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Ajith Kumar T G
Department of Botany,
St. Teresa's College, Ernakulam,
Kerala, India,
Email:
ajithgopalakrishnan81@gmail.com
Tel: +91-9895147858

Lizzy Mathew
Department of Botany,
St Teresa's College, Ernakulam,
Kerala, India
Tel: +91-9847540286

Correspondence:
Ajith Kumar T G
Department of Botany,
St. Teresa's College, Ernakulam,
Kerala, India,
Email: ajithgopalakrishnan81@gmail.com
Tel: +91-9895147858

Comparative account of the preliminary phytochemical aspects of *Helicanthes elastica* (Desr) Danser growing on two different hosts

Ajith Kumar T G, Lizzy Mathew

ABSTRACT
Preliminary phytochemical screening of the methanolic extract *Helicanthes elastica* belongs to Loranthaceae growing in two hosts *Nerium indicum* and *Hevea brasiliensis* revealed the occurrence of various constituents. The important constituents identified in the parasite were glycosides, saponins, tannins and phenols in both hosts. Qualitative chemical tests conducted in the present study were to create a standardized parameters that have an immense value for higher phytochemical quantification of the major compounds present in the plant. It also helped to find out the difference in the qualitative aspects in the parasite with respect to hosts.

Keywords: *Helicanthes elastica* (Desr) Danser, Loranthaceae, methanolic extract, glycosides.

1. Introduction
Investigations conducted among the traditional communities in different parts of the world have greatly helped the modern world benefit from the traditional and indigenous knowledge systems regarding the medicinal properties of the plants [9]. Use of traditional medicine is widespread and plants still contain a large source of structurally novel compounds that serves as leads for novel drugs [4] and the curative properties of medicinal plants are due to the presence of such various complex chemical substances of different composition which occur as secondary metabolites [8].

The members of the Loranthaceae family (about 74 genera), generally known as mistletoes and mostly distributed in the tropics, are semiparasitic shrubs attached to the hosts by modified root generally called as haustoria [2]. Some species of Loranthaceae from China have been used as medicinal materials for the treatment of cancer, bacterial infection, hypertension and rheumatics [3]. Various compounds have been found in Loranthaceae and some of them have been identified with antimicrobial and hypotensive properties [5]. Regarding the method of application and administrations, the whole plant, roots, branches, leaves, flowers, fruits etc are used in various forms like paste, dried powder, decotion, etc, by various tribes of Western Ghats.

Fig 1: *Helicanthes elastica* (Desr.)Danser

Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical and biological properties [1]. For this quality assessment should be interpreted by the various tests so that a visible path is available for higher analysis.

Helicanthes elastica (Desr) Danser commonly called as mango mistletoe is a parasitic shrub endemic to India and growing throughout Kerala in various hosts. It is investigated that the host trees influence the parasitic plants growing on them in their phytochemical constituents. The host and the parasite also share some common metabolites which may be either produced by the host or the parasite [10]. The present study aims to find out whether any difference in the occurrence of phytochemical contents in *Helicanthes elastica* with respect to host on which they grow.

2. Materials and methods

2.1 Collection of plant materials

Fresh plants of *Helicanthes elastica* (Fig 1) growing on *Nerium indicum* and *Hevea brasiliensis* were collected from Ernakulam district, Kerala. The plants were authenticated by Dr. M J Paul (Associate professor (Retd.), Dept. of Botany, Bharata Mata College Thrikkakara, Ernakulam, Kerala) and was deposited in the department herbarium (Specimen no: 2014/602). The plant parts were washed in running water for several times followed by distilled water and shade dried at room temperature for 30 days. Dried specimen was powdered and stored in airtight plastic covers in low temperature for the phytochemical investigation.

2.2 Preparation of the extract [6]

About 20 gm of the powdered sample was extracted with 200 ml hot methanol in a soxhlet apparatus at temperature 55 °C for 10 hours. The extract is then concentrated in a boiling water bath to a volume of 30 ml and stored in air tight small brown bottles. These extracts were subjected to phytochemical screening for the identification of various phytoconstituents.

2.3 Preliminary phytochemical screening [6, 7, 11]

2.3.1. Tests for alkaloids

2.3.1.1 Mayer's Test

One or two drops of the Mayer's reagent (Potassium mercuric iodide solution) were added to the small amount of extract. Formation of a cream coloured precipitate indicates the presence of alkaloids

2.3.1.2 Wagner's Test

Few drops of Wagner's reagent (Solution of iodine in potassium iodide) are added to the extract and observed for the formation of reddish brown precipitate which indicates the presence of alkaloids

2.3.1.3 Hager's Test

To 1 ml of the extract a few drops of Hager's reagent (Saturated Picric acid solution) is added. Formation of a yellow precipitate indicates alkaloids

2.3.1.4 Dragendorff's Test

A few drops of Dragendorff's reagent (solution of potassium bismuth iodide) are added to a small amount of the extract which gives orange yellow colour if alkaloids present

2.3.2 Test for Glycosides

2.3.2.1 Salkowski Test

Concentrated extract is mixed with 2ml chloroform. To this solution 2ml concentrated sulphuric acid added carefully and shake gently. Formation of a reddish brown colour indicates the presence of glycosides.

2.3.2.2 Keller-Kiliani Test

Extract is mixed with 2 ml of glacial acetic acid containing 1 or 2 drops of freshly prepared ferric chloride solution. The mixture is shaken well and carefully poured along the sides of test tube containing concentrated sulphuric acid. Brown ring formation at the junction indicates the presence of cardiac glycosides.

2.3.3 Test for flavonoids

2.3.3.1 Shinoda test

Crude extract was mixed with a few small pieces of Magnesium ribbon for a minute and concentrated HCl was added drop wise into this mixture. If pink scarlet colour or light red colour develops after a few minutes, it indicates the presence of flavonoids

2.3.3.2 Lead acetate Test

Small quantity of the extract was treated with few drops of lead acetate solution. Formation of a yellow colour or yellow cream precipitate indicates the presence of flavonoids.

2.3.3.3 Alkaline reagent test

Extract was mixed with 2% NaOH solution. Intense yellow colouration which loses the intensity on the addition of dilute acid.

2.3.4 Test for phenols and tannins

2.3.4.1 Ferric chloride test

2 ml of freshly prepared ferric chloride solution added to a few drops of concentrated extract. Formation of dark blue or green or black colour indicates the presence of phenols and tannins

2.3.4.2 Gelatin Test

2ml of the extract was mixed with 2 ml of 1% gelatin solution containing NaCl. Formation of white precipitate indicates the presence of tannins.

2.3.5 Test for saponins

2.3.5.1 Froth test

2 ml of the extract is mixed with 20 ml of distilled water in a graduated test tube and shaken well for 10 minutes. Formation of froth with 1 cm thickness indicates that the sample contains saponins.

2.3.6 Test for sterols and triterpenoids:

2.3.6.1 Liebermann-Burchard test

The extract was treated with a few drops of acetic anhydride. It is boiled and cooled, concentrated sulphuric acid was added from the sides of the test tube carefully. A brown ring at the junction of two layers and the upper layer turning green, which indicated the presence of sterols while the formation of deep red colour indicated the presence of triterpenoids.

2.3.6.2: Salkowski's test: Extract was treated with chloroform and a few drops of concentrated sulphuric acid, shaken well and allowed to stand for some time, red colour appeared in the lower layer indicated the presence of sterols while the formation of a yellow coloured lower layer indicated the presence triterpenoids

2.3.7 Test for diterpenes

2.3.7.1 Copper acetate test

1 or 2 drops of the extract was mixed with 2 ml of copper acetate solution and shaken well, formation of green colour indicated the presence of diterpenes.

2.3.8 Test for carbohydrates

2.3.8.1 Molish's test

2 ml of the extract was taken in attest tube and few ml of Molish's reagent is added along the sides of the test tube. Formation of violet ring at the junction may indicate the presence of carbohydrates.

2.3.8.2 Fehling's test: Extract was treated with equal volumes of Fehling's solution A and B and then boiled in a water bath. Formation of reddish brown colour indicated the presence of reducing sugar.

2.3.8.3 Iodine test

1 or two drops iodine solution added to 1ml of the extract. Formation of dark blue colour indicated the presence carbohydrates.

2.3.9 Test for proteins and amino acids

2.3.9.1 Ninhydrin test

Concentrated extract was boiled with 2% Ninhydrin solution. Formation of blue or violet colouration indicates the presence of amino acids.

2.3.9.2 Xanthoproteic test

The extract was treated with a few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins.

3. Results & Discussions

Table1: Phytochemical evaluation of *Helicanthes elastica* growing on *Nerium* and *Hevea.*

TEST	H-1	H-2
Tests for alkaloids		
Mayer's test	+	-
Wagner's test	+	+
Hager's test	-	+
Dragendorff's test	+	-
Test for glycosides		
Salkowski Test	+	+
Keller Kiliani test	+	+
Test for flavonoids		
Shinoda test	+	+
Lead acetate test	+	+
Alkaline reagent test	-	-
Test for phenols and tannins		
$FeCl_3$ test	+	+
Gelatin test	+	+
Test for saponins		
Froth test	+	+
Test for sterols and triterpenoids		
Liebermann- Burchard's test	+	+
Salkowski test	+	-
Test for diterpenes		
Copper acetate test	-	+
Test for carbohydrates		
Molish's test	-	-
Fehling's test	-	-
Iodine test	+	+
Test for proteins and amino acids		
Ninhydrin test	-	-
Xanthoproteic test	-	-

\+ Present H1= *Helicanthes elastica* on *Nerium indicum*

\- Absent H2= *Helicanthes elastica* on *Hevea brasiliensis*

The present preliminary investigations of phytochemicals in *Helicanthes elastica* growing on Nerium and Hevea revealed (Table 1) that glycosides, phenols, saponins and tannins are the major constituents. The Keller Kiliani test gave good result indicated the occurrence of cardiac glycosides. Some tests showed positive and some negative in the case of alkaloids, sterols, triterpenoids, proteins and carbohydrates which indicates the variation in the types and concentrations of these compounds in the present plant growing in the selected hosts. Out of the three tests for flavonoids, two gave the positive results and one negative, which showed that these compounds are also present in the plants but its occurrence was not reported yet in the case of *Helicanthes elastica*.

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