixed again. The colour changes were observed immediately and over a period of an hour.

EXTRACTION

The leaves of Datura alba were collected from Northern part of Sri Lanka. About 350 g of powdered leaves (dry) were extracted in a soxhlet extractor with petroleum ether (500 ml) as a solvent for 30 hours. The solvent was evaporated under pressure. A greenish brown colour semi solid (12.90 g) was obtained.

PREPARATION OF DIFFERENT CONCENTRATIONS

Chloroform extract (4.5 g) was dried well using hot air oven to remove the trace amount of chloroform. After drying, four different weights of residue were weighed. Each of them was dissolved well in distilled water using magnetic stirrer and transformed to 100 ml volumetric flask. Then each of them was made up to the mark with distilled water.

TOXICITY OF DATURA ALBA ON APHIDS

A clean well dried bottle was taken and it had ten aphids which had the facility of air and young leaves of beans pasted with water as control or extract having concentration 2500 ppm. The system was in rest for 10 minutes. Number of live aphids was counted. This was carried out in quadruplicates. Above procedure was repeated for different concentrations (5000, 10000 and 15000 ppm) without changing the amount of young leaves of beans.

TOXICITY OF DATURA ALBA ON ANTS

A clean, well dried bottle was taken and it had ten black ants which had the facility of air and small amount of D-Glucose for controls (0) and mixed with extract having concentration 2500 ppm. The system was in rest for 10 minutes. Number of black ants killed, was counted. This was carried out in quadruplicates. Above procedure was repeated for different concentrations (5000, 10000 and 15000 ppm) without changing the amount of D-Glucose.

STATISTICAL ANALYSIS

One-way ANOVA with Dunnett's post test was performed using GraphPad Prism version 5.00 for Windows, (GraphPad Software, San Diego California USA).

RESULTS

INORGANIC ANALYSIS

The moisture content of Datura alba was 86.23 ± 0.15. The carbon content as a measure of ash was 8.73 ± 0.30. The mean organic matter was 10.06 ± 0.11. (Table 1)

Figure 2

Table 1: Quantitative measure of moisture, ash and organic matter of

Contents	Amount (%)
Moisture	86.23 ± 0.15
Ash	8.73 ± 0.30
Organic matter	10.06 ± 0.11

Calcium, magnesium, ferric and phosphate contents were $(3.17 \pm 0.02) \times 10^4$, $(3.37 \pm 0.01) \times 10^4$, $(1.38 \pm 0.004) \times 10^4$ and $(4.20 \pm 0.03) \times 10^4$ ppm respectively (Table 2).

Figure 3

Table 2: Quantitative measure of inorganic cations and anions.

Ions	Amount (ppm)
Ca^{2+}	$(3.17 \pm 0.02) \times 10^4$
Mg^{2+}	$(3.37 \pm 0.01) \times 10^4$
Fe^{3+}	$(1.38 \pm 0.004) \times 10^4$
PO_4^{3-}	$(4.20 \pm 0.03) \times 10^4$

QUALITATIVE ORGANIC ANALYSIS

In the chloroform extract, five organic constituents were tested for out of which only two were present (Table 3). Analysis of alkaloids and steroids was positive.

Key: - Absence; + Presence.

Figure 4

Table 3: Organic constituents of

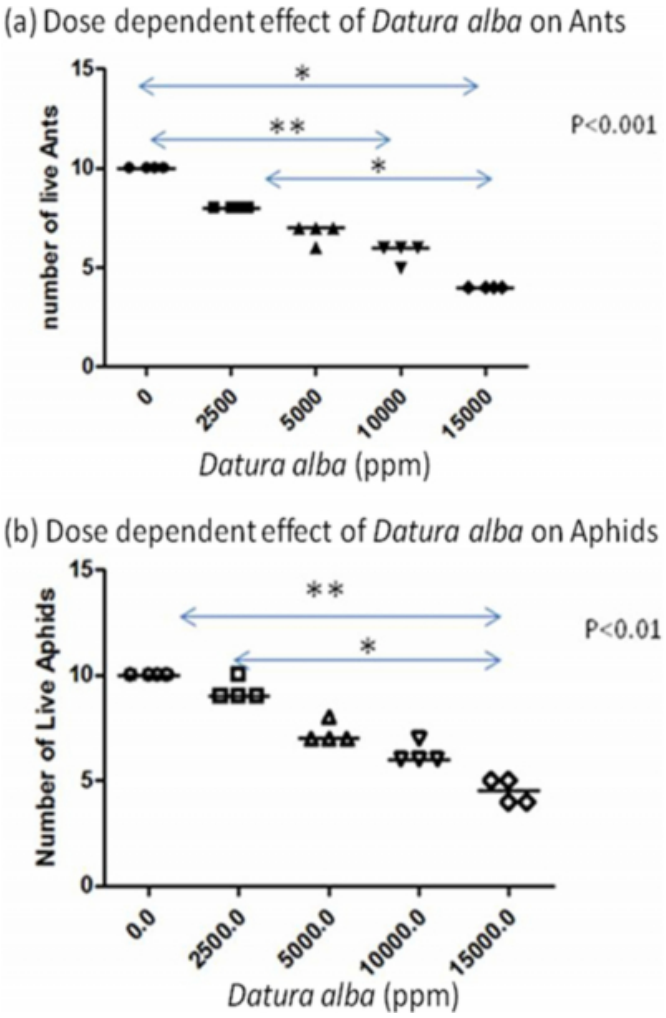
Organic compound	Chloroform extract
Saponins	-
Cardiac glycosides	-
Flavanoids	-
Alkaloids	+
Triterpenoids or steroids	+

TOXICITY OF DATURA ALBA ON ANTS AND APHIDS

There was a significant dose dependent effect of Datura alba on the number of living Ants (P<0.001) and Aphids (P<0.01) (figure 2). This indicates that high doses of leaf extracts are toxic.

Figure 5

Figure 2: Scatter plot of dose dependent effect of on the number of live (a) ants or (b) aphids in the experimental chamber. ANOVA ; *=P



The results showed the Effective Concentration 50% survival rate (EC_{50}) was 12000 ppm for aphids and 11600 ppm for ants.

DISCUSSION

Datura alba is a commonly used medicinal plant for inflammatory diseases such as asthma, rheumatism, muscle spasm and whooping cough and also used in wound healing as a disinfectant [16] among indigenous doctors in the Indian subcontinent. In the northern part of Sri Lanka, local farmers found that aphids feed young leaves of beans. Also, various size ants were found in valuable vegetable plants (ladies finger, aubergine) before they were ready for harvest damaging its stem that attached to the vegetable. In addition, at homes ants feed on variety of substances including sweets, starch and fats. Commonly available chemical insecticides are inorganic substances that are toxic to human and could

dissolve in water and pollute the environment. Since water is often obtained from underground wells in these parts of the world, farmers are greatly interested in natural insecticides as they are organic compounds that cannot dissolve completely in water and hence pollute the environment to a lesser degree. These are also easily available and cheap. People are reluctant to use chemical insecticides at home as they can be toxic to the human body. In this regard, Datura alba was suggested to be an ideal candidate by the local farmers that lead to this project.

In this study for the first time, we have isolated and confirmed the presence of chemical constituents in the leaf of this plant and tested its toxicity on aphids and ants. Inorganic cations, anions, moisture, ash and organic matter were also determined quantitatively. The concentration of Ca^{2+}, Mg^{2+}, Fe^{3+} and $PO_4^{3?}$ were found to be $(3.17 \pm 0.02) \times 10^4$, $(3.37 \pm 0.01) \times 10^4$, $(1.38 \pm 0.004) \times 10^4$ and $(4.20 \pm 0.03) \times 10^4$ ppm respectively. The Initial screening test in this study confirmed the presence of alkaloids in the leaf extracts. Alkaloids in excessive amount could be poisonous. Hence we investigated the toxicity of this plant on aphids and ants. Experiments suggest a statistically significant dose dependent decrease in the survival rate and an increase in the percentage mortality of ants and aphids in the presence of Datura alba. These experiments suggest that extract of Datura alba at suitable concentration could potentially be useful to local farmers.

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