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Research Article

PHYTOCHEMICAL SCREENING OF *STEVIA REBAUDIANA*-BERTONI A RARE SWEETNER

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ABSTRACT

Nature has store house of remedies to cure all ailments of mankind. Plants are valuable sources of a vast array of chemical compounds they synthesize and accumulate in various parts of plant body. From the ancient ages the plants have been used for medicinal uses and other useful proposes to humans. Phytochemicals known as metabolites present in the plants play a vital part in these processes. The present investigation was carried out to estimate the phytochemical constituents present in *Stevia rebaudiana* a plant which belongs to the Asteraceae family. *Stevia rebaudiana* is a herb with incredible sweetness that is gaining very high popularity amongst all type of sweetener users as most ideal substitute for sugar. It is a perennial and endemic, medicinal herb. It is also called as honey plant due to its sweetness. The fresh leaves have a nice liquorice taste. The main sweet component in the leaves of *Stevia rebaudiana* is stevioside. In the present study the methanol extract of the plant was subjected to qualitative phytochemical screening. The phytochemical analysis revealed that the presence of alkaloids and tannins in high concentration followed by flavonoids and glycosides, triterpenes and saponins are lesser amounts.

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INTRODUCTION

Plants are the richest source of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 BC and 800 BC give many references of medicinal plants. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials such as tannins, oils, gums, and precursors for the synthesis of complex chemical substances (Chhetri *et al.*, 2008).

Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerle, 1993). About 1500 plants are systematically used in the indigenous systems of medicine like Ayurveda, Unani and

+Siddha. However, the ethnopharmacologists, botanists, microbiologists, and natural-product chemists world over today are constantly in search of medicinal efficacy of plants and their phytochemicals, as the reported data so far available on plants are comparatively meagre considering the vast number of plant population (Joshi *et al.*, 2011). In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab *et al.*, 2003). Thus the present study is a preliminary attempt to identify some of the phytochemical constitutions of this rare and important medicinal plant.

Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibres to form an integrated part of defence system against various diseases and stress conditions (Dipak Koche *et al.*, 2010). The term phytochemicals is used to refer chemical compounds that occur naturally in plants metabolism or their development. In addition, all higher plants produce one or several representatives, called as secondary metabolites of *Stevia rebaudiana* are analysed in this study.

Stevia rebaudiana Bertoni is a new and versatile herb with incredible sweetness that is gaining very high popularity amongst all type of sweetener users as most ideal substitute for

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sugar. It produces sweet steviol glycosides. It is a high demanding antidiabetic medicinal plant belonging to Asteraceae family. It is perennial and endemic, medicinal herb (Sivaram and Mugundan, 2003). It is also called as honey plant due to its sweetness. The fresh leaves have a nice liquorice taste. It is recommended for diabetes and has been extensively tested on animals and has been used by humans with no side effects. The compounds obtained from *Stevia* are the best alternative natural sweetener for diabetes. Leaves of stevia contain sweetening compounds viz., Stevioside, Rebaudioside-A, Rebaudioside-B,C and six other compounds which are said to be having insulin balancing properties (Farooqi and Sreeramu, 2001). The purpose of this research work was to screen this plant for its phytochemicals. The demand of *Stevia* is increasing widely due to its non caloric nature and usages as natural supplement for sugar. The leaves are having commercial importance due to the presence of di-terpene sweet glycosides which are 300-400 times sweeter than sugar with any side effects.

MATERIALS AND METHODS

Stevia rebaudiana plant were obtained from Thiruvananthapuram, Kerala and was grown under protective conditions. The leaves are collected and dried under shade. After drying, the leaves were powdered using mixer grinder and then kept in well closed container. This power was extracted in the soxhlet using methanol and subjected to qualitative phytochemical screening for the identification of various chemical constituents using the method described by Trease and Evans (1987) and by Harbone (1973). The plant extract were screened for the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, triterpenes, glycosides, catechin, coumarin, quinone and xanthoprotein.

Test for Tannins: About 0.5g of the dried power was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and was observed for brownish green or a blue black coloration.

Test for Alkaloids: One millilitre of aqueous extract was stirred and placed in 1% aqueous hydrochloric acid on a steam bath. Then, 1 ml of the filtrate was treated with Dragendorff's and Mayer's reagent. Turbidity or precipitation with this reagent was considered as evidence for the presence of alkaloids.

Test for Glycosides: 0.5g extract of sample was dissolved in 1 ml water and then aqueous sodium hydroxide was added. Formation of yellow color indicated the presence of glycosides.

Test for Saponins: 0.5 g extract were dissolved in 10ml of distilled water in a test tube and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over a 30 minutes period of time. If a honey comb froth above the surface of liquid persists after 30 minutes the sample is suspected to contain saponins.

Test for Triterpenes: Ten millilitre aqueous extract was placed in a small beaker and evaporated to dryness. The residue was dissolved in 0.5 ml each of acetic anhydride and chloroform. The solution was transferred into a dry test tube and concentrated sulphuric acid was added. Brownish red or violet rings at the zone of the contact with the supernatant and green or violet coloration denoted the presence of sterols and triterpenes.

Test for Flavonoid (Shindo's test): To the test solution, a few magnesium turnings and a few drops of concentrated hydrochloric acid were added and boiled for five minutes. Appearance of red or orange red colour indicates the presence of flavonoids.

Test for Catechins

To the test solution, a few drops of Ehrlich reagent and concentrated hydrochloric acid were added. Appearance of pink colour indicates the presence of catechins.

Test for Coumarins

To 2 ml of the test solution, a few drops of alcoholic sodium hydroxide were added. Appearance of yellow colour indicates the presence of coumarin.

Test for Quinones

The test solution was treated with a few drops of concentrated sulphuric acid or aqueous sodium hydroxide solution. Colour formation indicates the presence of quinone compound.

Test for Xanthoproteins

To the test solution, a few drops of concentrated nitric acid and few ml of ammonia were added. Appearance of a red precipitate indicates the presence of xanthoprotein.

RESULTS

The qualitative analysis of *Stevia rebaudiana* was performed using the methanol solvent. The *Stevia* powder subjected to preliminary phytochemical screening using chemical methods showed the presence of biochemicals. Table 1 shows the results of this investigation. The most abundant compounds in the leaf extract were the glycosides. Alkaloids and tannins, were also seen in higher amounts but lesser than glycosides. Flavonoids and glycosides were seen in moderate levels, and triterpenes and saponins were seen in least amounts. The test for catechins, coumarins, quinones and xanthoproteins showed negative result.

Table 1 Phytochemical constituents of leaves of *Stevia rebaudiana*

| Phytochemical constituents | Results* |
|----------------------------|----------|
| Alkaloids | +++ |
| Flavonoids | ++ |
| Tannins | +++ |
| Glycosides | ++++ |
| Catechins | - |
| Coumarins | - |
| Saponins | + |
| Quinones | - |
| Triterpenes | ++ |
| Xanthoproteins | - |

+++ Strongly present, ++ Present, + Weakly present, - Absent

DISCUSSION

Phytochemicals are major source of dyes, flavours, sweetener, aromas, perfumes, insecticidous, ant parasitic drugs and many other substances. According to Komisserenko *et al.*1994 the dry extract from the leaves of *Stevia rebaudiana* contains sweet diterpene glycosides, flavonoids, alkaloids, water-soluble chlorophylls and xanthophylls, hydroxycinnamic acids caffeic, chlorogenic etc. neutral water-soluble oligosaccharides, free sugars, aminoacids, lipids, essential oils and trace elements. Preliminary phytochemical screening revealed the presence of

alkaloids, tannins, flavonoids, glycosides, triterpenes and saponins in methanol extract of *Stevia rebaudiana*. These results give a picture that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowera, 1993). Methanol is a good solvent for extraction of compounds which has a polarity index of 5.1. Mostly methanol is used for extraction various polar compounds but certain group of non polar are fairly soluble in methanol if not readily soluble. Therefore methanol is commonly used for extraction of bioactive compounds (Marcus, 1983). More over methanol among all the alcohols has low boiling point 0 first 65 degrees Celsius. This may be the reason for maximum expression of metabolites in methanol.

The presence of these phytochemicals in the investigated medicinal plant would be responsible for the antimicrobial activity of the plant too.

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