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Pharmacognostic and phytochemical evaluation of *Colubrina travancorica* Bedd.

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Abstract
Colubrina travancorica Bedd. is a member in Rhamnaceae family. The plant is endemic to southern Western Ghats. It is traditionally used to relieve pain. Eventhough several species of *Colubrina* has been used in traditional medicine and in industry, scientific experiments are lacking in *Colubrina travancorica*. The aim of the present study, as a part of quantitative microscopy, stomatal number, stomatal index, trichome length & breadth and veinislet number were determined. Two solvents viz; water and methanol was used to obtain extracts from powdered plant parts. The extracts were subjected to qualitative phytochemical screening using standard procedure. Water extract of stem, leaf and roots shows the presence of diverse group of phytochemicals. Powder analysis and reaction with different reagents can be used for the identification of the various parts of the plant. The present study, phytochemical evaluation of the leaf, stem and root extract has been undertaken as an initial step to understand the pharmaceutical potential of the plant.

Keywords: *Colubrina travancorica*, phytochemical analysis, powder analysis, quantative microscopy

Introduction
Phytochemicals are bio-active chemicals of plant origin. Phytochemical studies have attracted the attention of plant scientists to systematic problems on the one hand and in the search for additional resources of raw materials for pharmaceutical industry on the other hand. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances (Mojab 2003) [1].

The genus *Colubrina* is floristically the least specialized members of the Rhamnaceae (Buckthorn) family (Johnston 1971) [2]. Eventhough several species of *Colubrina* are scientifically studied, literature and experiments are lacking in *Colubrina travancorica.* From various species of *Colubrina*, numerous chemical compounds (Saponins, alkaloids, proteins, phenol etc.) with industrial and medicinal uses were identified. Three novel saponins, mabiosides C-E were isolated from the bark of *Colubrina elliptica* together with the mabioside B and [alpha-L-rhamnopyranosyl-beta-D-glucopyranosyl alpha-L-arabinopyranosyl] jujubogenin (Oulad Ali etal.1994) [3]. The methanolic extract of the *Colubrina greggii* shows an anti-microbial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Karlina Garcia-Sosa *et al*. 2006) [4]. The effect of regular consumption of tropical food drink mauby (*Colubrina arborescens*), on the control of hypertension (Alleyne *et al*. 2005) [5] was also studied. Essential oil isolated from the seed kernel of *Colubrina* contains terpenes and other unsaturated compounds and are responsible for inhibiting the growth of a number of pathogenic microbes. *Colubrina* is one of the most frequently a used plant in Yucatan traditional medicine for the treatment of asthma, tuberculosis, ulceration, and abscess is *Colubrina greggii* (Mendieta *et al*. 1981) [6]. Tolkavech *et al*. (1980) [7] isolated bisbenzylisoquinoline alkaloids and 2-benzylisouinoline compounds which possess anti-hypertensive and anti-arrhythmic effects in hypertensive mice and humans.

The plant *C. travancorica* is endemic to Southern Western Ghats. Flowering and fruiting takes place from November to March. In Kerala, *C. travancorica* is distributed mainly in Alappuzha, Pathanamthitta, Thiruvanathapuram, Ernakulum, and Thrissur (Sasidharan, 2004) [8]. *C. travancorica* is a glabrous, evergreen shrub capable of attaining heights of upto 5-7 meters. Bark is dark brown, leaves alternate conspicuous dark green and shiny above and dull, paler green below. Flowers reduced, orange to red in color (Figure 1 A), each with a necter disc, 5 sepals, 5 hooded petals, 5 stamens. Fruits a small, tri-carpellate, subglobose capsule

There is no scientific studies reported in *C. travancorica* hence the chemical analysis is more important.

The work in brief, envisaged to study pharmacognostic features of the stem, leaves and root, quantitative microscopic studies, powder analysis, preliminary phytochemical analysis.

Materials and Methods

Collection of plant materials

The plant materials were collected from the natural habitats, Mylapra, Pathanamthitta Dist, Kerala (Latitude 9.2725438, Longitude 76.798892, Altitude 22 meters). For micro characterization, fresh plant parts are used. Different plant parts are shade dried and the powdered material used for powder analysis.

Preparation of Extracts

Shade dried, coarsely powdered raw drug was used for powder analysis. Preliminary phytochemical studies were carried out using 5g powdered material and subjecting it to successive extraction in a beaker with 50 ml solvents viz, methanol and water. The extraction was continued upto 20 mins.

Quantitative Microscopy

As a part of quantitative microscopy, palisade ratio, stomatal index, vein islet number, trichome length &breadth were determined.

Powder analysis

Identification of organoleptic characters, determination of physiochemical characters, behavior of the powder with different chemical reagents/solvents according to methods (Anonymous 1985) [9] and fluorescence analysis (Chase *et al*. 1949 [10]; Kokashi 1958) [11] was made.

Phytochemical analysis

For phytochemical studies, fresh plant materials were collected and they allowed to shade dry and then homogenized to fine powder and stored in air tight bottles. Presence of various phytoconstituents viz., alkaloids (*Mayer's test)*, anthraquinones (*Borntragers test*), saponins (*Foam test*), coumarines (*Colour test*), tannins (*Ferric Chloride test*), steroids/ terpenoids (*Salkowski test, Liebermann-Burchard test*), quinines (Sodium hydroxide test), protein (Xanthoprotein test, Biurete test), Phenols (ferric chloride test), reducing sugar detection test (Benedict's reagent test, Fehlings test), resins (Turbidity test), glycoside (Benedict's reagent test), test for fixed oils (oil stain test), phlobatannins (Precipitate test),flavones, flavanones, (sodium hydroxide test), phytosterols, cardiac glycosides (Keller Kelliani's test),carbohydrates (Molisch's Test), amino acids and proteins (ninhydrin test) were qualitatively tested ([12, 13, 14]).

Results and Discussion

Quantitative Microscopy

Leaf peelings from both adaxial and abaxial side showed paracytic (rubiaceous) type of stomata, where the stoma is surrounded by two subsidiary cells; the long axis of which are parallal to the stoma. The palisade ratio, stomatal index, vein islet number, trichome length and breadth (Table 1) were calculated as a part of quantitative microscopy.

Table 1: Quantitative determinations of *C. travancorica*

Determinations	Plant part	Value (Mean±S.E)
Palisade ratio	Leaf	2.4±0.08
Stomatal index	Leaf	
Upper		26.4±0.33
Lower		21.5±0.34
Vein islet number	Leaf	14.0±0.47
Trichome length	Leaf	539.20±59.29 µm
	stem	812±46.33 µm
	petiole	874.0±37.86 µm
Trichome breadth	Leaf	15±0.00 µm
	stem	15±0.00 µm
	petiole	15±0.00 µm

Powder analysis

Powder microscopy: Organoleptic characters refer to evaluation of drugs by colour, odour, taste, size and consistence. These were identified by using the crude powder samples (Figure 1 B,C & D). The stem and root powders are moderately fine, aromatic and fibrous. Leaves are NATO green in colour whereas stem and roots are Deep buff and beige coloured. Stem and root samples show similarities in all cases except in taste (Table 2). Leaf powder is fine, smooth and bitter tasted.

Fig 1: *Colubrina travancorica* A) Flowering twig B) Powdered leaves C) Powdered stem D) Powdered root.

Table 2: Organoleptic characters of stem, root and leaf powders of *C. travancorica.*

Character	Leaf	Stem	Root
Colour	NATO green	Deep buff	Light beige
Odour	Mouldy	Aromatic	Aromatic
Taste	Bitter	Sour	Taste of tea
Size	Fine	Moderately fine	Moderately fine
Texture	Smooth	Fibrous	Fibrous

colour as per British Standared Colours. (Www British standarad colour.com).

Behavior of powder with Chemical reagents

Detection of colour variation of the powdered samples under daylight is a way to identify its purity. Green colour is prominent in powder of leaves. The stem shows yellow to orange colour shades in most of the reagents except HCl, H_2SO_4, aqueous ammonia and 5% NaOH where green colour is found. Root shows yellowish colour in most cases (Table 3).

Table 3: Behavior of *C. travancorica* powder with different chemical reagents

Powder	Leaf*	Stem*	Root*
Powder+D.W	Light Brunswick Green	Light Stone	Light Beige
Powder+5% $FeCl_3$	French Mustard/Florida	Apricot	Cannary Yellow
Powder+5% KOH	French Mustard/Florida	Cannary Yellow	Light Buff
Powder + Conc. HCl	Grass Green	NATO Green	Carnival Red
Powder+HNO_3	Rail Red/Azo Orange	Light Orange	Light Orange
Powder+H_2SO_4	Deep Bronze Green	Olive Drab	Maroon
Powder + aqueous ammonia	Grass Green	French Mustard/Florida	Bold Yellow
Powder + 5% Iodine solution	Middle Bronze Green	Light Beige	Light Beige
Powder + 5%NaOH	NATO Green	French Mustard/Florida	Golden Yellow /Amber/Gold Cup

*colour as per British Standared Colours. (www. British standarad colour.com).

Fluorescence Analysis

Table 4: Fluorescence analysis of various *C. travancorica* extracts in UV and visible light.

Treatment	Fluorescence analysis of extracts under UV light			Optical activity in visible light		
	Leaf*	Stem *	Root*	Leaf*	Stem*	Root*
Chloroform	Graas Green	Mineral Green/Opaline/ Moorland	Light beige	French mustard/ Florida	Vannila pollen	Light beige
Acetone	French Mustard/Florida	Sunflower Yellow/Canary/ Aztec	Misty red/ Tawny	Cannary Yellow	Light Buff	Salmon pink
Methanol	Sunflower Yellow/Canary/ Aztec	Wheat/Golden Bronze/Savannah	Light yellow	Golden Yellow	Mellow Apricot/ Apricot	Light orange
Water	Middle Bronze	Light Stone	Deep Buff	Golden Maize/ Banana/ Capricorn	Golden Yellow/ Amber	Deep buff

*colour as per British Standared Colours. (www. British standarad colour.com).

The optical activity of powdered samples and extracts under visible an UV light are observed and recorded. In UV light and visible light, yellow colour is most prominent in stem, but in the case of leaf green and yellow colours have equal chances in UV light, in visible light yellow colour is present in four treatments. In both UV and visible light, chloroform extract is light beige, methanol extract is yellow to orange and water extract is Deep buff (Table 4).

Powder analysis of *C. travancorica* leaf, stem and root

Powder analysis of *C. travancorica* leaf show green shades mostly under visible and UV light. Under visible light, 50% sulphuric acid, 1N sodium hydroxide in methanol and water, 5% potassium hydroxide are along with powder shows green shades. Under UV light powdered sample shows green shades when powder alone or with reagents 50% hydrochloric acid and 1N sodium hydroxide (water and methanol), and 5% potassium hydroxide shows green shades (Table 5).

Table 5: The optical activity of powdered leaf, stem and root under visible and UV light.

Treatment	Behaviour on Visible Light*			Behaviour on UV light*		
	Leaf	Stem	Root	Leaf	Stem	Root
Powder Alone	Misty Red / Tawny	Deep buff	Deep Buff	Bright Green/Goblin/ Garland	Sky	Mineral Green/ Opaline/ Moorland
Powder + 50%H_2SO_4	Grass Green	Camouflage beige	Dark Cherry/Russel/ Monarch	Misty Red/Tawny	NATO Green	Sable/Sierra
Powder +50% HNO_3	French Grey	Mellow apricot/apricot	Misty Red/Tawny	Light Orange	Mellow Apricot/Apricot	Golden Maize/Banana/ Capricorn
Powder + 50% HCl	Light Yellow	Light beige	Pale Roundel Red	Mineral Green/Opaline/ Moorland	Deep Bronze Green	Light Violet
Power +1 N HCl	Light Stone	Vannila pollen	Light Buff	French Grey	Deep Buff	Grass Green
Power +1 N NaOH	Midnight	Red oxide	Light orange	NATO Green	Deep Bronze Green	Middle Bronze

(Water)	Green/ Juniper					Green
Power +1N NaOH (Methanol)	Bright Green/Goblin/ Garland	Cannary Yellow	Mellow Apricot/Apricot	Emerald Green/Viridian	Bold Green	Light olive green
Powder + 5% KOH	Grass Green	Light Orange	Red Oxide	Salmon Red/Lobster/ Azalea	Red Oxide	Misty Red/Tawny

*colour as per British Standared Colours. (www. British standarad colour.com).

Powder analysis of stem shows yellow and beige colours predominantly in visible light whereas under UV light, it is green with most of the reagents. Powder along with 50% sulphuric acid, 50% hydrochloric acid, and 1N sodium hydroxide in methanol produce green colour in UV light. 5% potassium hydroxide (UV) and 1N sodium hydroxide in water (visible light) produce red oxide colour (Table 5).

Root of *C. travancorica* shows prominent red to orange colour under visible light and mostly green colour under UV light (Table 5). Root powder alone shows deep buff colour under visible light and mineral green colour under UV light.

Phytochemical Analysis

Phytochemical analysis in *C. travancorica* leaf powder

The qualitative screening by using prepared leaf samples revealed the presence of a wide range of phytochemicals (Table 6). Leaf extracts in water shows the presence of phytochemicals such as alkaloids, saponins, quinines, proteins and flavanones. Methanolic extracts have the presence of Tannins, proteins and carbohydrates. Both the water and methanolic extracts shows the presence of proteins under the xanthoprotein test for protein.

Phytochemical analysis in *C. travancorica* stem

Water extracts of *C. travancorica* stem showed the presence of phytochemicals such as saponins, coumarins, quinines, proteins (Biurete test), phenols, reducing sugar and resins. Methanolic extracts shows the presence of coumarins, phenols and reducing sugar (Table 6). Most of the phytochemicals are present in water extracts. Stem powder containing more phytochemicals as compared with leaf and root powders. Reducing sugar, coumarins and phenols are present in both water and methanolic extracts. Saponins, quinines, proteins and resins are only present in water extracts.

Water extracts of stem shows the presence of phytochemicals such as alkaloids, saponins, coumarins, quinines, proteins (Biurete test), phenol, reducing sugar, resin, oils, flavones, carbohydrate and cardiac glycosides. Methanolic extracts shows the presence of alkaloids, coumarins, phenol, reducing sugar and carbohydrate. Most of the phytochemicals are found in water extracts.

Phytochemical analysis in *C. travancorica* root

Resin is commonly present in the root extracts in both water and methanolic extracts. Water extracts have the presence of saponin and coumarins along with resin. In methanolic extract, alkaloids, steroids are present along with resin (Table 7).

Water extracts of root powder shows the presence of phytochemicals such as Saponins, coumarins, resins, oil, flavanones and carbohydrates. In methanolic extract alkaloids,tannins,steroids and resins are present. In the case of alkaloids, Mayers and Wagners reagent shows the positive results.

Table 6: Phytochemical analysis of *C. travancorica* leaf, stem and root powders.

Compound	Name of Test	Leaf Extract		Stem Extract		Root	
		Water	Methanol	Water	Methanol	Water	Methanol
Alkaloids	Mayers Test	_	_	_	_	_	+
	Wagners reagent	+	_	+	_	_	+
Anthraquinones	Borntragers Test	_	_	_	_	_	_
Saponin	Foam Test	+	_	+	_	+	_
Coumarins	Colour Test	_	_	+	+	+	_
Tannins	Ferric Chloride Test	_	+	_	_	_	_
Steroids	Salkowski Test	_	_	_	_	_	+
Quinines	Sodium hydroxide test	+	-	+	-	-	-
Protein	Xanthoprotein Test	+	+	_	_	_	_
	Biurete Test	-	-	+	-	-	-
Phenol	Ferric Chloride Test	_	_	+	+	_	_
Reducing Sugar	Benedicts Test	-	-	+	+	-	-
	Fehling's test	-	-	-	-	-	-
Resin	Turbidity Test	-	-	+	-	+	+
Glycoside	Benedicts reagent test	_	_	_	_	_	_
Fixed oils	oil stain test	-	-	+	-	+	-
Phlobatannins	Precipitate test	_	_	_	_	_	_
Anthocyanins	Sodium hydroxide test	-	-	-	-	-	-
Flavones	Sodium hydroxide Test	-	-	+	-	-	+
Flavanones	Sodium hydroxide test	+	-	-	-	+	-
Phytosterol	Salkowski's Test	-	-	-	-	-	-
Cardiac glycosides	Keller Kelliani's test	_	_	+	_	_	_
Carbohydrate	Molisch's test	-	+	+	+	+	-
Amino acids &proteins	Ninhydrin test	-	-	-	-	-	+

(-' indicates the absence and ' +' indicates the presence of phytochemicals).

The macroscopic and microscopic description of a medicinal plant is the first step towards identification and determination of purity. However, such an attempt has not been made on *C. travancorica*.

Qualitative screening confirmed the presence of the secondary metabolites in the plant *C. travancorica*. Alkaloids are known to contain a lot of pharmacological properties. They are mostly used as antidepressant, stimulants, anaesthetic, antitumor and antibacterial agents (Bruneton 1995[15]; Gurib-Fakim 2006[16]). Leaf, stem (water) and root (methanolic) extracts of *C. travancorica* also contain alkaloids so it may have these properties.

Saponins are known to be immune booster. Extracts of plants rich in saponins are said to be demonstrate antiflammatory, hemolytic, allelopathic, cholesterol lowering and anticancer properties (Sauvaire *et al*. 1996[17]; Mandeaau *et al*. 2005[18]). Saponins are abundantly present in water extracts of *C. travancorica* leaf, stem and roots.

Tannins are good antimicrobial agent which precipitate protein there by providing waterproof layer on the skin when used externally or protect the underlying layers of the skin and loss of fluid. They are also known to be good antiviral agents (Cowan 1999[19]). Methanol extract of *C. travancorica* leaf have the presence of tannins.

Alkaloids generally play some metabolic role and control development in living system. They also involved in protective function in animals and are used as medicine and especially the steroidal alkaloids. Tannins also inhibit pathogenic fungi. The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-carcinogenic etc. (Lalitha and Jayanthi 2012[20]). The *C. travancorica* may also have these properties because of the presence of these phytochemicals.

These results may help in standardization, identification and in carrying out further research in *C. travancorica* based drugs. Diverse group of Pytochemicals present in stem, leaves and roots of *C.travancorica* indicates their potential as a source of principles that may supply novel medicines. Saponins were present in the water extract of leaves, stem and roots of *C. travancorica*. The plant may play a vital role in the development of novel drugs and well defined pharmacognostic parameters and standards must be established before the inclusion of any crude drug in a herbal pharmacopoeia. Most of the phytochemicals obtained through water extracts. Saponin is the major component of the genus *Colubrina* and this saponin is abundantly present in this plant. Observations suggest that the presence of various important phytochemicals in *C. tavancorica*, so that this plant can be used in future medicines.

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