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IS *CLEISTANTHUS COLLINUS* BENTH. A POISONOUS PLANT?

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To evaluate traditional medicine, which is an important part of the health-care system, three different regions of Vidarbha, Gadchiroli, Chandrapur and Nagpur districts were surveyed for *Cleistanthus collinus* used by traditional healers. The present study highlights ethnomedicinal observations, major phytochemical screening like, alkaloids, anthracene derivatives, flavonoids, phenols and terpenoids etc., antimicrobial activities against human pathogenic strains such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Proteus vulgaris* and anti-inflammatory actions.

Keywords : *Cleistanthus collinus*; Poisonous plant.

Introduction

To cure many diseases, nature has gifted various plants to human and the knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature. Species of Euphorbiaceae have been used by local population of many countries in folk medicines as remedies against several diseases such as cancer, diabetes, hepatitis, jaundice, malaria and complaints related to diarrhoea, heart, skin and eye problems, hemorrhages, rheumatism etc[1-3]. Similarly, few of the plants have been categorized separately for their poisonous activities. Chopra et al.[4,5] have documented several plants of Euphorbiaceae in India, which have been used in the Ayurvedic system of medicine against different diseases.

Cleistanthus collinus predominantly included under the list of poisonous plants of India and its poisonous activities have been largely exploited by most of the Indian tribes for the homicidal and suicidal purposes[6]. Several other reports also reveal its toxic, piscicidal and insecticidal properties[4,5]. The major phytochemicals identified in this plant are leucoanthocyanidins[7], diphylline glycosides[8], lignans[9] and β-sitosterol, paulownin, diphyllin and cleistanthin-D[10].

Pharmaceutical studies of Rao and Nair[11] reported the cytotoxic properties of *Cleistanthus collinus*. Sarathchandra and Balakrishnamurthy[12] reported that *Cleistanthus collinus* deplete thiol and thiol containing enzymes in organs of animals, this depletion makes the plant toxic. Since ages studies have been restricted to only toxic nature of this plant. Present paper is an effort to bring about knowledge of different ethno medicinal uses, chemical constituents and pharmacological activities of aerial parts of this plant to elucidate the actual unexplored medicinal value.

Material and Methods

Fresh plant material was collected in bulk and preserved. Specimens collected from various localities were satisfactorily identified and confirmed with the help of the herbarium at Department of Botany, Nagpur University, Nagpur.

The ethnomedicinal survey was conducted according to the methods evolved and adopted by different ethnobotanists[13,14]. The basic methodology in the present investigation involved frequent visits to different villages of Gadchiroli, Chandrapur and Nagpur districts and collection of related information from *vaidu,* the medicine man, of the village.

Preliminary Phytochemical Screening : Preliminary phytochemical screening of plant was done according to the standard procedures adopted by the various workers[15-21]. Extraction procedures have been given in the Fig.1 and simple chemical tests were conducted for the chemicals such as, alkaloids, anthocyanins, anthocyanidins, anthracene glycosides, amino acids, coumarins, flavonoid, saponins, proteins, polyoses, polyuronoids, monosaccharides, reducing sugars, starch, other carbohydrates, gums and mucilages, steroids, triterpenoids, volatile oils, fatty acids, emodins, carotenoids and tannins.

Qualitative and Quantitative Phytochemical screening : Qualitative and quantitative phytochemical screening of plant was done according to the standard procedures[18, 19, 22, 23].

Qualitative analyses of various phytochemicals such as alkaloids, anthracene derivatives, anthraquinones, steroids, acubins, iridoids, triterpenoids, polyphenols, phenolic acids, coumarins, flavonoids and cardiac glycosides were done by employing Thin Layer Chromatographic technique. Whereas, quantitative chemical analyses of phenols[24], sugars[25] and proteins[26] were done by Spectrophotometrical procedures.

Pharmacological Screening

1. Antibacterial Activity : The alcoholic extracts were used for assaying antibacterial activity by using filter paper

disc diffusion method[27]. Different bacterial species used for the purpose were, *Staphylococcus aureus*, *Bacillus subtilis* (Gram +ve); *Escherichia coli* and *Proteus vulgaris* (Gram –ve) courtesy, Microbiology Lab, Department of Pharmacy, Nagpur University, Nagpur.

2. Anti-inflammatory Activity: The "Carrageenan Induced Rat Paw Odema Assay"[28] was adopted to study the anti-inflammatory action. The test compound was administered orally in the dose of 100mg/ 1kg body weight and 1% Acacia gum was used as control. After an hour carrageenan 0.05ml was injected into the planter tissue of right hind paw. The paw columns were measured plethysmographically at 1 and 3 hrs after the carrageenan injection. The percentage inhibition of the paw oedema was calculated using the equation:

Percentage inhibition = *(1 – Vt/Vc) X100*

Where, *Vt* and *Vc* are the volumes of the paw oedema in the treated and control animals respectively.

Results and Discussion

Ethno medicinal Uses : i) Fresh leaf paste applied as an antidote for snakebite (novel report). ii) Dried plant is used as insecticide and fish poison.

Phyto Chemical screening: A general screening conducted to characterize chemical composition of *Cleistanthus collinus* leaf and stem samples. The screening covered mainly nitrogenous compounds, isoprenoids, acetogenins and carbohydrates, are summarized in Table 1.

Screening for nitrogenous compounds was mainly concerned with alkaloids which are reputed to have dramatic physiological activities, mainly on central nervous system. Both leaf and stem samples showed positive test with 5 different alkaloids on the basis of their Rf values in TLC. Out of which 3 were observed in leaf and 2 in stem samples. Amino acids and proteins were observed in water extracts of leaf and stem samples.

Acetogenin screening included tannins, flavaonoids, coumarins, emodins, anthocyanidins, anthocyanins, anthroquinones, anthracene derivatives, poly phenols, phenolic acids and fatty acids. Stem samples only gave positive test for tannins, coumarins, emodins and anthocyanidins. Wheras, flavonoids, anthocyanins, anthracene glycosides were found in both stem and leaf. On the basis of different Rf values, Thin layer chromatogram showed abundant occurrence of few of these compounds, polyphenols (13) and phenolic acids (10), while, anthracene derivatives (2); flavonoids (2) and coumarins (1), showed less in occurrence (Table 2). Rest of the acetogenic compounds were not found in either of the samples (Table 1). 38.0 mg and 90.0 mg total phenol content appeared in leaf and stem samples respectively (Table 3).

Screening for isoprenoids was confined to steroids, diterpenoids, iridoids, tripernoids, saponins, cardiac glycosides and carotenoids. Total of six steroids were found to be present, five of which were observed in leaf and two in stem samples. The one common in both was found to have Rf value 0.31. Saponins are widely well known to have expectorant and anti tussive activity, was observed only in water. Carotenoids, Iridoids, volatile oils and cardiac glycosides were showed negative tests in both the samples. Further TLC results confirmed diterpenoids (1); triterpenoids (2); steroids (6) *in toto.* (Figures in parenthesis show the number of bands in Thin Layer Chromatography)

Carbohydrates screening included reducing sugars, polyuronoids, polyoses and starch. All these compounds, except starch were shown positive tests in both stem and leaf samples, where as starch and gums were seen in stem extracts only. Total carbohydrates and soluble proteins in per gram seed sample are distributed as 50.92 mg and 4.712 mg respectively (Table 3).

Antibacterial activity: These samples; were tested against 4 bacteria, pathogenic to humans. Two Gram +ve bacteria viz., *Staphylococcus aureus and Bacillus subtilis* and two Gram –ve viz., *Escherichia coli* and *Proteus vulgaris* were selected for antibacterial activity. It is found that the leaf and stem extracts of *Cleistanthus collinus* inhibited growth of all the four bacteria conforming their antibacterial activity (Table 4). However, leaf samples were found to have more potential activity than stem extracts, except in case of *Proteus vulgaris*. Both leaf and stem samples in case of *Escherichia coli* inhibit its growth with a greater range of disc diameter i.e 26.0 mm and 21.0 mm respectively (Table 4).

Anti-inflammatory Screening : Alcoholic extract of aerial parts of *Cleistanthus collinus* showed positive anti-inflammatory action. The percentage of anti-inflammatory activity was increasing from 28.57% to 46.13%, values correspond to percentage activity of 1st to 3rd hour after carrageenan injection respectively (Table 5).

Present investigation reported some of the novel uses, which are not observed by earlier workers used as anti dot against snake bite. This plant is warehouse of chemo-diversity which could be useful in medicine like steroids, alkaloids, terpenoids, phenols, flavonoids and some other chemicals. Anti bacterial activity screening concluded that this plant might be useful as a good source of medicine to stop these bacterial growth. Finally anti-inflammatory results proved that this is having medicinal properties other than poisonous activities. The results are encouraging, but scientific scrutiny is absolutely necessary before being put into practice.

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Fig.1. Schematic representation for preliminary Phytochemical screening.

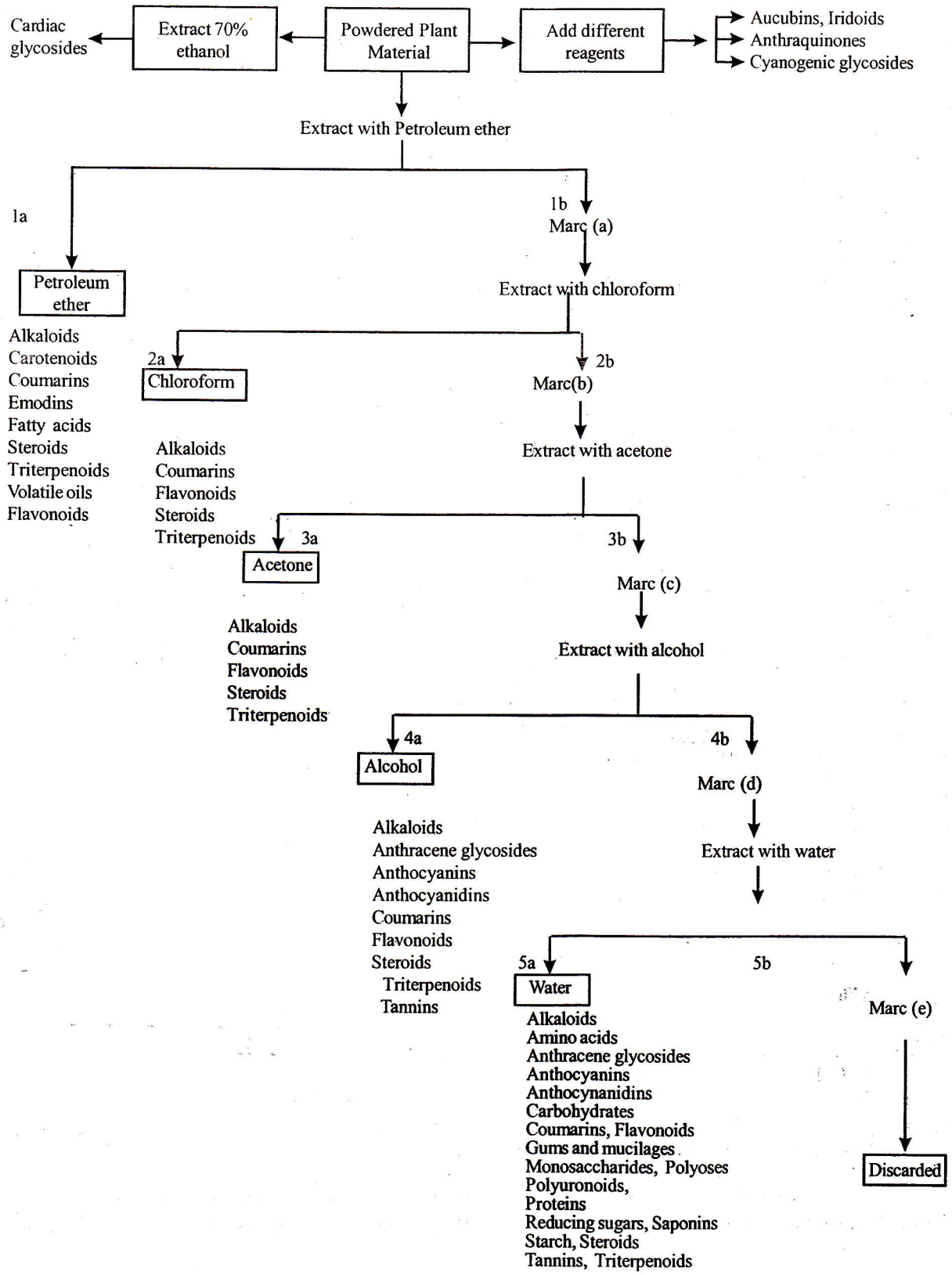

Table 1. Preliminary Phytochemical screening.

Tests with all five extracts						
Chemical name	**Part**	**P.ether**	**Chloroform**	**Acetone**	**Alcohol**	**Water**
Alkaloids	Leaf	+	+	+	+	-
	Stem	+	+	+	+	-
Steroids	Leaf	+	+	+	+	-
	Stem	+	+	+	-	-
Triterpenoids	Leaf	+	-	-	-	-
	Stem	+	+	-	-	-
Coumarins	Leaf	-	-	-	-	-
	Stem	+	-	-	-	-
Flavonoids	Leaf	-	-	+	+	+
	Stem	-	-	+	+	+

Tests with water extracts		
Amino acids	Leaf	+
	Stem	+
Proteins	Leaf	+
	Stem	+
Carbohydrates	Leaf	+
	Stem	+
Monosachharides	Leaf	+
	Stem	+
Reducing sugars	Leaf	+
	Stem	+
Polyoses	Leaf	+
	Stem	+
Polyronoids	Leaf	+
	Stem	+
Gums and mucilages	Leaf	-
	Stem	+
Starch	Leaf	-
	Stem	+
Saponins	Leaf	-
	Stem	+

Tests with 70 % ethanol extract		
Cardiac glycosides	Leaf	-
	Stem	-

Tests with alcohol and water extracts			
Chemical name	**Part**	**Alcohol**	**Water**
Anthocyanins	Leaf	-	-
	Stem	+	+
Anthocyanidins	Leaf	-	-
	Stem	+	+
Anthracene glycosides	Leaf	+	+
	Stem	+	+
Tannins	Leaf	-	-
	Stem	-	+

Tests with Petroleum ether extracts			
Emodins	Leaf	-	
	Stem	+	
Fatty acid	Leaf	+	
	Stem	-	
Volatile Oils	Leaf	-	
	Stem	-	

Tests with dry powder		
Acubins	Leaf	-
	Stem	+
Iridoids	Leaf	-
	Stem	-
Cynogenic glycosides	Leaf	-
	Stem	-
Anthraquinones	Leaf	-
	Stem	-

Note: '+' means positive test; '- ' means negative test

Table 2. Qualitative Chemical screening by Thin layer chromatography

Chemical name	Solvent system	Part	Rf values	Total bands	Spray reagent
Alkaloids	Methonol : Conc. NH_4OH (200:3)	L	0.40, 0.65	3	Dragendroff's reagent
		L	0.73	3	
		S	0.12, 0.31	2	
Phenolic acid	Toluene : Chloroform : Acetone (8 : 5 : 7)	L	0.32, 0.40, 0.59, 0.40, 0.80, 0.97, 0.21, 0.27,	6	Diazotized p-Nitro aniline reagent
		S	0.68, 0.80,	6	
Poly phenols	Toluene : Chloroform : Acetone (8 : 5 : 7)	L	0.15, 0.24, 0.28, 0.36, 0.45, 0.49, 0.56, 0.60, 0.70	9	Diazotized p-Nitro aniline reagent
		S	0.04, 0.15, 0.18, 0.36, 0.51, 0.60, 0.70, 0.78, 0.98	9	
Coumarins	Ethyl acelate : Formic Acid : Glacial acetic acid : Water (100 : 11 : 11 : 26)	L	Nil	0	Borntrager's reagent
		S	0.6	1	
Anthracene derivatives	Ethyl acetate : Methanol : Water (100 : 13.5 : 10).	L	0.53, 0.86	2	Borntranger's reagent
		S	0.86	1	
Flavonoids	Glacial acetic acid : Water (4 : 1 : 5), top layer	L	0.21	1	No reagent, UV light
		S	0.98	1	
Diterpenoids	n- Hexane : Ethyl acetate (17 : 3)	L	Nil	0	Libermann-Burchard reagent and Anisaldehyde-Sulphuric acid reagent
		S	0.55	1	
Triterpenoids	Touene : Chlorofor m : Ethanol (4 : 4 : 1)	L	0.78	1	
		S	0.62	1	
Steroids	Toluene : Ethyl acetate (9 : 1)	L	0.31, 0.45 0.47, 0.52, 0.69	5	Phosphoric acid reagent
		S	0.08, 0.31	2	

Note : L = Leaf; S = Stem

Table 3. Quantitative phytochemical analysis.

Name of the compound	Part	mg/gm sample
Total soluble proteins	Seed	4.712
Total carbohydrates	Seed	50.92
Total phenol	Leaf	38.0
	Stem	90.0

Table 4. Screening for antibacterial activity.

Part	*S. aureus*	*B. subtilis*	*E. coli*	*P. vulagaris*
L	16.2	15.6	26.0	9.2
S	10.0	10.4	21.0	16.4

Note : Diameter in mm along with disc diameter (6mm)
L = Leaf; S = Stem
S. aures = Staphylococcus aureus; E. coli = Escherichia coli
B. subtilis = Bacillus subtilis; P. vulgaris = Proteus vulgaris

Table 5. Screening for antiinflammatory action.
(All values represent average of 5 readings)

Drug	Dose (mg/Kg)	Mean edema		Percent inhibition	
		1hr	3hr	1hr	3hr
Acacia gum (negative control)	100	0.7±0.1	1.3±0.254	-	-
Clistanthus collinus	100	0.5±0.158	0.7±0.158	28.57	46.13

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