RESEARCH ARTICLE

Pharmaco-Chemical Characterisation of *Eugenia singampattiana* Bedd.

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ABSTRACT

The present study deals with the pharmaco-chemical characterisation of Eugenia singampattiana Bedd, the said plant species are used by the Kanikkar tribe of Agasthiarmalai Biosphere Reserve, Tamil Nadu for   treating rheumatic pain. Physico-chemical constants (ash and extractive values), fluorescence analysis, preliminary phytochemical analysis were carried out. The preliminary study will be helpful to study the active principles using modern techniques in the later part of this work.

KEYWORDS: Ethnomedicine, fluorescence analysis.

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. 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 studies [1]. 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 physico-chemical constant, fluorescence analysis and preliminary photochemical analysis [2]. These standards are of utmost importance not only in finding out genuity, but also in detection of adulterants in marketed drug. *Eugenia singampattiana* Bedd is one of the medicinally important plant belongs to Myrtaceae family. The leaf paste of *Eugenia singampattiana* is given to treat rheumatic pain by *Kanikkar* tribe. However, perusal of literature reveals that pharmaco-chemical information on *Eugenia singampattiana* is totally lacking, hence in the present investigation was undertaken. The objective of the present study is to evaluate pharmaco-chemical characteristics like ash values, extractive values, fluorescence analysis and preliminary phytochemical analysis of *Eugenia singampattiana* leaf.

MATERIALS AND METHODS

The leaf of *Eugenia singampattiana* Bedd were collected from well grown healthy plants inhabiting the natural forests of Agasthiarmalai Biosphere Reserve, South-eastern slope of Western Ghats, Tamil Nadu. With the help of local flora the plant material were identified. The air dried leaf samples were powdered in a Willy Mill 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 procedures [3]. 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 [4, 5].

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 [3, 6].

RESULT AND DISCUSSION

The results of the ash and extractive values of leaf of *Eugenia singampattiana* are depicted in Table 1. The total ash value of *Eugenia singampattiana* leaf is 11.63%. This ash value is indicative of the impurities present in the drug. Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug. In the present study, *Eugenia singampattiana* leaf has more water soluble ash than acid insoluble ash. The extractive value of water is more than in the solvents investigated.

Table 1: Ash and extractive values of the powdered leaf of *Eugenia singampattiana*[a]

Ash Values	
Type of Ash	% of Ash
Total ash	11.63 ± 0.17
Water soluble ash	6.56 ± 0.21
Acid insoluble ash	2.84 ± 0.07
Sulphated ash	6.14 ± 0.05
Extractive Values	
Nature of extract	% of extractive value
Benzene soluble extractive	4.26 ± 0.11
Petroleum ether soluble extractive	6.31 ± 0.12
Chloroform soluble extractive	5.04 ± 0.08
Acetone soluble extractive	5.76 ± 0.14
Methanol soluble extractive	7.96 ± 0.13
Ethanol soluble extractive	9.32 ± 0.07
Water soluble extractive	12.36 ± 0.34

[a]All values are means of triplicate determinations expressed on dry weight basis. ± denotes the standard error.

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

Experiments	Visible/Day light	UV light	
		254nm (short wave length)	365nm (long wave length)
Powder as such	Green	Dark green	Brown
Powder + 1N NaOH (aqueous)	Green	Brownish green	Dark green
Powder + 1N NaOH (alcoholic)	Green	Fluorescent green	Yellow
Powder +1N Hcl	Brown	Light green	Pale green
Powder + 50% H_2SO_4	Yellowish green	Fluorescent green	Greenish yellow
Powder +50% HNO_3	Green	Fluorescent green	Brown
Powder +40% NaOH + 10% lead acetate	Brown	Green	Greenish yellow
Powder + nitric acid	Light green	Pale green	Fluorescent green
Powder + acetic acid	Yellowish brown	Yellowish green	Yellowish green
Powder + ferric chloride	Yellowish green	Fluorescent yellow	Yellowish green
Powder + HNO_3 + NH_3	Pale yellow	Green	Green

The results of various types of ash and extractive values may provide a basis to identity the quality and purity of the drug. The results of the fluorescent analysis of leaf of *Eugenia singampattiana* are shown in Table 2. The powder from the leaf of *Eugenia singampattiana* fluoresced green under day light, dark green in UV short wave length (254nm) and brown in UV long wave length (365nm). The leaf powder of *Eugenia singampattiana* shows the characteristic fluorescence green colour when treated with 1N alcoholic NaOH, 50%H_2SO_4 and 50%HNO_3 under short UV light and HNO_3 under long UV light. Many phytocompounds 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. The fluorescent 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 samples [7].

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*

Tests	Benzene	Chloroform	Ethanol	Water
Alkaloid	+	-	+	+
Anthraquinone	-	+	-	-
Coumarin	-	-	+	+
Catechin	+	-	+	+
Glycoside	-	-	+	-
Flavonoid	-	-	+	+
Phenol	+	+	+	+
Quinone	+	-	-	-
Saponin	-	+	+	-
Steroid	-	-	+	-
Tannin	+	-	+	+
Terpenoid	+	+	+	-
Sugar	+	+	+	+
Xanthoprotein	+	+	+	+
Fixed Oil	-	-	+	+

The phytocompounds such as alkaloid, coumarin, catechin, glycoside, flavonoid, phenol, steroid, saponin, tannin, terpenoid, sugar, xanthoprotein, and fixed oil have been reported from the ethanolic extract of the *Eugenia singampattiana* leaf. 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. Several authors reported that flavonoids, sterols/terpenoids, phenolic acids are known to be bioactive antidiabetic principles [8, 9]. Flavonoids are known to regenerate the damaged beta cells in the alloxan diabetic rats [10]. Phenolics are found to be effective anti hyperglycemic agents [11]. Earlier studies have shown that drugs containing tannins and sterols posses' antidiabetic activity [12].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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