



RESEARCH ARTICLE

PHARMACOLOGY

PHARMACOGNOSTIC STANDARDISATION AND PHYSICO-CHEMICAL EVALUATIONS OF LEAVES OF *EUGENIA SINGAMPATTIANA* BEDDOME ENDANGERED SPECIES*Corresponding Author***P. PAVENDAN****Department of Botany, Bishop Heber College, Tiruchirappalli, Tamil Nadu, South India.***Co Authors***C. SEBASTIAN RAJASEKARAN AND V. ANAND GIDEON****Department of Botany, Bishop Heber College, Tiruchirappalli, Tamil Nadu, South India.****ABSTRACT**

The various pharmacognostical parameters of leaf of *Eugenia singampattiana* Bedd was carried out as per World Health Organization guidelines procedure. Physical constant values involving ash, extractives, Fluorescence, quantitative estimation of various phytochemicals (alkaloid, Anthraquinone, Coumarin, Catechin, Glycoside, Flavonoid, Phenol, Quinone, saponin, steroid, tannin, Terpenoid, Sugar, Xanthoprotein, and Fixed Oil some other chemical constituents are recorded) and morphological features have been recorded. This plant is used for treatment of rheumatism. In order to ensure the use of only genuine and uniform material in preparation of herbal formulation, work on standardization was carried out. The result of the pharmacognostical standardization of this plant serves as a reference piece and helps in future identification and authentication of this plant specimen.

KEY WORDS

Eugenia singampattiana, fluorescence analysis, pharmacognostic, phytochemical, macroscopical standardization. ethnomedicine,

INTRODUCTION

Eugenia singampattiana Beddome (Myrtaceae), locally known as 'Korandi' by Kanni tribes in Tirunelveli district, Tamil Nadu is one of the endemic and threatened tree species of the southern Western Ghats in Peninsular India with medicinal value. The species is known from the two collections made by Beddome from the abovementioned areas between 1864 and 1874¹. It had not been collected or reported again during the last 112 years till a collection was made by Daniel in 1986 and 1987 from Papanasam hills near Hope lake². The species is categorized as Endangered or Possibly Extinct by the Botanical Survey of India. Plants have been used as sources of medicine in virtually all cultures and according to World Health Organization (WHO), herbal medicines serve the primary health needs of about 80% of the world's population, especially for people in the rural areas³. The people of nation, especially the South Indian tribes are blessed with rich biological diversity of plants and a high degree of traditional knowledge about medicinal plants and their uses for various ailments and human health care system. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies⁴. These studies help in identification and authentication of plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in

standardization of plant material include its morphological features, physico-chemical constant, fluorescence analysis and preliminary photochemical analysis⁵. These standards are of utmost importance not only in finding out genuity, but also in detection of adulterants in marketed drug. The leaf paste of *Eugenia singampattiana* is given to treat rheumatic pain by *Kanikkar* tribe. However, perusal of literature reveals that pharmacological information on *Eugenia singampattiana* is totally lacking, hence in the present investigation was undertaken. The objective of the present study is to evaluate morphological features, pharmaco-chemical characteristics like ash values, extractive values, fluorescence analysis and preliminary phytochemical analysis of *Eugenia singampattiana* leaf.

MATERIALS AND METHODS

The species *Eugenia singampattiana* Bedd has been collected in the Western Ghats area, Tirunelveli district, Tamil Nadu. With the help of local flora the plant material were identified. Different parts on the plants, viz. leaf, stem, flower, fruit and bark sample were collected and morphological characteristic were studied. The air dried leaf samples were powdered and stored in screw cap bottles at room temperature for further analysis.

Physicochemical constant and fluorescence analysis

These studies were carried out as per the standard procedures⁶. In the present study, the leaf powder was treated with 1N aqueous



sodium hydroxide, and 1N hydrochloric acid, 50% sulphuric acid, nitric acid, ferric chloride, acetic acid and nitric acid with ammonia. These extracts were subjected to fluorescence analysis in visible/day light and UV light (254nm&365nm). Various ash types and extractive values were determined by following standard method ⁷.

Preliminary phytochemical analysis

Shaded dried and powdered leaf samples were successively extracted with benzene, chloroform, ethanol and water. The extracts were filtered and concentrated using vacuum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure ⁸.

RESULT AND DISCUSSION

Morphological characters

Eugenia singampattiana is a dense, evergreen tree, commonly 6-9 m in height. Bark grey, smooth; branchlets ferete. Leaves opposite, simple 6 - 12 x 3 - 8 cm, dark green above, light beneath. Ovate or elliptic-oblong, acute at apex, rounded at base, nerves and intramarginal nerves prominent; petiole short. Inflorescence terminal, racemes. Flowers white, crowded, actinomorphic, bisexual. Sepals four, oval-orbicular; petals four, nerved ovate. Bracts and bracteoles pubescent; calyx tube 3 mm long.

Fruit is almost spherical in shape (1.5 - 1.75 cm diameter and 5.5 - 6.5 cm circumference). Persistent calyx. Ripped fruit is yellowish-orange to crimson-red with fleshy pericarp. Seeds 2 - 3, planoconvex, 1.5-1.5 x 1.3 cm stony black. The plant starts flowering from middle of February, it continues up to end of July and fruiting starts from July and fruits ripen in September - October.

Physico-chemical constants

The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs by African Pharmacopoeia ⁹. Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica ¹⁰. The total ash content of *Eugenia singampattiana* leaf is 11.58% and sulphated ash is 6.18%. The acid insoluble ash is less than that of water insoluble ash at 2.84% and 6.42% respectively. The water extractive value of *Eugenia singampattiana* is more than in the solvents investigated in the table 1.

Table 1
Ash and extractive values of the powdered leaf of *Eugenia singampattiana*

Ash values		
Sl. No	Type of Ash	% of Ash in leaf
1	Total ash	11.58 ± 0.16
2	Water soluble ash	6.42 ± 0.25
3	Acid insoluble ash	2.84 ± 0.12
4	Sulphated ash	6.18 ± 0.10
Extractive value		
Sl. No	Name of extract	Extractive value (in %)of leaf
1	Benzene soluble extractive	4.28 ± 0.21
2	Petroleum ether soluble extractive	6.29 ± 0.16
3	Chloroform soluble extractive	4.96 ± 0.09
4	Acetone soluble extractive	5.80 ± 0.18
5	Methanol soluble extractive	7.79 ± 0.15
6	Ethanol soluble extractive	9.40 ± 0.10
7	Water soluble extractive	12.47 ± 0.31

* Values are Mean + S.E.

The fluorescence method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples¹¹. The results of various types of ash and extractive values may provide a basis to identify the quality and purity of the drug. The fluorescence analysis (fluoresced green under day light, dark green in UV short wave length (254nm) and brown in UV long wave length (365nm) of the powdered crude drug of *Eugenia singampattiana* leaf is presented in the table 2. The leaf powder of *Eugenia singampattiana* shows the characteristic fluorescence green colour when treated with 1N alcoholic NaOH, 50% H₂SO₄ and 50% HNO₃ under short UV light and HNO₃ under long UV light. Many phytochemicals fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent.

Table 2
Fluorescence analysis of the powdered leaf of *Eugenia singampattiana*

Sl. No	Treatment	Visible/Day light	Short UV light (254 nm)	Long UV light (365 nm)
1	Powder as such	Green	Dark green	Brown
2	Powder + 1N NaOH (aqueous)	Green	Brownish green	Dark green
3	Powder + 1N NaOH (alcoholic)	Green	Fluorescent green	Yellow
4	Powder +1N Hcl	Brown	Light green	Pale green
5	Powder + 50% H ₂ SO ₄	Yellowish green	Fluorescent green	Greenish yellow
6	Powder +50% HNO ₃	Green	Fluorescent green	Brown
7	Powder +40% NaOH + 10% lead acetate	Brown	Green	Greenish yellow
8	Powder + nitric acid	Light green	Pale green	Fluorescent green
9	Powder + acetic acid	Yellowish green	Yellowish green	Yellowish green
10	Powder + ferric chloride	Yellowish green	Fluorescent Yellow	Yellowish green
11	Powder + HNO ₃ + NH ₃	Pale yellow	Green	Green

Presence or absence of certain important compounds in an extract is determined by colour reaction of the compounds with specific chemicals which acts as dyes. This procedure is a simple preliminary pre-requisite before going

for detailed phytochemical investigation. The results of preliminary phytochemical screening of leaf of *Eugenia singampattiana* are presented in Table 3.

Table: 3
Phytochemical screening of powdered leaf of Eugenia singampattiana

Sl. No	Tests	Benzene	Chloroform	Ethanol	Water
1	Alkaloid	+	-	+	+
2	Anthraquinone	-	+	-	-
3	Coumarin	-	-	+	+
4	Catechin	+	-	+	+
5	Glycoside	-	-	+	-
6	Flavonoid	-	-	+	+
7	Phenol	+	+	+	+
8	Quinone	+	-	-	-
9	Saponin	-	+	+	-
10	Steroid	-	-	+	-
11	Tannin	+	-	+	+
12	Terpenoid	+	+	+	-
13	Sugar	+	+	+	+
14	Xanthoprotein	+	+	+	+
15	Fixed Oil	-	-	+	+

Preliminary phytochemical analysis

In the present investigation, ethanol extract of *Eugenia singampattiana* leaf was accounted for the presence of alkaloid, coumarin, catechin, glycoside, flavonoid, phenol, steroid, saponin, tannin, terpenoid, sugar, xanthoprotein, and fixed oil in the table 4. All the four extracts have phenol, sugar, terpenoid, and Xanthoprotein compounds. This could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because of pharmacological activity of any plant is usually traced to a particular compound. Flavonoids are known to regenerate the damaged beta cells in the alloxan diabetic rats¹². Phenolics are found to be effective anti hyperglycemic

agents. Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles¹³. Earlier studies have shown that drugs containing tannins and sterols possess antidiabetic activity¹⁴. Our study suggests that *Eugenia singampattiana* may be a potential resource of phenol, sugar, terpenoid, and Xanthoprotein compound and useful for the identification and preparation of a monograph of the plant. Thus this type of preliminary pharmaco-chemical analysis is the first step towards understanding the nature of active principles in medicinal plants and this type of study will be helpful for further detailed study.

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