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In vitro* investigation of anti-microbial and anti-helminthic activity of extract obtained from flowers of the shrub *Calliandra haematocephala

Jharna Tiwari and Dr. Gopal Rai

Abstract

The objective of preceding study was to investigate the *in vitro* anti-microbial and anti helminthic activity of extract obtained from flowers of the shrub *Calliandra haematocephala* against gram positive bacteria *Staphylococcus aureus* and *Eisenia foetida* (species of helminths) respectively. Extraction was done by soxhlet method using chloroform. Followed by preliminary phytochemical analysis, Agar well dilution method was used in anti microbial testing. And the antihelminthic activity was tested by using gradual concentration of crude ethanolic extract using Piprazine citrate syrup as reference solution. From the investigations it has been cleared that flower of *Calliandra haematocephala* likely to have phytoconstituents such as alkaloids, tannins, cardiac glycosides, saponins and flavonoids. And also the flowers are fruitful by having properties like antimicrobial and anti helminthic.

Keywords: *Calliandra haematocephala*, antihelminthic activity, anti microbial activity, phytochemical screening

Introduction

The plants have been known the vital source for obtaining variety of new herbal drugs. Establishment and practice of pharmacy is itself derived from use of herbaceous plant. Thus it is fruitful to investigate such plants and to study their chemical constituents behind their prior activities. *Calliandra haematocephala*, because of its distinct appearance as that of powder puff, it is commonly known as red powder puff. It is a rambling shrub with height of 1.5-4 meters has branched pinnates and silky leaves. It has been found that their leaves close at night hence they are kept in *Mimosaceae* family (Touch-me-not family). The red powder puff flowers are attractive to butterflies and hummingbirds but only appear from November -April. They are widely found^[1]. The presence of antibacterial and insecticidal properties in leaves and seeds from former researches has drawn attention in investigating activities found in flowers as no earlier significant researches are made on its flowers^[2].



Fig 1: *Calliandra haematocephala* shrub

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Taxonomical description

Kingdom: Plantae
 Phylum: Angiosperm
 Family: *Mimosaceae*
 Sub Family: *Fabaceae*
 Genus: *Calliandra*
 Species: *C. Haematocephala*



Fig 2: red powder puff

Plant Description

| | |
|--------------------|---------------------|
| Common Name: | powder puff tree |
| Type: | Broadleaf evergreen |
| Family: | Fabaceae |
| Zone: | 9 to 11 |
| Height: | 3.00 to 6.00 feet |
| Spread: | 2.00 to 3.00 feet |
| Bloom Time: | Seasonal bloomer |
| Bloom Description: | Red |

Plan of Work

- ✚ Literature Survey
- ✚ Collection Of Plant
- ✚ Extraction And Phytochemical Screening
- ✚ Assessment Of Pharmacological Activity
- ❖ Anti-Microbial Activity
- ❖ Antihelmintic Activity

Literature survey

- ❖ Moharram FA, Marzouk MS, Ibrahim MT, Mabry TJ.: Antioxidant galloylated flavonol glycosides from *Calliandra haematocephala*: Nat Prod Res. 2006 Aug; 20(10):927-34.
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- ❖ Michel Marlier, Gaston Dardenne, Jean Casimir: 2S,4R-carboxy-2-acetyl-amino-4-piperidine dans les feuilles de *Calliandra haematocephala* *Phytochemistry, Volume 18, Issue 3, 1979, Pages 479-481*
- ❖ I. Mattagajasingh, L. Acharya, A.K. Mukherjee, P.C. Panda, P. Das: Genetic relationships among nine cultivated taxa

of *Calliandra* Benth. (Leguminosae: Mimosoideae) using random amplified polymorphic DNA (RAPD) markers Original Research Article: *Scientia Horticulturae, Volume 110, Issue 1, 11 September 2006, Pages 98-103*

Collection, Identification and Authentication of Flower

The fresh flowers of plant were collected and was identified, confirmed and authenticated by the taxonomist from Jawaharlal Nehru Krishi Vishwa Vidyalay, Jabalpur (M.P.).

Extraction of flower

The flowers of *Calliandra haematocephala* were shade dried for 15 days, crushed in an electrical grinder and then powdered. Defatening was done by taking 150 gm of powder and soaked in chloroform for 24 hrs. The residue was then soxhletated for 2-3 days. The obtained content was used directly for phytochemical screening and for further studies.

Preliminary Phytochemical Analysis^[3-7]

Various quantitative preliminary phytochemical tests performed on crude powder are enlisted below:-

Tannins

Two hundred mg of flower powder extracted with 10.0 mL of d/w and filtered. Two mL of filtrate was treated with 2 mL ferric chloride. Formation of blue black ppt indicated the presence of tannins.

Steroids

Two hundred mg of powder was extracted with 10 mL of chloroform and filtered. Two mL of filtrate was treated with 2 mL of acetic anhydride, boiled for few minutes and cooled. To the solution conc. sulphuric acid was added foam the sides of test tube. No blue green ring was obtained

Alkaloids

10 mL of ethanol extract was treated with dil.HCl and filtered, and following test were made:

Test 1: Two drops of filtrate was treated with Dragendorff's reagent. Orange ppt formed indicated the presence of alkaloids.

Test 2: Two drops of filtrate was treated with Mayer's reagent. Formation of creamish ppt. confirmed the presence of alkaloids.

Cardiac Glycosides

Two mL of ethanol extract was mixed with 1 mL of glacial acetic acid with the addition of few drops of FeCl₃ and conc H₂SO₄. Formation of greenish colour indicated the presence of cardiac glycosides.

Flavonoids

Ethanol extract of flower was treated with 2 drops of conc. HCl and a piece of magnesium ribbon. Pink-tomato red colour formed indicating the presence of flavonoids.

Sapopnins

0.5 mL of ethanol extract was mixed with 5 mL of d/w. it was kept on a shaker for 10 minutes. After that, it was allowed to stand. Persistent frothing indicated the presence of saponins.

Table 1: Phytoconstituents Which Were Found to Be Present in *Calliandra haematocephala*

| S. No. | Name of Phytoconstituents | Inference |
|--------|---------------------------|-----------|
| 1 | Tannins | Present |
| 2 | Steroids | Absent |
| 3 | Alkaloids | Present |
| 4 | Cardiac Glycosides | Present |
| 5 | Flavonoids | Present |
| 6 | Saponins | Present |

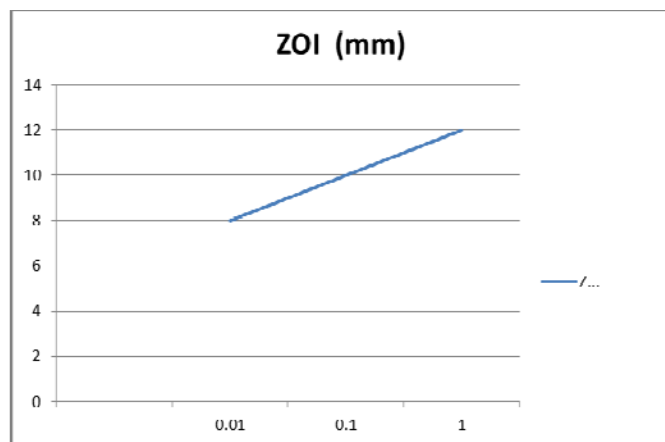
Detection of Antimicrobial Activity

The *in vitro* anti microbial study was made by using gram positive bacteria *Staphylococcus aureus*. The method adopted was Agar dilution method to determining the activity. Ciprofloxacin was taken as standard solution. Taking into consideration the safety measures, test sample were sent to Daksh Laboratories (An ISO 9001:2008 Certified Company) A unit of Excellent Bio-Research Solutions Pvt. Ltd. for detecting the activity. The results and observations observed are drawn below.

Method Used

Table 2: Antimicrobial Activity of *Calliandra haematocephala* against *Staphylococcus Aureus*

| S. No | Control (etoh) | Concentration | Zone of inhibition |
|-------|----------------|------------------|--------------------|
| 1 | -- | stock | 12 mm |
| 2 | -- | 10 ⁻¹ | 10mm |
| 3 | -- | 10 ⁻² | 8 mm |



Anthelmintic Activity

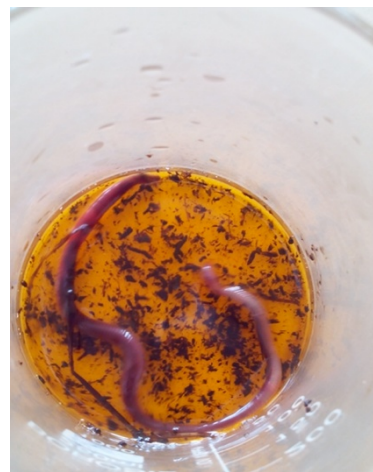
The anthelmintic activity was conceded by using gradual concentration of crude ethanoic extract in concentration of (10, 50, 100) mg/mL in d/w. And three worms of nearly same type per concentration were placed in it. Paralysis time was recorded by ensuring no movement of any sort with the exception of when the worms were shaken vigorously. Time of death of worms were recorded after ascertaining that the worms does not possessed any sort of movement even when they were shaken vigorously or when they dipped in hot water(50 °C).^[8]

Standard

Piperazine citrate syrup (10 mg/mL approx) was used as reference standard.

Worm Collection and Authentication

The earthworms *Eisenia foetida* were collected and authenticated From Department of Agronomy College Of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidhyalaya, Jabalpur (M.P.), India

**Fig 5:** activity in standard reference 10mg/mL**Fig 6:** showing activity of earthworms in two different dilutions of extract.**Fig 7:** ensuring death of earthworms by dipping into 50 °C warm water (no movement was observed)**Table 4:** activity of standard reference as observed in study

| S. No | Concentration of Drug (Mg/MI) | Time of Paralysis | Time of Death |
|-------|-------------------------------|-------------------|---------------|
| 1 | 10 | 25.50 ± 0.82 | 40 ± 0.53 |

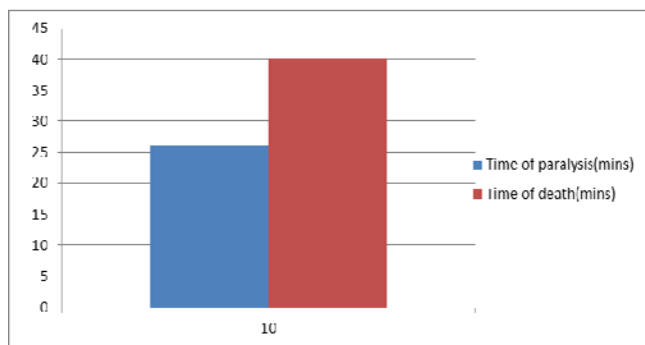
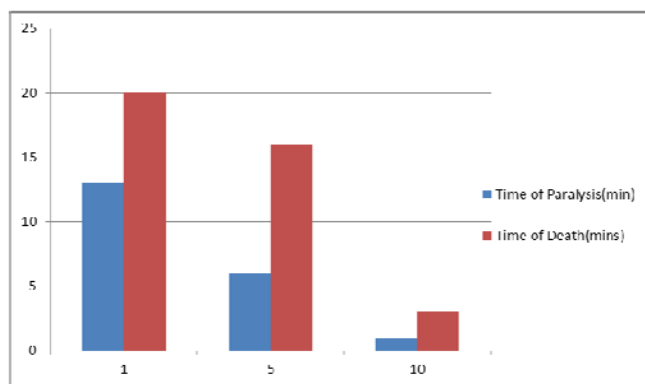


Table 5: Anti helminthic activity of extract as observed in the study

| s.no | Concentration of eth. extract in d/w | Time of paralysis | Time of death |
|------|--------------------------------------|-------------------|---------------|
| 1 | 1 in 10 ml | 12±76 | 20±02 |
| 2 | 5 in 10 mL | 5±93 | 15±84 |
| 3 | 10 ml | 1±30 | 2±55 |



Result and Discussion

Preliminary Phytochemical Anaysis

Preliminary phytochemical analysis exposed the presence of alkaloids, tannins, flavonoids. The secondary metabolite such as cardiac glycosides was also found to be present. The variation seen in the different activities may be due to phytochemical properties of constituents. Possibly the effectiveness of the constituents may not be because of only one active constituent but may be due to several chemicals compound present in it.

Antimicrobial Activity

The activity observed in any plant extract is because of various phytoconstituents present in it. Antimicrobial activity found in extract of *Calliandra haematocephala* was thought to be effective in higher concentration as the zone of inhibition observed from the stock solution was much estimable than other dilutions.

Anthelmintic Activity

As piperazine citrate is much likely be super effective for pin worms and round worms, but its effectiveness on earth worms had also been recorded. Because of lack of crude piperazine citrate, marketed formulation was used (piperazine citrate syrup manufactured by Glaxo Smith Kline Pharmaceutical Limited). Hence there might be chances of little variations in the results. Remarkable anthelmintic activity has been observed from the preliminary investigation.

Conclusion

The potential for developing antimicrobials and antihelminthic from plants appears rewarding in development of phytomedicine. From the investigations it has been cleared that flower of *Calliandra haematocephala* likely to have phytoconstituents such as alkaloids, tannins, cardiac glycosides, saponins and flavonoids. And also the flowers are fruitful by having prosperities like antimicrobial and anti helminthic. The present study of *in vitro* evaluation of flowers of *Calliandra haematocephala* forms a primary platform for further phytochemical and pharmacological studies.

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