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INVESTIGATION OF LARVICIDAL ACTIVITY OF CAESALPINIA BONDUC L. ROXB BARK

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ABSTRACT

The dengue virus, yellow fever, and other contagious diseases are spread by the mosquito Aedes aegypti. New solutions, such as bio-larvicides, have been created since this mosquito has a high level of resistance to the organophosphorus larvicides used to prevent its growth. Utilising plant extracts is quite common. The aim of this study was to evaluate the larvicide activity of Azadirachta indica leaf ethanolic extract against Aedes aegypti and to characterise its main components using GC-MS. The experiment used three different concentrations of ethanolic extract: 10 mg L-1, 20 mg L-1, and 50 mg L-1. A commercial larvicide consisting of Bacillus thuringiensis var. israelensis Serotype H-14 spores and endotoxic crystals was used as a positive control, while water was used as a negative control, three times. Higher larval mortality (93%) was reported after 72 hours of incubation in the ethanolic environment 50 mg L-1 of extract was utilised; extracts at 10 mg L-1 and 20 mg L-1 resulted in 47% and 70%, respectively, larval death. The primary substance discovered by the GC-MS research was phytol (14. 4% area). The results of this study demonstrated that A. indica's ethanolic extract might kill A. aegypti larvae. **Objectives:** To carry out qualitative analysis of crude extract for the presence of phyto constituents. • To investigate the larvicidal activity of the test extract bark of Ceasalpinea bonduc. L. Roxb. **Result:** The medicine Ceasalpine bonduc's mortality rate. When the concentration was raised, L. Roxb bark demonstrated an increase in mortality. When compared to low doses, high doses (mention concentration) had a higher death rate, indicating that the action was dose-dependent. Standard medicine has a high death rate. **Conclusion:** The larvicidal action of our test substance, an extract of Ceasalpinia bonduc. L. Roxb, is dose-dependent and exhibits a high death rate at high concentrations and a low mortality rate at low concentrations.

KEYWORDS: Larvicidal, Caesalpinia bonduc l. roxb, contagious diseases, mortality.

INTRODUCTION

Worldwide, malaria causes significant rates of illness and mortality, with sub-Saharan Africa seeing the worst effects.[1] The main species found here include Anopheles gambiae, An. coluzzii (previously An. gambiae M form[2]), An. arabiensis, and An. funestus, which are among the most effective malaria vectors. The latter is the sole important vector and nominal member of the Anopheles funestus species group, while the first three species belong to the Anopheles gambiae species complex.[3, 4] Over the past ten years, the prevalence of malaria has dramatically decreased thanks to insecticide-based vector control measures, with long-lasting insecticide-treated bednets (LLINs) and indoor residual insecticide spraying (IRS) being particularly important.[1] Larval source management (LSM) is also becoming more crucial to vector control, particularly in view of the rising incidence of pesticide resistance in target vector populations.[5] [6] Despite notable gains gained by larviciding in the early-to-mid 1900s, larval source management as a vector control approach has substantially diminished since the transition towards the use of synthetic mosquito adulticides, primarily DDT and subsequently pyrethroids.[7] Only 38 of the 97 countries with continuous malaria transmission use LSM, which is presently only acknowledged by the World Health Organisation (WHO) as an adjunct to the main therapies of IRS and LLINs.[1] Although there is limited recent data, larviciding may once again play a significant role in integrated vector control, according to the WHO, if conditions are favourable and it is technically practicable.[8,7] Due of these factors, research on possible larviciding methods is still ongoing. Interest in naturally occurring pesticides, especially those with a botanical origin, has grown over the past few years. Over many millennia, plants and insects have coevolved, as have the defensive systems they developed in response to insect predation. Compared to their synthetic equivalents, plant

allelochemicals are far more beneficial as insecticidal actives since they are biodegradable and frequently exhibit decreased or no side effects.

Review of literature

Plant profile

Fig no 01: Bark of *Caesalpinia bonduc* L. Roxb.

Fig no 02: Flower of *Caesalpinia bonduc* L. Roxb.

Fig no 03: Fruit of *Caesalpinia* Roxb.

Fig no 04: Leaf of *Caesalpinia bonduc* L.

bonduc

Fig no 05: Seed coat of *Caesalpinia bonduc* L. Roxb.

Fig no 06: Fruit with seed of *Caesalpin bonduc* L. Roxb.

Plant Profile[12]

Kingdom	**:** Plantae
Phylum	**:** Magnoliophyta
Division	**:** Magnoliopsida
Class	**:** Angiospermae
Order	**:** Fabales
Family	**:** Fabaceae
Genus	**:** Caesalpinia
Species	**:** bonduc
Part used	**:** Bark

Vernacular Names

English Name	**:** Fever nut,
Hindi Name	**:** Kantkarej,
Sanskrit Name	**:** Kakachika,
Kannada Name	**:** Gajjiga,
Telugu Name	**:** Mulluthige,
Tamil Name	**:** Kalarci ver,
Urdu Name	**:** Akitmakit

Plant Description

An extensive climber; very thorny shrub, branches finely grey-downy, armed with hooked and straight hard yellow prickles.

Stem: Vine stem diameters to 5 cm recorded. Usually grows as a vine but also flowers and fruits as a shrub. Occasional spines or numerous spines present on the stems. Blaze odour resem- bles that of fresh green beans (Phaseolus vulgar-is). Pith white, quite large in diameter.

Leaves: Leaves are with large, leafy, branched, baal appendages: 30 – 60 cm. Long: petioles : stipulus a pair of reduced pinnaebipinnate, large, stipules a pair of reduced pinnae at the base of the leaf each furnished with a long mucronate point; pinnae 6-8 pairs; 5-7.5 cm. long, with a pair of hook stipulary spines at the base, main leaf axis armed with stout, sharp, recurved spines, divided into 4-8 pairs of secondary branches.

Flowers: Flowers in dense (usually) long- peduncled, terminal and supraaxillary racemes dense at the top, looser downward, 15-25 cm. long; pedicels very short in buds, elongating to 5 mm. in flower and 8 mm. in fruits, brown- downy; bracts squarrose, linear, acute, reaching 1 cm. long, fulvous hair, Calyx 6-8 mm. long, fulvous and hairy; lobes obovate-oblong, obtuse. Petals about 10-12 mm long, oblanceolate, yellow, filaments declinate, flattened at the base, clothed with long white silky hairs. Ovary on a stalk (stipe) about 1 mm. long, 2 ovules.

Principle Constituents

The detailed survey of literature revealed that the species of *Caesalpinia bonduc* L. Roxb., exhibited anti-diabetic, anti-asthmatic, anti-oxidant, anti-inflammatory, anti-filarial, anti-bacterial, immunomodulatory, anti-tumor, and anxiolytic activity. Several chemical constituents including Steroidal Saponin, Fatty Acids, Hydrocarbons, Phytosterols, Isoflavones, Aminoacids and Phenolic are present in the plant.

Medicinal Uses

Caesalpinia bonduc L. Roxb. (Caesalpiniaceae) is a large scan dent prickly shrub found throughout the interior parts of India, Sri Lanka and West Indies. It is common in southern parts of India and is often grown as a hedge plant.[13] This plant has profound medicinal use and is a proved anti-inflammatory[14] anti-helminthic and anti-malarial drugs.[15] It has also been an effective stomachic, digestive and is used as liver tonic in the treatment of jaundice and various liver disorders. It is attributed to be an aphrodisiac and general tonic helping in the rejuvenation of body.[16] The roasted seed powder is used as an anti-leprotic. The seeds are useful as anti-diabetic, anti-periodic, anti-pyretic, etc.

Common Larvicidal agents and mechanism of action

Abate 500E[17]

Active material: Temephos (organophosphate)

MOA: Abate acts by ingestion or contact. It inhibits an enzyme that is important to the normal function of the insect larvae`s nervous system, killing the insect larvae before the mature and preventing new generations of disease-carrying insects from developing.[29]

Imicon 17.8%.

Active material: Imidacloprid

Imidacloprid is a broad-spectrum neonicotinoid insecticide, Imidacloprid is the most well-known and widely used representative of the neonicotinoid insecticides. Neonicotinoid insecticides are designed to act on nicotinic receptors to control insect pests and, at the same time, to express low toxicity to vertebrate species, The activity ofneonicotinoid insecticides on the central nervous system of vertebrates is further reduced by poor penetration of the blood–brain barrier.

METHODOLOGY

Preliminary Phytochemical screening (Qualitative Analysis)

The preliminary phytochemical studies were performed on the ethanolic extract of the bark *of caesalpinia bonducL. Roxb.*.

1. Alkaloids

a) Dragendorff's test

To 2 mg of the ethanolic extract 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

b) Hager's test

To 2 mg of the ethanolic extract taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow precipitate confirmed the presence of alkaloids.

c) Wagner's test

2 mg of ethanolic extract was acidified with 1.5%v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.

d) Mayer's test
To a few drops of Mayer's reagent, 2 mg of ethanolic extract was added. Formation of white or pale yellow precipitate indicated the presence of alkaloids.

2. Carbohydrates
a) Anthrone test
To 2 ml of Anthrone reagent solution, 0.5 ml of alcoholic extract wasadded. Formation of green or blue colour indicated the presence of carbohydrates.
b) Benedict's test
To 0.5 ml of aqueous extract, 5 ml of Benedict's solution was added and boiled for 5 minutes. Formation of brick red coloured precipitate indicated the presence of carbohydrates.
c) Fehling's test
To 2 ml of aqueous extract, 1 ml mixture of equal parts of Fehling's solutionA and B were added and boiled for few minutes. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.
d) Molisch's test
In a test tube containing 2 ml of alcoholic extract, 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added. 2 ml of Conc. Sulphuric acid was added so as to form a layer below the mixture. Red-violet ring appeared, indicating the presence of carbohydrates, which disappeared on the addition of excess of alkali.

3. Flavonoids
a) Shinoda's test
In a test containing 0.5 ml of the ethanolic extract, 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

4. Triterpenoids
a) Liebermann - Burchard's test
2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of thetest tube. Formation of a violet coloured ring indicated the presence of triterpenoids.

5. Proteins
a) Biuret's test
To 1 ml of hot aqueous extract, 5-8 drops of 10%w/v sodium hydroxide solution, followed by 1 or 2 drops of 3%w/v copper sulphate solution were added. Formation of violet red colour indicated the presence of proteins.
b) Millon's test
1 ml of aqueous extract was dissolved in 1 ml of distilled water and 5-6 drops of Millon's reagent were added. Formation of white precipitate, which turns red on heating, indicated the presence of proteins.

6. Resins
1 ml of ethanolic extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicated the presence of resins.

7. Saponins
In a test tube containing about 5 ml of an ethanolic extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

8. Steroids
a) Libermann -Burchard's test
1 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of thetest tube. Formation of green colour indicated the presence of steroids.
b) Salkowski reaction
2 mg of dry extract was shaken with chloroform, to the chloroform layer; sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

9. Tannins
To 1-2 ml of the ethanolic extract, few drops of 5%w/v $FeCl_3$ solution was added. A green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudotannins.

10. Starch
0.01g of Iodine and 0.075 g of potassium iodide were dissolved in 5 ml of distilled water and 2-3 ml of ethanolic extract was added. Formation of blue colour indicated the presence of starch.

Pharmacological investigation
Preparation of stock solutions or suspensions and test concentrations. Larvicidal activities of Caesalpinia bonduc bark. Ethanol fractions were evaluated against A.aegypti mosquitoes following the WHO standard protocol for testing the mortality of mosquitoes with slight modifications (WHO 2005). disposable Pasteur pipettes were used to dispense a homogeneous population of third and early fourth instar larvae. A water depth in the test cups between 5.00 cm and 10.0 cm (average of 7.50 cm) was maintained to prevent undue larval mortality when soaked at deeper levels. All test cups used in the assay was prepared prior to dose The technical materials of many organic compounds are insoluble in water. These materials were dissolved in appropriate organic solvents such as ethanol (the manufacturer should be consulted) in order to prepare to dilute solutions for laboratory testing. The formulated materials are, however, miscible with water. Suspending or mixing these formulations in water requires no special equipment– homogeneous suspensions can be obtained by gentle shaking or stirring.

The stock solution is then serially diluted (two-fold) in ethanol or other solvents Stock solutions of Caesalpinia bonduc extract is prepared in alcohol at an initial concentration of 160 mg in 2 ml (80 mg/ml). Then, two-fold dilutions were prepared in 1ml alcohol in each of the

test cups to get a total of five different concentrations rangingfrom 80, 40, 20, 10, and 5 mg/ml of the Caesalpinia bonduc extract. It should be kept in a screw-cap vial, with aluminum foil over the mouth of the vial, shake vigorously to dissolve or disperse the material in the solvent. Twenty third or fourth-instar larvae were placed in plastic cups containing distilled water for 1 h to reduce the stress. five different concentrations ranging from 80, 40, 20, 10, and 5 mg/ml of the Caesalpinia bonduc. L. Roxb. extract is added to their respective plastic cups containing the 20 test larvae each.

Larvicidal Activity

Standard procedure for investigation of larvicidal activity is used according to WHO guidelines, in this procedure the larvae are divided in to five groups and one group for negative control and another group for positive control, here the different range of concentrations of our test drug is prepared according to guidelines, Initially, the mosquito larvae are exposed to a wide range of test concentrations and a control to find out the activity range of the materials under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range (of 4–5 concentrations, yielding between 10% and 95% mortality in 24 h or 48 h) is used to determine LC50 and LC90 values.

Batches of 20 third or fourth instar larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups or vessels, each containing 100– 200 ml of water. Small, unhealthy or damaged larvae were removed and replaced. The depth of the water in the cups or vessels should remain between 5 cm and 10 cm; deeper levels may cause undue mortality.

The appropriate volume of dilution was added to 100 ml or 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration. Four or more replicates were set up for each concentration and an equal number of controls were set up simultaneously with tap water, to which 1 ml alcohol (or the organic solvent used) was added. Each test will run three times on different days. For long exposures, larval food were added to each test cup, particularly if high mortality is noted in control. The test containers are held at 25–28 C0 and preferably a photoperiod of 12 h light followed by 12 h dark (12L:12D).

After 24 h exposure, larval mortality was recorded. For slow-acting insecticides, 48 h reading may be required. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed. The results were recorded on the form provided where the LC50, LC90 and LC99 values, and slope and heterogeneity analysis was also noted. The form will accommodate three separate tests of six concentrations, each of four replicates, Larvae that have pupated during the test period was negate the test. If more than 10% of the control larvae pupate in the course of the experiment, the test was discarded and repeated. If the control mortality is between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott's formula.

$$\text{Mortality (\%)} = \frac{X - Y}{X} 100$$

where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

RESULTS

Fresh stem bark was collected from the tree and dried at room temperature to remove moisture. The bark was coarsely powdered and extracted with 95% ethanol in a Soxhlet extractor, further, the extract was filtered and concentrate on Rotaevaporator to get extract that is Ethanolic extract of bark of Caesalpinia bonduc L. Roxb. bark. The percentage yield of ethanolic extract of Ceasalpinea bonduc. L. Roxb bark was found to be 19%.

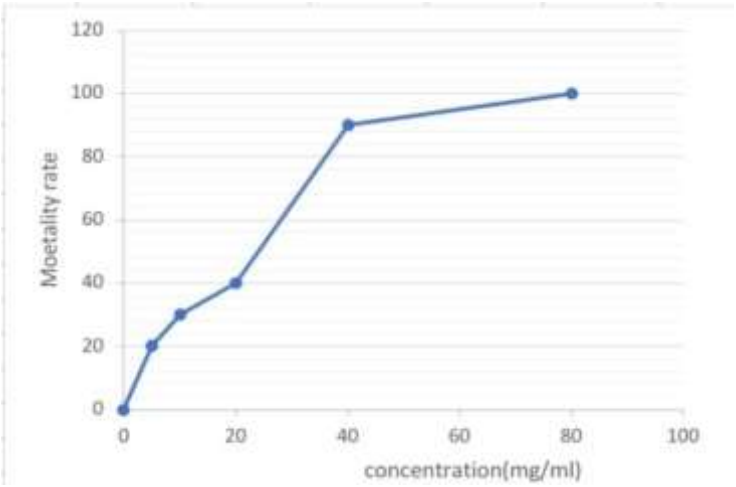
Preliminary phytochemical screening of bark of Caesalpinia bonduc L. Roxb.

It is observed from the preliminary phytochemical screening of bark of Caesalpinia bonduc L. Roxb. that alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenoids, resins, Preliminary qualitative phytochemical screening was carried out for the ethanolic extract of bark of Caesalpinia bonduc. The presence or absence of active constituents in extract was mentioned in Table 03.

Sl no.	Phyto-constituents	Alcoholic extract
1	Carbohydrates	-
2	Proteins	-
3	Tannins	+
4	Saponins	-
5	Triterpenoids	+
6	Flavonoids	+
7	Resins	-
8	Starch	-
9	Alkaloids	+
10	Steroids	-

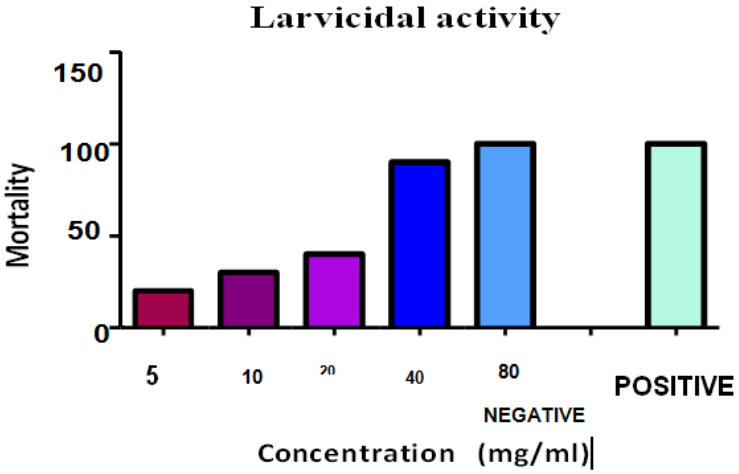
The preliminary phytochemical studies revealed the presence of Alkaloids, Flavanoids, Triterpenoids, Steroids and Tannins from the ethanolic extract of bark of Ceasalpinea bonduc L. Roxb.

DETERMINATION OF LC50 & LC90



Determination of LD50 & LD90 by plotting a graph mortality rate v/s concentration.

LD50 = 24mg/ml LD90 = 40mg/ml



DISCUSSION

Medicinal plants are a great source of economic value all over the world. Nature has given us a very rich botanical wealth and large number of diverse types of plants growsin different parts of the country.

The present study has been designed to investigate the larvicidal activity by ***Caesalpinia bonduc. L. Roxb bark*** on mosquito larvae. For this study ***Caesalpinia bonduc. L. Roxb bark.*** was collected in the local market of the chitradurga. The preliminary investigation of ***Caesalpinia bonduc. L. Roxb bark*** shows the presence of volatile and fixed oils, alkanoids, flavonoids, triterpionoids and tannins for which several bioactivities like glucose and lipid lowering effects, anti- diabetic, anti- periodic and anti-helminthic actions.

The findings of result revealed that the bark extract has been shown significant larvicidal activity. Thus from the results of the current investigation it may be inferred that the bark extract of ***Caesalpinia bonduc. L. Roxb bark*** possess larvicidal activity.

For this experiments we used the mosquito larvae, ***Caesalpinia bonduc. L. Roxb bark*** extract, the mosquito larvae were divided as five gropus and one for negative control and another one for positive control, by using the ***Caesalpinia bonduc. L. Roxb bark*** extract is added to the cups contains 20 3rd & 4th instar larvae. the experiment was monitored for 12 hours day and 12 hours night, then observe the state of the larvae in the test cup and taken the readings of the different concentration for calculation of mortality followed by the determination of LD50 and LD90. The activity in positive control and negative controls are compared to theactivity of test drug.

CONCLUSION

In the present study the test sample of bark extract of Caesalpinia bonduc was exhibited significant larvicidal activity on aedes aegypti mosquito larvae and it has the phytochemical constituents that may be responsible for the results shown by the plant

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