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ORIGINAL RESEARCH ARTICLE

Pharmacognostic study of *Clerodendrum colebrookianum* Walp. plant used for medicinal food by Adi tribe of Arunachal Pradesh, India

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ABSTRACT

Clerodendrum colebrookianum Walp.is used as a medicinal food plant among tribal communities of Arunachal Pradesh, India. The shoot of the herb is used as vegetable as well as a medicine to control high blood pressure. This herb is one of the widely used and well-known medicinal food plants in North East India. The present study was carried out to discourse the Pharmacognostic characters of the *Clerodendrum colebrookianum* shoot. The anatomical discourse revealed up the main vascular bundle and lateral vascular bundle with well developed central pith, secondary xylem, and conspicuous endodermis with the outer surface covered by numerous multicellular trichomes. The fluorescence study of powder shows dull green to brownish in daylight and ash colour to dark brown under UV light. The plant sample contains total ash of 11.15%, the acid in-soluble ash is 1.7% and water-soluble ash is 8%. Methanol gave the highest extractive value with 12.56% while petroleum ether gave the lowest extract of 1.40%. Alkaloids, flavonoids, phenols, saponin, glycosides, carbohydrates, proteins and amino acids, fixed oils and fats were found positive but volatile oil was not recorded in all six different solvents used in the phytochemical screening. The present study characterises the diagnostic Pharmacognosy features of *C. Colebrookianum*, and would give useful data to differentiate the authentic drug sample from the adulterated sample.

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INTRODUCTION

Let food be your medicine, once said Hippocrates (c. 460 – c. 370 BC) over 2500 years' ago (Guarrera and Savo, 2013; Ramalingum and Fawzi, 2014). Galen "the father of observational medicine" believed that the fundamentals of good medicine lay in the diet. Such medicinal food concepts and belief is still observable in the indigenous food system practices among indigenous people in various pockets of the world in general and Arunachal Pradesh in particular; Food without dal, wheat, potato, oil and spice are the chief features of the indigenous food system in Arunachal Pradesh (Payum *et al*., 2015). In the word of (Etkin and Ross, 1982), wild plants that are retained in local food cultures are inseparable from traditional therapeutic systems. Moreover, Pieroni and Price (2006) remarked that it is difficult to draw a line between food and medicine; food may be medicine and medicine may be food. Domesticated and non-domesticated green leafy vegetables have numerous dietary and health benefits and rich in macro and micronutrients (Lakshmi and Vimla, 2000; Addis *et al*., 2013), and also Medicinal plants are one of the sources of natural products for the treatment and management of debilitating diseases (Onyekere *et al*., 2000). In the word of (Benzie, 2011), herbal medicines are of course, used for their reputed beneficial effects, however, scientific studies for validation are also important and Pharmacognosy is one of the basic methods to characterize and validate

the drugs of natural origin to give correct and authentic identity. Despite various modern techniques, identification of plant drug by Pharmacognostic study is very reliable (Shah *et al.*, 2013). Besides, De Pasquale (1984) termed "Pharmacognosy" as one of the oldest modern sciences. Pharmacognosy encapsulates medicinal plants and related fields of inquiry with various methods of analysis into drug discovery and serves as a tool of plant identification (Balunas and Kinghorn, 2005; Balasubramaniam *et al.*, 2020; Abu-Al-Futuh, 2020). Present study was carried out to discourse the Pharmacognostic characters of *C. colebrookianum* shoot. And the traditional knowledge has been the main clue to lead the search of bioactive compound for phytochemical scientists; And, *Clerodendrum colebrookianum* Walp. (Figure 1) is used widely used and reported medicinal food plant from Eastern Himalayan states, India. This plant has been reported to be traditionally used in lowering of high blood pressure from almost all states of the NE region by numbers of workers (Nath and Bordoloi, 1991) have reported medico-ethnobotanical use of *C. colebrookianum* leaves for the treatment of hypertension in North-eastern India. Yang *et al.* (2000) have isolated two new Sterols from *C. colebrookianum* (C29 sterols, colebrin A (1) and colebrin B (2). Kotoky *et al.* (2005) have studied potential of *C. colebrookianum* as hypotensive plant. Majaw *et al.* (2008) have studied the effect of *C. colebroolianum* leaf extract on mice cold-resistant stress and reported the administration of 100 mg/kg dose significantly prevented the cold-restraint stress. Namsa *et al.* (2011) have also reported use in High blood pressure, stomach disorder, among Monpa ethnic group of Arunachal Pradesh. Devi *et al.* (2011) studied the antiperoxidative and lipid lowering activity of the crude extract of *C. colebrookianum* leaf and reported encouraging effectiveness. Lokesh and Amitsankar (2012) have studied antihypertensive role of *C. colebrookianum* on mice. But there is no study on Pharmacognosy of this widely known and used medicinal food plant. Thus, this investigation was conducted to discourse the pharmacognostic characteristics of *Clerodendrum colebrookianum* (Walp.) plant used for food and medicine by Adi tribe of Arunachal Pradesh, India.

MATERIALS AND METHODS

Plant material

Clerodendrum colebrookianum Walp., a Verbenaceae family is a shrub of about 15 ft. high with a characteristic smell. Bark shining light grey; Leaves: 3.5-10 by 2.5-8.5 in., broad, ovate, acute, entire, membraneous, almost glabrous; lateral nerves 6-9 on either half; base shallow cordate; petiole 0.5-6.5 in. long with a cluster of glands near the apex. Flower white in a broad terminal compact, corymb biform compound cymes; bract caduceus. Calyx: pubescent, often bearing a few glands; calyx: teeth short; Corolla: tube slender, 1-1.25 in. long; style exerted; Fruit: Bluish -green to deep green when fully ripe, glossy, 0.3 in. across, globose, compressed above, of 4 duprels seated on an accrescent cup-shaped calyx about 0.4 inch across Kanjilal and Borthakur, 1997). The material was collected from Renging Village of East Siang District of Arunachal Pradesh, India.

Parameters

Pharmacognostic parameters, extractive value, ash contents, Phytochemical studies were carried out by using methods described in (Shah and Seth, 2010; Wallis, 2011; Kokate *et al.*, 2012) and by standard methods described in (Iswaran, 1980; Thimmaiah, 1999; Raghuramulu *et al.*, 2003).

Figure 1. *Morphological view of C. colebrookianum.*

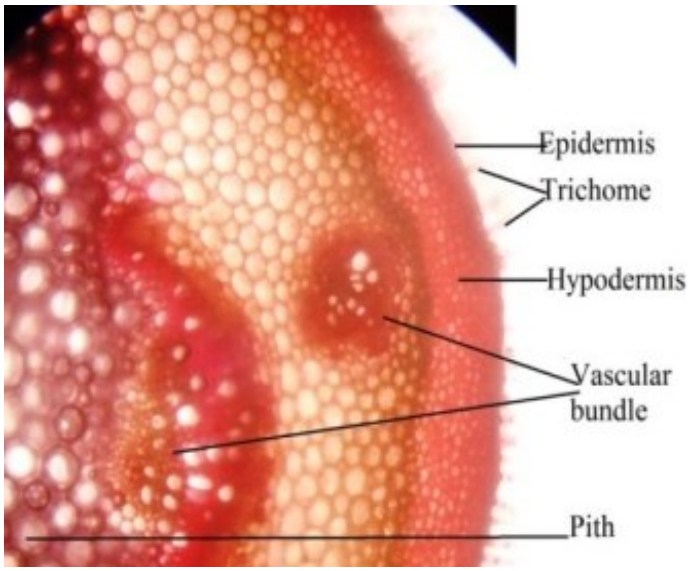

Figure 2. T.S. of Petiole: *C. Colebrookianum*.

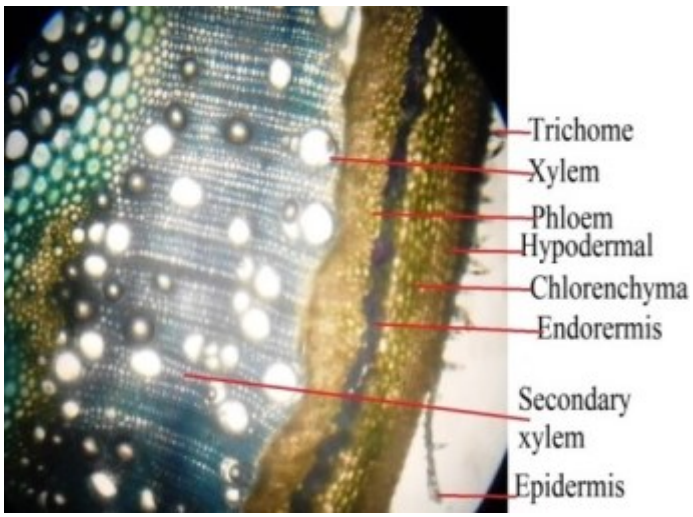

Figure 3. T.S. of stem of C. *Colebrookianum*.

RESULTS AND DISCUSSION

Ethnobotany of *C. colebrookianum*

Indigenous people of Arunachal Pradesh use this herb as a vegetable as well as medicine. As a food, the tender shoot is boiled or steamed; some tribes prefer to consume with bamboo shoot and rice powder while other tribes prefer to consume simple boiled preparation. The best season to eat as a vegetable is from the month of February to April before the shoot starts to develop a bitter taste. As a medicine, decoction (1-2ml) of the leaf is taken daily to bring down high blood pressure before food.

Leaf / Petiole

Outer surface of the rachis is coved by the epidermis, numerous multicellular trichome outgrows from the epidermal layer. Petiole bear lateral as well as main vascular bundle. Vascular bundles are collateral and open (Figure 2).

Stem anatomy

The outline of the stem is quadrangular with a vascular bundle at each angle. Vascular bundles are open and collateral. Epidermis with numerous multicellular trichomes covers the outer surface. The endodermis is conspicuous and central portion is occupied with well-developed pith (Figure 3).

Powder study of shoot

When the powdered drug is treated with different chemicals, a specific colour is observed under UV and visible light respectively for specific drug sample. Fluorescences studies of drug powder plays a crucial role in the identification of drug adulteration. The fluorescent powder study of *C. Colebrookianum* is given in Table 1.

Extractive value

Different solvents have different capacities to extracts a dissimilar percentage of chemical constituents from the given sample. Based on the chemical nature and properties of contents of drugs, various solvents are used for determination of extractives and extractive value is one major qualitative examining characteristic to authenticate a given sample. The extractive values of *C. colebrookianum* are given in Table 2 and Figure 4.

Phytochemical screening

The plant is a biosynthetic laboratory for secondary metabolites such as alkaloids, glycosides, and volatile oils, *etc.* which are therapeutically useful. Phytochemical screening is an important parameter to get an idea of the phytochemical composition of the drug samples. Phytochemical screenings of the *C. colebrookianum* is given in Table 3.

Ash content

Ash content of a biological sample is one of the important studies in which the burnt and ash content of a sample can be studied, it plays crucial role in understanding any given biological sample. The ash study of *C. colebrookianum* is given in Table 4.

In term of ethnobotany, *C. colebrookianum* is one of the most preferred and daily consumed vegetables in Arunachal Pradesh, it is commonly found to be grown in secondary forest and jhum field and also widely cultivated in the kitchen garden. The herb is also consumed to control high blood pressure. Organoleptically, the stem is quadrangular, dark green in colour, the plant has a characteristic aroma, the petiole is long and leaf is almost ovate in shape, flower white and seed is black on mature. The vein terminal is 6 and the vein islet is 17-20. Anatomical discourse of this medicinal food plant reveals that petiole has *the* main vascular bundle and lateral vascular bundle. The outer surface is covered with numerous multicellular trichomes. Pith is well developed, secondary xylem is well developed and endodermis is conspicuous.

Table 1. Fluorescence powder study.

***C.colebrookianum* (Shoot)**	**Colour**		
	Day light	**UV light**	
Powder as such	Light green	Pale green	
Powder + NaOH	Dark green	Light green	
Powder + Acetic acid	Dark brown	Black	
Powder + HNO_3	Orange	Pale green	
Powder + H_2SO_4	Black	Black	
Powder + HCl	Light ash colour	Deep ash colour	
Powder + $FeCl_3$	Bright ash	Aluminium colour	
Powder + water	Deep dark green	Bright dark green	

Table 2. Extractive value of *C. Colebrookianum*.

Sample		**(%=w/w) & n=3**					
		Methanol	**Acetone**	**Ethyl acetate**	**Chloroform**	**Benzene**	**Petroleum ether**
C. colebrookianum	Extractive value	12.56±0.06	2.32±0.08	5.63±0.061	4.97±0.13	2.82±0.24	1.40±0.05
	Extract colour	Greenish	Greenish	Blackish	Yellowish	Yellowish	Yellowish

The study of fluorescence activity of powder of *C. Colebrookianum*, it appears dull green in daylight and when observed under UV light, the powder appears ash colour, Powder + NaOH appears brown in daylight while it is black when observed under UV light, Powder + Acetic acid appears brownish- black in daylight while it appears black under U V light, Powder + HNO_3 a pears brownish orange in day light while it appears yellowish-green, Powder+ H_2SO_4 appears black in daylight but brown under UV light, Powder + HCl appears greenish black in daylight but appear black under UV light, Powder+$FeCl_3$ appears brown in daylight but appears black when observe under UV light, Powder + water appears ash brown in daylight while it appears light brown under UV light. The plant sample contains total ash of 11.15%, with acid insoluble ash of 1.7% and 8% water soluble ash. The Methanol extract was 12.56% with green extract colour, Acetone extract was 2.32 % with green extract colour, Ethyl acetate extract was 5.63 % with black extract colour, while Chloroform extract was 4.97% with extract colour of yellow, Benzene extract was 2.82% with yellow extract colour and Petroleum ether extract was 1.40% with yellow extract colour.

Table 3. Phytochemical test.

C. colebrookianum (shoot)	Extract reaction tests				
	Benzene	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids					
Mayer's test	–	-	–	+	+
Wagner's test	+	+	+	+	+
Hager's test	+	+	+	+	+
Flavonoids					
Alkaline reagent test	–	+	+	+	+
Phenols					
Ferric chloride test	–	–	+	+	+
Gelatin test	–	–	–	+	+
Lead acetate test	–	–	–	+	+
Detection of volatile oil	–	–	–	–	–
Saponins					
Foam test	–	–	–	+	+
Glycosides					
Borntrager's test	–	–	–	–	+
Legal's test	–	–	–	–	+
Carbohydrates					
Molish's test	–	–	–	+	+
Fehling's test	+	+	+	+	+
Barfoed's test	–	–	–	+	+
Benedict's test	–	–	–	+	+
Detection of proteins and amino acids					
Millon's test	–	–	–	+	+
Biuret test	–	–	+	+	+
Ninhydrin test	–	–	–	–	–
Detection of fixed oils and fats					
Spot test	–	–	–		–
Saponification test	–	–	–	+	+

Table 4. Ash study in g/100g dry sample.

Sample	(% = w/w) & n=3		
	Total ash	Acid insoluble ash	Water soluble ash
C. coolebrookianum	11.15± 0.63	1.7± 0.32	8± 0.21

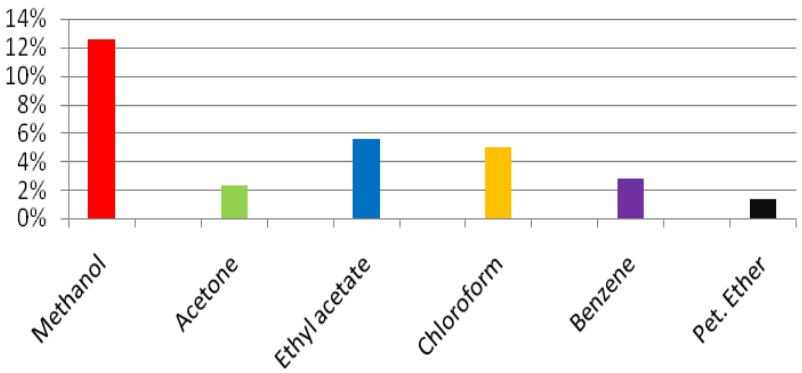

Figure 4. *Extractive value of C. Colebrookianum.*

Conclusion

C. colebrookianum is a widely consumed medicinal food vegetable, primary and secondary metabolites including alkaloids, flavonoids, phenols, saponin, glycosides, carbohydrates, proteins and amino acids, fixed oils and fats were found positive but volatile oil was not recorded in all six different solvents. Presence of large central pith in the stem, vascular bundles and trichomes including greenish to dark green fluorescence characteristics of powder studies may be useful to distinguish the authentic sample from adulterated one.

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Conflict of interest

Authors does not claim any conflict of interest.

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