

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *CLERODENDRUM INERME*Chethana G.S<sup>1</sup>, Hari Venkatesh K.R.<sup>2</sup>, S.M Gopinath<sup>3\*</sup><sup>1</sup>Research Associate, Sri Sri Ayurveda Trust, Udayapura, Bangalore, Karnataka, India<sup>2</sup>Head, R & D, Sri Sri Ayurveda Trust, Udayapura, Bangalore, Karnataka, India<sup>3</sup>HOD, Department of Biotechnology, Acharya Institute of Technology, Bangalore, Karnataka, India

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**ABSTRACT**

The aim of the present study was to determine phytochemical analysis and the total alkaloid and flavonoid contents of the leaf part of *Clerodendrum inerme* plant. The general techniques of medicinal plant extraction are done by soxlet extraction from polar to non polar i.e. petroleum ether, chloroform, ethyl acetate, methanol & aqueous extraction. The ethyl acetate extract has been shown the presence of most of the active components. These extracts were tested for alkaloids, terpenoids, flavonoids, Phenol, Steroids, Diterpene test, Flavonoids, Flavanones, Quinones, coumarins and Tannins. The total alkaloid and flavonoid content is 13.96% and 6.2% respectively. This proves its application in wide areas of medicine.

**Keywords:** Alkaloids, flavanoids, terpenes, steroids, coumarins.

**INTRODUCTION**

Plants have the secondary metabolites which are in useful for mankind in many aspects. It is new trend of approaching any problem with the herbal extracts. It is rudimentary that any prior to the study of the plant, phytochemical analysis is done to know its phytoconstituents, which makes the task easier for further studies to carry out. The importance of *Clerodendrum inerme* plants is well known as folk medicine which is used for applying wounds etc. It is known for its antimicrobial activity.<sup>1</sup> Evergreen sprawling shrub is 1-1.8m tall. Stems are woody, smooth. Leaves are ovate to elliptical (5-10cm) long, acute to acuminate tip, green, smooth, slightly shiny upper surface, pinnate venation, margins entire, leaves opposite, simple. Cyme or umbel usually comprised of 3 flowers joined at a common base point; corolla white, fused, with 5 lobes; stamens 4, reddish to purple and upwardly curved. Fruit is green turning black, 1 to 1.5cm long; obovoid<sup>2</sup>. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent. The part of the plant used for the extraction and phytochemical analysis is the leaf part.

**Phytochemical Analysis****Alkaloids**

200µl of extract was added in a test tube. Take few drops of Wagner's reagent added to the sides of the test tube. A reddish brown precipitate formed confirms the presence of Alkaloids.<sup>3</sup>

**Proteins**

200µl of extract was added in a test tube. Take few drops of Ninhydrin reagent added to the test tube. A purple colour formed confirms the presence of Proteins.<sup>3</sup>

**Diterpene test**

Extracts were dissolved in water and treated with 3-4drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.<sup>4</sup>

**Steroids**

A test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H<sub>2</sub>SO<sub>4</sub> and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.<sup>5</sup>

**Terpenoids**

200µl of extract was added in a test tube. 1ml of chloroform reagent and 1ml of sulphuric acid was added to the test tube. A colour change to greyish colour confirms the presence of terpenoids.<sup>5</sup>

**Flavonoids**

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.<sup>5</sup>

**Phenol and Tannins test**

Crude extract was mixed with 2ml of 2% solution of FeCl<sub>3</sub>. A Blue-green or black coloration indicated the presence of phenols and Tannins.<sup>5</sup>

**Test for Quinones**

To 1ml of the extract, 1ml of concentrated sulphuric acid was added. Formation of red color shows the presence of Quinones.<sup>6</sup>

**Test for Flavanones**

To the substance concentration sulphuric acid was added, orange to crimson red color confirms the presence of flavanones.<sup>6</sup>

**Test for Coumarins**

To 1ml of extract, 1ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.<sup>6</sup>

**Determination of Alkaloids and Flavanoids****Determination of Alkaloids**

The method followed is in convenience with reference<sup>7</sup>. A measured weight of the sample i.e. 3g was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28°C. It was later filtered via Whatman No. 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH<sub>4</sub>OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution dried in the oven at 80°C.

Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

**Determination of Flavonoids**

The method followed is in understanding with reference<sup>7</sup>. 3g of the sample was boiled in 30ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through Whatman No. 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

**RESULTS****Table 1: Preliminary phytochemical analysis of *Clerodendron inerme* leaf extracts**

Phytoconstituents	Leaf				
	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids	+	+	+	+	+
Phenol & Tannins test	-	+	+	-	-
Steroids	+	+	+	+	-
Proteins	+	+	+	+	+
Diterpene test	+	+	+	-	-
Terpenoids	+	+	+	-	-
Flavonoids	+	+	+	+	+
Flavanones	+	+	+	+	+
Quinones	+	+	+	+	+
Coumarins	-	-	-	-	-

+ means present; - means absent

From the above followed method in estimation of alkaloids and flavonoids is as below

**Table 2: Total Alkaloids & Flavanoids**

The estimation of the presence of Alkaloids & Flavanoids	
Total Alkaloids (%)	13.96
Total Flavanoids (%)	6.2

**CONCLUSION**

The results of the phytochemical test carried out on the various extract, were the preliminary photochemical screening revealed the presence of alkaloids, steroids, phenol, flavonoids, diterpenes, triterpenes, flavonones, coumarins and the presences of active constituents were found more in ethyl acetate extract. The studies of following extract were selected for the antimicrobial activity. The quantitative analysis of the alkaloids & flavanoids are done in intention with the antimicrobial activity.

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