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Pharmaco-Phytochemical Characterization of *Clitoria ternatea* Linn.

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Abstract: The roots of *Clitoria ternatea* Linn., (Fam: Papilionaceae) is used as one of the ingredient in the preparation of an anti – leprosy drug "SULAK" .* Since the studies on its Pharmaco – Phytochemical Characterization are scanty, this species is studied with the following parameters : Taxonomy of the plant, anatomy of the root, powder studies, pertaining to organoleptic, microscopic, fluorescence, powder with chemical reagents, ash values, extractive values, phytochemical screening and geochemical estimation. The plant is considered as a good brain tonic and is useful for throat and eye infections, skin diseases, urinary troubles even in cattle's, ulcer and anti dote properties[1]. Besides its medicinal property, it is also a good source of phytochemical substances. It contains anti fungal proteins and has been shown to be homologous to plant defense (ct-AMP1)[2]. Further the ethanolic extract of the powder is compared with the standard allopathic anti – leprosy drugs like dapsone, clofazimine and rifampicin by chemical reaction to chromatographic methods (IP method) to assess their activity equivalence.
Keywords :*Clitoria ternatea,* leprosy, SULAK, Pharmaco- Phytochemical, dapsone, clofazimine and rifampicin.

INTRODUCTION

C. ternatea is commonly known as *shankupushapam*, is widely used in traditional Indian system of medicine as a brain tonic and is believed to promote memory and intelligence.The study conducted on rat revealed that root extracts increase rat brain acetyl choline content and esterase activity in a similar fashion to the standard cerebro drug pyritinol[3]. The present study is to report the pharmaco-chemical characterization of *C. ternatea*. The root powder is used as one of the ingredients in the preparation of the drug "SULAK" and its ointment to treat leprosy[4,5]. Chemical identity comparison is made with standard allopathic anti-leprosy agents like clofazimine, dapson, and rifampicin to assess their activity equivalence to treat leprosy by adopting Indian pharmacopeal (IP) methods.

MATERIALS AND METHODS:

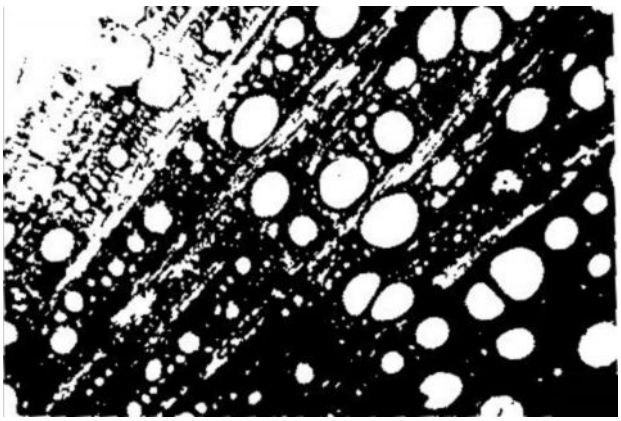
C. ternatea is a green climber, growing all over the Coimbatore districts .Plant was collected at their flowering stage the voucher specimen has been deposited in the Herbarium, Botany Department, Bharathiar University, Coimbatore. The material was dried, powdered, by Wiley Mill (50mm) for Pharmaco-phytochemical characterization of plant[6]. The powder was sieved through 40 mesh sieve plate for organoleptic, microscopic[7-9] and fluorescence evaluation[10]. The powder as such used in phytochemical characterization.

OBSERVATION AND RESULTS:

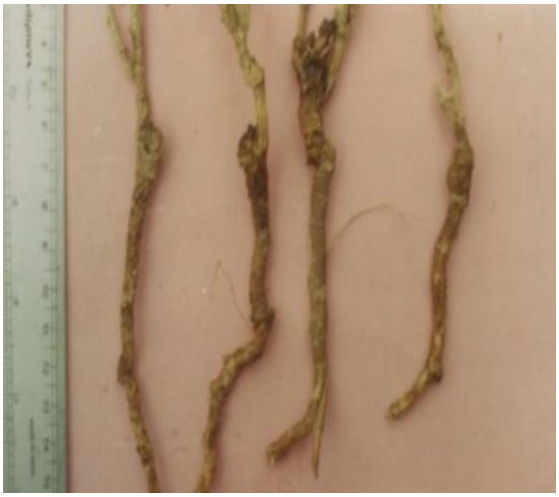
TAXONOMY

C. ternatea is a twining shrub; branchlets appressed-tomentose. Leaves alternate imparipinnate, 2-3 pairs; leaflets opposite,ovate,2.5-4-5x2-3.5cm obtuse at base, entire obtuse at apex, strigose. Flowers white or blue, solitary or in pairs (Plate-1). Calyx lobes lanceolate. Petals: Standard orbicular; wings oblong 9-10 cm long. Seeds 5-9, subglobose compressed.

Plate -1



***Clitoria ternatea* T.S of root .**



***Clitoria ternatea* roots**

***Clitoria ternatea* – Habit with flower**

VERNACULAR NAMES

Hindi	**: Aparafit**
Malayalam	**: Kakkanam**
Sanskrit	**: Aparajita**
Tamil	**: Sangupushpam**
Telugu	**: Nalla-ghentana**

MICROSCOPIC EVALUATION

C. ternatea (roots) fragment of cork, osteo sclereids, elongated phloem fibres and wood parenchyma, long and septate and short wood fibres, tracheids (spiral), long narrow and broad short vessels, simple starch granules are seen (Plate-1).

FLUORESCENCE EVALUATION

The fluorescence property of the powder and their extract of the species as such and after treatment with chemical reagents, under day and UV lights are summarized in Table 1, 2 & 3 respectively.

PHYTOCHEMISTRY

BIOCHEMICAL EVALUATION (PERCENTAGE ON DRY WEIGHT BASIS).

The root powder has sugar 24%, protein 11.7%, free amino acids 3.6%, and oil 1.3%. Among free amino acids, serine, aspartic acid threonine, tyrosine, isoleusine , leusine and hydroxy proline are distributed. The values obtained are average of 3 replicates.

GEOCHEMICAL EVALUATION

Amount of mineral from the root powder is as follows: copper 16.67 ppm, ferrous 800 ppm, manganese 66.64 ppm, potassium 51.50 ppm, sodium 5 ppm, zinc 19.04, phosphorous 4.8 ppm, sulphur 525.73 ppm .The values are average of 3 replicates.

CHEMICAL IDENTITY COMPARISON

The results of chemical identity of plant powder with that of standard antyleprosy agents like clofazimine, dapsone and rifampicin for their chemical activity. Equivalence by chemical reaction method (IP) is summerised (table 7).

TABLE 1: Behaviour of Powder of *C. ternatea* (Roots) with chemical reagents

SI.No	Treatment of Powder	Colour under day light
1	Powder as such	Off White
2	1N NaOH (aqueous)	Mid Buff
3	1N NaOH (in methanol)	Olive Green
4	Picric acid (Saturated solution)	Pistachio Green
5	Acetic acid (concentrated)	Mid Buff
6	Hydrochloric acid (concentrated)	Deep Green
7	Nitric acid (concentrated)	Golden Brown
8	Sulphuric acid (concentrated)	Truck Brown
9	Saliwanoff's reagent	Eucalyptus Green
10	Ferric chloride (5% solution)	Deep Green
11	40% NaOH (aqueous) +10% Lead acetate	Pale Cream
12	Iodine solution (5%)	Sand Stone
13	Sudan III (in ethanol)	Signal Red
14	Nitric acid (conc.) +Ammonia solution	Dawn Pink

TABLE 2: Behaviour of Powder of *C. ternatea* (Roots) with Chemical Reagents Under UV Light

SI. No.	Treatment of Powder with	Colour under UV Light		
		200-280nm	280-320nm	320-400nm
1	Powder as such	Pistachio Green	Off White	Sand Stone
2	Nitrocellose (in amyl acetate)	Pistachio Green	Pistachio Green	Pistachio Green
3	1N NaOH(aqueous)	Clear Green	Olive Green	Olive Green
4	1N NaOH (aqueous) + Nitro-Cellulose in amyl acetate	Brown	Olive Green	NewOlive Green
5	1N NaOH (in methanol)	Brown	Sand Stone	Olive Green
6	NaOH (in methanol) + Nitro-Cellulose in amyl acetate	Brown	Olive Green	NewOlive Green
7	1N.Hydrochloric acid	Pistachio Green	Stain Blue	Stain Blue
8	1N Hydrochloric acid + Nitro- Cellulose (in amyl acetate)	Pistachio Green	Pistachio Green	Lt.bs Grey
9	50% Nitric acid	Leaf Brown	Brown	
10	50% Sulphuric acid	Clear Green	Pistachio Green	Clear Green
11	Methanol	Olive Green	Clear Green	Clear Green
12	Reagent Saliwanoff's	Clear Green	Opaline Green	Dark Grey

TABLE 3: Behaviour Of Powder Extractives Of *C. ternatea* (Roots) Under Day and UV Light .

SI.NO.	Name of Extractive	Day Light	Uv light		
			200 – 280 nm	280 – 320 nm	320 – 400 nm
1.	Petroleum ether	Clear green	Olive green	Olive green	Golden yellow
2.	Solvent ether	Olive green	Olive green	Deep green	Olive green
3.	Benzene	New Olive green	Olive green	Olive green	Golden brown
4.	Chloroform	Olive green	New Olive green	New Olive green	Truck brown
5.	Acetone	Olive green	Aqua – marine	Mint green	Olive green
6.	Ethyl Acetate	Apple green	Olive green	Olive green	Olive green
7.	Ethanol	Brown	Olive green	Olive green	Brown
8.	Water pH 5	Brown	Golden yellow	Golden yellow	Golden brown
9.	Water pH 7	Golden yellow	Golden yellow	Brown	Brown
10.	Water pH 9	Lemon yellow	Golden yellow	Golden yellow	Lemon yellow

TABLE :4 Phytochemical Evaluation of *C. ternatea* (Roots) Powder

Active principle	Name of test	Degree of colouration/ precipitation
Leucoanthocyanins	--	3 +
Flavones	--	3 +
Glycones	--	+
Aglycones	--	7 +
Unsatural Sterols and Triterpenes	Liebermann –Burchard Test Salkowski Test	4 + 7 +
Anthraquinone Heterosides	Borntrager Test BZ AZ	3 + 3 +
	Modified Borntrager Test BZ AZ	3 + 3 +
Saponin	Froth Test Liebermann – Burchand Test	3 + 3 +
2 deoxy – sugar	Killer – Killiani Test	3 +
Tannin		+
Phenol		3 +
Cardic Glycosides	Keddi Reagent Test	3 +
	Modified Keddi Reagent Test	4 +

+ = indicates positive response - = indicates negative respons, +_ = indicates incondusive No.+ = Degree of intensity of colouration / precepitation, Red, Red – Violet = positive for Leucoanthocyanins, Red, Green-Red = positive for Flavones , Colouration in acetyl alcohol layer = positive for aglycones, Colouration in aqueous layer = positive for glycones, Blue or Green = positive for sterols, Red, Brown or Red to Brown = positive for triterpenes .

TABLE 5: Alkaloids Evaluation of *C. ternatea* (Roots) Powder

Extract	Degree of precipitation				
	DRO	HAG	SCH	VAL	WAG
Petroleum ether	2+	-	-	-	-
Solvent ether	2+	-	-	-	-
Benzene	+	-	-	-	-
Chloroform	+	-	-	-	-
Acetone	+	-	+	-	-
Ethyl acetate	+	-	-	-	-
Ethanol	+	-	+	-	-
Water	+	-	+	+	-

+ = indicatespositive - = indicates negative, No. + = indicative intensity of precipitation

TABLE 6: Alkaloid Estimation by titration from ethanolic Extract of *C. ternatea* (Roots) Powder

Alkaloid group	Amount in m Eq/100g.dry weight
Tertiary	0.130
Quaternary	0.036

The values are average of three replicates

TABLE 7: Chemical Comparison of *C. ternatea* (Roots) Powder with Anti-Leprosy Agents.

Anti – leprosy Agents	**Preliminary tests**				**Confirmatory test**
	A	**B**	**C**	**D**	**Amount in percentage by titration**
Clofazimine	3+	3+	0	0	3.5
Dapsone	5+	4+	3+	3+	7.2
Rifampicin	2+	0	0	0	1.8

*The values are average of three replicates. ABCD – Colouration / precipitation reactions as per IP method. Dapsone – have ABCD reaction / Tests. Clofazimine – have A and B. Rifampicin – have A only.

DISCUSSION

The sugar content is rich (24%) in the sample. The following free amino acids are located in chromatographic study viz., serine, aspartic acid, threonine, tyrosine, isoleusine, leusine and hydroxypriline. Among them hydroxyproline is high. Mineral analysis reveals the high amounts of ferrous (800 ppm) and sulphur (525.73ppm). The tannin content of the root bark is determined by Chopra, *et al*[11]. Present phytochemical screening reveals the presence of all active components. Among them aglycones, unsaturated sterols, tri-terpenes, cardiac glycosides and saponin are high (Table-4). The alkaloid precipitating agents give maximum precipitation with all extractives and that of Wagner's gives no precipitation with any extractive (Table 5).The high amount of tertiary alkaloid content was found (Table 6). The total solid and ash content are determined as 91% and 7.43% respectively. Insolubility of ash is high in ethanol and low in 50%HCl .The sample gives high extractive value in water (43%). Chemical reaction of the sample with standard anti-leprosy agents reveals that activity equivalent to the presence of clofzimine (3.5%) dapsone (7.2%) and rifampicin (1.8%) (Table-7).

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