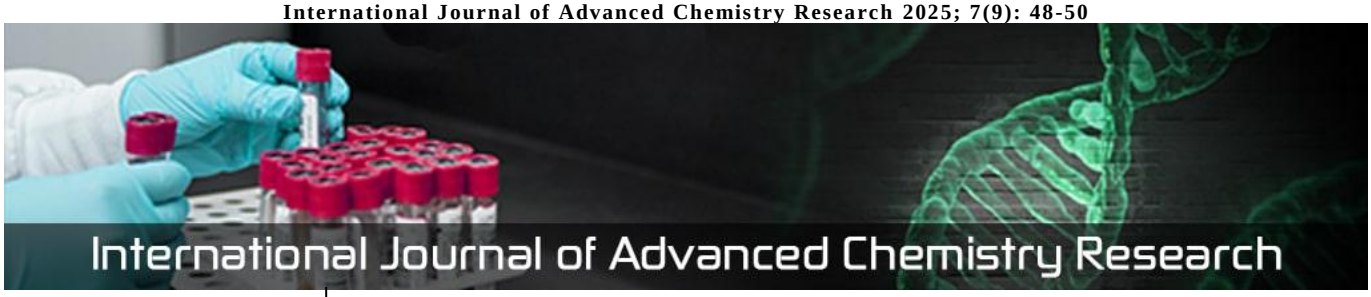

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Extraction of various nutraceuticals of *Stevia rebudiana* from tissue culture system: An innovative business idea for young scientists

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Abstract
Stevia rebudiana is commonly known as Candy leaf, Sweet leaf or Sugar leaf or Madhu Patrika due to its sweetened leaves. It is a native plant of Paraguay in South America having humid & wet environment. Various nutraceuticals like Steviol glycosides, antioxidants & flavonoids are found in this plant. Steviol gylcosides possess sweet taste so it can be helpful in diabetes, phenolic compounds possess antioxidant property which can be useful in cosmetics and flavonoids possess antimicrobial, antiobesity, anticancer & anti-inflammatory properties. The plant can be grown through tissue culture technique which produces large number of plants in short time period and during adverse climatic conditions (non-seasonal). Tissue culture can help the plant in producing phytochemicals as Secondary metabolites. Secondary metabolites are bioactive compounds produced by plants through their metabolism. During plant tissue culture these secondary metabolites accumulate into the medium. Alkaloids, phenolic compounds, glycosides are secondary metabolites which accumulate during plant tissue culture of *Stevia rebudiana*. So, plant tissue culture of *Stevia rebudiana* can be a profitable venture for young scientists.

Keywords: *Stevia rebudiana*, nutraceuticals, steviol gylcosides, antioxidants, flavonoids, antimicrobial, antiobesity, anticancer, anti-inflammatory, plant tissue culture, sugar alternative

Introduction

***Stevia rebudiana*:** *Stevia rebudiana* or sugar leaf belongs to the Asteraceae family (ragweed family). The leaves of this South American plant *Stevia rebudiana* are used to make, a sugar alternative because they possess Steviol glycosides that have a strong sweet taste. Leaf of Stevia is 100–300 times sweeter than sugar and it is a natural, low-calorie sugar alternative. It can be used in a wide range of items, such as fruit nectars, cereals, yoghurts, flavored drinks, dairy desserts, jams, and carbonated water. Stevia also possess bioactive substances such as polyphenols, chlorophylls, carotenoids, and tannins which can be utilized to create functional meals and nutraceuticals. It may offer anti-inflammatory, antidiabetic, antihypertensive, and chemopreventive actions, among other potential medical benefits. Additionally, it may aid in the treatment of cancers, microbiological infections, obesity, liver pathologies, and renal ailments. Stevia can lower sugar intake, which may help to prevent cavities.

Additionally, Stevia can be added to food as an emulsifier, foaming agent, or solubilizing agent. It can also supply chlorophyll for medications and dental care products.

Stevia leaves have been used in folk medicine and to sweeten teas for more than 1,500 years by the Guaraní people. Stevia was first documented scientifically in 1899. Stevia sweeteners received approval from the FDA in 2008 and the European Union in 2011.

Several research have demonstrated that Stevia leaf preparations are natural, calorie-free sugar alternative and are safe for those with high blood pressure, diabetes, and other conditions.

Plant tissue culture: The process of cultivating plant cells, tissues, or organs on a nutritional medium in sterile conditions known as plant tissue culture. There are numerous uses of plant tissue culture, such as:

i) Enhancement of crops
To create disease-free plants which yield more, and stress tolerant plant, plant tissue culture technique is utilized.

ii) Preservation of plants
Elite germplasms and endangered plant species are preserved through the use of plant tissue culture.

iii) Production of secondary metabolites
Plant cells are grown in liquid culture using plant tissue culture to create secondary metabolites that can be used in food, medicine, and cosmetics.

iv) Fundamental research
Cell biology, genetics, and biochemistry are all studied using plant tissue culture.

v) Concerns about the environment
Conservation strategies are developed through the use of plant tissue culture.

vi) Breeding
Dihaploid plants, the crossing of distantly related species, and the screening of cells for beneficial traits are all accomplished through plant tissue culture.

vii) Elimination of viruses
By proliferating from meristematic tissues, viruses can be eliminated through plant tissue culture.
Scientists can employ plant tissue culture technique for business purposes for producting secondary metabolites.

viii) Propagation of plants
Large numbers of plants can be produced quickly using plant tissue culture. For the large-scale production of crops like grains, fruits, and vegetables, this is helpful.

ix) Plants free of disease
Diseased plants can be transformed into disease-free plants using plant tissue culture. This may lessen the transmission of illness and enhance crop quality.

Secondary metabolites of stevia: Stevia plant possess many secondary metabolites which are products of metabolism such as alkaloids, phenolic compounds, glycosides etc. These secondary metabolites are utilized in a variety of industries such as food, cosmetics, and pharmaceuticals industries and can be produced by plant tissue culture. Phenolic compounds which are bioactive secondary metabolites present in Stevia possess numerous health advantages such as:

Food additive: Stevia leaves polyphenol components (or PPS) can be added to foods as an antioxidant or in medications that have antidiabetic effects.
Reactive Oxygen Species (ROS) are neutralized and cellular damage is avoided by the antioxidant qualities of the phenolic compounds.

Antibacterial: It has antibacterial, antiviral and antitumor properties.

Reduced inflammation: Stevia reduces inflammation.

Antidiabetic: Stevia has the ability to lower blood sugar levels and it also possesses antilipidemic properties.

Elicitation: Elicitation is a technique that increases the synthesis of secondary metabolites by introducing biotic or abiotic elicitors into the media. The elicitors like Salicylic acid, paclobutrazol, gibberellic acids, methyl jasmonate, and various auxin and cytokinin combinations are utilized *in vitro* stevia cultures.
Micropropogation of *Stevia rebudiana* is a cost-effective method. The production cost can be reduced by about 34% by doing away with the rooted culture media. The ratio of stevioside to rebudioside A, the primary steviol glycosides, was used to establish the sensory attributes. Using 0.02 mg/l NAA and 0.05 mg/l BAP, *Stevia rebudiana* tissue with a rebudioside A to stevioside ratio of 3.76 was created in a bioreacter. Compared to shoots produced in the field, this plant tissue had a greater flavor. Stevioside was more abundant in cell suspension than rebudioside A, and there was no discernible increase in steviol glycosides. The best outcomes for generating calluses from internodal and leaf segments have been found on media containing one cytokinin (BAP, Kn, or Z, 200 µg/L) and 2, 4-D (800 µg/L). Large-scale cultivation of *Stevia rebudiana* to increase the supply of its highly sought-after low-calorie sweetener glycosides is made possible by the research for plant-based therapy to cure diabetes. A sustainable and environmentally friendly method for producing secondary metabolites is plant cell and tissue culture, and recent advancements in gene editing and genome engineering offer new opportunities to enhance both the qualitative and quantitative aspects of these phytochemicals.
A plant tissue culture provide rapid clonal propagation of plants, disease free plants, high yield quality production and propagation of rare plants. It also offers a sustainable and efficient method for mass propagation and higher yields of the valuable natural sweetener, steviol glycosides. So, plant tissue culture of *Stevia rebudiana* can be a profitable venture for young Scientists.

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