Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(2): 40-43
Received: 20-04-2015
Accepted: 24-05-2015

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Quantitative analysis of selected primary metabolites in aqueous hot extract of *Eugenia uniflora* (L.) leaves

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Abstract
Purpose of present study was to evaluate the presence of selected primary metabolites in aqueous hot extract of *Eugenia uniflora* leaves. Primary metabolites like lipids and amino acids were estimated using standard procedures. Quantitative analysis is very essential for identifying the compounds present in the medicinal plants. The results obtained from the present study provides evidence that aqueous hot extract of *Eugenia uniflora* leaves contains various primary metabolites and this justifies the use of this plant species as traditional medicine for treatment of various diseases. The results are very much encouraging but scientific validation is necessary before being put into practice.

Keywords: Primary metabolites, Lipids, Amino acids, Aqueous extract, *Eugenia uniflora.*

1. Introduction
Plants have been an integral part of traditional medicine across the continents since time immemorial. Medicinal plants have their values in the substances present in various plant tissues with specific physiological action in human body. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. India is endowed with a rich wealth of medicinal plants. India recognizes more than 2500 plant species which have medicinal values [1]. Plants are like natural laboratories where a great number of chemicals are biosynthesized and in fact they may be considered the most important source of chemical compounds.
The identification of plants is useful to human beings from natural strands commenced in prehistoric studies. Experiments and trails are the two main ways through which humans have learnt various uses of the plants [2]. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period [3]. Over three-quarters of the world population relies mainly on plants and plant extracts for health care.
According to the Food and Agriculture Organization (FAO), more than 50,000 plant species are used in the traditional folk medicine throughout the world [4]. The drugs are derived from the whole plant or from different parts like leaves, stem, bark, root, flower, tuber and seed etc. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing country such as India, the contribution is as much as 80% [5].
Since ancient times, people have been exploring the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 80% of the world population for their basic health needs. India is the birth place of renewed system of indigenous medicines such as Siddha, Ayurveda and Unani. Traditional systems of medicines are prepared from a single plant or combinations of more than one plant. This efficacy depends upon the current knowledge about taxonomic features of plant species, plant parts and biological property of medicinal plants which in turn depends upon the occurrence of primary and secondary metabolites [7].
Plant synthesizes a wide range of chemical compounds which are classified based on their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites directly involved in growth and development while secondary metabolites are not involved directly and they have been worked as biocatalysts. Primary metabolites are widely distributed in nature, occurring in one form or another in

virtually all organisms. They are like chlorophyll, amino acids, nucleotides, carbohydrates etc., which have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are used as industrial raw materials and food additives.

Eugenia uniflora L. is a widely distributed tree in South American countries, mainly in Brazil, Argentina, Uruguay, and Paraguay [8]. Its leaves are used in popular medicine as infusion in the treatment of fever, rheumatism, stomach diseases, disorders of the digestive tract, hypertension, yellow fever, and gout. It may also reduce weight, blood pressure, and serve as a diuretic [9]. Pitanga fruits, also known as Brazilian cherry or Suriname cherry, contain various volatile compounds that are also found in the essential oil of pitanga leaves [10, 11]. Like the leaves, pitanga fruits may also have health benefits. In the Brazilian food industry, pitanga fruits have mostly been used to produce juice and frozen pulp. Pulp production has high economic potential because the product has consumer appeal and high concentrations of antioxidant compounds, such as anthocyanins, flavonols, and carotenoids [12].

Plants produce the majority of the world's lipids, and most animals, including humans, depend on these lipids as a major source of calories and essential fatty acids. Like other eukaryotes, plants require lipids for membrane biogenesis, as signal molecules, and as a form of stored carbon and energy. In addition, soft tissues and bark each have distinctive protective lipids that help prevent desiccation and infection. Plant lipids also have a substantial impact on the world economy and human nutrition. More than three-quarters of the edible and industrial oils marketed annually are derived from seed and fruit triacylglycerols. These figures are particularly impressive given that, on a whole organism basis, plants store more carbon as carbohydrate than as lipid. Since plants are not mobile, and since photosynthesis provides fixed carbon on a regular basis, plant requirements for storage lipid as an efficient, light weight energy reserve are less acute than those of animals.

The amino acids have several roles in plants, for example they act as osmolytes, detoxify heavy metals, regulate ion transport, stomatal opening, affect synthesis and activity of enzymes, gene expression and redox homeostasis [13]. Positively charged polyamines are involved in the stress response through their interaction with the negatively charged macromolecules, such as DNA, RNA, proteins and phospholipids, resulting changes in the physical and chemical properties of the membranes, in the structure of nucleic acids and in the enzyme activities [14]. In addition, polyamines are able to detoxify the reactive oxygen species accumulating during abiotic stress.

Considering the potential pharmacological benefits of *Eugenia uniflora*, the aim of the study was to quantitatively estimate primary metabolites like lipids and amino acids in aqueous hot extract of *Eugenia uniflora* leaves.

Materials and Methods

Plant material

Fresh leaves of *Eugenia uniflora* (Linn), Family- Myrtaceae, were collected from Wayanad district, Kerala during the month of April 2014. Taxonomic authentication was done by Dr. V.S Ramachandran, Taxonomist, Department of Botany, Bharathiar University, Coimbatore, Tamil nadu, India.

Sample Processing

The leaves were washed, shade dried at room temperature and powered in a mixer grinder.

Hot Water Decoction*:* 10g of the powdered sample was dissolved in 100ml of distilled water which was boiled for one and half hours and filtered. The decoction was stored at 4 °C for further usage.

Quantitative estimation of Lipids

Lipids are an essential constituent of all plant cells. The vegetative cells of plants contain 5 to 10% lipid by dry weight, and almost all of this weight is found in the membranes. Membrane lipids are important for improvement of photosynthesis against high temperature stress and improved photosynthesis means improved stress tolerance as well [14]. In the present study free fatty acids, total cholesterol, phospholipids, triglycerides were estimated using standard procedures.

Table 1: Qunatitative estimation of Lipids

Parameters	References
Free fatty acids	Horn and Mehanan,1981[15]
Total cholesterol	Parekh and Jung,1970 [16]
Phospholipids	Rouser,1970 [17]
Triglycerides	Rice,1970 [18]

Quantitative estimation of Aminoacids

Amio acids have traditionally been considered as precursor and constituents of proteins. Many amino acids also acts as precursor of other nitrogen containing compound eg:-nucleic acids [19]. Amino acids such as tryptophan, methionine, histidine, proline and arginine were quantitatively estimated using standard procedures.

Table 2: Quantitative estimation of Amino acids

Parameter	References
Tryptophan Methionine Proline	Sadasivam and Manickam, 1996 [20]
Histidine	Kapeller and Adler,1933 [21]
Arginine	Sakaguchi,1925 [22]

Statistical Analysis

All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation (SD).

Results

Lipids are the major form of carbon storage in the seeds of many plant species. Lipids are the most effective source of storage energy, function as insulators of delicate internal organs and hormones and play an important role as the structural constituents of most of the cellular membranes.

Free amino acids and polyamines take part in several metabolic processes and they are involved in the protection against abiotic stresses.

The results obtained from the present study are shown in Table 3 and Table 4.

Table 3: Quantitative estimation of lipids

Primary metabolites	Aqueous hot extract of Eugenia uniflora leaves(mg/g)
Triglycerides	1.88 ± 0.08
Total cholesterol	0.67 ± 0.07
Free fatty acids	2.70 ± 0.10
Phospholipids	0.93 ± 0.07

Values are expressed by mean ± SD of 3 Samples

Table 4: Quantitative estimation of Amino acids

Primary metabolites	Aqueous hot extract of Egenia uniflora leaves(mg/g)
Tryptophan	0.70 ± 0.04
Methionine	0.19 ± 0.04
Histidine	1.06 ± 0.05
Proline	0.38 ± 0.04
Arginine	1.84 ± 0.08

Values are expressed by mean ± SD of 3 Samples

Discussion

In the present study hot water extract of *Eugenia uniflora* shows higher amount of free fatty acids (2.70 ± 0.10 mg/ g) followed by triglycerides (1.88 ± 0.08 mg/g).The fatty acid is found in every cell of the plant and is essential to growth. As part of complex lipids, fatty acids are also important for thermal and electrical insulation, and for mechanical protection. Triglycerides make up the structure of all vegetable oils and fats found in nature. It acts as energy reserve when stored as adipose tissue also acts as insulator, shock protection. Phospholipids are important components of the lipid bi-layer of the cell membrane of all cells. The cell membrane has an essential general role of maintaining cell order and integrity and a number of disease control mechanisms involve compounds that directly (by partitioning into the membrane and inducing disorder) or indirectly (by inhibiting fatty acid biosynthetic pathways) target the phospholipids of the cell membrane [23]. The level of Phospholipids in the aqueous hot extract of *Eugenia uniflora* leaves was 0.13 ± 0.07mg/g. The level of cholesterol was very low compared to others 0.67 ± 0.07 mg /g.

Tryptophan is an essential amino acid which acts as building blocks in protein biosynthesis. In addition tryptophan functions as a biochemical precursor for many compounds [24]. The level of tryptophan in aqueous hot extract of *Eugenia uniflora* leaves was 0.70 ± 0.04mg/g.

Methionine is needed to produce two sulphur containing amino acids cysteine and taurine which helps the body to eliminate toxins, build up strong, healthy tissue and promote cardiovascular health [25]. The level of methionine in the aqueous hot extract of *Eugenia uniflora* leaves was 0.19 ± 0.04 mg / g.

Histidine is found abundantly in haemoglobin.It has been used in the treatment of reheumatoid arthritis, allergic diseases, ulcers and anemia. Deficiency can cause poor hearing [26]. The level of histidine in the aqueous hot extract of *Eugenia uniflora* leaves was 1.06 ± 0.05 mg/g.

Proline is a proreogenic aminoacid with an exceptional conformational rigidity and is essential for primary metabolism [27]. Proline accumulation has been reported during conditions of drought [28] high salinity [29] high light and UV irradiation [30], heavy metals [31], oxidative stress [32] and in response to biotic stresses [33, 34]. The level of proline in aqueous hot extract of *Eugenia uniflora* leaves was 0.38 ± 0.04mg/g.

Arginine play an important role in the healing of wounds, in muscle growth and in fetal and child development. It turns into nitric oxide in the body and cause vasodilation, a relaxant of arterial walls and facilitiates blood flow. The level of arginine was more compared to other amino acids in the aqueous hot extract of *Eugenia uniflora* leaves was 1.84 ± 0.08 mg / g.

The values of amino acid concentration in this study showed that aqueous hot extract of *Eugenia uniflora* leaves contains considerable amounts of lipids and amino acids and confirmed that the nutritional quality of the sample was commendable and may included in treating various diseases.

Conclusion

Plants and its products are used as medicine from the ancient time1. Recently there has been a shift in universal trend from synthetic to herbal medicine [35]. It is estimated by the World Health Organization that approximately 75-80% of the world's population uses plant medicines either partly or entirely as medicine. Interest in plant derived drug increases mainly due to the increasing use, and misuse, of existing synthetic drugs. This poses the need for search and development of new drugs to cure diseases. The chemical substances of the medicinal plants which have the capacity of exerting a physiologic action on the human body are the primary features. The bioactive compounds of plants compounds are considered to be most important. The phytochemical research that has been done based on the ethno-pharmacological information forms the effective approach in the discovery of new medicinal agents from higher plants.

The results obtained in the present study indicate *Eugenia uniflora* leaves have the potential to act as a source of useful drugs because of presence of various phytochemical components such as various lipids and amino acids. The results are very much encouraging but scientific validation is necessary before being put into practice.

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