

TRADITIONAL KNOWLEDGE, PHYTOCHEMICAL INVESTIGATION AND TRACE ELEMENTS OF CLERODENDRUM GLANDULOSUM COLEB

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ABSTRACT

Clerodendrum glandulosum coleb is an important medicinal plant of Manipur. It belongs to verbenaceal family of plant kingdom. It is small tree or shrub perennial and wild in nature. In Manipuri local dialect, it is known as “Kuthap”. The “Kuthap” is a combination of two words “Ku” means “Wooden Coffin” and “Thap” “distant” which literally means that a person using the plant on food or medicine is shielded or kept away from ills and ailments. The plant has various traditional applications. It is used in the preparation of special menu called “Uti” naturally carbonated mixed vegetable porridge for the treatment of stomach. This plant are used in the treatment of diabetes, hypertension and fresh leaf juice is also used in the treatment of ringworm, skin

diseases. Dry powdered leaves have insecticidal vermifugal properties. But the claim has no scientific support or evidences. The objective of present study is to find out scientific support or evidences for using this ethnomedicinal plant with the help of modern techniques. The plant was collected from Manam Yang hill of Sadar hill district of Manipur during the month of June and identified with authenticated herbarium. In the present study, the leave, bark and root of clerodendrum glandulosum coleb were screened for presence of the physiochemical ingredients and analysed trace elements. Leaves are found to contain many bioactive

compounds such as alkaloid, Anthraquinone, Amino acid, flavanoids, glycosides, protein, reducing sugar, steroid, saponin and tannins. In case of bark and root of *clerodendrum glandulosum*, some of bioactive compounds were absent. Leaves of this species are found to contain all the seven elements. But Bark and root of this species did not contain vanadium element. The present study may be helpful for discovery of new bioactive compounds and trace elements in the field of modern chemical research especially in modern drug discovery.

KEYWORDS: *Clerodendrum Glandulosum* Coleb, Drug Discovery, Ethnomedicinal, Vermicidal Phytochemical.

INTRODUCTION

Verbenacea is a large plant family consisting of trees, shrub and herbs (Jrease and Evan, 1983). Modern research carried on the verbenaceous plants revealed and most of the plants belonging to this family are medicinnially important as they contain biologically active compounds^[1] *Clrodendrum glandulosum* coleb is an important medicinal plant of Manipur. It belongs to verbenaceae (Lamiaceae) family of the plant kingdom.^[2] It is small tree shrub perennial and wild in nature. In Manipuri local dialect, it is known as “Ku” means “Wooden coffin” and “thap” distant which literally means that a person using the plant as food or medicine is shielded or kept away from ills and ailments. It has a long association with culture and tradition of Manipur since early 16th century.^[3,4] The plant has various traditional applications. It is used in the preparation of special menu called “Uti” naturally carbonated mixed vegetable pourice for the treatment of stomach. It is also used in the preparation of natural hair care remedy locally known as “Chinghi” to kill lice. The decoction of diabetes hypertion and fresh leaf juice is also used in the treatment of ringworm skin diseases. Dry powdered leaves has insecticidal or vermicidal properties. Now local markets, sell Kuthap for various purposes including household consumption as a food additive.^[5,6] But the claim has no scientific support or evidences. Hence the plant has been selected for scientific study.

Medicinal plants are part of human medicine. Since the dawn of civilization. These plants are making backbone of traditional medicinnial system of India (Nayak 1994). The therapeutic basis of herbal medication are by the presence of diverse bioactive compounds like alkaloid terpeniod, flavanoid, phenolic compounds and proteins etc in plants and also for the treatment of diseases which are source of noval therapeutic agent.^[10] The trace elements in the medicinal plants play an important role in the treatment of diseases. There has been remarkable expansion in the knowledge of the significance of trace element and the effects of

its absence on human health. The deficiency or excess of trace elements leads to various complications and metabolic disorder in human being.^[12]

OBJECTIVE

The objective of present study is to find out scientific support as evidence of phytochemical compounds and trace elements present in the clerodendrum glandulosum coleb with the help of modern scientific techniques.

MATERIAL AND METHOD

The plant was collected from Manam yang hill of Sadar hills district of Manipur during the month of June and identified with a authentic herbarium.



Clerodendrum glandulosum Plant.



Clerodendrum glandulosum Flower.



Clerodendrum glandulosum bark.



Clerodendrum glandulosum root.

Fig Clerodendrum glandulosum coleb studied

Phytochemical Screening of Clerodendrum Glandulosum Coleb Leaves,

Barks, Roots: Fresh plant leaves, barks and roots were washed under running tap water, air dried and then homogenized to find powdered and subjected to solvent extraction using various solvents like petroleum ether, chloroform and methanol.

Crude Extraction

250 gm of each of fine clerodendrum glandulosum powders of leaves, barks, roots were extracted in 1 litre of each of petroleum ether, chloroform and methanol in cold conditions for 10 days with occasional shaking. The solvent from total extract were filter through ordinary filter paper and then distilled at low temperature and dried under shaded. Extracts were used for phytochemical analysis. Each extract were subjected to the identification test for various active chemical constituents. Phytochemical identification test were carried out adopting standard protocols (Trace, et-al 1983, Kokate et-al 1997, Mukherji 2000 Hegde et-el 2010, Mohammad Nisur et-al 2011; R.S. Sawant et-al 2013).

Alkaloid

About 3 ml of concentrated extract was taken in a test tube and about 1 ml HCl was added the mixture was heated gently for about 20 min cooled and filter, the filtrate was used for the following test of alkaloids.

a) Wagner Test: Wagner reagent was prepared by dissolving 2 gm of iodine and 6 gm of KI in 100 ml of water (Altolami Olubunni, Nigerea) 1 ml of the extract was taken in a test tube, it is then treated with Wagner's reagent, reddish brown precipitate identified in presence of alkaloid.

b) Mayer's Test: Preparation of Mayer's reagent. In 60 ml of distilled water, 1.36 gm of mercuric chloride was dissolved and 0.5 gm of KI in 10 ml of water. The two solution were mixed and diluted to 100 ml with distilled water. About 1.2 ml of palant extract was taken in a test tube to this 0.2 ml of dilute HCl and 0.1 Mayer's reagent was added. Yellowish buff coloured precipitate confimed the presence of alkaloid.

Anthraquinones

Ammonia test: 5ml of plant extract was hydrolysed with dilute H₂SO₄ and then add 1 ml of benzene and 1 ml of NH₃, formation of rose pink colouration suggest the presence of anthraquinone.

Amino acid

Extract was treated with 2 ml of dilute HCl. To this clear solution added 2 ml of 2% NaNO₂ solution. Copious evolution of nitrogen occurs while the solution remain clear indicated the presence of amino acid.

Flavanoids

Shinoda test: 0.5 ml of plant extract taken in a test tube. 5-10 drops of dilute HCl and small piece of $ZnCl_2$ or $MgCl_2$ were added and the solution was boiled few minutes. Reddish pink colour or red colour formation indicated the presence of flavanoids.

Glycoside

Keller Killiani test: Plant extract was treated with glacial acetic acid, few drops of 5% of ferric chloride and concentrated H_2SO_4 acid were added. Observation of reddish brown colouration at the junction of two layers and bluish green colour in the upper layer.^[7]

Protein

Biuret test: Extract was treated with 1 ml of 40 % NaOH and mixed with 2 drops of 1% copper sulphate, violet colouration indicated the presence of protein.

Reducing sugar

a) **Felhing test:** 5 ml of the extract solution and 5 ml of felhing's solution were mixed and boiled for 5 minutes, formed brick red ppt indicated the presence of reducing sugar.

b) **Benedict's test:** 5 ml of the extract solution and 5 ml of Benedict's solution were mixed in a test tube and boiled for few minute, formed brick red ppt indicated the presence of reducing sugar.

Saponins

Lead acetate test: 5 ml of the plant extract was mixed with 20 ml distilled water in graduated cylinder, shake vigorously about 15 minutes, formation of foam indicated the presence of saponin.

Steroid

a) **Salkowski test:** 10 ml of chloroform was mixed with 1 ml extract of the test sample. To this solution equal volume of concentrated H_2SO_4 acid was poured carefully from side of the test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence indicated the presence of steroid.

b) **Liebermaun – Burchard Test:** 1 ml of plant extract was dissolved in 10 ml of chloroform, 1ml of acetic anhydride was added in that, following 2 ml of conc. H_2SO_4 acid, a reddish violet colour developed, indicated the presence of steroid.

Tannin

a) **FeCl₃ test:** 4 ml plant extract and 4 ml FeCl₃ was treated together, greenish black colour formation indicated the presence of tannin.

b) **Lead acetate test:** 5 ml of the plant extract taken in a test tube, a few drops of 1% lead acetate solution was added. Formation of yellow ppt indicated the presence of tannin.

RESULTS AND DISSUSSION

Table. 1: Phytochemical analysis of clerodenum glandulosum coleb.

Sl. No.	Phytochemicals		Name of Extracts (Leave)			Name of Extracts (Bark)			Name of Extracts (Root)		
			Petroleum ether	Chloroform	Methanol	Petroleum ether	Chloroform	Methanol	Petroleum ether	Chloroform	Methanol
1.	Alkaloids										
	a)	Wagner's test	-	+	+	-	+	+	-	+	+
	b)	Mayer test	-	+	+	-	+	+	-	+	+
2	Anthraquinones Ammonia test		+	-	+	-	-	-	-	-	-
3	Amino acid		+	-	+	-	-	-	-	-	-
4	Flavanoids Shimoda test		+	+	+	-	-	-	-	-	-
5	Glycosides Keller Killani test		+	+	+	-	-	-	-	-	-
6	Protein Biuret test		+	+	+	-	-	-	-	-	-
7	Reducing sugar										
	a)	Felhing's test	-	+	+	-	-	-	-	-	-
	b)	Benedict's test	-	+	+	-	-	-	-	-	-
8.	Sapnin Lead acetate test		-	+	+	+	+	+	+	+	+
9.	Steroid										
	a)	SalKowski test	+	+	+	-	-	-	-	+	+
	b)	Libermann – Burchard test	+	+	+	-	-	-	-	+	+
10.	Tannin										
	a)	FeCl ₃ test	-	+	+	-	-	-	-	-	-
	b)	Lead acetate test	-	+	+	-	-	-	-	-	-

Key: + = Present and – = absent

In this present study, leaf, bark, root of chrodendrum glandulosum coleb were screened for the presence of phytochemical ingredients. From table number 1,2 and 3, leave of chrodendrum glandulosum coleb are found to contain many bioactive compounds, such as alkaloids, anthraquinones, amino acids, flavanoids, glycosides, proteins, reducing sugars, sponins steroids and tanneins. However barks and roots of chrodendrum glandulosum are not

found all the compounds but contain some compounds. These bioactive compounds have many pharmacological activities including antihypertensive effects, antirhythmic effect, antimalarial activity anticancer actions, anti-allergic, anti-inflammatory and antimicrobial activity^[8,9] etc.

Elemental Analysis

Trace element analysis was done by the powdered of the above samples.

Sample preparation: For acid digestion of the above sample, 1 g sample was digested for 3 hr at 85⁰C with conc. HNO₃ : HCl (3:1 ml) mixture. The conc. HClO₄ (1ml) was added. The solution was filtered and diluted to 50 ml with distilled water. The blanks solution was taken at the same procedure. Following the above methods, all samples for concentration of trace elements Fe, Zn, Cu, Mo, Cr, V and Mn (in ppb) were determined by Graphite Furnace Atomic Absorption Spectrometer (GF-AAS) Model : Analytic vario – 6.

The concentration of trace elements in leaf, bark and root of clerodendrum glandulosum coleb are shown in table – 2.

Table. 2: Concentration of trace elements of leaf, bark, root of clerodendrum glandulosum coleb by Graphite Furnace Atomic Absorption (GF-AAS) Model Analytical Jena Vario – 6.

Clerodendrum glandulosum coleb	Trace elements						
	Fe	Zn	Cu	Mo	Cs	V	Mn
Leaf	0.904±0.010	0.243±0.008	1.32±0.002	0.01±0.004	7.42±0.731	2.52±0.57	0.017±0.003
Bark	2.96±0.09	0.48±0.06	0.62±0.03	0.28±0.01	4.50±0.71	-	1.96±0.05
Root	3.05±0.05	0.35±0.01	0.25±0.01	0.189±0.03	1.55±0.01	-	2.05±0.12

(All concentration are in ppb).

The elements analysed in the samples are Fe, Zn, Cu, Mo, V and Mn. These elements were found to present in considerable concentrations. Out of these elements Cr and Fe were present in high concentration Cr 7.42 ppb to 1.55 ppb and Fe 3.05 ppb to 0.904 ppb. The concentration of Cr and Fe was highest in leaf of clerodendrum glandulosum 7.42±0.010 ppb and root of clerodendrum glandulosum 3.05± 0.05 ppb respectively. The element vanadium 2.52±0.57 ppb was found only in leaf of clerodendrum glandulosum. This element vanadium was not found in bark and leaf of clerodendrum species.

The essential trace elements like Fe, Zn, Cu, Mo, Cr, V and Mn take definite and specific roles in the metabolism of human body. They also balance toxicity levels. Some of elements have anti-oxidant property at some extents.

CONCLUSION

The ethnomedicinal applications of *clerodendrum glandulosum* coleb were quite meaningful and logistic in view. The different concentration of elements in leaf, bark and root leads of the *clerodendrum glandulosum* to the conclusion that these plants have different specific roles in the treatment of different diseases. The present study may be helpful for discovery of new bioactive compounds and trace elements in the field of modern chemical research specially in modern drug discovery.

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