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

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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 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).
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 covered 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 | UV light |
|--------------------------|--------|---------|
| Powder as such           | Light green | Pale green |
| Powder + NaOH            | Dark green | Light green |
| Powder + Acetic acid     | Dark brown | Black |
| Powder + HNO₃            | Orange | Pale green |
| Powder + H₂SO₄           | Black | Black |
| Powder + HCl             | Light ash colour | Deep ash colour |
| Powder + FeCl₃           | Bright ash | Aluminium colour |
| Powder + water           | Deep dark green | Bright dark green |

Table 2. Extractive value of C. Colebrookianum.

| Sample               | Methanol (%)=w/w & n=3 | Acetone (%)=w/w & n=3 | Ethyl acetate (%)=w/w & n=3 | Chloroform (%)=w/w & n=3 | Benzene (%)=w/w & n=3 | Petroleum ether (%)=w/w & n=3 |
|----------------------|------------------------|-----------------------|-----------------------------|--------------------------|----------------------|-----------------------------|
| C. colebrookianum    | 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 UV light, Powder + HNO$_3$ appears brownish-orange in daylight while it appears yellowish-green, Powder+ H$_2$SO$_4$ 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                  | Total ash (%) | Acid insoluble ash (%) | Water soluble ash (%) |
|-------------------------|---------------|------------------------|-----------------------|
| *C. colebrookianum*     | 11.15±0.63    | 1.7±0.32               | 8±0.21                |

![Figure 4. Extractive value of C. Colebrookianum.](image)
**Conclusion**

*C. colebrookianum* is a widely consumed medicinal food vegetable, primary and secondary metabolites including alkaloids, flavonoids, phenols, saponins, 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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**REFERENCES**

Abu-Al-Futuh, I.M. (2020). Study on Pharmacognosy Curricula in UAE. B Pharm Programmes and Possible Implications. Pharmacognosy Journal, 12(3): 478-84.

Addis, G., Asfaw, Z., Singh, V., Woldu, Z., Baidu-Forson, J.J. and Bhattacharya, S. (2013). Dietary values of wild and semi-wild edible plants in Southern Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, 13 (2): 7485-7503.

Balasuhramaniam, G., Sekar, M. and Badami, S. (2020). Pharmacognostical, Physicochemical and Phytochemical Evaluation of *Strobilanthus kunthianus* (*Acanthaceae*). *Pharmacognosy Journal*, 12(4): 731-741.

Balunas, M.J. and Kinghorn, A.D. (2005). Drug discovery from medicinal plants. *Life Sciences*, 78 (5): 431-41.

Benzie, I.F. and Wachtel-Galor, S. (Eds.). (2011). Herbal medicine: biomolecular and clinical aspects. CRC press.

De Pasquale, A. (1984). Pharmacognosy: The oldest modern science. *Journal of Ethnopharmacology*, 11 (1): 1-16.

Devi, R., Boruah, D.C., Sharma, D.K. and Kotoky, J. (2011). Leaf extract of *Clerodendrum colebrookianum* inhibits intrinsic hypercholesterolemia and extrinsic lipid peroxidation. *International Journal of PharmTech Research*, 3(2): 960-967.

Etkin, N. L. and Ross, P.J. (1982). Food as medicine and medicine as food. An adaptive framework for the interpretation of plant utilization among the Hausa of Northern Nigeria. *Social Medicine*, 16: 1559-1573.

Guerrera, P.M. and Savo, V. (2013). Perceived health properties of wild and cultivated food plants in local and popular traditions of Italy: A Review. *Journal of Ethnopharmacology*, 146: 659-680.

Iswaran, V. (1980). A Laboratory Handbook for Agricultural Analysis. Today and tomorrow’s Printers and Publishers, New Delhi-110005.

Kanjilal, U. and Borthakur, S. (1997). Flora of Assam. Vol. IV. Osmonos Publications, New Delhi; (reprint). pp: 291-192.

Kokate, C.K., Purohit, A.P. and Okhale, S.B. (2012). Pharmacognosy (47th edn.). Vol I, Narla Prakashan, Pune. 411005: pp. 6.22.

Kotoky, J., Dasgupta, B. and Deka, N. (2005). Pharmacological studies of *Clerodendrum colebrookianum* Walp. A potential hypotensive plant. *Indian Journal of Physiology and Pharmacology*, 49(3): 289-296.

Lakshmi, B. and Vimala, V. (2000). Nutritive Value of dehydrated green leafy vegetables powders. *Journal of Food Science and Technology*, 37: 465-471.

Lokesha, D. and Sankaran, D. (2012). Evaluation of mechanism for antihypertensive action of *Clerodendrum colebrookianum* Walp., used by folklore healers in north-east India. *Journal of Ethnopharmacology*, 43 (1): 207-12.

Majaw, S., Kurkalang, S., Joshi, S.R. and Chatterjee, A. (2008). Effect of *Clerodendrum colebrookianum* Walp. Leaf extract on mice cold-resistaints tree in mice. *Pharmacology Online*, 2: 742-753.

Namsa, N.D. Mandal, M. Tangjang, S. and Mandal, S.C. (2011). Ethnobotany of the Monpa ethnic group at Arunachal Pradesh, India. *Journal of Ethnobiology and Ethnomedicine*, 7:31.

Nath, S.C. Bordoloi, D.N. (1991). *Clerodendrum colebrookianum*, a Folk Remedy for the Treatment of Hypertension in North-eastern India. *Pharmaceutical Biology*, 29(2): 127-129.

Onyekere, P.F., Odoh, U.E., Peculiar-Onyekere, C.O., Nwafor, F.I. and Ezugwu, C.O. (2020). Pharmacognostic and phytochemical studies of leaves *Psidioxorizontalis* Schum. &Thonn Pncoq.com (Rubiaceae). *Pharmacognosy Journal*, 12 (3): 541-50.

Payum, T. Das, A.K and Shankar, R. (2015). Phytochemistry, pharmacognosy and nutritional composition of *Allium hookeri*: An ethnic food plant used among Adi tribe of Arunachal Pradesh, India. *American Journal of Pharmtech Research*, 5 (1): 465-477.

Phillipsen, J.D. (2007). Phytochemistry and Pharmacognosy. *Phytochemistry*, 68: 2960-2972.

Pieroni, A. and Price, L.L. (2006). Eating and Healing: Traditional Food as Medicine. *Food Products Press*, 10 Alice Street, Binghamton, New York; pp: 1-3.

Raghuramu, N., Nair, K. and Kalyanasundaram, S. (2003). *A Manual of Laboratory Techniques*. National Institute of Nutrition, ICMR, Hyderabad-500007.

Ramalingum, N. and Mahonoodally, M. (2014). The Therapeutic potential of medicinal foods. *Advances in Pharmacological Sciences*, Retrieved May, 2020 from https://www.hindawi.com/journals/aps/2014/354264/.

Shah, B. and Seth, A.K. (2010). *Textbook of Pharmacognosy and Phytochemistry*. Reed Elsevier India Private Limited, New Delhi-110019, pp: 110.

Shah, G., Chawla, A., Baghel, U.S. and Rahar, S. (2013). Pharmacognostic standardisation of leaves of *Melaleucaleucadendron*: *Pharmacognosy Journal*, 5: 143-148.

Thimmiah, S. R. (1999). *Standard Methods of Biochemical Analysis*. Kalyani Publishers, New Delhi.

Wagner, H. and Bladt, S. (1996). *Plant Drug Analysis*. Springer (India) Private Limited: New Delhi-110001.

Wallis, T.E. (1985). Text book of Pharmacognosy, 5th edition, CBS Publishers & Distributors, 4596/1A, 11 Darya Ganj, New Delhi-110002; pp.1.

Wallis, T.E. (2011). Practical Pharmacognosy. PharmaMed Press, sultan Bazar, Hyderabad-500095; pp: 1.

Yang, H., Xi Mei, S., Jiang, B., Wen Lin, Z., Dong Sun, H. (2000). Two New C29 Sterols from *Clerodendrum colebrookianum*. *Chinese Chemical Letters*, 11(1): 57–60.