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Pharmacognostic and Phytochemical Investigations on Cleistanthus Collinus (Roxb.) Benth. Ex Hook. F.

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Abstract:

The pharmacognostic profile of crude drug has key role in standardization for quality, purity and drug identification. Pharmacognostic studies of Cleistanthus collinus (Roxb.) Benth ex Hook f. were carried out in order to establish parameters for its identification and to check adulteration by other species of Cleistanthus. Morphological characteristic of the plant was studied by organoleptic evaluation. Powder drug study of the plant parts were also carried out and various structures of the powder drug were observed. This study also includes quantitative leaf microscopy, extractive values and quantitative data. The results of this study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of this plant.

Key words: Crude drug standardization, Phytochemistry, Cleistanthus collinus.

Introduction:

As a result of the adverse effects associated with synthetic drugs, people started looking back at the ancient healing systems like Ayurveda, Siddha and Unani. In the present scenario, the demand for herbal product is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential of medicinal value. (Mukharjee, 2002). Herbal drugs play an important role in health care programs especially in developing countries. However, obstacle behind the acceptance of alternative medicines in developed countries is the lack of documentation and stringent quality control. So the documentation and standardization of the raw materials used in herbal medicine is very essential for the worldwide acceptance of this system of medicine (Anonymous, 1989). Correct identification and quality assurance of plant material is indispensable to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy (Chopra et al., 1956).

In earlier days, only the external morphological characters were used to identify a drug. As late as the beginning of present century, pharmacognosy had developed mainly on the botanical side being particularly concerned with the description and identification of drug both in their whole state and in powder form. This technique can be established for the correct botanical identification of plant (Kokate et al., 2005).

Cleistanthus collinus (Roxb.) Benth ex Hook f. commonly called as Garavi belongs to family Euphorbiaceae. This plant is having pharmacological potential (Arivoli et al., 2011 and

Suman et al., 2013). Roots and fruits of this plant are traditionally used to treat gastrointestinal disorder, antifungal activity (Remya, 2018). In SriLanka, leaves of this plant are used for homicidal and Suicidal purposes (Jain et al., 2010 and Suman et al., 2014). So, the present work thus attempts to analyze the wide potential traditional plant Cleistanthus collinus (Roxb.) Benth ex Hook f. which includes the standardization of crude materials with the use of different modern techniques based on the available literature and knowledge.

Materials And Methods:

Collection and identification of plant material: The selected plant for the study i.e. Cleistanthus collinus (Roxb.) Benth ex Hook f. was collected during the period of flowering and fruiting from Mandev forest region district Yavatmal during June - December 2017. The herbarium specimen of selected plant was prepared, identified with the help of standard floras (Cooke, 1967, Naik, [illegible]8; Almeida, 2001; Singh and Karthikeyan, 2001) and the voucher specimen was deposited in Department of Botany, Bharti Mahavidyalaya Arni, district Yavatmal (MS) India.

Pharmacognostical studies: The organoleptic evaluation, anatomical study, powder microscopy, stomatal study, extractive values and chemical analysis was carried out by using standard methods mentioned in (Pratt and Chase, 1949; Anonymous, 1966; Mukharjee, 2002; Trease & Evans, 200[illegible] Kokate et. al., 2005).

Qualitative and quantitative phytochemical analysis: It involves testing of different classes of compou[illegible] The methods used for detection of various phytochemicals were followed by qualitative che[illegible] test to give general idea regarding the nature of constituents present in crude drug (Wa[illegible] Harborne, 1998; Kokate, 2005; Sadashivan and Manickam, 2005). The extracts were [illegible] for the presence of phytoconstituents like carbohydrates, cardiac glycosides, alkal[illegible] vonoids, tannin, phenolics, steroids, coumarins and saponin. The crude quantifications of m[illegible] phytochemicals were done using standard method (Mukharjee, 2002). Each sample was an[illegible] in triplicates. Only tannin and saponin from the plant under study were [illegible] ntified.

Results And Dis[illegible]

The phar[illegible]ical study is the major and reliable criteria for identification of plant drugs. The pha[illegible]tic parameters are necessary for confirmation of the identity and determination of [illegible]y and purity of the crude drug. The detail and systematic pharmacognostical e[illegible]ation of Cleistanthus collinus (Roxb.) Benth ex Hook f. would give valuable information for the future studies.

Organoleptic evaluations: The organoleptic evaluations of Cleistanthus collinus (Roxb.) Benth ex Hook f. shown in table 1.

Table 1: Organoleptic evaluation of powder of C. collinus.

S.N.	Particulars	Plant parts		
		Leaves	Stem	Petiole
1	Colour of Powder	Green	Pale Brown	Pale Green
2	Odour	Mild	Mild	-----
3	Taste	Bitter	Bitter	Slightly bitter
4	Texture	Smooth	Rough	Rough

Powder microscopy: The leaf powder of Cleistanthus collinus (Roxb.) Benth ex Hook f. was containing epidermal cell with stomata, trichome, cortical cells and spongy parenchyma cells. While in stem powder, Cork cell, collenchymatous cells, sclerenchymatous cells, pitted vessels and tracheids were found. In petiole powder, cortical cells and xylem parenchyma tissue were found.

Stomatal Study: Quantitative microscopical study also yielded valuable information regarding specific leaf constant. Stomata frequency is one of the most widely used characters in taxonomy and pharmacognosy. Mostly paracytic stomata are pre-dominant and anisocytic type of stomata is also found. In lower surface of leaf, stomata number is higher than the upper surface (table - 2).

Table 2: Quantitative leaf microscopic analysis of C. collinus

S. N.	Parameters		Range	Mean ± SD
1	Stomatal Number	lower surface	21 - 24	22±0.04
		upper surface	17 - 22	19.8±0.30
2	Stomatal Index	lower surface	37.2 – 42.0	40.2±0.48
		upper surface	32.2 – 37.0	34.08±0.06

Extractive values: The extractive values of the drugs are an important parameter for detecting adulteration in the drugs. However, on the basis of polarity of solvents, successive solvent extractive values of Cleistanthus collinus (Roxb.) Benth ex Hook f. in various organic solvents was observed as shown in table 3. The extractive values determine the amount of active constituents in given amount of crude plant material extracted with respective solvent (Mukharjee, 20 The extraction of any crude drug with particular solvent yields a solution containing dif phytoconstituents in that particular solvent depends upon the nature of drug and solvent which reflect the extractive values of crude drugs. The maximum extractive value obser more polar solvent. C. collinus leaf showed higher extractive values than stem and petiole xtractive value of crude powder was maximum in water followed by ethanol and chloro minimum in petroleum ether which is non-polar solvent.

Table 3: Extra values of C. collinus

S	Parameter studied	Leaf (% w/w)	Stem (% w/w)	Petiole (% w/w)
	Petroleum ether	2.12%	2.09%	2.02%
	Benzene	4.86%	4.04%	3.18%
	Chloroform	10.85%	7.8%	6.22%
	Acetone	10.9%	8.09%	8.08%
	Ethanol	12.60%	10.8%	10.22%
6	Water	17.8%	15.48%	14.24%

Chemical behavioural analysis: The crude drug of various parts i.e. stem, leaf and petiole of C. collinus showed different colours when it was treated with various chemicals. The crude drugs consist of various phyto-constituents in different forms shows characteristic reactions with various reagents. This phenomenon is used for qualitative examination of crude drugs. This technique can be used for the standardization and detection of adulterant in crude drug (Wallis,

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1990). The crude powder of plant under study reacts with various chemicals and behavioral characteristic of powder was observed as shown in (table-4).

S.N.	Reaction	Stem	Leaf	Petiole
1	Powder as such	Pale brown	Green	Pale Green
2	Powder + Conc. H_2SO_4	Red	Orange Red	Red
3	Powder + Conc. HNO_3	Pale Yellow	Light green	Pale Yellow
4	Powder + Conc. HCl	No change	Pale Green	No change
5	Powder + 10% NaOH	No change	No change	No change
6	Powder + Iodine solution	No change	No change	No change
7	Powder + 5% $FeCl_3$	No change	Dark Green	No change
8	Powder + KI	Brown	Green	Brown
9	Powder+ Ethyl acetate	Brown	Green	Brown

Phytochemical analysis:

Qualitative phytochemical screening: Phytochemicals are also known secondary metabolites of plants which play important role in herbal crud drugs. Biologically, diverse active phyto-constituents present in the plant sample which were investigated by phytochemical screening of various plant parts like leaf and stem indicated the presence of different constituents as shown in (table 5). The result of preliminary phytochemical screening of leaf in six different extracts i.e. petroleum ether, benzene, chloroform, acetone, ethanol and water revealed the presence of carbohydrate, proteins, glycosides, tannins, saponin and phenolic compounds. The majority of phytoconstituents were found in ethanol and water extracts. Flavonoids are present only in ethanol extract. Steroids & alkaloids were totally absent in all the six extracts. Saponin is present dominantly in all the extract of the leaf. While, in stem showed that there were presence of carbohydrate, proteins, cardiac glycoside, anthraquinone glycosides, tannins, Saponin, flavonoid and phenolic compounds. The majority of phytoconstituents were found in Water extracts. Alkaloids, steroids and Anthroquinones glycosides were totally absent in all the extracts (table- 5).

Table 5: Qualitative phytochemical screening of Leaf and stem of C. collinus

S. N.	Constituents	Chemical Tests	Extracts											
			Leaf						Stem					
			P	B	C	A	E	W	P	B	C	A	E	W
1	Alkaloids	Mayer's Test	--	--	--	--	--	--	--	--	--	--	--	--
		Dragendroff's	--	--	+	+	+	+	--	+	+	+	+	+
2	Carbohydrates	Fehling's Test	--	--	+	+	+	+	--	+	+	+	+	+
		Benedict's test	+	--	+	+	+	+	--	--	+	+	--	+
		Molisch's Test	--	+	+	+	+	+	+	+	+	+	--	+
3	Steroids	Salkowski Test	--	--	--	--	--	--	--	--	--	--	--	--
4	Saponin	Foam Test	+	+	+	+	+	+	--	--	+	+	+	+
5	Phenolics &	$FeCl_3$ Sol. Test	--	--	+	+	+	+	--	+	+	--	+	+

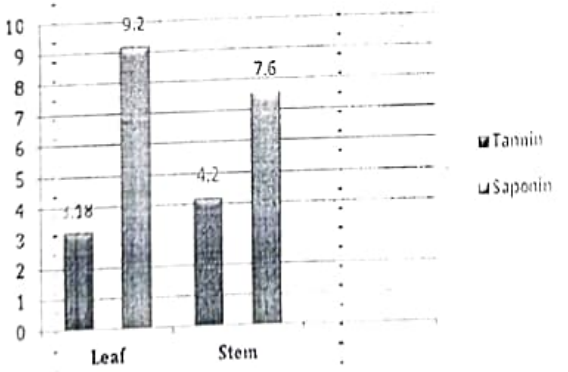
	Tannin	Lead Acetate	+	--	+	+	+	+	+	--	+	+	+	+
6	Oil & Fats	Spot Test	--	--	--	+	+	--	--	+	--	+	--	--
7	Proteins	Biuret Test	--	--	--	+	+	--	+	+	+	--	+	+
		Million's Test	--	--	--	+	+	+	--	--	--	+	+	+
8	Anthraquinone glycosides	Borntrager's Test	--	--	+	+	--	+	--	--	--	--	--	--
9	Cardiac glycosides	Keller-Killiani	--	+	--	--	+	+	+	--	+	+	+	+
10	Flavonoids	Lead Acetate	--	--	--	--	+	--	--	--	--	--	+	--

[Where, P= Petroleum ether, B= Benzene, C= Chloroform, A= Acetone, E= Ethanol and W= Water.]

Quantitative phytochemical analysis: The secondary metabolites in plant under study were found in appreciable concentrations (Table-6). The quantification of secondary metabolites like tannin and saponin in C. collinus leaf was showed percentage of tannin 3.18 ± 0.21 g/100g and Saponin 9.20 ± 0.10 g/100g. While, stem was showed percentage of tannin 4.20 ± 0.12 g/100g and saponin 7.60 ± 0.22 g/100g. Quantitative analysis of plant showed highest quantity of tannin was recorded in the stem and saponin in leaf. Out of two quantified phytochemicals, concentration of saponin was higher than the tannin.

Table 6: Quantitative phytochemical screening of C. collinus

S. N.	Phytochemical	Leaf (g/100g)	Stem (g/100g)
1.	Tannin	3.18 ± 0.21	4.20 ± 0.12
2.	Saponin	9.20 ± 0.10	7.60 ± 0.22



Conclusion:

A Pharmacognostic study on C. collinus has brought to light certain microscopic features as well as preliminary phytochemical data of diagnostic value. Collective powder microscopical data of plant parts have proved to be simple technique of identification. Quantitative microscopic data such as stomatal index have been highly relied upon by pioneer pharmacognocists. It is believed that these features are constant for given species and can be employed for inter specific identity of drugs. Physico-chemical constants such as successive extractive values of the drug are corroborative evidences in drug standardization. Chemical behavioural analysis of the drug

powder as well as drug extract is other test for standardization of the drug. Thus, the powder microscopical characters coupled with preliminary phytochemical results are specific for the identification of therapeutically used potent drug C. collinus. Qualitative and quantitative parameters can serve as an important possible source of information for the identity and to determine the quality and purity of the plant material. These information will also be helpful to differentiate Cleistanthus collinus from the closely related other species and varieties of Cleistanthus. Also, this work could be useful for the adulterants resolution of doubtful materials of C. collinus and compilation of a suitable monograph for its proper identification.

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