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PHYTOCHEMICAL AND PHARMACOGNOSTIC STUDY OF COLEBROOKEA OPPOSITIFOLIA SMITH.

Darshan Kathait*, Bahuguna Yogendr, Tailor Chandra Shekhar

School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun (Uttarakhand) 248001.

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***Corresponding Author**
Darshan Kathait
P.G. Research Scholar, School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, (U.K.).
darshankathait95@gmail.com

ABSTRACT
The species *Colebrookea oppositifolia* smith is one of the largest family member of dicotyledons, in plantae kingdoms and highly aromatic, due to the external glandular structures which produce volatile oil.[1] The volatile oil of the plants is importants in pharmaceutical, pesticide, and others usese. The investigation present deals with importantsphyto-constituents which is usese as various disorder such as eplipepsy,[3,4] ulcer, hepatitis, antibacterials, antifungals, wound healing, bleeding[6] (heamostatic) anti-inflammatory[7] etc.*C.oppositifolia* Sm indicated the presence of various phyto-constituents such as alkaloids, flavonoids, glycosides, steroids and saponins terpenoids, tannins, and cardiac glycosides are showed only in different medium.

KEYWORDS: Binda, fungitoxic, Indian squirrel, wound healing.

INTRODUCTION

The selected plant, *Colebrookea oppositifolia* Sm., is one of the most important plant of the family Lamiaceae. Its other name in Garhwali language is ***Bhinda*** or ***Bhindu*** & Marathi is ***Bhaman*** or ***dosul*** and also known as ***Indian Squirrel*** Tail in English.[8] *Colebrookea* is a monotypic genus of plants in lamiaceae, first described in 1806.[9] The plant has extensively medicinal potential and generally used in the traditional system of Indian medicine to cure epilepsy, urinary problems, dysentery, peptic ulcer, and hepatitis. It is also used as anti-microbial agent. The essential oil of Colebrookea possesses fungitoxic property.[10] Furthermore work various part of plant like root, leaves, and stem are being extensively used as epilepsy, haemostatic,[18] treat dermatitis, wounds, nose bleeds and leaves paste is utilize for wound healing. There are various folk medicine practicing communities such as nomadic Gujjars, Tharu, and Bhoxa in sub-himalayan regions of India, from many decades and even nowdays.[12]

The plant has immense medicinal potential and is generally used to cure epilepsy,[3,4] urinary problems, dysentery, peptic ulcer,[18] and hepatitis. It is also used as antimicrobial agent. The essential oil of Colebrookea possesses fungitoxic property. Leaves are used in the treatment of wounds, bruises and fracture. Leaves are warmed in boiling water and applied on the sprains, given to cattle to eat in anthrax.

CLASSIFICATION

Kingdom: Plantae
Phylum: Eudicots
Class: Asterids
Order: Lamiales
Family: Lamiaceae
Genus: *Colebrookea*
Species: *oppositifolia*

Fig: *Colebrookea oppositifolia* plant.

GLOBAL DISTRIBUTION

Colebrookea oppositifolia is evergreen plant densely woolly shrub or small tree, 1.2 to 4.5 meter distributed in subtropical Hilly regions of the world such as India, Pakistan, Nepal, Thailand, Myanmar, china and mainly in south, south east asia.

INDIAN DISTRIBUTION

In India widely in northen slopes of Himalayan range of Himachal, Uttarakhand, Sikkim at the elevation of 3500 - 5500 ft and it grows wild on hills and plains mainly in subtropical M.P and deccan peninsula. In souther state kerala (palakkad, iduki, malappuram, kannur, thrissur, kozhikkode) & Karnataka (chikmaglur, coorg, mysore) and Tamil nadu (Coimbatore, Nilgiri, Salem).

HABIT & HABITATE

Colebrookea oppositifolia sm. Plant is semi-green and evergreen forests. Flowering period December to April. It is large shrubs, stem and leaves densely tomentose. The leaves are densyhairy like, elongated, finely

notched, 12-15 cm long. Large number of tiny white flowers occurs in raceme of upright spikes, 5-10 cm long. The plant is gernally found in dreid areas of hilly.

BOTANICAL DESCRIPTION

The plant is 1-4 meter in height and highly branched. Branches are pale and densely hairy when young and stems are stout and light in colour. Leaves of the plant, light green in colour are oppositely arranged and flocked at branch ends. Sometimes, the leaves are present in whorls of the leaves are lance like, elongated, finely notched, 12-15 cm long, darkish green above and whitish hairy below.[23] Large number of tiny white flowers occurs in raceme of upright spikes, 5-10 cm long. The spikes bearing flowers look hairy resembling squirrel's tail, hence the name, Indian squirrel tail.

PHARMACOLOGICAL IMPORTANCE

- The plant has immense medicinal potential and is generally used to cure epilepsy, urinary problems, dysentery, peptic ulcer, and hepatitis.
- It is also used as antimicrobial agent. The essential oil of Colebrookea possesses fungitoxic property.
- Leaves are used in the treatment of wounds, bruises and fracture. Leaves are warmed in boiling water and applied on the sprains, given to cattle to eat in anthrax.

OTHER USE

- It is tradition folklore medicinal plant to used by hilly ancient people for various remedies such as wound healing, antibacterial, antifungal, epilepsy.
- It also quickly stop bleeding (coagulation) if there is cuts on the skin.
- Leaf juice can be used to treat fever and has been applied topically to ease headache. Leaves can also be made into a poultice to treat dysentery.
- Root extracts can be used as a remedy against epilepsy while the decoction of the roots is used to treat individuals with peptic ulcers.

OTHER NAME OF THE PLANT

English: Indian squirrel tail
Hindi: Binda, Kala-bansa, Pansra, Bhirmoli
Telugu: Jolidi, Tolisi
Kannad: Thuggigida
Marathi: Bhaman, Bhamini, Bhamni
Tamil: Vitupucittalai
Oriya: Tulia
Lepcha: Umhyemkung

ETHNO MEDICINAL SIGNIFICANCE

Various species of Colebrookea oppositifolia sm. have been used in the traditional system of medicine across the world. *C.* oppositifolia is accredited for diverse medicinal properties and finds therapeutic applications in traditional medicines antimicrobial, blood clotting, antifungal, and antifertility. Conventionally the leaf of plant is used to stop bleeding of cut and wounds, forming clots. A decoction of the leaf has been recommended to treat ulcers and epilepsy diseases.

METHEDOLOGY

The plant was collected from hilly areas of gopeshwar chamoli. It generally grow wild in the forest area and local. The plant was identified and authenticated from Botanical Survey of India, Dehradun.

PHYTOCHEMICAL INVESTIGATION

The Investigation of *Colebrookea oppositifolia* smith plant revealed the presence of following phytochemical: terpenoids, sterols, glycosides, flavonoids, alkaloids, carbohydrates, tannins, phenols, proteins (amino acid), saponnins, fixed oil and fats.

ALKALOID TEST

Dragendroff's test

- Extracted drug treated with potassium iodide solution (dragendroff's reagent).
- Formation of orange red precipitate presence of alkaloids.

Hager's test

- Filtrate was treated with saturated aqueous solution of picric acid (hager's reagent)
- Yellow colour shows the presence of alkaloids.

CARBOHYDRATES TEST

Dissolve 2 gm extract in 5 ml distilled water & filter it.

Molicsch's test

- Filtrate was treated with 2drops of alcoholic alpha-napthol solution in attest tube,& shaken
- Add conc. Sulphuric acid from the side of test tube.
- Development of a violet ring at the junction of two liquid confirmed the presence of carbohydrates.

DETECTION OF SAPONNINS

- Extract was diluted with 20 ml of distilled water & shaken in a graduated test tube for 10 minutes.
- Formation of1cm foam layer indicate the presence of saponnins.

DETECTION OF PHYTOSTEROLS

Small quantity of extract dissolved in 5ml of chloroform.

Salkowski's test

- Add a few drops of conc. Sulphuric acid with extract, allow the solution to stand.
- Formation of brown rings indicates the presence of phytosterols.

Libermann Burchard's test

- The chloroform extract treated with acetic anhydride, boil & cool.
- Add conc. Sulphuric acid. Formation of a bluish green colour solution confirmed the presence of phytosterols.

DETECTION OF PHENOLS

Ferric chloride test

- Treat the extract with 2, 3 drops of ferric chloride solution.
- Formation of bluish black colour indicates the presence of phenols.

Lead acetate test

- Treat the extract of 3 ml of 10 % lead acetate solution.
- A bulky white precipitate indicates the presence of phenols.

DETECTION OF TANNINS

- Take 0.5 gm of the dried powdered plant, boil 0.5gm sample in 20 ml of water in a test tube & filter the above mixture.
- Add few drops of 0.1 % ferric chloride.
- Development of brownish green or a blue-black colouration indicated the presence of tannins.

DETECTION OF FLAVONOIDES

Alkaline reagent test

- Treat the extract of few drops sodium hydroxide solution.
- Formation of intense yellow colour, which becomes colourless on further addition of dilute acid, indicate the presence of flavonoids.

Lead acetate test

- Treat the extract with few drops lead acetate solution.
- Formation of yellow precipitate indicates the presence of flavonoides.

DETECTION OF PROTEINS & AMINO ACIDS

Million's test

- Treat the test solution of few drops of millions reagents.
- When warmed, a white precipitate is formed which change to a brick red or disappears indicate the presence of proteins & A.A.

Ninhydrin test

- Add ninhydrin reagent to the test solution & boiled for few minutes.
- Formation of blue colour indicates the presence of amino acids.

DETECTION OF TERPENOIDS

Salkowski test

- Mix 2 ml of chloroform to extract solution carefully added conc. Sulphuric acid (3ml) to form a layer.
- A reddish brown colouration of the interface indicates the presence of terpenoids.

DETECTION OF GLYCOSIDES

Keller-killani test

- Treat the extract of 2 ml of glacial acetic acid containing one drop of ferric chloride solution, add 1 ml of conc. Sulphuric acid.
- Appearance of brown ring at the interface indicate the deoxy sugar characteristic of cadenolides

TEST FOR FIXED OIL AND FATS

Spot test

- Place small quantity of the extract in between two filter papers.
- Oil stain produce with any extract showed the presence of fixed oils and fats in the extracts.

THIN LAYER CHROMATOGRAPHY

It involves a stationary phase consisting of a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose immobilized onto a flate, inert carrier sheet.

A liquid phase consisting of the solution to be separated which is dissolved in an appropriate solvent and is drawn up to the via capillary action, separating the solution based on the polarity of the components of the compound.

Procedure

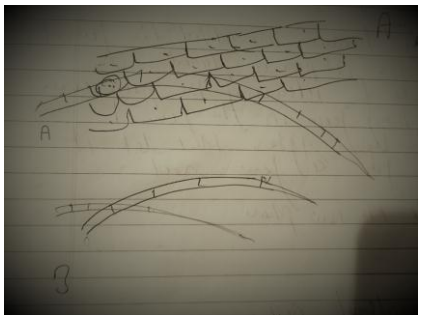
- Made a thick slurry of silica gel and spread on inert carrier sheet, usually glass or plastic.
- Make a thin mark on bottom of TLC plate
- Applied sample solution are on spot marked & dried
- Place TLC plate into the TLC chamber
- Allow to dry the plate
- Visualisation of spot under UV rays or iodine chamber

RESULT

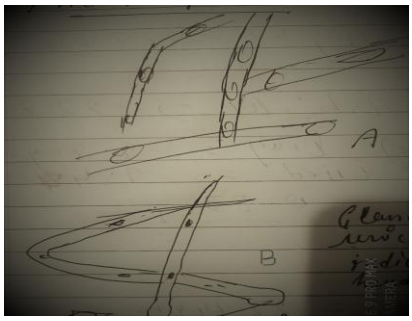
MACROSCOPIC EVALUATION

Powder microscopy

The powder microscopy character shows below Capitate glandular trichomes in surface view and part of view underlying palisade, Trichome base epidermis, Lignified fibro-vascular structure in sponge mesophyll, Glandular trichome with unicellular stalk & radiate bladder.



A: Capitate glandular trichomes in surface view and part of view underlying palisade B: Trichome base epidermis, underlying palisade.



C: Lignified fibro-vascular structure in sponge mesophyll, D: Glandular trichome with unicellular stalk & radiate bladder.

The C.oppositifolia smith is commonly traditional folklore herbal drugs used to treat various diseases such as epilepsy, coagulation, antibacterial, antifungal etc practicing by various communities such as tharu, boxa and gujjar in Himalayan regions from many decades. This drug may have various advantages. The investigation of *Colebrookea oppositifolia* smith has done. The current investigation reveals the pharmacognostical feature and various physiochemical properties of C.oppositifolia smith. All the different extracts such as petroleum ether, alcohol and water are similar but the chloroform extract has less effective than other three.

The solvents system was taken for TLC is chloroform (100%), alcohol (100%), petroleum ether (100%) and alcohol-chloroform (1:1), chloroform-pet.ether (1:1), alcohol-pet-ether (1:1). The R.f. value of different solvent system is following the table 1.

$$\text{R.f value} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by solvents front}}$$

Table 1: R.F. Value of Different Extract.

Solvents	Extract	R.f. value
Alcohol	Pet. Ether Chloroform Alcohol Aqueous	0.42 0.17 0.55 0.25
Pet. Ether	Aqueous Pet. ether Chloroform Alcohol	0.9 0.18 0.22 0.21 0.11
Chloroform	Pet. ether Alcohol Aqueous Chloroform	0.15 0.19 0.16 0.21
Alcohol - Chloroform (1:1)	Pet. ether Alcohol Aqueous Chloroform	0.14 0.17 0.11 0.23
Chloroform - Pet. Ether (1:1)	Pet. Ether Alcohol Aqueous Chloroform	0.13 0.20 0.8 0.24
Pet. Ether-Alcohol (1:1)	Pet. Ether Alcohol Aqueous Chloroform	0.15 0.19 0.16 0.21

PHYSICOCHEMICAL CONSTANTS

Loss on Drying

Loss on drying is the loss of mass expressed as percent w/w. The test for loss on drying determines both water and volatile matter in crude. Moisture is inevitable component of crude drug, which must be eliminated as far as possible.

An accurately weighed quantity of about 5g of powdered drug was taken in tarred glass Petridis. The powder was distributed evenly. The Petridis kept open in vacuums and the sample was dried at a temperature "between" 100 to 105^0 C for 2 hour until a constant weight was recorded. Then it was cooled in desiccators to room temperature, weighed and recorded % loss on drying was calculated using following formula[31].

% Loss on drying = Loss in weight of the sample × w

Table No. 2: Loss on drying.

Parameter	% yield
Loss on drying	8.46

Ash values

Total ash is useful in detecting the crude drugs that are mixed with various mineral substances like, sand, soil, calcium oxalate, other drugs with different inorganic contents to improve their appearance.

Physiological ash: which is derived from the plant tissue itself?

Non-Physiological ash: Which of the adhering material to the plant e.g. sand & soil.

$$\% \text{ water soluble ash} = \frac{\text{Weight of water soluble ash}}{\text{Weight of sample}} \times 100$$

$$\% \text{ water insoluble ash} = \frac{\text{Weight of acid insoluble ash Value of}}{\text{Weight of sample}} \times 100$$

Table 3: Ash value of *C.oppositifolia* sm.

Parameter	Values % (w/w)
Water soluble ash	6.5
Acid insoluble ash	25
Total ash	12

Extractive values: It is the amount of extractive a drug yields to a given solvents on dry weight basis. It helps to give approximate measure of a certain chemical constituent or group of related constituents that the drug contains.

Water soluble extractive value: water soluble extractive value is applied for the drugs which contain water soluble constituents such as tannins, sugars, plants acids and mucilage.

Alcohol soluble extractive value: Alcohol soluble extractive value is applied for the drugs which contain alcohol soluble constituents such as tannins, resins and alkaloids. Extractive value of the drug in follows the table no. 3.

Table 4: Extractive values of *C. oppositifolia* sm.

Parameter	% yield
Water extractive value	26
Alc. extractive value	12

PHYTOCHEMICAL SCREENING

Preliminary qualitative tests

Phyto-chemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens. The name comes from Greek (phyton), meaning plant. Some phyto-chemicals have been used as poisons and others as traditional medicine.

Table 5: Phytochemical analysis of *C. oppositifolia* sm.

S. N.	Group	Name of Test	Extract				Inflorescnce
			Alc.	Chlo.	Pet.eth.	Aq.	
1	Terpenoids	Salkowski test	+	+	+	+	+
2	Phytosterol	Libermann Burchard's test	+	+	+	+	+
3	Glycoside	Keller-killiani test	+	+	+	+	+
4	Flavonoids	Alkaline reagent test	+	+	+	+	+

5	Alkaloids	Dragondroff's test	+	+	+	+	+
6	Proteins	Million's test	-	-	-	-	-
7	Carbohydrates	Molisch's test	+	+	+	+	+
8	Saponins	Foam test	-	-	-	-	-
9	Lipids	Soap formation test	-	-	-	-	-
10	Phenols	Ferric chloride test	+	+	+	+	+
11	Tannins	Braymer's test	+	-	+	+	+
12	Fixed oils		+	+	+	+	+

CONCLUSION & DISCUSSION

The current investigation reveals the pharmacognostical features and physicochemical properties of *Colebrookea oppositifolia*. The present study findings are associated with standardization of parameters like phytochemical screening, and physicochemical quantification of *C. oppositifolia*. The R.f value was found in different solvents system like chloroform, alcohol, pet. Ether and aqueous extracts of leaf. Ash values added more strength to crude drug standardization with prominent results indicating the involvement of extraneous matter. Such study on the preliminary phytoconstituent screening and physicochemical parameters is important information which may be useful in verification and contamination for quality control of this therapeutic plant afterwards. The results of phytochemical tests indicate the presence of glycosides, alkaloids, tannins, flavonoids, terpenoids, carbohydrates, phenols and phytosterol. Physiochemical standards like total ash, acid insoluble ash, water & alcohol soluble extractive values, loss on drying, phyto-chemical analysis, and safety evaluation were carried out.

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