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Research Article

QUANTITATIVE ANALYSIS OF PRIMARY AND SECONDARY METABOLITES IN AQUEOUS HOT EXTRACT OF *EUGENIA UNIFLORA* (L.) LEAVES

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ABSTRACT

Objective: In the present study, laboratory evaluations were made to quantitatively assess primary metabolites and secondary metabolites in aqueous hot extract of *Eugenia uniflora* leaves.

Methods: Primary metabolites like total soluble carbohydrates, proteins, total amino acids and secondary metabolites such as flavonoids, total phenols, and tannins were estimated using standard procedures.

Results: 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 *E. uniflora* leaves contains various primary and secondary metabolites and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Conclusion: The finding of this study suggests that this plant leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing various diseases. The results are very much encouraging but scientific validation is necessary before being put into practice.

Keywords: Phytochemicals, Primary metabolites, Secondary metabolites, Aqueous extract, *Eugenia uniflora.*

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to treat disease all over the world [1]. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness [2,3]. Natural remedies derived from herbs [4], food or raw materials are used by pharmaceutical and food industries. It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant-derived components were discovered by following ethano medicinal use of the plants [5]. World Health Organization estimated that 80% of the population of developing countries like India and China still relies on traditional medicines mostly plant drugs, for their primary healthcare needs [6]. The medicinal value of these plants lies in the chemical substances that produce a definite physiological action in the human body.

India has a rich, vibrant and diverse cultural history. An important component of this culture and tradition is that of health and healing. India is the largest producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance, which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% of the medicinal needs of the third world countries [7].

India is one of the leading countries in Asia in terms of the wealth of traditional knowledge systems related to the use of plant species. India is also known to harbor a rich diversity of higher plant species (about 17,000 species) of which 7500 are known as medicinal plants [8]. Such a huge number of medicinal plant species has allowed the evolution of many systems of herbal medicine. In India almost all medicinal plants especially in traditional medicine are currently well-acknowledged and established as a viable profession [9]. In developing countries like India synthetic drugs are not only expensive and inadequate for treatment of diseases but are also often with adulterations and side-effects. Therefore, it is of great interest to carry out a screening of these plants, in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents.

The traditional herbal medicines are receiving great importance in the health care sector.

India has a century's old tradition of using medicinal plants and herbal medicines for the alleviation of various diseases and ailments [10]. About 500 plants with medicinal uses are mentioned in ancient literature, and around 800 plants have been used in indigenous systems of medicine. In recent decades there is manifold increase in medicinal plant based industries due to the increase in the interest of the use of medicinal plants throughout the world which are growing at a rate of 7-15% annually [11]. The evaluation of new drugs especially phytochemically obtained materials has opened a vast area for research and development. Therefore, the evaluation of the rich heritage of traditional medicine is essential. Many medicinal plants contain a broad range of natural antioxidants such as phenolic acids, flavonoids and tannins, which possess remarkable antioxidant activity [12]. Several investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases [13].

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds [14]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [15-17]. Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions.

The phytochemicals are grouped into two main categories [18] namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc. [19,20]. Majority of phytochemicals have been known to bear valuable therapeutic activities such as insecticidals [21], antibacterial, antifungal [22], anti-constipative [23], spasmolytic, anti-plasmodial and antioxidant [24] activities etc. The plants thus find their medicinal value due to respective phytochemical constituents they contains.

Eugenia uniflora L., commonly known as "pitangueira" or Brazilian cherry tree, is a species belonging to the family Myrtaceae, which is native to South America and common in regions with tropical and subtropical climate [25]. In Brazil, it is used in the treatment of digestive disorders and is thought to have anti-inflammatory and anti-rheumatic activities [26]. It is an aromatic species and its essential oil has pharmacological properties that are well characterized in the literature as antioxidant and antimicrobial [27]. *E. uniflora* has known antihypertensive [28], antitumor [29], and anti-nociceptive properties, and it shows good performance against microorganisms, demonstrating antiviral, antifungal [30], anti-*Trichomonas gallinae* [31], and anti-*Trypanosoma cruzi* properties [32].

Scientific classification
Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Eugenia
Species: *E. uniflora*
Binomial name: *E. uniflora* L.

Considering the potential pharmacological benefits of *E. uniflora*, the aim of this study is to investigate the presence of various primary and secondary metabolites in aqueous hot extract of *E. uniflora* leaves (Fig. 1).

METHODS

Plant material
Fresh leaves of *E. uniflora* (Linn), Family-Myrtaceae, were collected from Wayanad district, Kerala during the month of February 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.

Fig. 1: *Eugenia uniflora*

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

Quantitative determination of primary metabolites and secondary metabolites
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 [33].

Determination of primary 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 of prime importance and essentially required for growth of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs [34,35].

1. Determination of total soluble carbohydrates
   The total soluble carbohydrate content was determined according to the method described by Hedge and Hofreiter [36]. 1 ml of sample was mixed with 4 ml of anthrone reagent. Incubated in boiling water bath for 8 minutes after which the absorbance was read at 630 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.
2. Determination of proteins
   Protein content was determined according to the method of Lowry *et al*. [37]. 1 ml of sample was mixed with 0.5 ml of 0.1 N NaOH and 5 ml of alkaline copper reagent, incubated the mixture in room temperature for 30 minutes. Added 0.5 ml of Folin–Ciocalteau reagent and incubated again for 10 minutes at room temperature. Absorbance was read at 660 nm against a reagent blank. The analysis was performed in triplicates and the results were expressed mg/g sample.
3. Determination of total free amino acids
   Total free amino acids (ninhydrin method) was determined according to the procedure given by Moore and Stein [38]. 1 ml of the sample was mixed with 1 ml of Ninhydrin in a test tube. Tubes were kept in boiling water bath for 20 minutes and then added 5 ml of diluent (equal volume of water and n-propanol) incubated at room temperature for 15 minutes and absorbance were read at 570 nm against a reagent blank. The analysis was performed in triplicates, and the results were expressed as mg/g sample.

Quantitative determination of secondary metabolites
Drug discovery from the medicinal plants has played a significant role in the treatment of various diseases and indeed, most new clinical applications of plants secondary metabolites and their derivatives over the last century. Secondary metabolites are important mediators of ecological interactions between plants and their environment.

1. Determination of total phenols
   Total phenol content were estimated in the aqueous hot extract by the procedure given by Bray and Thorpe [35], Folin–Ciocalteau method. To 1 ml of sample added 0.5 ml of Folin–Ciocalteau reagent and incubated at room temperature for 3 minutes. After 3 minutes 2 ml of 20% Na_2CO_3 was added, mixed well and incubated the tubes in boiling water bath for 1 minute. Cooled rapidly and read absorbance at 650 nm against reagent blank. The analysis was performed in triplicates and the results were expressed as mg/g sample.
2. Determination of flavanoids
   Flavanoids in aqueous hot extract of *E. uniflora* was estimated according to the procedure given by Jia *et al*. [39]. 1 ml of the was mixed with 0.075 ml of 5% Sodium nitrite solution and incubated at room temperature for % minutes. Then added 10% aluminum chloride and incubated at room temperature for 6 minutes. Then added 1 N NaOH and absorbance was read at 510 nm against a

reagent blank. The analysis was performed in triplicates and the results were expressed as mg catechin equivalent/g sample.

3. Determination of tannins
   Estimation of tannins in the extract was done by the procedure given by Bray and Thorpe [35]. 1 ml of the sample was mixed with 5 ml of vannilin hydrochloride reagent and incubated at room temperature for 20 minutes. Absorbance was read at 500 nm against a reagent blank. The analysis was performed in triplicates, and the results were expressed as catechin equivalents.
4. Determination of alkaloid
   Estimation of alkaloids in the extract was done by the procedure given by Harborne [40]. 10 mg of plant material was homogenized in a motor and pestle. Added around 20 ml of methanol:ammonia (68:2). Decanted the ammonicial solution and after 24 hrs added fresh methanolic ammonia. Repeated the procedure thrice and pooled the extracts. The extracts were evaporated using a flash evaporator. Treated the residue with 1 N HCl and kept it overnight. Extracted the acidic solution with 20 ml of chloroform thrice, pooled the organic layers and evaporated to dryness, basic fraction. Basified the acidic layer with concentrated sodium hydroxide to pH 12 and extracted with chloroform (20 ml) thrice, Pooled the chloroform layers, dry over absorbent cotton and evaporated to dryness. Weighed the fraction that contains alkaloids expressed as mg/100 g.

Statistical analysis

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

RESULTS

Medicinal plants are of great importance to health of individual and communities. The medicinal values of a plant lie in some chemical substances that produce a definite physiological action on the human body. Phytochemical analysis is of paramount importance in identifying a new source of therapeutically and industrially valuable compounds having medicinal plants have been chemically investigated. In the present investigation primary and secondary metabolites were qualitatively and quantitatively analyzed using *E. uniflora* leaves. The results are presented in Tables 1 and 2.

DISCUSSION

Quantitative analysis of primary metabolites shows that (Table 1), protein content was found high (1.80±0.05 mg/g) followed by amino acid (1.71±0.06 mg/g) and then carbohydrate (1.14±0.13 mg/g). Plant sugars can be used as artificial sweetener and they can even help in diabetes by supporting the body in its rebuilding [41]. The presence of higher protein level in the plant parts towards their possible increase food value or that a protein base bioactive compound could also be isolated in future [42].

Table 1: Quantitative analysis of primary metabolites in aqueous hot extract of *E.uniflora* leaves

Primary metabolites	Aqueous hot extract of *E uniflora* leaves (mg/g)
Total soluble carbohydrates	1.14±0.13
Proteins	1.80±0.05
Total free amino acids	1.71±0.06

Values are expressed by mean±SD of three samples, *E. uniflora: Eugenia uniflora*, SD: Standard deviation

Table 2: Quantitative analysis of secondary metabolites in aqueous hot extract of *E. uniflora* leaves

Secondary metabolites	Aqueous hot extract of *E. uniflora* leaves (mg/g)
Total phenols	1.74±0.05
Flavanoids	2.11±0.07
Tannins	1.45±0.49
Alkaloids	2.05±0.06

Values are expressed by mean±SD of three samples, SD: Standard deviation, *E. uniflora: Eugenia uniflora*

Secondary metabolites analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. The hot water extract showed higher level of flavonoids (2.11±0.07 mg/g) than the other secondary metabolites. Flavonoids have been reported to exert wide range of biological activities. These includes: Anti-inflammatory, antibacterial, antiviral, anti-allergic [43-45], cytotoxic anti-tumour, treatment of neurodegenerative diseases, vasodilatory action [46-48]. In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation [45,48,49]. These are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase [50].

Alkaloids protect against chronic diseases [51] and earlier recorded that bitter leaf contains an alkaloid that is capable of reducing headaches associated with hypertension. Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase [52]. The level of alkaloids was (2.05±0.06).

The level of phenol was (1.74±0.05 mg/g), the higher amount of phenol is important in the regulation of plant growth, development and disease resistance. Consumption of diets rich in plant polyphenols offers protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.

Tannin contributes various medicinal properties such as antimicrobial, anti-inflammatory and astringent activity. They have been also reported to have anti-viral [53] antibacterial [54,55] and anti-parasitic effects, the level of tannins in the aqueous hot extract of *E. uniflora* was (1.45±0.49).

The phytochemical analysis of the medicinal plants are important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for the treatment of various diseases. Thus, we hope that the important phytochemical properties identified in the present study with aqueous hot extract of *E. uniflora* leaves will be helpful in the treatment of various ailments.

CONCLUSION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

Some traditional medicines are highly equipped with more qualities in therapeutical basis, majority of the people in developing countries have resorted to the use of medicinal plants as an alternative treatment. Herbal medicines are rich in the active ingredients and are safe with the body chemistry of man [56]. Furthermore, the presence of the active ingredients lends credence to the claims of the use of plants for the treatment of diseases by trado-medical doctors. However, plants have forever been a catalyst for our healing. In order to halt the trend of increased emerging and resistant infectious disease, it will require a multipronged approach that includes the development of new drugs. Using plants as the inspiration for new drugs provides an infusion of novel compounds or substances for healing disease.

Phytochemical, natural compound occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases. The results obtained in the present study indicates *E. uniflora* leaves have the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrate, protein, lipids, phenols, flavonoids and tannin. The results are very much encouraging but scientific validation is necessary before being put into practice.

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