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A Review: Natural Sweetener from Stevia (*Rebaudiana bertoni*) Leave

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ABSTRACT

Stevia (*Rebaudiana bertoni*) leave is an ancient perennial shrub mostly found in South America (especially Brazil and Paraguay). It contains a low-calorie sweetener, which is about 300 times sweeter than sucrose. Natural sweeteners extracted from plant and artificial or synthetic sweeteners produced from industires, are the two types of sweeteners that are available. Sweetening agents induce and enhance sweet taste which makes it favorable for consumption. Natural sweetening agents are more advisable to use than artificial sweetening agents because they do not have any side effect and is of no harm to health. In this work, the background of stevia leave, uses, and extraction of natural sweetener is reviewed.

INTRODUCTION

Natural sweetening agents are low calorific, harmless and super sweet (100 to 10,000 times sweeter than sugar), capable of overcoming the problems of artificial sweeteners. Natural sweeteners are useful with sugar and alternatives for diabetic patients. The active sweet principles stored in plants can be grouped under: terpenoids, steroidal saponins, dihydroisocoumarins, dihydrochalcones, proteins, polyols, and volatile oils etc. (Priya *et al*., 2011). Stevia (*Rebaudiana bertoni*) is an ancient perennial shrub mostly found in South America, especially in Paraguay and Brazil (Chang *et al*., 2005). It contains a glycoside that are low calorie sweeteners, which is about 300 times sweeter than sucrose (Ilca *et al*., 2017; Lemus-mondaca *et al*., 2012; Witono and Chandra, 2020). The leaf extract of Stevia possesses many phytochemicals, which include austroinullin, β-carotene, dulcoside, nilacin, rebaudioxides, riboflavin, steviol, stevioside, and tiamin, with known antimicrobial properties against many pathogens (Theophilus *et al*., 2015). Stevia extracts have therapeutic properties, antioxidant effect, antimicrobial and antifungal activity. Lemus-mondaca *et al*., (2012) reported different techniques for the extraction of stevioside from stevia leaves which include solvent extraction, chromatographic adsorption, ion exchange, selective precipitation, membrane processes and supercritical fluids. This study is limited to the review of stevia (*Rebaudiana bertoni*) leaves, its usage and extraction of natural sweetener.

LITERATURE REVIEW

Sweetness was considered as determinant of food safety to primitive people as they developed the desire for sweet taste as a means of survival. Decades in the history of Homo sapiens, sweetness could only be satisfied by consuming sweet-tasting fruits and vegetables. Sweet-tasting foods were found to be safe, while bitter-tasting foods were mostly toxic (Sardesai and Waldshan, 1991). For a long time, food was made more flavorful and attractive to consumers, especially when sweeteners are added to the food. Sweeteners were used for their high-calorie, and and help increase the body weight of infants and children (Saraiva *et al*., 2020). Sweetening agents are materials that promote sweet taste in food, thereby motivating a consumer to take certain food. Sweetening agents are classified into two types:

a. Synthetic sweetening agents
b. Natural sweetening Agents

Research shows that many synthetic sweeteners that are used are found to be unsafe, non-nutritive, carcinogenic, and a causative agent for weight gain, dental caries and chronic diseases. Hence, the demand for natural sweetening agents increased (Priya *et al*., 2011). In addition, sweeteners must be non-toxic in nature, it must have sugar-like taste, have low calorific value, heat and pH stability (Zhang *et al*., 2000). Sugar is mostly used as sweetening agent in food industries. However, the need for other sweeteners is increasing in response to consumer preference (Zhang *et al*., 2000). Sugar is a natural sweetener extracted from sugar cane or beet that provides 4 calories/gram. An excess consumption of sugar increases energy, but in turn, can lead to weight gain and chronic diseases associated with obesity and dental care. Obesity is a risk factor for heart disease, cancer, diabetes, and some other diseases. Therefore, there is a need for sugar substitutes, which can help to reduce caloric intake, particularly in overweight individuals (Sardesai and Waldshan, 1991). According to Priya *et al*., (2011), uses of natural sweetening agents includes;

Pharmaceutical Uses

1. In pharmaceutical industries these are used in liquid, oral preparations, lozenges, pills and tablets.
2. In liquid orals, sugar is used to prepare syrup base, to maintain the consistency and viscosity of the preparation and to mask the bitter taste of the drug.

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3. Sugar is also employed in the coating of pills and tablets.

4. Honey plays an important role in Ayurvedic system of medicine. It is used as an important vehicle for many preparations.

Food Industry

Sweetening agents are used to prepare jams, chocolates, sweets, ice-creams, cakes, candies, juices, soft-drinks, beverages, chewing-gums and many other food items.

Several sweet plants were used commercially as sucrose substitute (Kinghorn *et al.*, 1998). Over 140 plant materials have been investigated and were found to taste sweet because they contain large amounts of sugars, polyols or other sweet constituents. Although, the sweeteners may contain calorie similar to carbohydrate, their contribution to energy intake is negligible in the amounts used (Sardesai and Waldshan, 1991). Figure 1 presents some typical plants that were traditionally used as natural sweeteners. Priya *et al.*, (2011) presented thirty-seven different plants with their family name, sweetening part, sweetening principle, chemical class and sweetening concentration compared to conventional sugar as shown in Table 1.

Properties of Sweetening Agents

Figure 1: Typical plants that were used as natural sweetener

Table 1: Various sources for different natural sweetening agents with their sweetening properties

Plant	Family	Part	Sweetening Principle	Chemical Class	Times Sweeter
Abrus Precatoris	Leguminosae	Leaves, roots	Abrusosides and glycyrrhizin	Triterpene glycosides	30-100
Achras sapota	Sapotaceae	Latex and fruit	Glycyrrhizin	Triterpene glycosides	100
Baccharis gaudichaudiana	-	Aerial parts	Gaudichaudioside-A	Diterpene Glycosides	100
Beta vulgaris	Chenopodiaceae	Roots	Sucrose	Disaccharide	-
Cinnamomum osmophloeum	Lauraceae	Leaves	Trans-Cinnamaldehyde	Aromatic aldehyde	50
Citrus aurantium	Rutaceae	Peels of the fruits	Neohesperidin dihydrochalcone	Dihydrochalcone	1000
Citrus limoni	Rutaceae	Peels of the fruits	Hesperidin dihydrochalcone	Dihydrochalcone	300
Citrus sinensis	Rutaceae	Peels of the fruits	Hesperidin dihydrochalcone	Dihydrochalcone	300
Citrus paradise	Rutaceae	Peels of the fruits	Naringin dihydrochalcone	Dihydrochalcone	1000
Cyclocarya palirus	-	Leaves	Cyclocaryoside	Steroidal saponins	250
Cynara scolymus	Asteraceae	Leaves and flowers	Cynarin	Protein	-
Dioscoreophyllum Cuminsii	Menispermaceae	Fruit pulp	Monellin	Protein	2500

Eremophila glutinosa	-	Entire plant	-	Dihydroflavonols	400
Foeniculum vulgare	Umbelliferae	Fresh aerial Parts	Trans-anethole	Phenylpropanoid	-
Glycyrrhiza glabra	Leguminosae	Roots and stolons	Glycyrrhizin	Triterpene glycosides	100
Hemsleya carnosiflora	-	Rhizomes	Carnosiflosides-V, VI	Triterpene glycosides	-
Hydrangea macrophylla	Saxifragaceae	-	Phyllodulcin	Dihydroisocoumarin	300-400
Illicium verum	Illiciceace	Dried fruits	Trans-anethole	Phenyl propanoid	-
Lippia dulcis	Verbenaceae	Herb	Hernandulcin	Sesquiterpene	1000-1500
Myrrhis odorata	Apiaceae	Fresh roots	Trans-Anethole	Phenyl propanoid	-
Osmorhiza longistylis	Apiaceae	Fresh roots	Trans-Anethole	Phenyl propanoid	-
Perilla frutescens	Labiatae	Leaves,seeds and flowering tops	Perillartine	Monoterpenoid	400-2000
Periandra dulcis	-	Roots	Periandrin V	Triterpene glycosides	100-200
Piper marginatum	Piperaceae	Dried leaves	Trans-anethole	Phenyl propanoid	-
Polypodium glycyrrhiza	Polypodiaceae	Rhizomes	Polypodoside	Steroidal saponin glycosides	600
Polypodium vulgare	Polypodiaceae	Rhizomes	Osladin	Steroidal saponin glycosides	50-100
Pterocarya paliurus	-	Leaves and stem	Pterocaryoside A and B	Secodammaranoid saponin	50-100
Rubus suavissimus	Rosaceae	Leaves	Rubusoside and Sauvioside A	Diterpene glycosides	-
Saccharum officinarum	Poaceae	Canes	Sucrose	Disaccharide	-
Smilax glycyphylla	Liliaceae	All parts	Glycyphyllin	Dihydrochalcone glycosides	100-200
Staurogyne mergunsis	-	Leaves	Strigin	Steroidal saponin Glycosides	-
Stevia rebaudiana	Asteraceae	Leaves	Steviosides	Tricyclicditerpenoid Glycosides	200-300
Siraltia grosvenorii	-	-	Mogroside V	Triterpene glycosides	250
Symplococos paniculata	Symplocaceae	Leaves	Trilobatin	Dihydrochalcone Glycosides	400-1000
Synsepalum dulcifucum	Sapotaceae	Fruits	Miraculin	Protein	-
Tessaria dodoneifolia	-	Aerial parts	Dihydroquercetin-3-Oacetate 4-(methyl ether)	Dihydroflavonol	-
Thamatococcus	Marantaceae	Aril of the fruit	Thaumatin	Protein	3000

Source: Priya et al., 2011

According to Priya *et al*., (2011), sweetening agents should possess the following ideal properties:

1. Effectiveness when used in small amount.
2. Nontoxic
3. Low or non-calorific.
4. Compatibility with other ingredients in formulations.
5. It should be cheap and available.

Stevia (*Rebaudiana bertoni*)

Stevia (*Rebaudiana bertoni*) is commonly known as candyleaf, sweetleaf or sugarleaf (Jamil *et al*., 2020). In other terms, stevia is also called sweet lead flowering plant in the aster family (asteraceae), normally grown for its sweet taste (Ahmed, 2020). Stevia is also known as honey leaf because of its powerful sweet taste, the typical stevia plant and zoom portion of it's leaves is shown in Figures 2 and 3, as reported by Ferrazzano *et al.,* (2015).

Stevia (*Rebaudiana bertoni*) is a small perennial shrub that grow up to 65-80 cm in height, with directly, oppositely arranged

Figure 2: Stevia (*Rebaudiana bertoni*) plant

Figure 3: Zoom portion of stevia (*Rebaudiana bertoni*) leave

leaves. Stevia is a semi-humid subtropical plant that can be grown easily even in kitchen garden just like any other vegetable crop. The shrub grows in a well-drained red sandy loam soil with pH range of 6.5-7.5. Saline soils should be avoided during cultivation of this plant (Goyal *et al.*, 2010). As reported by Gupta *et al.*, (2013), stevia flowers are small in size, white in color, with a light purple throat and arranged in the form of small corymbs as shown in Figure 4.

According to Kobus-Moryson and Gramza-Michalowska (2015) and Yadav *et al.*, (2011), stevia (*Rebaudiana bertoni*)

Figure 4: Stevia leave flowers

grows naturally at subtropical meadows at an altitude ranging from 200–500m above sea level, with temperatures ranging from –6°C to 43°C, and average temperature of 23°C. The rainfall requirement of stevia is between 1500–1800 mm, the shrub possesses ellipse-shaped leaves and blooms in white. The conditions for the cultivation of stevia may vary. Stevia grows both on dark, damp, sandy soils and on loamy, highly-permeable soils. In addition, it is found on the banks of swamps, on infertile, acidic sands or muck soils.

Origin of Stevia

The genus, steivia, consists of about 200 species of herbaceous, shrub and sub-shrub plants (Shivanna *et al.*, 2013), and is one of the most distinctive genera within the tribe eupatorieae, because of the morphological uniformity of its flowers and capitula, which consist of five tubular flowers and five involucral bracts. It is distributed in the southwestern United States southward through Mexico and Central America. It is also found in non-Amazonian South America, southward to Central Argentina. In Brazil, more than 30 species have been found, distributed mainly in southern and central regions. Stevia (*Rebaudiana bertoni*) originated in the highland regions of northeastern Paraguay (on the Brazilian border), between latitudes 23^0 and 24^0, where the unique sweetening power of its leaves and its medicinal properties have been known by the local Guarani Indians many centuries ago. The first seeds were exported to the United Kingdom where it could not be brought under cultivation. In 1968, it was exported to Japan, and from there, awareness of and cultivation of the plant spread throughout the world. Subsequently, the crop has been introduced to many countries, including Brazil, Korea, Mexico, the United States of America, Indonesia, Tanzania, Canada and India (Claudio Gardana *et al.*, 2003; Ferrazzano *et al.*, 2015; Jamil *et al.*, 2020; Yadav *et al.*, 2011).

Botanical Classification

Yadav *et al.*, (2011) reported the botanical classification of stevia (*Rebaudiana bertoni*) as given in Table 2.

Uses of Stevia

Table 2: Botanical descriptions of stevia

	Description
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Group	Monochlamydae
Order	Asterales
Family	Asteraceae
Subfamily	Asteroideae
Tribe	Eupatorieae
Genus	Stevia
Species	Rebaudiana

Source: Yadav et al., 2011

According to Wolwer-Rieck (2012),, stevia (*Rebaudiana bertoni*) is a perennial herb of significant economic value due to its high content of natural, dietetically valuable sweeteners in its leaves. For many years, dried stevia leaves have been used as natural sweeteners, and the extract are approved as food additives in many countries throughout the world. The use of stevia (*Rebaudiana bertoni*) has a vital role in treatment of numerous diseases. There is no evidence of harmful effects from the consumption of stevia (*Rebaudiana bertoni*) extracts. Stevia contains a high percentage of phenols and flavonoids, which makes it possess a high antioxidant activity (Asrani and Thakur, 2020).

Health Benefit of Stevia

Many researchers have reported that stevia (*Rebaudiana bertoni*) has many health benefits. Among them;

Effect on Obesity

The excess consumption of sugar has led to several nutritional and medical problems, such as obesity and other diseases (Abou-Arab *et al*., 2009). Obesity is one of major health challenge, mostly affecting adults and children. Too much intake or consumption of sugar-rich foods and beverages has been one of the main causes of obesity. Elnaga *et al*., (2016) reported that, overweight and obesity are major health challenge worldwide and its risk factor, results in heart disease, diabetes, cancer, hypertension, arthritis, and other musculoskeletal problems. Hence, the need for substitution of those sweeteners with a non-nutritive effect that promotes body weight reduction, is paramount.

Stevia can take the role of a non-nutritive sweetener, and also take the place of other more calorie-dense sweeteners (e.g., sucrose). Simply because, high-purity stevia leaf extracts are considered as having zero calories while providing a taste which is 100–300 times as sweet as that of sucrose (Jamil *et al*., 2020).

Effect on Diabetes Mellitus

Diabetes mellitus is a metabolic disorder caused as a result of insulin resistance, a defect in insulin secretion or both, characterized by hyperglycemia (Jamil *et al*., 2020). Apart from hyperglycemia, other factors including dyslipidemia or hyperlipidemia are involved in the development of micro and macrovascular complication of diabetes, which are the causes of morbidity and death. Research on plant products to treat diabetes mellitus is growing by the day, as they contain many bioactive substances with therapeutic potential (Vishnu *et al*., 2010).

According to World Health Organization (WHO), diabetes mellitus is projected to become the seventh leading cause of death worldwide by 2030 (Ahmad and Ahmad, 2018). Stevia leaf extract has been used traditionally in the treatment of diabetes (Gupta *et al*., 2013). Jamil *et al*., (2020) reported that the stevioside (Stevia leave extract) not only regulate the blood glucose levels through the enhancement of insulin secretion in a dose-dependent manner, thereby increasing the glucose utilization, but also decrease the gene expression of phosphoenol pyruvate carboxykinase (PEPCK) - a rate-limiting enzyme for gluconeogenesis expressed mainly in the liver-in a dose-dependent manner (Jamil *et al*., 2020). Research shows that the use of stevia (*Rebaudiana Bertoni*) for the treatment of diabetes, is safe and non-carcinogenic compared to the use of synthetic drugs for the treatment of diabetes which result in many complications (Ahmad and Ahmad, 2018).

Effect on Hypertension

Hypertension is often referred to as a "silent killer", it leads to congestive heart failure, myocardial infarction, aneurysm, left ventricular hypertrophy, atrial fibrillation, peripheral vascular disease, stroke, hypertensive nephropathy and hypertensive retinopathy. Mild to moderate hypertension can remain asymptomatic for many years. Both hypertension and its related disabling conditions could be reduced if it is detected early and treated effectively through anti-hypertensive medications and adequate lifestyle changes (Jamil *et al*., 2020). A mechanism for treating hypertension was reported by Jamil *et al*., (2020), which is the inhibition of angiotensin-converting enzyme activity. A study of ethanol extract of stevia leaves, steviol glycosides (with 95% purity) and stevia leaf protein hydrolysates, strongly inhibited angiotensin converting enzyme activities. As such, they are helpful for regulating hypertension.

Effect on Dental Caries

Dental caries is an infectious disease of the mouth that affect the teeth resulting in the growth of harmful bacteria in the mouth that promote the formation of plaque such as making holes, cavities and decay in the teeth. The common cause of dental caries is the ingestion of diet (carbohydrates), mainly sucrose, which can be fermented into acids, leading to the demineralization of the tooth. Hence, an important step to preventing dental caries is by finding a healthy and non-toxic substitute to sucrose which does not promote formation of caries (Gupta *et al*., 2013; Jamil *et al*., 2020). The use of stevia leaf extracts does not cause cavities and tooth decay. Rather, it shows potential antimicrobial activities against pathogenic bacteria. Stevia can play a beneficial role in preventing dental caries, as it is used in dental care products to decrease plaque acidogenicity and cariogenic microflora (Asrani and Thakur, 2020).

Forms of Stevia Usage

According to Kumar *et al*., (2015), stevia appears as fresh leaves that can be dried, processed into powder, liquid, alcohol based extracts, or even tablets. While according to Singh and Rao, (2005), the forms for the usage of stevia depends upon the amount of sweetness required; either sweeter, moderate or sweetest, even though the white crystals are the sweetest.

Fresh Stevia Leaves

The herb in this form of stevia is mostly natural at unrefined state. A leaf taken from a stevia plant and chewed, will impart an extremely sweet taste sensation reminiscent of licorice that may last for quite a while. To have a more practical application as a tea or sweetener, the leaves must be dried or put through an extraction process, which makes the sweet taste even more potent. Figure 5 shows a typical fresh stevia leave.

Figure 5: Fresh stevia leaves

Dried Leaves

Drying and crushing of stevia leaf is necessary in order to get more flavour and sweet constituents. A dried leaf is considerably sweeter than a fresh one, and is the form of stevia used in brewing herbal tea. Dried stevia leaf may come in bulk or packaged like tea bags. It can also be finely powdered. In this form, it is 15-20 times sweeter than sugar (one teaspoon of stevia is approximately equivalent to one cup of sugar). It has a greenish colour and can be used in a wide variety of foods and beverages, including coffee, sweets, confectionaries and several edible dishes. It can also be used to make herbal tea blend. Its distinctive flavour is reminiscent of licorice, which will blend very well with different aromatic spices, such as cinnamom and ginger. Figure 6 (a), (b) and (c) shows stevia leaves before dehydration, after dehydration, and the crushed form (powder).

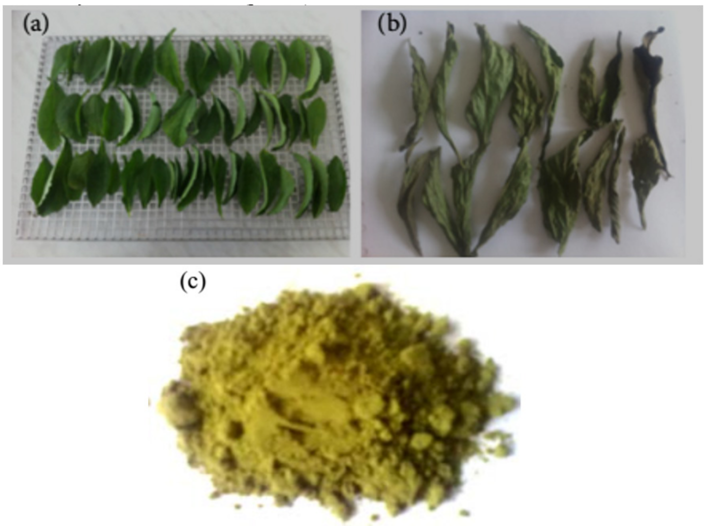

Figure 6: (a) Stevia leaves before dehydration process (b) Stevia leaves after dehydration (c) Crushed dried stevia leave (powder)

Stevia Extracts

The form in which stevia is primarily used as a sweetener in Japan is that of a white powdered extract. In this form, it is approximately 200 to 300 times sweeter than sugar (by weight). This white powder (as in Figure 7), is an extract of the sweet glycosides (natural sweetening agents) in the stevia leaf. Not all stevia extract powders are the same. The taste, sweetness and cost of the various white stevia powders will likely depend on their degree of refinement and the quality of the stevia plant used. Companies have also been planning to isolate the active compound of sweet glycosides in purest form for different commercial and medicinal uses.

Figure 7: Stevia leave crystals

Liquid Concentrates

These come in several forms. They are syrupy black liquid (as shown in Figure 8) which results from boiling the leaves in water, also capable of enhancing the flavour of many foods. Steeping stevia leaves in distilled water or a mix of water and grain alcohol makes another type. A liquid can also be made from the white and preserved mixed with water, and preserved with grapefruit seed extract.

Figure 8: Liquid Concentrate of stevia leave

Extraction of Stevioside

Several techniques were used by different researchers for the extraction of sweetener from stevia (*Rebaudiana bertoni*) leaves among which are;

Conventional Extraction Method

Stevioside is extracted from stevia leaves using an aqueous or alcohol as solvent, followed by precipitation, coagulation and crystallization. Abou-Arab *et al*., (2009) uses dried stevia leaves sample and extracted stevioside by using water, methanol and methanol/water (4:1) extraction.

For the first extraction solvent (water), the aforementioned author mixed the dried ground leaves with hot water (65ºC) at different percentage of powder leaves/water ratio of 1:15, 1:25, 1: 35, 1:45, 1:55, 1:65 and 1:75 (w/v). The extraction took 3hours, and extract containing stevioside was filtered through Whatman No. 4 filter paper, which was purified by addition of 5% $Ca(OH)_2$. The addition of $Ca(OH)_2$ was repeated twice and the filtrates was collected and passed through ion exchange column (packed with Amberlite IR-4B resin) to remove the undesirable colors at a rate of 1ml/sec at 25ºC. The pigments were adsorbed on resin and eluted (clear and colorless solution), containing stevioside was collected, then concentrated using rotary evaporator at 45 ºC to the maximum concentration value.

For the second extraction solvent (methanol), methanol was added to dried leaves at ratio (4:1 v/w) and remained for 7 hours before it was filtered through Whatman No. 4 filter paper. The filtrate was evaporated to dryness by using rotary evaporator at 45 ºC. The residue was washed with ether and then extracted 3 times with butanol. The organic phase was evaporated and the residue was recrystallized at -5 ºC for whole night.

For the third extraction solvents (methanol/water 4:1), the solvents were mixed at the ratio of (4:1), added to the dried leave at (4:1 v/w), left for 7 hours and then filtered through Whatman No. 4 filter paper. The filtrate was then evaporated to dryness by using rotary evaporator. The residue was washed with ether and then extracted 3 times with butanol. The author reported that methanol/water extraction was found easier and simple compared to water extraction.

Pressurised Fluid Extraction

Pressurized fluid extraction (PFE) is now the popular substitute to Soxhlet extraction because of its multiple rate and lower solvent consumption (Karasek *et al*., 2007). The aforementioned author, uses PFE method to extract stevioside from stevia, using both water and methanol (as different solvents), where they compared the extraction effectiveness of both. If water is used as a substitute to organic solvent in PFE, the method is often referred to as "pressurized hot water extraction" (PHWE) or subcritical water extraction; which implies that the extraction temperature employed is below the critical temperature of water.

Karásek *et al*., (2007) reported the result of a comparative study of the two extraction methods, PFE employing

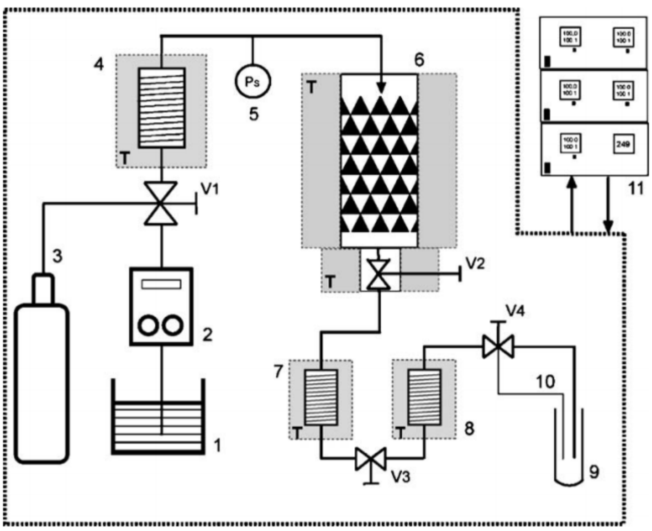

Figure 9: High-pressure extraction apparatus for pressurized fluid extraction (PFE) and pressurized hot water extraction (PHWE)

methanol and (PHWE), for stevia leaves.

A laboratory constructed apparatus was designed for both PFE and PHWE by Karasek *et al.*, (2007), as shown in Figure 9, where its description is presented in Table 3. The solvent reservoir was equipped with a purging unit for removing residual oxygen by gently stripping with helium. The water or methanol was delivered to the extraction vessel using an LC1120 high-performance Liquid Chromatography (HPLC) and a pump working in constant flow rate mode. The solvent enters the extraction vessel, where it was preheated by an electronic controlled heater. The extraction vessel was placed in a thermostatic block that allowed heating up to a temperature of 350 °C. Microprocessor unit with proportional-integrated-derivative (PID) regulation is used to regulate temperature of the thermostatted block with an accuracy of ±0.1 °C. This means, the extract was moved from the extraction vessel to thermostatted decompression units independently in a pair of two, that can either heat or cool the extract.

Each thermostatted decompression unit consisted of stainless steel tubing (0.03-in. inner diameter) tightly wound on a thermostatted aluminum block. The temperatures of the decompression units depended on the extraction temperature and the boiling point of the solvent at ambient pressure.

Table 3: Description of Figure labelled instruments

Label	**Description**
1	Solvent reservoir
2	High-performance liquid chromatography pump
3	Nitrogen tank
4	Preheating unit,
5	Pressure sensor,
6	Extraction vessel placed in a thermostatted block
7, 8	Thermostatted decompression units,
9	Collection vial for extract,
10	Fused-silica restrictor,
11	Proportional-integrated-derivative control units,
V2, V3	Two-way valves
V1, V4	Three-way SSI valves
T	Thermoinsulation covers

Figure 9 represents the High-pressure extraction apparatus for PFE and PHWE and Table 3 describes the figure label instrument of High-pressure extraction apparatus for PFE and PHWE.

Microwave-Assisted Extraction

Yildiz-ozturk *et al.*, (2015) uses the microwave assisted extraction method, where the authors used about 2g of dried aerial parts of stevia and transferred it into a container with ethanol using a microwave. The extraction experiment was performed according to a 4 × 3 full factorial design. Temperature (40, 50, 90 ◦C), time (5, 30, 45 min), solid/liquid ratio (1:5, 1:10, 1:15 g/ml) and power (300, 400, 500 W) were the independent variables. The set process parameters were automatically controlled throughout the extraction process. The system was allowed to cool down to room temperature at the end of the extraction time for an additional 5 min before opening the containers. The extract was then concentrated to dryness at 55 ◦C in rotary vacuum evaporator.

Ultrasonically-Assisted Extraction

Yildiz-ozturk *et al.*, (2015) also reported the ultrasonic assisted extraction, where about 2 g of dried aerial parts of stevia was poured into a centrifuge tube containing ethanol by using ultrasonic bath. The independent variables were temperature (40, 50, 90 ◦C), time (30, 60, 90 min), solid/liquid ratio (1:5, 1:10, 1:15 g/ml) and power (50, 75, 100W). The extract was concentrated to dryness at 55 ◦C in rotary vacuum evaporator.

Enzymatic Extraction

Puri *et al.*, (2012) utilizes enzymatic extraction, where three different enzymes were used; pectinase, cellulase and hemicellulase, at five different concentrations (0.5%, 1%, 2%, 3% and 4%, w/v), different extraction temperatures (35, 45 and 55°C for pectinase; 40, 50 and 60°C for cellulase; 50, 60 and 70°C for hemicellulase) and extraction time of 15, 30 and 45 min. The authors placed 100 g of dried leaf powder in a 500 ml conical flask with different enzyme concentrations in 100 ml of acetate buffer at pH 5.0. The contents were shaken at 150 rpm after the addition of methanol (200 ml, 100%), which was repeated for each time and temperature. The resultant extract was filtered with Whatman filter paper. The filtrate was collected and the above procedure was repeated three times for each condition. Toluene (100 ml) was added to the resultant filtrate and the resultant mixture was passed through an alumina column preconditioned with isopropyl alcohol (150 ml). The eluent was concentrated by boiling for half an hour before cooling to room temperature. Chilled acetone was added in small amounts until precipitation occured, before being refrigerated for 24 h. The solution (25 ml) was centrifuged at 10,612g for 30 min at 4 °C and was filtered through a syringe filter (pore size 0.22 µm).

CONCLUSION

From the foregone, stevia (*Rebaudiana bertoni*) leave was observed to be a shrub/leaf with numerous importance and usage. It is hereby recommended that government should collaborate with agencies, industries and companies to commercialize the production of stevia leave which has many medicinal benefits. Other methods of extraction should be compared with the previous research for improvement. Also, the yield of sweetener should be optimized during extraction of sweetener.

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