Phytochemical Analysis and Acute Toxicity/Lethality Study of Ethanol Extract of *Eugenia uniflora* Pulp.

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**ABSTRACT:**
The phytochemical analysis and acute toxicity (LD50) studies were carried out on the ethanol extract of *Eugenia uniflora* pulp. The phytochemical results showed the presence of alkaloids, glycosides, flavonoids, tannins, saponins and terpenoids. Results of the acute toxicity study gave LD50 value of 2408.3 mg/kg.

**KEYWORDS:** Phytochemical analysis, Acute Toxicity, *Eugenia uniflora*, Ethanol.

**INTRODUCTION:**
*Eugenia uniflora* is an evergreen shrub that is about 6-8m high. It is a hardy species than can thrive in a variety of habitats both in its native and introduced forms. *Eugenia uniflora* can quickly reach thick densities which affect light penetrations, subsequently changing microenvironments. It is also known to host recognized pests and pathogens.

*Eugenia uniflora* is considered to be effective in treating many diseases and are popularly used in the Brazilian medicine. A leaf infusion of pitanga is used in Brazil for stomach pain, and as an astringent. In Surinam, the leaf decoction is taken as a cold remedy and, in combination with lemongrass, as a febrifuge. In Java, fruits are used to reduce blood pressure.

Extract from pitanga leaves are considered to be effective against many diseases and are therefore used in popular Brazilian medicines. In a study, where the volatile constituents of pitanga fruits (*Eugenia uniflora* L) were trapped on to pompak-Q and eluted with ethyl acetate, and the chemical composition of the extract was analysed by gas chromatography/mass spectrometry. Fifty four compounds were detected, and twenty-nine of those were identified by close matches with standard Ms Spectra. Monosterpenes (75.3% in mass) were found to comprise the largest class of the pitanga fruit volatiles, and some therapeutic constituents of pitanga leaf extract, such as selina-1,3,7(11)-trien-8-one were also found to be present in the fruit volatile extract, suggesting that the fruit may display therapeutic properties similar to those of the leaf extract.

Generally, *Eugenia uniflora* is economically important in its endemic range as pioneer specie in the resting ecosystem. They prefer fertile, moist soils and partial shade. They are grown in Brazil for its edible fruit which can be consumed fresh as food or made into jam or jelly with preserved whole in syrup. They are often made into jam, relish or pickles. Brazilians ferment the juice into vinegar or wine.

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The leaves could be used as poison; the crushed leaves release pungent oil which is used as insect repellent. *Eugenia uniflora* is appreciated in ice creams and liquids and are also used as phytocosmetic by the Brazilian cosmetics industry to develop shampoos, hair conditioners, face and bath soaps and perfumes. The bark contains 20 to 28.5% tannins and can be used for tanning leather. The seeds are extremely resinous and toxic and should not be eaten. However, sesquiterpenes are identified as the main class of compounds in *Eugenia uniflora*.

Phytochemicals are naturally and biologically active plant compounds that provide health benefits. They are found in plant foods and they work together with nutrients and dietary fiber to protect against disease. Current studies on phytochemicals and nutrients found in fruits, vegetables and nuts, show that they help in aging process and also reduce the risk of many diseases, like cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis and urinary tract infection.

Phytochemicals have complement any overlapping mechanisms of action in the body, including antioxidant effects, stimulation of the immune system, modulation of hormone metabolism, as well as antibacterial and antiviral effects.

New phytochemicals are being discovered on a daily basis, and plants contain hundreds of thousands of different phytochemicals. Researchers have long known that phytochemicals provide health benefits for plants, but it is only recently that certain phytochemicals have been recommended for the purpose of disease prevention and treatment for humans.

There are literally thousands of different phytochemicals found throughout the plant kingdom. These phytochemicals exhibit a wide range of biological activities, arising mainly from their antioxidant properties, anti-inflammatory strengths, and ability to boost, the body’s natural detoxification system. They have been recognized to exert anti-bacterial, anti-viral, anti-cardiovascular disease and anti-cancer activities as well as analgesic, anti-allergic, liver protective, estrogenic and anti-estrogenic effects.

Hundreds of phytochemicals are currently being studied for their human health benefits. To better understand the scope of these studies, it might be helpful to know that carrots, orange juice and apples contain 217,170, and 150 phytochemicals in their disease fighting make up. Scientific research is helping us to understand how, and why, the phytochemicals found in color-laden produce, herbs and super foods have such positive effect on health.

The Phytochemical database states, “Cancer, in many cases, is a deficiency of antioxidants”. So is heart disease. Scientist is starting to think of these diseases as a shortage of phytochemicals.

This study aims at determining the phytochemical constituents and the acute toxicity analysis of ethanol extract of *Eugenia uniflora* fruit pulp.

**MATERIALS AND METHODS:**

**Plant Material:**
Fresh fruits of *Eugenia Uniflora* plant were obtained from Anambra State, and identified by Mr. Ozioko of the Department of Botany, University of Nigeria Nsukka.

**Animals:**
Eighteen (18) experimental mice of between 8 and 12 weeks old with average weight range of 30 – 45 g were used. The mice were purchased from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka.

**Chemicals/Reagents:**
All chemicals used in this study were of analytical grade and products of Sigma GMBH, England and Merck, Darmstadt, Germany.

**Extraction Procedure:**
The fresh fruit pulps of *Eugenia uniflora* were separated carefully from the seed. The pulps were grounded using mortar. A weighed quantity, 174 g were then Macerated in absolute ethanol, which was left to stand for 24 hours, after which the extractive was filtered out with cheese cloth and filter paper. The resulting ethanol extract was concentrated to obtain a semi-solid extract using a rotary evaporator at an optimum temperature of between 40 to 45°C to avoid denaturation of the active ingredients. The weight of the semi-solid extract was determined and the extract subjected to phytochemical and toxicological analyses.

**Determination of Yield of Extract:**
The percentage yield of the extract was determined by weighing the *Eugenia uniflora* pulp extract before extraction and after concentration and then calculated using the formula:

\[
\text{Percentage (\%) yield} = \frac{\text{Weight (g) of Extract Evaporated}}{\text{Weight (g) of Grounded Pulps}} \times 100
\]

**Phytochemical Analysis:**
The Phytochemical tests were carried out based on the procedures outlined by Harborne, Trease and Evans.

**Acute toxicity and lethality (LD₅₀) Test:**
Investigation on the acute toxicity study (LD₅₀) of the ethanol extract was determined using the Lorke method.
RESULTS:
Percentage Yield of the Extract:

Table 1: The percentage yield of the alcoholic extract from the Eugenia uniflora pulp.

<table>
<thead>
<tr>
<th>Initial weight of extract (g)</th>
<th>Final weight of Extract (g)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>174</td>
<td>25.32</td>
<td>14.55</td>
</tr>
</tbody>
</table>

From the result in Table 1 the (%) yield of the ethanol extract of Eugenia uniflora pulp was found to be 14.55%.

Phytochemical Analysis of the Extract:
The results of the phytochemical analysis show that the alcoholic Eugenia uniflora pulp extract contains alkaloids, glycosides, flavonoids, tannins, saponins, terpenoids and reducing sugar. Resins were not detected during the test.

Table 2: Results of the phytochemical analysis

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugar</td>
<td>++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>ND</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
</tbody>
</table>

Key: ++ = Relative abundance of compound.
++ = Moderate abundance of compound.
+ = Relative low presence of compound.
ND = Not detected.

Acute Toxicity Studies of Eugenia uniflora Pulp
The acute toxicity (LD₅₀) of the ethanol extract of E. uniflora showed toxicity at 2408.3mg/kg.

Table 3: Results of the acute toxicity (LD₅₀) of Eugenia uniflora Pulp.

<table>
<thead>
<tr>
<th>Dose (mg/kg body weight)</th>
<th>No of animals before Administration</th>
<th>No of deaths after Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>1000</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>2000</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>2900</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

DISCUSSION:
The reactive oxygen species (ROS) formed in the body is due to exogenous and endogenous factors. They are found to be responsible for many diseases. Current research is revealing the potentials of phytochemical antioxidants as health benefactors due to their ability to neutralize the oxidant responsible for the onset of cell damage. Synthetic antioxidants are harmful to the body and most of the natural antioxidants from plants sources are safer to health and have better antioxidant activity.

The phytochemical screening of the alcoholic E. uniflora pulp extract revealed the presence of alkaloids, glycosides, tannins, saponins, flavonoids, terpenoids and steroids. Flavonoids and glycosides are the main constituent found from the alcoholic extract while tannins, saponins and terpenoids were also found to be present in little quantity. This result compared well with the phytochemical screening found on Eugenia jambolana another member of the Myrtaceae family. From the study it was found out that Eugenia jambolana contains flavonoids, glycosides, alkaloids, saponins, steroidal tannins and terpenoids. Saponins cause haemolysis of the red blood cell and their medicinal value is due to their expectorant effect. They have been found to have a hypercholesterolemic and anti-diabetic property. Tannins are often perceived as detrimental because of their potential to affect protein digestibility or on metal ion availability. Current studies have shown that saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effect. Steroids and saponins are equally responsible for central nervous system activities. The terpenoids have also been shown to decrease blood sugar level in animal studies.

Acute toxicity studies of Eugenia uniflora pulp in mice established an LD₅₀ of 2408.3mg/kg. These LD₅₀ levels are relatively low. It could be suggested that the alcoholic pulp extract of E. uniflora could be generally regarded as safe. However further studies are needed to ascertain prolonged feeding effect on the organs and of tissues of experimental animals. This again may suggest why people who take cherry do not suffer from any known toxicity.

In conclusion, the results of this research, has shown that the alcoholic extract of E. uniflora fruit pulp has tremendous phytochemicals that have a lot of bio-pharmacological functions. It could be inferred that the alcoholic pulp extract of E. uniflora could be generally regarded as safe as a result of its relatively low LD₅₀ value.

REFERENCES:


