J Acupunct Meridian Stud 2016;9(4):207–212

Available online at www.sciencedirect.com

Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com

RESEARCH ARTICLE

Phytochemical Profile of *Erythrina variegata* by Using High-Performance Liquid Chromatography and Gas Chromatography-Mass Spectroscopy Analyses

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Available online 11 June 2016

Received: Dec 1, 2015
Revised: May 26, 2016
Accepted: Jun 1, 2016

KEYWORDS
Erythrina variegata;
flavonoids;
GC-MS;
HPLC;
phytochemicals

Abstract
Natural products derived from plant sources have been utilized to treat patients with numerous diseases. The phytochemical constituents present in ethanolic leaf extract of *Erythrina variegata* (ELEV) were identified by using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS) analyses. Shade dried leaves were powdered and extracted with ethanol for analyses through HPLC to identify selected flavonoids and through GC-MS to identify other molecules. The HPLC analysis of ELEV showed the presence of gallic and caffeic acids as the major components at concentrations of 2.0 ppm and 0.1 ppm, respectively, as well as other components. GC-MS analysis revealed the presence of 3-eicosyne; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; butanoic acid, 3-methyl-3,7-dimethyl-6-octenyl ester; phytol; 1,2-benzenedicarboxylic acid, diundecyl ester; 1-octanol, 2-butyl-; squalene; and 2H-pyran,

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pISSN 2005-2901 eISSN 2093-8152
http://dx.doi.org/10.1016/j.jams.2016.06.001

2-(7-heptadecynyloxy) tetrahydro-derivative. Because pharmacopuncture is a new evolving natural mode that uses herbal extracts for treating patients with various ailments with minimum pain and maximum effect, the results of this study are particularly important and show that ELEV possesses a wide range of phytochemical constituents, as indicated above, as effective active principle molecules that can be used individually or in combination to treat patients with various diseases.

1. Introduction

Biologically active compounds derived from plants have become an important source of drugs due to the increasing recognition of herbal medicine as an alternative form of health care [1]. Many plants contain a variety of phytopharmaceuticals, which have been found to have very important applications in the fields of agriculture, and human and veterinary medicine for the prevention of diseases [2—4]. Medicinal plants provide a large number of molecules that can be screened to find potential compounds that might lead to the discovery of new drugs [5,6]. Secondary plant metabolites that are phenolic in nature exhibit antiallergenic, antimicrobial, antiatherogenic, antithrombotic, antiinflammatory, vasodilatory, and cardioprotective effects [7,8], and medicinal properties and pharmacological actions have been observed in different parts of the medicinal plants used in folk medicine [9,10]. For example, the leaves of *Erythrina variegata* (Indian coral tree; family: *Fabaceae*) eaten as a pot herb are used as an antiobesity drug in Siddha medicine [11].

Pharmacopuncture is a treatment that combines herbal medicine and acupuncture and is characterized by injections at acupoints, and most pharmacopuncture contains certain amounts of herbal extracts that are injected intramuscularly [12]. Even though lifestyle change through diet and moderate intensity exercise is an essential strategy for improving all features of the metabolic syndrome, acupuncture therapy has been shown to reduce the body mass index and abdominal fat significantly by reducing the abdominal visceral adipose tissue content, which leads to decreases in several atherogenic and metabolic complications [13,14]. In addition, nonpharmacological interventions, including acupressure and acupuncture, have been advocated as major nonmedical interventions for the relief of pain [15,16], and acupuncture has been reported to be successful in treating patients with primary dysmenorrhea [17,18].

The juice of the crushed leaf of *E. variegata* has been shown to be helpful in relieving pain and inflammation in rheumatic joints. Moreover, the juice of a fresh whole plant has been used to cure chronic dysmenorrhea and sterility in heavy women by gradually reducing abdominal fat and inducing natural menstrual flow [19]. In traditional medicine, different parts of *E. variegata* have been used to produce nervine sedation, febrifuge, antiasthmatic, and antiepileptic effects [20], and its leaves have been used for the treatment of patients with various conditions, such as liver trouble, convulsions, arthritis, etc. [21,22]. It has also been shown to have potential for treating patients with conditions such as fever, inflammation, bacterial infection, insomnia, helminthiasis, coughing, cuts, and wounds [23—26]. In the present study, we used high-performance liquid chromatography (HPLC) and gas chromatography-mass spectroscopy (GC-MS) to identify the phytocomponents present in the ethanolic leaf extract of *E. variegata* in order to determine the medicinal properties of the plant.

2. Materials and methods

Leaves of *E. variegata* were collected from Kolli hills in the Namakkal district, Tamil Nadu, India, and the plant material was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, India (Identification number: PARC/2012/1297). The collected *E. variegata* leaves were washed well with distilled water and allowed to shadow-dry at room temperature. The dried leaves were ground into fine powder in an electric blender. A Soxhlet apparatus was used to extract 25 g of powdered leaves with 250 mL of ethanol (1:10 w/v). After extraction had been completed, ethanol was removed by evaporation in a water bath, which gave a solid mass.

A HPLC system (LC-10AD VP, Shimadzu, Kyoto, Japan) equipped with a binary gradient pump with an online degasser and capped with a C18 reverse-phased chromatographic column was used for the analyses of flavonoids. The mobile phases used were distilled water with 0.1% trifluoroacetic acid (A) and methanol with 0.1% trifluoroacetic acid (B). Gradient elutions were as follows for solvent (B): 0 minutes — 33%; 35 minutes — 50%; 80 minutes — 90%; 85 minutes — 95%; 90 minutes — 95%; 91 minutes — 33%; and 111 minutes — 33% in a flow-rate of 1 mL/min. A sample volume of 4 μL per injection was obtained by using an autoinjector, and detection was done by using a UV detector at 280 nm. The standards of gallic acid, caffeic acid, rutin, quercetin, and ferulic acid were of the highest purity and grade. Analyses were done in a facility at the Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu, India.

The phytochemical constituents were determined by using a GC-MS system (Clarus 500, Perkin Elmer, CT, USA) containing Elite-5MS (5% diphenyl/95% dimethyl polysiloxane), 30 × 0.25 mm × 0.25 μm df. Helium gas was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was maintained at 110°C for 2 minutes and then increased to 280°C in 9 minutes. The injector temperature was 250°C, and the total analysis time was 36 minutes. Two-μL aliquots of ethanolic extract were injected into the chromatographic column after a clear baseline had been obtained. Major constituents were identified with the aid of a computer-driven algorithm and were identified by

using the mass spectrum library (NIST version 2.0, 2005). The Turbomass 5.2 software program was used in the analyzer. Analyses were carried out in a facility at the Indian Institute of Crop Processing Technology.

3. Results

The ELEV extracted through the Soxhlet apparatus was dried using a hot water bath, and an extract with a solid mass was recovered. The yield was found to be 2.1 mg/g of dry leaves. An analysis of the flavonoids in the ELEV extract was performed by using HPLC, and the chromatogram is presented in Fig. 1. Quantitative data for gallic acid and caffeic acid were determined, and the concentrations were 2.0 ppm and 0.1 ppm with retention times of 5.858 minutes and 9.525 minutes, respectively. No clear peaks corresponding to rutin, quercetin, and ferulic acid were observed, so the concentrations of those components in the ELEV extract were below the lower quantification limit for the current HPLC protocol. All the HPLC data, i.e., the analyte names, retention times, areas of the peaks, heights of the peaks, and concentrations, are presented in Table 1.

The GC-MS analysis was done as per the standard protocol and manufacturer instructions under the conditions listed in the Materials and methods section. Results are presented in Fig. 2 (chromatogram) and in Table 2, which reveal the presence of eight major compounds in the ELEV extract. Table 2 presents the retention time, the compound's name, molecular formula, and molecular weight, and the peak area percentage for each major compound found in the ELEV extract.

Table 1 High-performance liquid chromatography (HPLC) analysis data for ethanolic leaf extract of *Erythrina variegata*.

Name	Retention time (min)	Area (%)	Height (mV)	Concentration (ppm)
Gallic acid	5.858	13,237	576	2.0
Caffeic acid	9.525	681	107	0.1
Rutin	10.317	60	0	Below detection limit
Quercetin	12.325	90	75	Below detection limit
Ferulic acid	24.067	130	32	Below detection limit

4. Discussion

Traditional medicinal plants, spices, vegetables, and fruits have been found to protect human beings from acute and chronic diseases such as cancer and cardiovascular disease [27]. Different bioactive compounds and microelements from different dietary functional foods can be used as preventive and therapeutic medicines [28,29]. Polyphenolic flavonoids occurring as glycosides were found to have strong antioxidant properties, to be effective in scavenging free radicals and reactive oxygen species with their phenolics hydroxyl groups [30], and to decrease the risk of diseases such as cardiovascular and degenerative diseases by counteracting macromolecular oxidation [31,32].

Gallic acid (3,4,5-trihydroxybenzoic acid) is an endogenous plant phenolic compound that is found in many phytomedicines [33]. Many gallic-acid-rich plants exhibit protective effects against liver injury [34—36], and they have been found to have various pharmacological properties, including neuroprotective, antioxidant [37], antiinflammatory [38,39], antiobesity [40—42], and anticancer [43] properties. Several studies have demonstrated that gallic acid and its derivatives can selectively induce cancer cell death by apoptosis without harming healthy cells [44—46].

A major class of phenolic compounds is hydroxycinnamic acids, which are found in almost every plant, and the major representative of hydroxycinnamic acids is caffeic acid [47,48]. Caffeic acid possesses both antioxidant and prooxidant properties [45]. The prooxidant action of

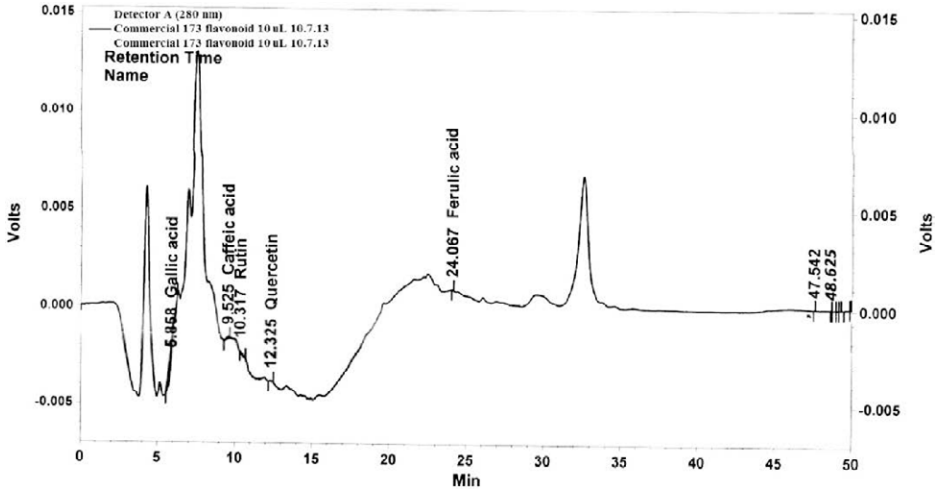

Figure 1 High-performance liquid chromatography (HPLC) chromatogram of ethanolic leaf extract of *Erythrina variegata*.

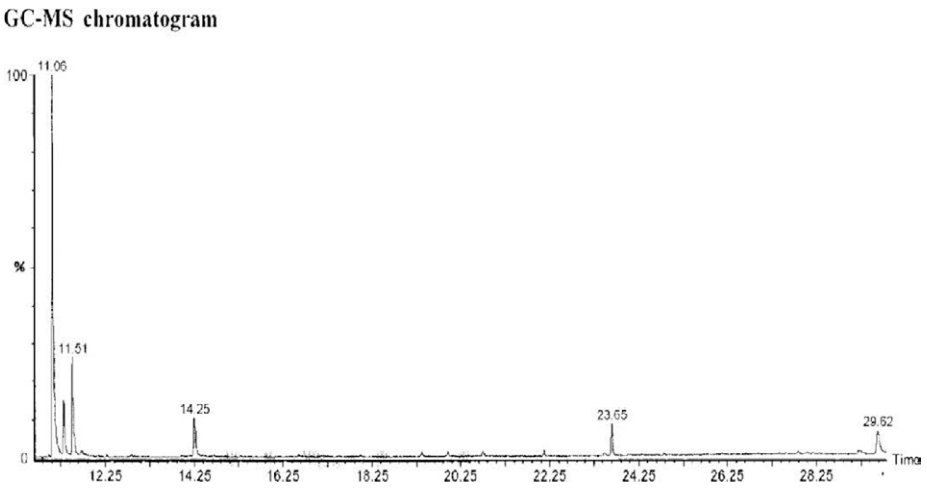

Figure 2 Gas chromatography-mass spectroscopy (GC-MS) chromatogram of ethanolic leaf extract of *Erythrina variegata*.

Table 2 Gas chromatography-mass spectroscopy (GC-MS) analysis of phytocompounds present in the ethanolic leaf extract of *Erythrina variegata*.

No.	RT	Name of the compound	Molecular formula	MW (g/mole)	Peak Area (%)
1	11.06	3-eicosyne	$C_{20}H_{38}$	278	54.93
2	11.31	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	7.96
3	11.51	butanoic acid, 3-methyl-, 3,7-dimethyl -6-octenyl ester	$C_{15}H_{28}O_2$	240	14.95
4	14.25	Phytol	$C_{20}H_{40}O$	296	8.02
5	19.95	1,2-benzenedicarboxylic acid, diundecyl ester	$C_{30}H_{50}O_4$	474	1.20
6	22.12	1-octanol, 2-butyl-	$C_{12}H_{26}O$	186	0.91
7	23.65	Squalene	$C_{30}H_{50}$	410	5.15
8	29.62	2H-pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	6.89

MW = Molecular Weight; RT = Retention Time.

polyphenols may be an important mechanism in their anticancer and apoptosis-inducing properties [49,50].

Plant monographs and pharmacopoeia describe only the physicochemical characteristic features of the materials. However, the use of modern methods for the identification and the quantification of bioactive principles with appropriate standards might be useful for the standardization of medicinal plants and their formulations, as has been emphasized by the World Health Organization [51,52]. GC and LC with different detection techniques are relatively expensive and can be used to identify the phytoconstituents that are largely present in herbal medicines and formulations [53]. In recent years, GC with MS detection (GC-MS) has become a key technology for the profiling of metabolites in almost all species [54—56], so phytocomponents are being identified based on mass spectrum fragmentation patterns with an updated database, and quantitative estimates are being obtained by using the relative peak-area percentage method [57,58].

The key identified components found in the ELEV extracts were 3-eicosyne; butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester; phytol; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; 2H-pyran, 2-(7-heptadecynyloxy) tetrahydro derivative; squalene; 1,2-benzenedicarboxylic acid, diundecyl ester; and 1-octanol, 2-butyl-. The component 3-eicosyne belongs to the class of organic compounds known as terminal alkynes. It is found in green vegetables and acts as a nutrient [59]. Butanoic acid, 3-methyl-, 3,7-dimethyl-6-octenyl ester, has acidulant and arachidonic acid inhibitor activities. It also inhibits the production of uric acid and increases the activity of the aromatic amino acid decarboxylase [60]. Phytol, a diterpene, is widely used as antimicrobial, antioxidant, antitumor, anticancer, antiarthritic, immunestimulatory, antidiabetic, chemopreventive, pesticidal, and diuretic agents and has sunscreen properties [61—63]. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol is a terpene alcohol, and it has antimicrobial and antiinflammatory activities [64]. 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro derivative, is a herbicide safener and an inducer of hemolytic, hemostatic, hepatoprotective, hemopoietic, and hemoglobin activities [60]. Squalene (triterpene) is a phenolic compound and a natural antioxidant found in a majority of plants [65]. It possesses antimicrobial and chemopreventive activities against colon carcinogenesis [66] and has also been reported to have anticancer, gastropreventive and hepatoprotective effects, as well as pesticide, antitumor and sunscreen properties [67—69].

The extract of ELEV was found to possess valuable, safe natural therapeutics that should find use in traditional folk

medicinal practice. The characterization protocol deduced from the ELEV extract indicates it to be an ample potential source of natural remedial chemical compounds, such as gallic acid, caffeic acid, alkynes, diterpenes, triterpenes, alcohols, hydrocarbons, and carboxylic acids. These compounds will certainly find applications in combating various diseases when combined with or transformed into a form suitable for pharmacopuncture.

Disclosure statement

The author declares to have no conflicts of interest and no financial interests related to the material of this manuscript.

Acknowledgments

Suriyavathana Muthukrishnan and Subha Palanisamy (ICMR-SRF) are grateful to the Indian Council of Medical Research (IRIS ID - 2014-20140) for providing financial support to carry out this part of the work, and we thank the Department of Biochemistry, Periyar University, Salem-11, Tamil Nadu, India.

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