



# EVALUATION OF PRELIMINARY PHYTOCHEMICALS, LARVICIDAL AND BLOOD COAGULATIVE PROPERTIES OF SPHAGNETICOLA TRILOBATA (L.) PRUSKI & LANTANA CAMARA (L.)

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## ABSTRACT

The present study was carried out to evaluate the preliminary phytochemical analysis, larvicidal and blood coagulative properties of *Sphagneticola trilobata* (L.) Pruski & *Lantana camara* (L.). The leaves were selected for this study. Aqueous extract used for phytochemical analysis. In plants the secondary metabolites are responsible for metabolic activities and defensive in purpose. Phytochemical analysis shows the presence of carbohydrates, alkaloids, phenols, saponins, tannins and flavonoids in both of these plants. Therefore they contain medicinally active phyto components. These plant materials have potent larvicidal activity against the *Culex* larvae. *L. Camara* showed higher larvicidal activity. These plant extracts shows brilliant blood coagulating property. So these invasive plant extracts are very useful as coagulant in bleeding diseases and wound healing.

**KEYWORDS:** Lantana camara, Sphagneticola trilobata, Uses

## 1. INTRODUCTION

Invasive species are the non native species introduced to an area by man. In the new area, the invasive species have little or no threat from their natural enemies. IAPS is regarded as destructive to the ecosystem. Some of these IAPS are utilized by a substitute for scarce indigenous plants. Phytochemicals are chemical compounds that occur naturally in plants, have biological significance & act as essential nutrients. Blood coagulation is the basic process for wound healing. Mosquitoes are the major threat to health. Larval stages are appropriate to control it. The larvicidal studies help to measure the ability of plants to kill larvae.

## 2. MATERIALS AND METHODS

*Lantana camara* (L.) leaves are used for the study. *Lantana camara* belongs to verbenaceae, native to Central and South America. *Sphagneticola trilobata* (L.) Pruski belongs to asteraceae, native to Mexico.

## PHYTOCHEMICAL ANALYSIS

Standard protocols were practiced for the identification of phytochemical constituents of *Lantana camara* and *Sphagneticola trilobata* (Santhi and Sengottuvel., 2016)

## TEST FOR CARBOHYDRATES

**Benedict's test:** Take 5 ml of Benedict's reagent and add the plant extract to boil it. Boil for 2 minutes and cool for some times. The appearance of reddish brown precipitate indicates a positive test for sugar.

**Molisch's test:** To 2ml of the plant extract, two drops of Molisch's reagent were added. Shake well and few drops of concentrated sulphuric acid added slowly along the sides of test tubes. A violet ring indicates the presence of carbohydrates.

## TEST FOR PROTEIN AND AMINO ACID

**Xanthoprotein test:** Take about 2 ml of the sample in a test tube and add few

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drops of  $\text{HNO}_3$  into it and heat the test tube. A yellow precipitate is formed.

**Ninhydrin test:** To 2 drops of Ninhydrin solution is added to 2 ml of aqueous filtrate. The appearance of purple color indicates the presence of amino acid.

#### TEST FOR ALKALOIDS.

Extract were dissolved individually in dilute HCl and filtered. The filtrate was used to test the presence of alkaloids.

**Mayer's test:** Add one or two drops of Mayer's reagent (Potassium Mercuric Iodide) to the plant extract. A yellow/cream precipitate indicates the presence of alkaloid.

**Wagner's test:** Dissolve a little extract to its solvent and add Wagner's reagent. Alkaloid gives the brown flocculent precipitate.

#### TEST FOR PHENOLS

**$\text{FeCl}_3$  test:** Add 3 drops of  $\text{FeCl}_3$  to 2ml of sample solution taken in the test tube. Dark blue-green color indicates the presence of phenols.

**Lead acetate test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow/cream colour precipitate indicates the presence of phenols.

#### TEST FOR SAPONINS

**Foam test:** The extract is diluted with distilled water and shaken well. A 2 cm layer of foam indicates the presence of saponins.

**Froth test:** Take 1 ml of sample in a test tube. Add 2 ml of distilled water and shake well. The appearance of froth on the shaking of the mixture shows the presence of saponins.

#### TEST FOR STEROIDS

**Lieberman Burchard test:** Add freshly prepared Lieberman Burchard reagent to the extract. A green color shows the presence of steroids.

#### TEST FOR TANNINS

**$\text{FeCl}_3$  test:** Add  $\text{FeCl}_3$  to 1 ml of sample solution taken in a test tube. A brownish green or blue black

colouration indicates a positive test for tannin.

**Lead acetate test:** A small amount of extract was dissolved in distilled water. To this solution, few drops of lead acetate solution were added. Formation of green colour precipitate indicates the presence of tannins.

#### TEST FOR FLAVONOIDS

**NaOH test:** 3 ml of dilute NaOH is added to 1 ml of sample, if it turns to yellow color indicates the presence of flavonoids.

**$\text{H}_2\text{SO}_4$  test:** 1 ml extract taken in the test tube, add 1 ml of con.  $\text{H}_2\text{SO}_4$ . Formation of an orange colour indicates the presence of flavonoids.

#### TEST FOR TERPENOIDS

**Salkowski's test:** Take 1 ml of the sample solution in a test tube. Add a few drops of chloroform along the sides of the test tube. Then add carefully a drop of concentrated  $\text{H}_2\text{SO}_4$ . Reddish brown colouration at the interphase indicates the presence of terpenoids.

#### TEST FOR PHLOBETANNIN

**HCl test:** Boil 1 ml extract taken in a test tube with 1 % HCl. A red precipitate indicates the Presence of phlobetannin.

#### TEST FOR GLYCOSIDES

**Con. $\text{H}_2\text{SO}_4$  test:** 1 ml of con. $\text{H}_2\text{SO}_4$  was added to 1 ml of test solution and allowed to stand for 2 minutes. The formation of reddish colour indicates the presence of glycosides.

**Salkowski's test:** extract was taken in a test tube; 2 ml of chloroform and 2 ml of con. $\text{H}_2\text{SO}_4$  was added carefully and shake well. Appearance of reddish brown colour indicates the presence of glucosides.

#### LARVICIDAL ACTIVITY

- **COLLECTION OF MOSQUITO LARVAE:** *Culex quinquefasciatus*.
- **LARVICIDAL ASSAY:** Larval bioassays were performed according to the standard guidelines of WHO. In the first trail about 14 ml of water is taken in a petriplate & 1 ml of plant extract is added to the petriplate. In the second petriplate

5 ml of the extract was added to 10 ml of the water. In the third petriplate the concentration of extract was increased to 10 ml. In every case the number of larvae maintained to a constant number of 5. In each petriplate the mortality rate were recorded in every hour. The final volume of larval water and extract is maintained to 15 ml.

- $\text{LARVICIDAL RATE} = (X-Y/Y) \times 100$  ( X= number of larvae died, Y= total larvae )

### **BLOOD COAGULANT ACTIVITY**

**COLLECTION OF BLOOD SAMPLES:** from healthy human volunteers.

**PROCEDURE:** The haemostatic activity were studied on clotting time (CT) of blood, by using **Lee-White method**.

The venous blood was collected from volunteers in a clean and dry slide without the addition of an anticoagulant. Add a little amount of each extract in the corresponding slides and examined at 30 second intervals to see if clotting had occurred.

Stop-watch was started as soon as the blood spotted in a slide. The time required for clotting was noted. The watch was immediately stopped when clotting occurred and the time was noted. Likewise, CT was recorded for the remaining samples.

### **3. RESULT AND DISCUSSION**

Phytochemical analysis conducted on aqueous leaf extracts of *Lanatana camara* and *Sphagneticola trilobata* revealed the presence of carbohydrates, alkaloids, phenols, saponins, tannins and flavonoids in both of these plants. Proteins and amino acids are altogether absent in both plant extracts. There is a presence of glycoside in *Sphagneticola trilobata*.

In this study larvicidal activity of *L.camara* and *S.trilobata* investigated within a time period of 24 hours.

The ethanolic extract of the plants facilitate the blood clotting time, within 1-2 minutes.

### **Phytochemical analysis**

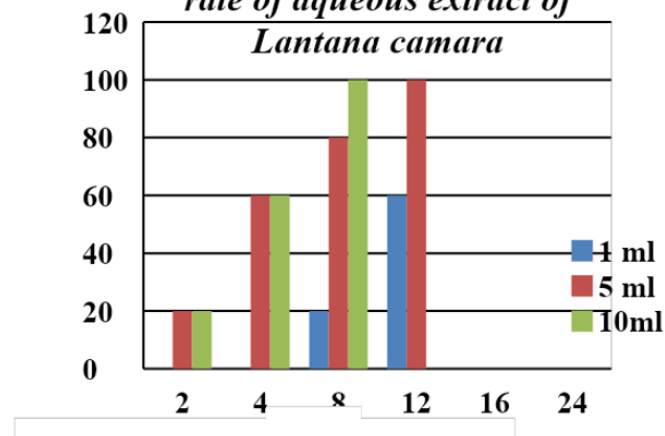
**Table 1**

| Sl. No | Phytocomponents |                                     | Aqueous solution of            |                       |
|--------|-----------------|-------------------------------------|--------------------------------|-----------------------|
|        |                 |                                     | <i>Sphagneticola trilobata</i> | <i>Lantana camara</i> |
| 1      | Carbohydrates   | Molish's test                       | +                              | +                     |
|        |                 | Benedict's test                     | +                              | +                     |
| 2      | Protein         | Xanthoprotein test                  | -                              | -                     |
| 3      | Amino acids     | Ninhydrin test                      | -                              | -                     |
| 4      | Alkaloids       | Mayer's test                        | +                              | +                     |
|        |                 | Wagners test                        | +                              | +                     |
| 5      | Phenols         | Ferric chloride test                | +                              | +                     |
|        |                 | Lead acetate test                   | +                              | +                     |
| 6      | Saponin         | Foam test                           | +                              | +                     |
|        |                 | Froth test                          | +                              | +                     |
| 7      | Tannin          | Ferric chloride test                | +                              | +                     |
|        |                 | Lead acetate test                   | +                              | +                     |
| 8      | Steroid         | Salkowski test                      | -                              | -                     |
| 9      | Flavonoid       | NaOH test                           | +                              | +                     |
|        |                 | H <sub>2</sub> SO <sub>4</sub> test | +                              | +                     |
| 10     | Phlobetanin     | Hcl test                            | -                              | -                     |
| 11     | Glycoside       | H <sub>2</sub> SO <sub>4</sub> test | +                              | -                     |
|        |                 | Salkowski test                      | +                              | -                     |
| 12     | Terpenoid       | Liebermann burchard test            | -                              | -                     |

### **LARVICIDAL ACTIVITY**

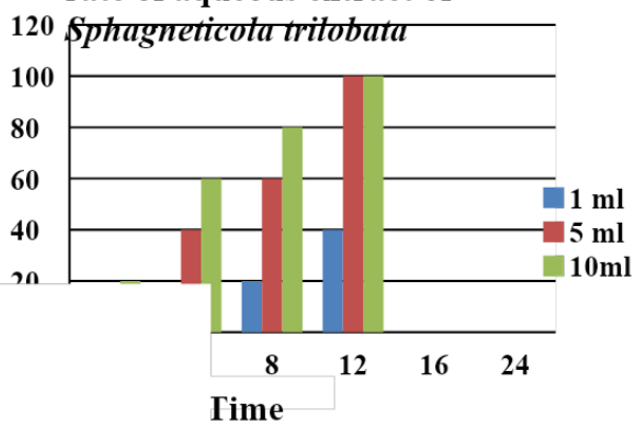
| Plant extract           | Concentration | 2 hrs | 4 hrs | 8 hrs | 12 hrs | 16 hrs | 24 hrs |
|-------------------------|---------------|-------|-------|-------|--------|--------|--------|
| Lantana camara          | 1 ml          | -     | -     | 1     | 3      | -      | -      |
|                         | 5 ml          | 1     | 3     | 4     | 5      | -      | -      |
|                         | 10ml          | 1     | 3     | 5     | -      | -      | -      |
| Sphagneticola trilobata | 1 ml          | -     | -     | 1     | 2      | -      | -      |
|                         | 5 ml          | -     | 2     | 3     | 5      | -      | -      |
|                         | 10ml          | 1     | 3     | 4     | 5      | -      | -      |

**Bar diagram showing the mortality rate of aqueous extract of *Lantana camara***



**Graph 1**

**Bar diagram showing the mortality rate of aqueous extract of *Sphagneticola trilobata***



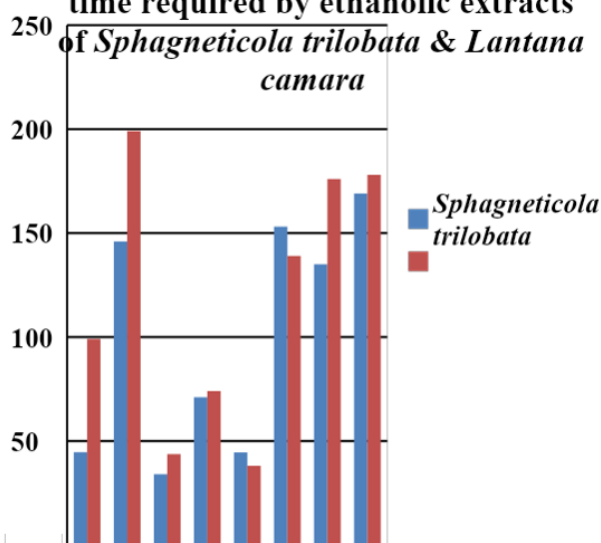
**Graph 2**

### **BLOOD COAGULANT PROPERTIES**

**Table 3: The Blood coagulant properties of Ethanolic extract of *Sphagneticola trilobata* and *Lantana camara***

| Blood group | Time taken to clot the blood group (sec) |                       |
|-------------|------------------------------------------|-----------------------|
|             | <i>Sphagneticola trilobata</i>           | <i>Lantana camara</i> |
| A +ve       | 44.6 sec                                 | 99 sec                |
| A -ve       | 146 sec                                  | 199 sec               |
| B +ve       | 34 sec                                   | 43.7 sec              |
| B -ve       | 71 sec                                   | 74 sec                |
| AB +ve      | 44.5 sec                                 | 38.1 sec              |
| AB -ve      | 153 sec                                  | 139 sec               |
| O +ve       | 135 sec                                  | 176 sec               |
| O -ve       | 169 sec                                  | 178 sec               |

**Bar diagram showing blood clotting time required by ethanolic extracts of *Sphagneticola trilobata* & *Lantana camara***



**Graph 3**

### **4. CONCLUSION**

- Plants have an immense potential for management and treatment of wounds.
- These primary and secondary metabolites in the plants facilitate the blood coagulation time. This property can be used in modern drug industry.
- The blood clotting property may correlates with presence of tannin and flavanoid levels.
- Eradication of invasive weed plant is a difficult task
- So these can be used in manufacture of bio-insecticidal industries.
- Larvicidal efficacy of *Lantana camara* is higher than *Sphagneticola trilobata*. It shows an acceptable pathway to reduce the invasiveness of weed plants.
- It becomes a low cost, eco-friendly, biodegradable and non toxic to control mosquitoes.
- Advanced researches in the field of invasive phytochemical and pharmacological studies shall open a sustainable control and inventions in the field of weed management.
- That would have a significant role in our society.

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