



Evaluation of Phytochemical constituents, Proximate and Fluorescence analysis of ethanolic extract and its fractions of *Clerodendrum philippinum* Schauer found in Wayanad region of Kerala, India

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Abstract

Clerodendrum philippinum Schauer, a member of the family Verbenaceae, is a semi-woody shrub widely distributed in the tropics and subtropics of India. The present study was conducted for phytochemical evaluation, proximate analysis and fluorescent characteristics of leaves of *C. philippinum* collected from Western Ghats of Wayanad. The ethanolic extract and its different fractions subjected to preliminary phytochemical analysis by standard procedures. The extract revealed alkaloids, saponins, glycosides, steroids, phenolic compounds, tannins, flavonoids, fixed oils and fats. The proximate analysis of the plant leaves revealed dry matter (94.54%), moisture (5.47%), carbohydrates (56.86%), crude fibre (11.36%), ash (8.80%), crude protein (26.25%) and crude fat (2.62%). Fluorescence analysis showed difference in characteristic colour when examined with specific chemical reagents in powdered plant leaves, crude extract and fractions of *C. philippinum* under visible light and UV light.

Keywords: *Clerodendrum philippinum*, ethanolic extract, fractions, phytochemical analysis, proximate analysis, fluorescence analysis.

Introduction

Traditional medicine continue to play a pivotal roles in the healthcare system of large proportion of the world's population because of their cost-effective, eco-friendly attributes, and true relief from disease condition^{1,2}. Scarcity and high costs of orthodox medicine are also attributed to this³. The medicinal plants constitute a rich source of pharmacologically active secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are available abundantly at low cost and easily biodegradable⁴.

The plants of the genus *Clerodendrum* have more than five hundred member species and are widely distributed in the tropics and subtropics especially in the southern Asia. Morphologically, the plant is characterized by opposite and stipulate leaves, terminal or axillary cymose inflorescence or panicles, hypogynous bisexual flowers and persistent calyx⁵ (figure 1).

Clerodendrum philippinum Schauer, commonly called as Chinese Glory Bower and locally as Kattumulla in Wayanad is an ornamental plant. It has a semi-woody shrub of approximately¹⁰ feet height and morphologically characterised by broad leaves up to 1 feet long and wide, toothed margins, white with pink or red tinged fragrant flowers in tight clusters.

Traditionally the extracts of root and leaves of *C. philippinum* were used for the treatment of rheumatism, asthma and inflammatory diseases. It is also used for the treatment of jaundice, syphilis and typhoid⁶. Daily external application of *C. philippinum* leaf paste, after bath in the morning on the affected area of the stem was reported to relieve from all types of skin diseases⁷. Srivastava *et al*⁸ reported that this plant is used externally as a poultice for rheumatism and decoction of leaves for scabies.

The plant also has many scientifically validated pharmacological activities like antibacterial as well as anti-inflammatory activity. The ethanolic extract of plant showed anti bacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiell sp.*, and *Bacillus substillus*⁹. The aqueous extract exhibited significant anti inflammatory activity in acute and chronic inflammatory animal models¹⁰.

In the present study, an attempt is made to explore the phytochemical constituents, proximate analysis and fluorescence characteristics of the plant *C. Philippinum* found in Wayanad regions of Kerala. The fluorescence and proximate analysis and information on phytochemical constituents of *C. Philippinum* revealed its identification, standardization and diverse medicinal and pharmacological properties respectively.



Figure-1

Photograph of *Clerodendrum philippinum* Schauer in wild

Material and Methods

Plant materials: The leaves of *Clerodendrum philippinum* Schauer (figure 1) were collected from the campus of College of Veterinary and Animal Sciences, Pookode, and were authenticated and a voucher specimen was deposited at Department of Botany, University of Calicut (Accession No: CALI 6770). The plant leaves were cleaned, dried in shade and ground in a temperature controlled plant sample pulveriser. The powdered plant material was used for extraction using ethanol in soxhlet extraction apparatus (M/s Buchi, Switzerland) under reduced vapour temperature. Solvents were evaporated off by rotary vacuum evaporator (M/s Buchi, Switzerland) at 175 mbar at a temperature in the range of 40°C to 50°C. The weight of the dried extract was recorded and the extractive yield was calculated as,

$$\text{Extractive yield} = \frac{\text{Weight of the extract}}{\text{Weight of the sample taken}} \times 100$$

Fractionation of crude ethanolic extract: The ethanolic extract was fractionated using four solvents of ascending polarity index namely hexane, chloroform, n-butanol and water. The crude extract (200 g) was transferred to a separating funnel and extracted with hexane to obtain a hexane soluble fraction and a hexane insoluble fraction. After collecting the hexane soluble fraction, the hexane insoluble fraction was extracted with chloroform to yield chloroform soluble fraction and insoluble fraction. After collecting the chloroform soluble

fraction, the chloroform insoluble fraction was further extracted with n-butanol and subsequently with water to yield n-butanol soluble and aqueous fractions. Solvents were initially removed using a rotary vacuum evaporator (M/s Buchi, Switzerland) and completely dried by keeping at room temperature.

Phytochemical analysis: The crude ethanolic extract and its four fractions were subjected to phytochemical analysis for secondary metabolites like steroids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes, saponins and alkaloids¹¹⁻¹³.

Proximate analysis: The analysis of total ash, crude fibre, crude protein, total carbohydrate, crude fat, dry matter and moisture content of the leaves of *C. philippinum* were carried out using standard techniques of proximate analysis¹⁴ as briefly mentioned below.

Determination of dry matter: The dry matter was determined using the weight difference method and estimated by deducting per cent moisture from hundred as described by James¹⁵.

$$\text{Dry matter (\%)} = 100 - \% \text{ of moisture}$$

The moisture content was determined by using the formula,

$$\text{Moisture Percentage} = \frac{\text{Weight of the sample} - \text{Weight of the dried sample}}{\text{Weight of the sample}} \times 100$$

Determination of total ash: Ash represents the inorganic content of the sample which is determined by the method as described by Association of Official Analytical Chemists.¹⁶ For this, dried sample approximately one gram was taken in a crucible, charred over low flame and was kept in a muffle furnace set at 550-600°C for 2-3 hours. It was cooled in a desiccator to prevent re-absorption of moisture and the procedure repeated till constant weight of ash was obtained after three repeated weighing when the ash becomes white or greyish. The total ash content was calculated using the formula,

$$\text{Ash (\%)} = \frac{\text{Weight of the ashed sample}}{\text{Weight of the sample taken}} \times 100$$

Determination of crude fat: One gram of dried crushed sample was taken in the filter paper thimble and kept in a pre-weighed flask of the soxhlet extractor. 80 ml of petroleum ether was poured into the flask and the system was refluxed for 8 hours. The flask was cooled in a desiccator and the weight of crude fat extracted was taken. The per cent crude fat was determined by using formula,

$$\text{Percentage of Crude fat} = \frac{\text{Weight of the flask with fat} - \text{Weight of empty flask}}{\text{weight of the original sample}} \times 100$$

Determination of Crude fibre: One gram of the de-fatted plant material was taken in a spout-less beaker and boiled with 200 ml of 1.25% sulphuric acid for 30 minutes. The boiled content was then filtered and washed with hot distilled water to neutralize and transferred again into the beaker and boiled with 200 ml of 1.25% sodium hydroxide for 30 minutes followed by filtration and the filtrate was washed with hot distilled water for neutralization. The crucible was dried in a hot air oven at $100 \pm 5^{\circ}\text{C}$ overnight (10-12 hr) and cooled in a desiccator to a constant weight. Subsequently, the desiccator along with its content was put in a muffle furnace at $550-600^{\circ}\text{C}$ for 2-3 hour for complete burning of organic matter and cooled in a desiccator and weighed to a constant weight. The per cent of crude fibre was determined from the formula,

$$\text{Crude fibre (\%)} = \frac{(w_1 - w_2)}{\text{weight of the sample}} \times 100$$

where, w_1 - The crucible having crude fibre was cooled and weighed, w_2 - The content of the crucible was ignited over a low flame until charred and then kept in a muffle furnace and weighed.

Determination of crude protein: Total nitrogen (N) content was determined with the help of Kjeldahl method described by Pearson¹⁷ which consisted of digestion, distillation and titration steps.

Digestion: Approximately 5g of dried plant material was taken in a 50 ml digestion flask and digested with 1 g of digestion mixture (copper sulphate and sodium sulphate) and 15 ml of concentrated sulphuric acid. The solution was heated until the frothing was ceased and the solution became clear. The solution was gently boiled for another 2 hours, cooled and digested with 30 ml of water with constant stirring. The final digest was transferred to 250 ml standard flask and required amount of distilled water (up to the mark of the flask) was added.

Distillation: The distillation was done in a Kjeldahl distillation apparatus. Four per cent boric acid (20 ml) was taken with one drop of methyl red as indicator. Digested material (10 ml) was transferred to the distillation assembly and mixed with 20 ml of 40 per cent sodium hydroxide solution. The distillation was stopped in 6 minutes as indicated by the change of colour of boric acid from pink to blue.

Titration: The boric acid having trapped the ammonia from the nitrogen of protein was titrated with 0.1N hydrochloric acid, changing the colour back to pink.

The per cent of protein was calculated by the formula.

$$\text{Protein (\%)} = \frac{V \times 1.4 \times 6.25 \times 0.1\text{N HCl} \times \text{Vol (used)}}{W \times A \times 1000} \times 100$$

Where; V= Titre value, 1.4 = Weight of nitrogen expressed in gram in formula, 6.25 = Protein factor, W= Weight of sample, A= Aliquot digested sample used for distillation.

Determination of carbohydrate: Determination of available carbohydrate in the sample was calculated from the above parameters by using the formula.

$$\text{Carbohydrate (\%)} = 100 - (\text{moisture} + \text{crude fat} + \text{ash} + \text{protein}) \%$$

Fluorescence analysis: The fluorescence analysis of the plant was done by placing dry powdered leaves on a slide and observing the colour changes under visible and UV lights after treating with several drops of specific reagents (acetic acid, 10% copper sulphate, 1N hydrochloric acid, 1N nitric acid, 1N sulphuric acid, 10% ferric chloride and 1N sodium hydroxide). Similarly, the crude ethanolic extract and its fractions along with the respective solvent were observed without adding any reagents under visible light and UV light. The development of colour was noted within 1-2 minutes in order to avoid drying and resultant colour change^{18,19}.

Results and Discussion

Extractive yield: The extractive value of the ethanolic crude extract of leaves of *C. Philippinum* was 6.13 per cent whereas for n-hexane, chloroform, n-butanol and aqueous fractions, the extractive yield varied from 11.24, 27.96, 25.58, 25.02 per cent respectively.

Phytochemical Analysis: The results of qualitative phytochemical analysis are summarized in the table 1. The crude ethanolic extract of *C. philippinum* revealed the presence of flavonoids, steroid, glycosides, phenolic compounds, tannins, saponins, carbohydrates, alkaloids, fixed oils and fats. The hexane fraction of *C. philippinum* was positive for the presence of alkaloids, carbohydrates, flavonoids, fixed oils and fats. The chloroform fraction was positive for alkaloids, carbohydrates, flavonoids, saponins, fixed oils and fats. The n-butanol fraction revealed the presence of steroids, glycosides, flavonoids, saponins and carbohydrates. Steroids, glycosides, saponins and carbohydrates were detected in the water fraction. Previous reports on *C. Philippinum* revealed that the leaves of the plant was quite rich in alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, anthraquinones, lignin, glycosides, phytosterols, quinines, amino acids and proteins^{6,20,21}. Terpenoids, proteins and amino acids were not found in the present study.

Proximate analysis: The dry matter showed the highest percentage of 94.54%, the moisture content 5.47%, carbohydrate has 56.86% while crude fibre has a percentage of 11.36%, ash 8.80%, crude protein 26.25% and crude fat 2.62% (table 2). In another study the proximate analysis of *C. volubile* revealed energy value of $3.98 \pm 0.12\%$, dry matter $93.3 \pm 0.28\%$,

crude fibre $5.31 \pm 0.3\%$, ash $12.14 \pm 0.23\%$, crude protein $11.2 \pm 0.36\%$, crude fat $4.87 \pm 0.2\%$ and NFE $59.78 \pm 0.2\%$ ²².

presented in table 3. On exposure to UV light, the crude ethanolic extract of *C. philippinum* and its fractions elicited characteristic colours as given in table 4.

Fluorescence analysis: The different colours shown by the powdered leaf in presence or absence of chemical reagents are

Table-1
Qualitative phytochemical analysis of ethanolic extract of leaves of *C. philippinum* and its four fractions

| Phytochemicals | Inferences | | | | |
|--------------------------------|------------|--------|------------|---------|-------|
| | Ethanol | Hexane | Chloroform | Butanol | Water |
| Alkaloids | ++ | ++ | + | - | - |
| Carbohydrate | + | + | + | + | + |
| Saponin | ++ | - | + | ++ | ++ |
| Steroids | + | - | - | + | ++ |
| Phenolic compounds and Tannins | ++ | - | - | - | - |
| Terpenoids | - | - | - | - | - |
| Flavanoids | + | + | + | ++ | - |
| Protein and amino acids | - | - | - | - | - |
| Fixed oils and fats | + | ++ | + | - | - |
| Glycosides | + | - | - | + | + |
| Gums and mucilages | - | - | - | - | - |

Note: (+) Present and (-) Absence

Table-2
Proximate composition of leaves of *C. philippinum*

| Plant | Proximate contents (%) | | | | | | |
|-----------------------|------------------------|----------|-------------|---------------|-----------|------|--------------|
| | Dry Matter | Moisture | Crude fibre | Crude protein | Crude fat | Ash | Carbohydrate |
| <i>C. philippinum</i> | 94.54 | 5.47 | 11.36 | 26.25 | 2.62 | 8.80 | 56.86 |

Table-3
Fluorescence characteristics of powdered leaves of *C. philippinum* treated with different chemical reagents

| No. | Reagents | Visible light | Long UV (356 nm) | Short UV (254 nm) |
|-----|---|----------------------|------------------|-----------------------|
| 1 | Powder | Green | Black | Green |
| 2 | Powder + Water | Light green | Black | Yellowish green |
| 3 | Powder + CH ₃ COOH | Yellowish green | Black | Yellowish green |
| 4 | Powder +10% CuSO ₄ | Green | Black | Green |
| 5 | Powder +1NHCl | Grey | Black | Pale green |
| 6 | Powder +1N HNO ₃ | Grey | Black | Pale green |
| 7 | Powder +1N H ₂ SO ₄ | Yellowish grey | Black | Yellowish light green |
| 8 | Powder +10% FeCl ₃ | Dark green | Black | Dark green |
| 9 | Powder + 1N NaOH | Yellowish dark green | Black | Yellowish dark green |

Table-4
Fluorescence characteristics of crude ethanolic extract of leaves of *C. philippinum* and its four fractions

| No. | Fractions | Visible light | Long UV (356 nm) | Short UV (254 nm) |
|-----|---------------------|-----------------|------------------|-------------------|
| 1 | Crude extract | Dark brown | Black | Dark brown |
| 2 | Hexane fraction | Lightbrown | Dark brown | Dark brown |
| 3 | Chloroform fraction | Dark green | Black | Black |
| 4 | n-butanol fraction | Light green | Brownish black | Light green |
| 5 | Water fraction | Yellowish green | Black | Light green |

The information on proximate and nutrient contents of herbal products are required for their standardization and oral use²³. Results of proximate analysis indicated high content of mineral and crude protein in leaves of *C. philippinum*. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the range of visible light or when exposed to ultra violet radiation¹⁹. The organic molecules absorb light over a specific range of wave length and reemit radiations and hence it can be used for the identification of the powdered drug, extract or fractions of herbs²⁴. Taking in to consideration of existing medical importance, the plant can be explored for its additional pharmacological properties and explanations for existing activities.

Conclusion

The present study revealed the phytochemical constituents, proximate and fluorescence characteristics of ethanolic extract and its fractions of *Clerodendrum philippinum* Schauer. found in Wayanad region of Kerala, India. Further studies are required to characterize the bioactive compounds by suitable techniques.

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