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Research Article

QUALITATIVE ANALYSIS OF LAGERSTROEMIA SPECIOSA AND EVALUATION OF REPELLANT ACTIVITY AND MORTALITY RATE OF HAEMADIPSA SYLVESTRIS (BLANCHARD, 1894)

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Abstract

The largest and toughest species of Indian land leeches is *Haemadipsa sylvestris*. Leeches are hermaphrodites that live in moist soil, rotting leaves and on the bodies of animals. They are hermaphrodites and can result in bacterial infections that are both painful and secondary. Commonly, chemical medications are used to treat them. The projects objectives include biocontrol of the leech and research on its repellent and mortality rate. The experimental plant contains bioactive component is revealed by the phytochemical analysis. Poomaruthu is the plants common name in Tamil. It is a member of the genus *Lagerstroemia* and the family Lythraceae and its scientific name is *Lagerstroemia speciosa*. Crape myrtle is the common name for *L.speciosa*. The ornamental plant contained steroids, phenolic compounds, amino acids and other chemicals, according to a preliminary phytochemical analysis. The control and standard groups show the mortality of experimental animals within 20 minutes 20.17±2.75d. and 35.00±3.00c the repellent activity with collapsing rate is 95.00±2.00b and 145.00±3.00b. The group IV consisting of *L. speciosa* bark shows the highest fatal rate within 20.33±2.52d and the disgusting activity is 90.00±3.00c, which is equivalent to the positive control and standard. The death rate of group VI is 70.00±3.00b and the collapsing rate is 105.00±2.00d. This is will compare to the control, and the standard rate of mortality is subordinate. The overall significant (P<0.05) mortality rates of *H. sylvestris* is 2.12±4.62 and 2.35±5.13.

Keywords: Land leech, *Lagerstroemia speciosa,* Repellent activity, Mortality Rate.

INTRODUCTION

The phylum Annelida contains the class Hirudinea, which includes leeches. It lives on land must be around a lot of moisture. Land leeches become active under a moderate drizzle but remain dormant in periods of intense rain and heat. Since the majority of leeches consume animal blood, they are referred to as "Hematophagous". Leeches are naturally aggressive and can infect a variety of animals with various diseases. Leeches secrete hirudin and histamine in their saliva . Hirudin, a thrombin inhibitor, is responsible for prolonged bleeding at sites of leech bites. Bleeding at the bite sites results in purpura, visible hemorrhage into the skin burning, fever, intense itching, swelling, tiredness, delirium and finally can cause unconsciousness by the bite of poisonous leeches (Chandra, 1991). They have ability to ingest small quantity of blood. Venomous leeches are thick in the middle part, they have elongated body with low locomotion; looks dull (Chandra,1991).Leech bite is very painful and also lead to secondary bacterial infections (Dhottamma *et al.,* 2021).Leech saliva also contains histamine, causing bite sites to be pruritic. Leech bites can also be complicated by trypanosome infection. Plants are rich source of alternative agents for control of insects, because they possess bioactive chemicals, which act against limited number of species including specific target insects and are eco-friendly (Sukumar, 1991).*Lagerstroemia speciosa* belongs to the family Lythraceae and the genus of *Lagerstroemia*. Crape myrtle is the common name for *Lagerstroemia speciosa*. Poomaruthu is the plant's common name in Tamil. This ornamental plant contained steroids, phenolic compounds, amino acids and other chemicals, according to a preliminary phytochemical analysis. Primary metabolites such as glycosides and tannins exist abundantly in the leaves, while sugars and proteins can also be found but in lesser concentrations. Secondary metabolites that were found to be present were steroids, anthraquinone glycosides, flavonoids, saponins and tannins. Bahmani *et al.,*(2012) shows the severity effect of the compounds was recorded for 720 min and categorized in 5 groups (1to5) based on the time to paralyze or kill the leech. This project objective includesbio control of the leech and research on its repellent and mortality rate of *Haemadipsa sylvestris*.

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MATERIALS AND METHODS

Collection and Identification of Experimental Plant

In this study, *Lagerstroemia speciosa* leaf, flower, bark and root were collected from PG girls hostel Government Arts college, Coimbatore-18, Southern India. The identification of the plant was authentified at BSI, Coimbatore. (No: BSI/SRC/5/23/2020/Tech/58).

Processing of Experimental Plant

The collected plant material was brought to the laboratory. The plant leaf, flower, bark and root were observed carefully for any kind of diseases or infection and if any were found, those parts were separated and not used for the experiment. The leaf, flower, bark and root of the plant were washed with distilled water, dried with a clean towel and the plant material was cut into small pieces and dyed at an ambient room temperature (27± 2ºC) for about 2 weeks. Afterwards, they became brittle. Dried plant materials were powdered using an electric blender. The raw powder was preserved in clean plastic containers, kept away from light, heat and moisture until use. No preservative

or colouring agents were used during the preservation of the trial drug.

Phytochemical Analysis

Qualitative phytochemical analysis of leaf, flower, bark, root of *L. speciosa* aqueous extracts was carried out according to the methodology of Horborne (1984) and Trease and Evans (1989). The preliminary phytochemical investigation of *L. speciosa* leaf showed that they contained steroids, phenolic compounds, α-amino acids, saponins, starch, alkaloids, Phytosterols, organic acids, reducing sugars and tannins (Laruan *et al.,* 2013). The preliminary phytochemical investigation of *L. speciosa* flowers showed that they contained Alkaloids, Flavonoids, saponin, Glycosides, Phenols, Saponins, Tannins, Reducing sugar (Faisal *et al.,* 2020). The preliminary phytochemical investigation of *L. speciosa* bark showed that they contained Flavonoid, Reducing sugar, Protein and Amino acid, Tannin, Phenols (Pal*et al.,* 2020). The preliminary phytochemical investigation of *L. speciosa* root showed that they contained Alkaloids, Phenols, Tannins, reducing sugar, Flavonoids, Reducing sugar and Saponins (Hussain *et al.,* 2014).

Collection of Experimental Animals

Leeches are collected from the Estates of O' Valley, Gudalur Taluk with latitude and longitude of 11º27'19''N and 76º28'79''E. O'Valley or Ouchterlony Valley is a Town panchayat in the Gudalur Taluk of the Nigiri's district in the Indian state of Tamil Nadu. The place is located between Mudumalai National Park and New Amarambalam Reserved Forest, is famous for its flora and fauna. It was mainly dominated by *Haemadipsa sylvestris and Haemadipsa montana.* The moist and humid climate causes heavy infestation of land leeches in jungles, hill slopes and pathways; leeches remain inactive during hot weather but become active during light drizzle (Nath *et al.,* 2002).

Maintenance of Experimental Animals

In this study, the number of experimental animals is used around 35. *H. sylvestris* leech with uniform sizes, are the largest and most robust species of the Indian land leeches. The colour is plain yellow or brown of varied shades, the mid-dorsal field somewhat paler, with three black or dark-brown stripes. The median line is narrower, broken or absolute. Marginal stripes are bright orange or yellow. It is gathered from its natural environment, specifically from decaying leaves and under rocks. Leeches were manually picked and transferred into a plastic container, and kept wet by adding a small amount of water and moist soil.The collected leeches were transported to the laboratory.

Identification of Animal

The adult leeches are subjected to morphological characteristics like colour, size, position of the anterior and posterior the sucker and location of the eye through microphotography method. A handbook and the book "Leeches of India" were used to identify the test leeches (Chandra, 1991).

Repellent Activity

Raw powders of the *L. speciosa* plant leaf, flower, bark, and root were used in the experiment. The methodology described by Eftekhari *et al.,* 2011 was adopted for the evaluation of repellents using containers in the experiment. For the anti-repellent and mortality activity, 4 plastic containers (30 cm length 12 cm width) were taken, and in total, a total of 25 leeches were used for this experiment. There are 4 types of experimental samples that were taken. For repellent activity, 0.3 g of each sample of leaf, flower, bark and root as a raw powder were added to the plastic container. For the inoculation of five experimental animals into a plastic container using size - one paint brush. The observation was noted. During the first 15 minute period of observation, there is no death occurs, but the blood suckers move very quickly from the experimental sample.

Experimental Design

The evaluation of the death of a leech was based on immobility after stimulation with a needle. The low average paralyzing or killing time of these compounds reflects anti - leech properties. The severity effect of these compounds/drugs based on the time was categorized into seven groups as follows:

Group I -ISP(0.3gm/1.025gm BW/Iodized Salt Powder)
Group II -BSP(0.3gm/1.025gm BW/Black Snuff Powder)
Group III -LLP (0.3gm/1.025gm BW/ *Lagerstroemia speciosa* Leaf Powder)
Group IV -LFP(0.3gm/1.025gm BW/ *Lagerstroemia speciosa* Flower Powder)
Group V -LBP(0.3gm/1.025gm BW/ *Lagerstroemia speciosa* Bark Powder)
Group VI -LRP(0.3gm/1.025gm BW/ *Lagerstroemia speciosa* Root Powder)

The powerful anti-leech properties of *L. speciosa* active compounds were noted during the experiment. It consists of seven groups as follows:

1. Standard group I - The paralysis and death of each leech within 20 mins
2. Standard group II - The paralysis and death of each leech within 24 mins
3. Standard group III - The paralysis and death of each leech within 27mins
4. Standard group IV- The paralysis and death of each leech within 135 mins
5. Standard group V - The paralysis and death of each leech within 20 mins
6. Standard group VI - The paralysis and death of each leech within 70 mins

The effect of active plant ingredients of *L. speciosa* raw powderkills the *H. sylvestris* within 20 minutes.

Statistical Analysis

The number of observation deaths in the study population is divided by the number of expected deaths (calculated from indirect adjustments) and multiplied by 100.The rate of mortality was calculated according to the formula adopted by Philimon, 2016.

Exposure mortality:

$$\text{Mortality rate} = \left(\frac{Number\ of\ dead\ leeches}{Total\ number\ of\ leeches}\right) \times 100$$

$$\text{Mortality rate} = \left(\frac{5}{5}\right) \times 100$$

$$\text{Mortality rate} = 100\%$$

The differences between the control and treated groups were analysed using differences ANOVA and the sigma state two programs.

RESULTS

Results 1 - Phytochemical Analysis: Qualitative phytochemical analysis of leaf, flower, bark, root of *L.speciosa* aqueous extracts was carried out according to the methodology of Horborne (1984) and Trease and Evans (1989). The table - 1 shows the active compounds of *L. speciosa* raw powder.

Table 1. Phytochemical Analysis of *Lagerstroemia speciosa*

S. No	Phytocompounds	Leaf	Flower	Bark	Root
1	Alkaloids	++	++	-	+
2	Flavonoids	-	+++	+++	++
3	Saponins	+++	+	-	+
4	Phenols	++	+++	+++	-
5	Tannins	+++	++	+++	++
6	Protein and Amino acids	+++	-	+	-
7	Reducing sugar	+	++	++	+
8	Steroids	++	-	-	-
9	Glycosides	-	++	-	-
10	Phytosterols	++	-	-	-

'+' indicates the presence of phytoconstituents.
'++' indicates the phytoconstituents present in a moderate level.
'+++' indicates the phytoconstituents present abundantly.
'-' indicates the absence of active constituents.

Table 2. Mortality Rate of *Haemadipsa Sylvestris* (240 Mins Observation)

Groups	Leech 1	Leech 2	Leech 3	Leech 4	Leech 5
ISP	35.00±2.00 d	95.00±2.00 b	40.00±3.00 e	20.17±2.75 d	35.17±1.76 e
BSP	99.67±3.51 bc	24.67±2.52 e	59.67±2.52 c	35.00±3.00 c	145.00±3.00 b
LLP	101.67±3.51 b	27.33±3.51 e	60.33±2.52 c	40.00±3.00 c	140.00±3.00 c
LFP	135.33±1.53 a	210.00±3.00 a	180.00±3.00 a	150.00±3.00 a	177.67±3.51 a
LBP	30.00±3.00 e	90.00±3.00 c	45.00±2.00 d	20.33±2.52 d	35.17±1.76 e
LRP	95.33±2.52 c	75.33±2.52 d	90.33±2.52 b	70.00±3.00 b	105.00±2.00 d
SEd±	2.2690±	2.2852±	2.1344±	2.3551±	2.1213±
CD (P<0.05)	4.943	4.9791	4.6504	5.1313	4.6220

The different subscripts (a, b, c and d) in column represents existence of significant differences between groups (P<0.05).

Results 2 - Repellent Activity

Plant-based chemicals act as a repellent activity. The experimental plant *L. speciosa* bark consists of more repellent activity when compared to other samples and it is also equivalent to the positive control and standard.

Results 3 - Mortality Rate

The mortality rate of *H. sylvestris* is compared to positive control and standard. The control and standard group show the mortality of the experimental animal within 20 minutes, 20.17±2.75d. and 35.00±3.00c.The repellent activity with collapsing rate is 95.00±2.00b and 145.00±3.00b. The group III *L.speciosa* leaf shows 27.33±3.51e and moderate timing for demise and the rate of collapse 140.00±3.00c. *L.speciosa* flower powder shows a poor repellent and mortality rate of 135.33±1.53a and the delayed collapsing activity is 210.00±3.00a. The group IV consisting of *L. speciosa* Bark shows the highest fatal rate within 20.33±2.52d and the revolting activity is 90.00±3.00c, it is equivalent to 1`for the positive control and standard. The death rate of group VI is 70.00±3.00b and the collapsing rate is 105.00±2.00d. This will compare to the control and the standard rate of mortality is subordinate. The overall significant (P<0.05) mortality rate of *H.sylvestris*is 2.12±4.62 and 2.35±5.13.

Results 4 - Statistical Report

As per the statistical report, the rate of mortality is higher in *L.speciosa* bark raw powder and lower in the *L. speciosa* flower powder. This shows the powerful compounds present in bark powder. The flower of *L. speciosa* phyto compounds does not actively participate in the mortality activity. The positive control of salt powder and the standard group's Snuff powder show highly commendable results in the death rate of *H.sylvestris.* Group V was compared and concluded that it is equal to the mortality rate of the same.

DISCUSSION

Polyphenols are one of the largest and most widespread groups of secondary metabolites in the plant world. The repellent activity is higher in the bark of *L. speciosa*; it consists of high amounts of phenols and flavonoids. The main sources of phenolic compounds are fruits and vegetables, but lately, more and more studies refer to woody vascular plants, especially to bark, as an important source of phenolic compounds with a potential biological effect (Tanase *et al*., 2019). The death rate is also high in Group V. The maximum rate is within 20 minutes of time intervals. The flavonoids are the natural source of different fragrances. This may evoke a feeding habit. Sometimes it causes toxicity to the organism. The flower of *L. speciosa* consists of furfural in the form of flavonoids. In experimental group IV, the collapsing rate is 210 minutes and the death rate is 135 minutes. The least power of the compound is responsible for the late time interval of demise. The group III and group VI show delayed repellent activity and delayed death rates. Plants synthesized different types of phenolic with diverse functions that they perform against biotic stresses (Dar *et al*., 2017). Tannins, natural products extracted from many plants, have been reported (Hoste *et al*., 2006). Tannins protect several plants against herbivores (Feeny, 1976) and are toxic to a wide range of fungi, bacteria and yeasts (Scalbert, 1991). Taylor and Murant (1966) found a reduction of the number of worms in soil treated with two powdered tannin extracts from *mimosa*. From the result, the tannins are responsible for the repellent and mortality of land leeches.

Conclusion

Dangerous Toady – It is commonly called "Indian Land Leech" or "Sina jook" or "Ramar attai" in Tamil. Five pairs of eyes and the sucker rays vary in different species. Its bite is very painful. The parasitic leech bite causes skin irritation and it forms excision wound. It must be control for the welfare of the land workers to prevent them from different health issues.

From the above results the *L. speciosa* is used as ornamental as well as for medicinal properties. We strongly recommend the plant being used for quick control and to kill the harmful bitter.

Authors' contributions

Author 1* designed the study, wrote the protocol, and wrote the first draft of the manuscript, Author 1 did the experimental work, performed the statistical analysis, Author 2managed the analyses of the study, managed the literature searches. All authors read and approved the final manuscript.

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