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RESEARCH ARTICLE

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

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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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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