



## SCREENING OF *Eugenia uniflora* (L.) LEAVES IN VARIOUS SOLVENTS FOR QUALITATIVE PHYTOCHEMICAL CONSTITUENTS.

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### ABSTRACT

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. The current study focuses on qualitative phytochemical screening of primary and secondary metabolites in *Eugenia uniflora* leaves with different solvents like methanol, ethanol, chloroform, petroleum ether, ethyl acetate, acetone, aqueous cold and hot extracts. Primary metabolites like carbohydrates, proteins, amino acids and secondary metabolites like alkaloids, flavonoids, saponins, tanins, phenols, fatty acids, anthraquinone, acids, terpenoids, triterpenoids, sterols, cardiac glycosides, coumarins, carbohydrates, protein, resins, gum & mucilages, phlobatanins, quinones, oxalate, anthocyanin, anthracenoids, emodins, chalcones, anthocyanosides and volatile oils were screened successfully using standard methods. The results obtained in the present study indicate *Eugenia uniflora* leaves have the potential to act as a source of useful drugs because of the presence of various primary and secondary metabolites. The results are very much encouraging but scientific validation is necessary before being put into practice.

**KEYWORDS:** *Eugenia uniflora*, Aqueous extract, Phytochemical constituents, Primary metabolites, Secondary metabolites.

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## INTRODUCTION

India is rich in its diversified flora and fauna. Plants are integral part of nature. Nature reflects the creative power of living god. Plants have an almost endless variety of metabolites which is very useful to human beings<sup>1</sup>. The importance of plants is well known to us. Plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects<sup>2</sup>. Medicinal plants are rich source of bioactive phytochemicals or bio nutrients. Studies carried out during the past 2–3 decades have shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes and coronary heart disease. There is, however, much scope for further systematic research in screening Indian medicinal plants for these phytochemicals and assessing their potential in protecting against different types of diseases. The use of traditional medicines is increasing and getting popularity throughout the developed and developing world. Herbal medicines are the finished labeled medicinal product that contains active ingredients, aerial or underground parts of the plant or other plant material or combinations<sup>3</sup>. About 80% of the marginal people in developing countries rely on traditional medicine for their primary health care<sup>4</sup>. With the increase in people's preference and demand, worth of herbal product industry is increasing day by day<sup>5-7</sup>. 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. *Eugenia uniflora* L. is a widely distributed tree in South American countries, mainly in Brazil, Argentina, Uruguay, and Paraguay<sup>8</sup>. 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<sup>9</sup>. 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<sup>10, 11</sup>. 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<sup>12</sup>.

### Scientific Classification

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

The phytochemical research based on ethno-pharmacological information is generally, considered as an effective approach in the discovery of new anti-infective agents from higher plants. The qualitative analysis is very essential for identifying and quantification of active principles present in the medicinal plants which is important for medicinal action and drug preparation. Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans, further those attributed to macronutrients and micronutrients<sup>13</sup>. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals<sup>14, 15</sup>. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and

are classified by protective function, physical characteristics and chemical characteristics<sup>16</sup> and about 150 phytochemicals have been studied in detail. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds<sup>17</sup>. Keeping this in view, this study aims at qualitatively determining the phytochemical constituents of *Eugenia uniflora* leaves in various solvents

## 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, Tamilnadu, India.

### **Sample Processing**

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

### **Sample extraction**

The purpose of standardized extraction procedure for crude drugs (medicinal plant parts) is to attain the therapeutically desired portion and to eliminate unwanted material with a selective solvent. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

### **Solvents used**

- Ethanol
- Methanol
- Chloroform
- Petroleum ether
- Ethyl acetate
- Acetone
- Aqueous Cold and Hot

### **Soxhlet extraction**

Soxhlet extraction is required when the desired compound has a limited solubility in a solvent

and the impurity is insoluble in that solvent. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. Accurately 10g of the dried powdered material was weighed and tied in a muslin cloth. The material was then put in a soxhlet apparatus and 100ml of solvent was added. Extraction was carried out for 8 hours with methanol, ethanol, chloroform, petroleum ether, ethyl acetate, acetone, after which the extract was collected and concentrated using rotary evaporator to remove the solvent. The residue was collected and kept at room temperature for 16 hours for complete evaporation of solvent. The extract was stored in cold room at a temperature of 4°C for further use.

### **Cold Water Decoction**

10g of the powdered sample was dissolved in 100ml of distilled water which was continuously shaken for 24 hours in a mechanical shaker at 40°C. After 24 hours, it was filtered and used. The decoction was stored at 4°C for further usage

### **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.

### **Determination of Yield of Extract**

The percentage yield of the extract was determined by weighing *Eugenia uniflora* leaf extract before extraction and after concentration, and then calculated using the formula.

$$\text{Yield \%} = \frac{\text{Weight of extract (g)}}{\text{Weight of fresh powdered sample (g)}} \times 100$$

### **Preliminary Phytochemical Screening of Primary metabolites and Secondary metabolites.**

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. Secondary metabolites are synthesized during secondary metabolism of plants. They are the basic source for the establishment of several pharmaceutical industries since they have enormous medicinal properties<sup>18</sup>. The most important secondary metabolites are alkaloids, tannins, flavonoids, phloblatannins, saponin and cardiac glycosides. All secondary metabolites have specific function as like saponins have antifungal activity<sup>19</sup>, some alkaloid may be useful against HIV infection<sup>20</sup>, flavonoids have strong anticancer activity<sup>21</sup> and tannin have antimicrobial activity. Leaf extract in different solvents was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures<sup>22-25</sup>. Tests for alkaloids, flavonoids, saponins, tanins, phenols, fatty acids, anthraquinone, acids, terpenoids, triterpenoids, sterols, cardiacglycosides, coumarins, carbohydrates, protein, resins, gum & mucilages, phlobtanins, quinones, oxalate, anthocyanin, anthracenoids, emodins, chalcones, anthocyanosides, volatile oils were conducted using standard methods

## RESULTS AND DISSCUSSION

Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources<sup>26</sup>. Medicinal value of plants have assumed an important dimension in the past few decades. Results of work had indicated that phytochemicals were responsible for medicinal effects of this plant. The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. The yield percentage of present study shows that percentage yield was more in hot water extract compared to others. (Table1). Different phytochemicals have been found to possess a wide range of activities (Table 2), which may help in protection against chronic diseases. Primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs<sup>27,28</sup>.

Plants have the ability to produce a large variety of secondary metabolites such as saponins, tannins, phenols, alkaloids, triterpens and phytosterols. For example, alkaloids protect against chronic diseases.<sup>29</sup> and earlier recorded that bitter leaf contains an alkaloid which 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<sup>30</sup>. Saponins, present in plants, have been suggested as possible anti-carcinogens. Saponins protect against hypercholesterolemia and antibiotic properties. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycemia, and to act as antifungal and antiviral agents<sup>31-33</sup>. Tannins reduce the risk of coronary heart diseases<sup>34</sup>. Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidal compounds. The anti-inflammatory effects of tannins helps to control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Diarrhea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine<sup>35</sup>. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by<sup>36, 37</sup>. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites<sup>38</sup>. A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers<sup>39</sup>, phenols are involved in defense against UV radiation or aggression by pathogens. Consumption of diets rich in plant polyphenols offer protection against development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. Flavanoids are used to treat many important common diseases due to their proven ability to inhibit specific enzymes to stimulate some hormones and neurotransmitters and to scavenge free

radicals. Flavonoids have been reported to exert wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic<sup>40-42</sup>, cytotoxic antitumour, treatment of neurodegenerative diseases, vasodilatory action<sup>41, 43-45</sup>. Anthraquinone helps in digestion as a laxative, to reduce inflammation and to inhibit the growth of cancer cells. Terpenoids have medicinal properties such as Anticarcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial or diuretic activities. Cardiac glycosides involves in the treatment of cardiac failure. Treatment with cardiac glycosides is still the only safe inotropic drug for oral use that improves haemodynamics

in patients with a compromised cardiac function<sup>46</sup>. Steroids and triterpenoids shows analgesic properties. Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents<sup>47</sup>. Phlobatannins have diuretic property<sup>48</sup>. Anthocyanins act as powerful antioxidant. Mucilages are most commonly used adjuvant in pharmaceutical preparations. Emodins possess antimicrobial activity against skin infecting pathogenic organisms. In present qualitative analysis of *Eugenia uniflora* leaves in various extracts, all the phytochemical constituents were found to present more in hot water extract compared to other extracts. (Table 2).

**Table 1**  
**Yield percentage of *Eugenia uniflora* Leaves in various solvents**

Solvents	% Yield (w/w)
Aqueous (Hot)	48.6%
Aqueous (Cold)	32.6%
Ethanol	29.4%
Methanol	23.5%
Acetone	18.5%
Petroleum ether	6.5%
Ethyl acetate	3.2%
Chloroform	2.1%

**Table 2**  
**Preliminary phytochemical screening results of *Eugenia uniflora* Leaves in various solvents.**

	Methanol	Ethanol	Chloroform	Petroleum Ether	Ethyl Acetate	Acetone	Aqueous (Cold)	Aqueous (Hot)
Alkaloids	+	++	-	+++	+++	+	+	+++
Flavonoids	+++	++	-	-	++	++	++	+++
Saponins	++	++	++	+	+	-	+	+++
Tanins	++	+++	-	++	+	++	+++	++
Phenols	+++	+++	-	+	++	+++	+++	+++
Fatty acids	++	++	++	+	-	+	+	+
Anthraquinone	++	++	-	-	-	-	-	++
Acids	+++	+++	-	-	-	-	-	++
Terpenoids	-	+++	-	++	+	+++	-	+++
Triterpenoids	++	+++	+++	++	+	+++	-	+
Sterols	++	++	+	++	+	++	++	+++
Cardiac glycosides	-	+++	-	+	+	++	++	+++
Coumarins	++	+++	-	++	+	++	++	+++
Thiols	+++	++	+	++	+	+	+	+++
Carbohydrates	++	+++	+++	-	+++	+++	+++	+++
Protein	+	-	-	+	-	++	+++	+++
Resins	+++	+++	-	++	-	-	+++	+++
Gum & Mucilages	+++	++	-	++	-	-	++	+++
Phlobatannins	-	-	-	-	+	+	-	++
Quinones	+++	+++	-	-	-	+++	+++	+++
Oxalate	++	+++	-	+	+++	++	-	+
Anthocyanin	-	+	-	-	-	-	+	+++
Anthracenoids	++	++	-	+++	+++	++	+++	+++
Emodins	+	+++	+	-	+	-	+	+++
Chalcones	+	+++	+	-	+	-	+	+++
Anthocyanosides	++	++	-	++	+	++	+	+++
Volatile oils	+++	+++	++	++	++	+	-	++

KEY: + = Presence, - = Absence.

++ = Plenty, +++ = Abundance

## CONCLUSION

Plants have an almost limitless ability to synthesize aromatic substances. Most of them are secondary metabolites, of which at least 12,000 have been isolated<sup>49</sup>. Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade<sup>50</sup>. The medicinal value of the plant lies, in some chemical substance that produces a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic flavonoids, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins. Plants can be used to discover and screen these bioactive natural organic compounds which may be beneficial for the development of new pharmaceuticals that address today's therapeutic needs. The phytochemical characterization of the extracts, the identification of responsible bioactive compounds and quality standards are necessary for future study. The phyto

constituents are the major important compounds which are responsible for the medicinal properties of the herbs. Most of the researchers suggest that the medicinal plants contain many of the phyto constituents, could be used for therapeutic purposes as they often exhibit a huge amount of medicinal properties such as antioxidant, anticarcinogenic, antitumor, antidiabetic, anti-inflammatory activities that are non-lethal and most valuable to the living system. In this study *Eugenia uniflora* leaves screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various primary metabolites and secondary metabolites that are vital for good health. 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. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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