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A Review of the Comparative and Qualitative Analysis and Examination of Stevioside in Stevia Using Different Evaluative Techniques with Pharmacological & Pharmacognostics Effects

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Abstract- The perennial herb Stevia rebaudiana, which belongs to the Asteraceae family, is a well-liked natural sweetener that performs better than traditional high-potency sweeteners in terms of both sensory and functional aspects. Sweet glycosides found in stevia rebaudiana leaves have physiologic effects. Measurements of the other glycosides present in extracts are necessary since, besides the main glycosides, they could potentially be involved in activity. The identity of dulcoside A, steviolbioside, rebaudioside C, and rebaudioside B was confirmed using the isocratic HPLC technique. The International Conferences on Harmonisation (ICH) was utilised inthe creation and approval of the HPLC method for the concurrent evaluation of steviolbioside, stevioside, and rebaudioside-A (harmonisation) standards in Stevia rebaudiana leaves. Using liquid chromatography with high (High-performance liquid) as the reference method, Utilising near-infrared reflectance spectroscopy (NIRS), the amount of glycosides of (SGs that are) percentage (weighted average drying basis in Stevia leaves was measured. Steviol-glycosides are calorie-dense, noncariogenic sweeten found in stevia rebaudiana stems, which may be beneficial to human health. Animal studies have revealed that the aglycone steviol can have unfavourable effects, even though steviol-glycosides are typically thought to be harmless. Stevioside were identified by using ultra-high performance liquid chromatography coupled with electrospray the ionisation mass chromatography (UHPLC-MS) and 2D NMR methods. Next, an internal standard was used to measure the steviol glycosides using NMR of anthracene.

Keywords: Stevioside, the high-performance liquid HPTLC, and The steviol Sugars

Introduction-The little perennial Stevia rebaudiana grows to a height of 65 to 80 cm and has sessile, oppositely orientated leaves. It's a semihumid subtropical plant that grows well in a kitchen garden. Early in the 1970s, stevioside—the primary sweetener present in the leaf and stem tissues of stevia—was given considerable attention as a sugar substitute thanks to a Japanese partnership whose purpose was to commercialise stevioside and stevia extracts. Because stevia leaves have better sensory and functional properties to many other high-potency sweeteners, they are an important supply An alternative sweetened for the expanding culinary market. The dried extract of stevia leaves contains flavonoids, alkaloids, hydroxycinnamic acids, chlorophylls, xanthophylls, oligosaccharides, free sugars, amino acids, lipids, and trace minerals.

(A)Stevia Leaf (B) Dry Stevia Leaf (C) Dry Stevia Leaf with Powder (D) Dry Stevia Leaf Powder




About 300 times sweeter than sucrose, stevioside, a stevia glycoside, may have health advantages. Among other health advantages for humans, The seeds of S. rebaudiana saccharine preparations have been used linked to anti-human rotavirus, anti-hypertensive, and anti-hyperglycemic effects. Steviol, the most common aglycone backbone, was analysed using liquid chromatography in conjunctionto identify the dulcet stevia glycosides using UV, MS, and ELS detection (2010) Accciola et al. One of the stevia glycosides called stevioside is about 300 times sweeter than saccharose, thus people who suffer from cardiovascular disease, type 2 diabetes, being overweight, or dental cavities may find it very helpful. Tea, milk shakes, biscuits, grapefruit juice, hamburger buns, the chikki, basen ladu, grain ladu, jelly, and fruit custard included stevia. Increased quantities of the antinutritional chemical oxalic acid were discovered in it, which may lower the gastrointestinal absorption of iron, calcium, and other minerals. Glycosides such as steviolbioside, stevioside, rebaudiosides A–E, and dulcoside A are responsible for the sweet taste sense. All eight entkaurene glycosides, including stevioside, are present in the sweetest species, S. rebaudiana Bertoni. Together with campesterol, b-sitosterol, stigmasterol, and steviol—an enzymatic hydroxylation result obtained from plants—it also contains other compounds. Some species of stevia include other, non-sweet-tasting chemicals, Several of them could even be disagreeable. Two kinds of seeds exist. in stevia: tan and black. Tan seeds are nonviable because they are not fertilised; black seeds have a higher chance of germination and viability. [1,2,3,4,5,6,7] . **Extraction Method-** The leaves of S. rebaudiana (Bertoni) were pulverised, filtered through a screen that was about 380 metres in diameter, and after which it was dried at 80degrees Celsius up till the mass did not change. Five grammes of the powder and extraction solvent were mixed together in an extraction container. The samples were homogenised using High Speed Shear Homogenization after being warmed for five minutes in a water bath at a steady temperature. For extraction, an FA25 system was employed. The solid-to-liquid ratio was adjusted from 1:10 to 1:30, the extraction time was extended from 2 to 12 minutes, and the The amount of ethanol present was increased from 0% to 100%.After the process of extraction, medium-speed filter paper was used to vacuum-filter the mixture, and the waste product was gathered. The mixture for extraction experienced a 0.22 millimetre process HPLC was used to determine the nature of the membrane as well as the values for STV & RA. Three extractions were made for each step. 60 minutes of extraction time, a 1:1 solid-liquid ratio, 50% ethanol concentration, and 50°C temperature, and a single extraction were the ideal parameters for SE extraction.[8,10]

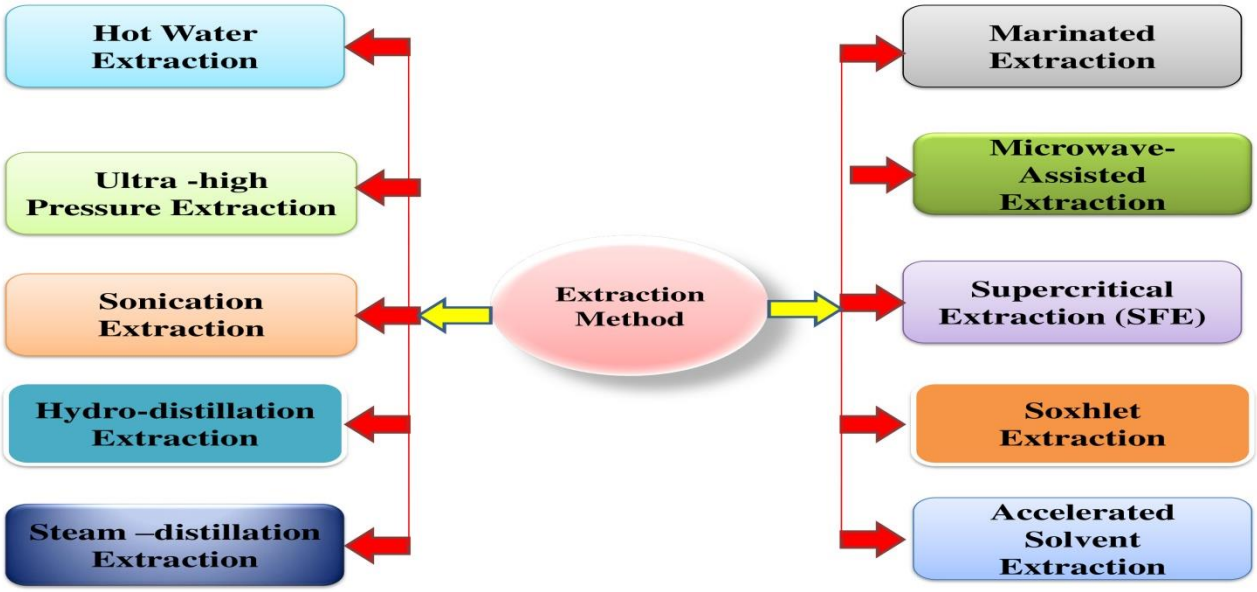


Fig. Type of Extraction Method

Chemical Composition- Of the eight Stevia glucosides found, stevioside, dulcosides A through E, and steviobioside were found. Along with These include stigmasterol, and sitosterol in, and campesterol as well as the triterpene amyrin alcohol, the leaves also contained three esters of lupeol. Stevia flowers have been used to extract jhanol, austroinulin, 6-O-acetylaustroinulin, 7-Oacetylaustroinulin, stevioside, and rebaudioside A. This compound, which is quercetin-3-O, kaempferol-3-O-rhamnoside, luteolin-7-O-glucoside, and apelin-4'-O-glucoside -glucoside are among the six flavonoid glycosides.

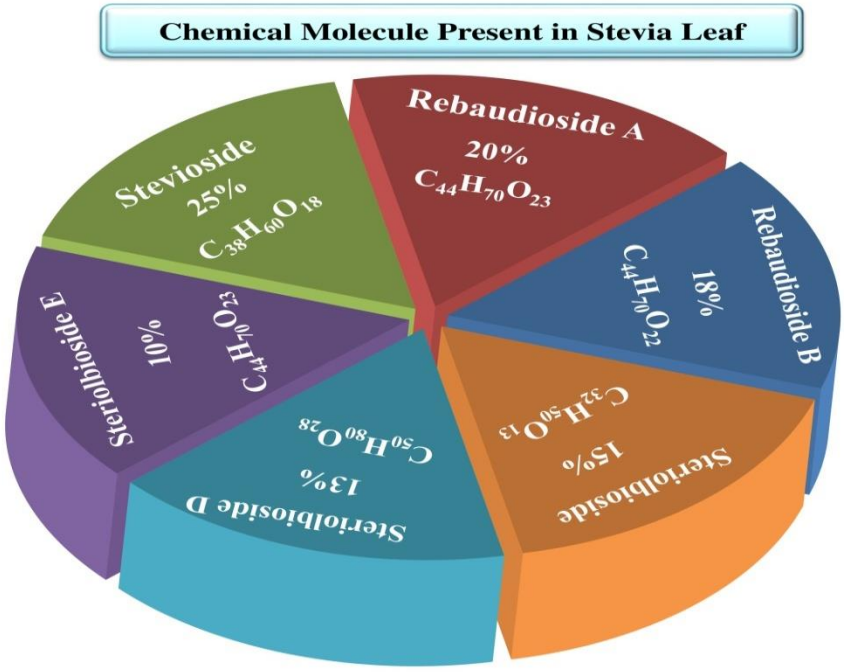


Fig. Chemical Molecule in Stevia

glucoside, Aqueous centauredin is a trimethoxy flavone from which6,4'-d-galactopyranoside and 5,7,3'-trihydroxy-3, and quercetin-3-O-arabinoside have been extracted.The essential oil included the sesquiterpenes a compound called car, trans-farnesene, humulene, candinene, a substance called car oxide, and nerolidol in addition to the substances linalool, terpinen-4, and terpineol. From S. rebaudiana I extracts, two glucosyltransferases (GTases I and IIB) acting on steviol and steviol-glycosides were found. On steviol, a unique transferase called GTase IIA was seen to be at work. The conversion of the UDP glucose to steviol glycol monoside (steviol-13-O-glucopyranoside) and the substance glycol involves a glucose transfer.was catalysed by purified GTase I (subunit Mr 24,600), but not to additional steviol-Glycosides. Mr. 30,700, subunit GTase IIB.acted About steviol, steviolbioside (13-O--sophorosyl-Steviol), steviolmonoside, & stevioside, demonstrating broad substrate specificity. The two main stevioside constituents are rebaudiosides A and C. Additionally, the organoleptic characteristics of dulcoside A, a Different diterpene glycosides made of steviol (ent-13-hydroxykaur-16-en19-oic acid) exist.[6,7,5]

USES[20]

Potential Reducing Diabetes Effects - Stevia leaf extract can lower plasma glucose levels and significantly enhance glucose tolerance because it has the ability to stimulate insulin's effect on cell

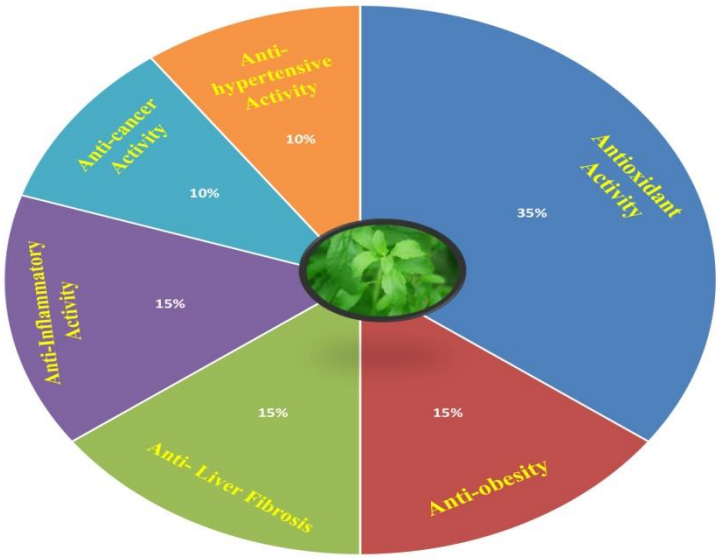


Fig. Application of Steviosides

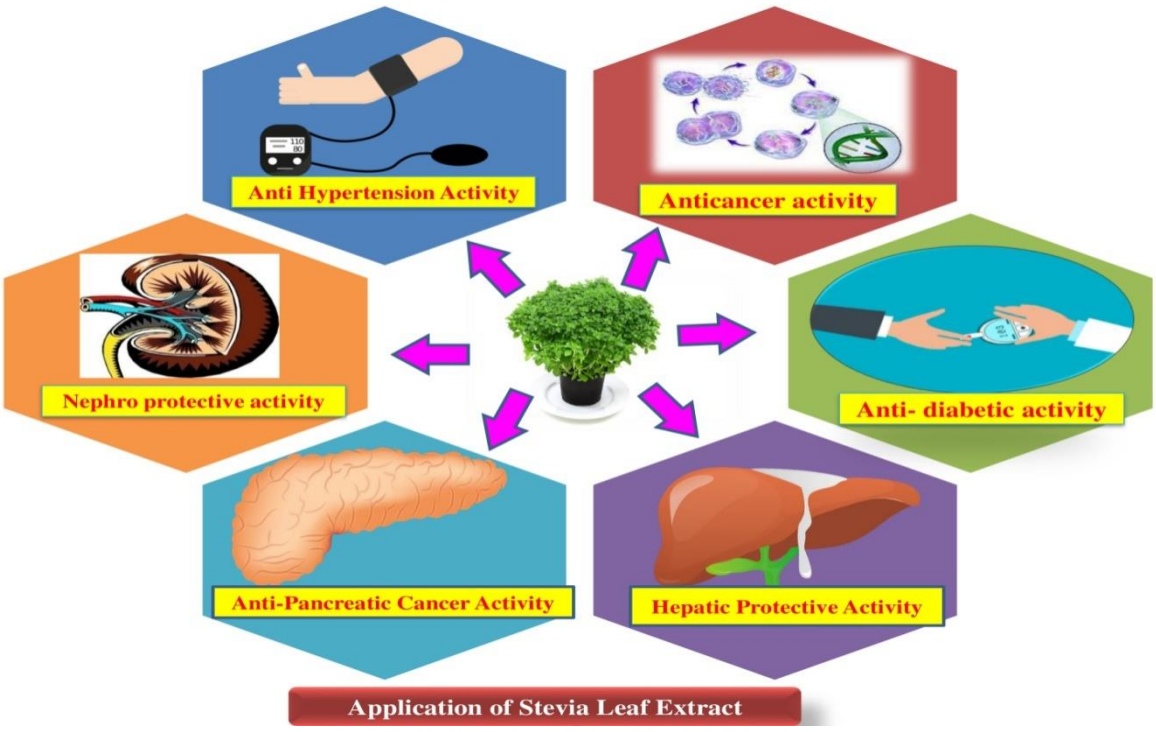


Fig. Application of Stevia Leaf

Blood Pressure Regulation- As a cardiac tonic, stevia regulates blood pressure, heart rate, and other cardiopulmonary functions. An extract of stevia leaf dissolved in hot water lowers The arterial blood pressure's moderate and maximum values in humans. Steviolglycosides, the sweeteners without calories present in S. rebaudiana leaves, have been shown to have a number of positive health effects, including lowering blood cholesterol levels, enhancing blood coagulation and cell regeneration, preventing the development of Cells associated with cancer & artery reinforcement (Atteh et al., 2008). By allowing the arteries to relax, they lower blood pressure.

Anti-Obesity Property- The natural sweetener found in stevia leaves, called ent-kaurene diterpene glycosides (stevioside and rebaudiosides), contains no calories and 300 times more sweetness than

sucrose. Thus, stevia keeps people from shedding the same amount of weight.

Function of the Renals - Melis (1992) investigated the results of the cultivation of S. rebaudiana stevioside leaves on renal function. It produces hypotension, diuresis, and natriuresis by acting as a conventional systemic vasodilator. Steviol and its counterpart are used in the treatment of polycystic kidney disease.

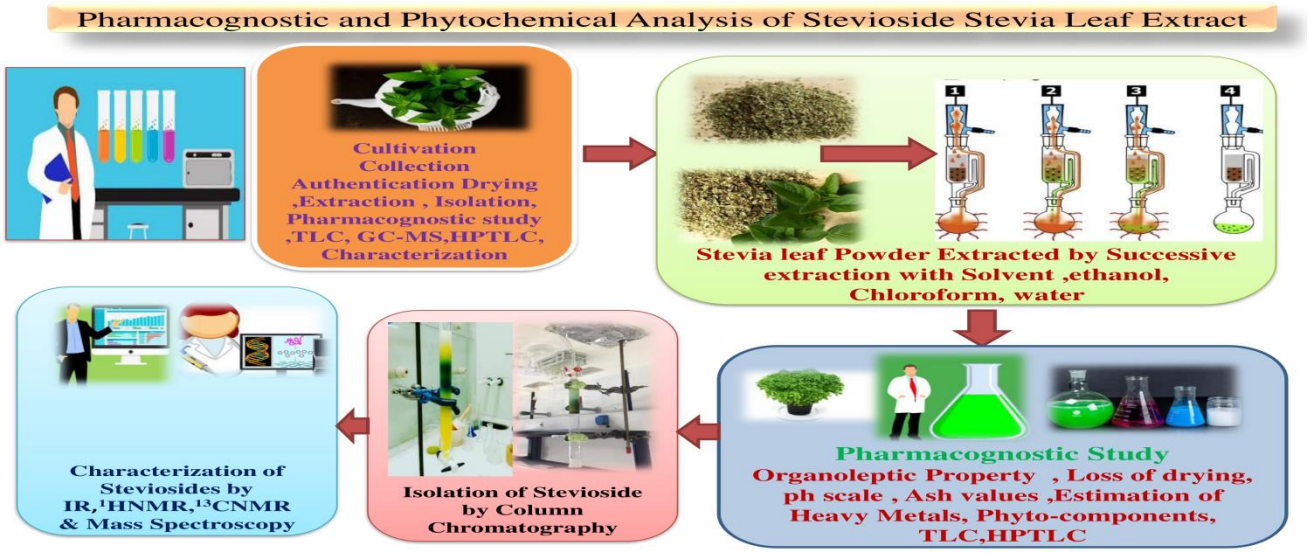
Fig. Application of Stevia Leaf

Other Uses-Stevia possesses antibacterial and antifungal properties. Stomach problems can be eased with stevia leaf tea. Stevia leaves are used to sweeten food. It also has antioxidant, antimutagenic, and anti-proliferative properties.

Analytical Methods

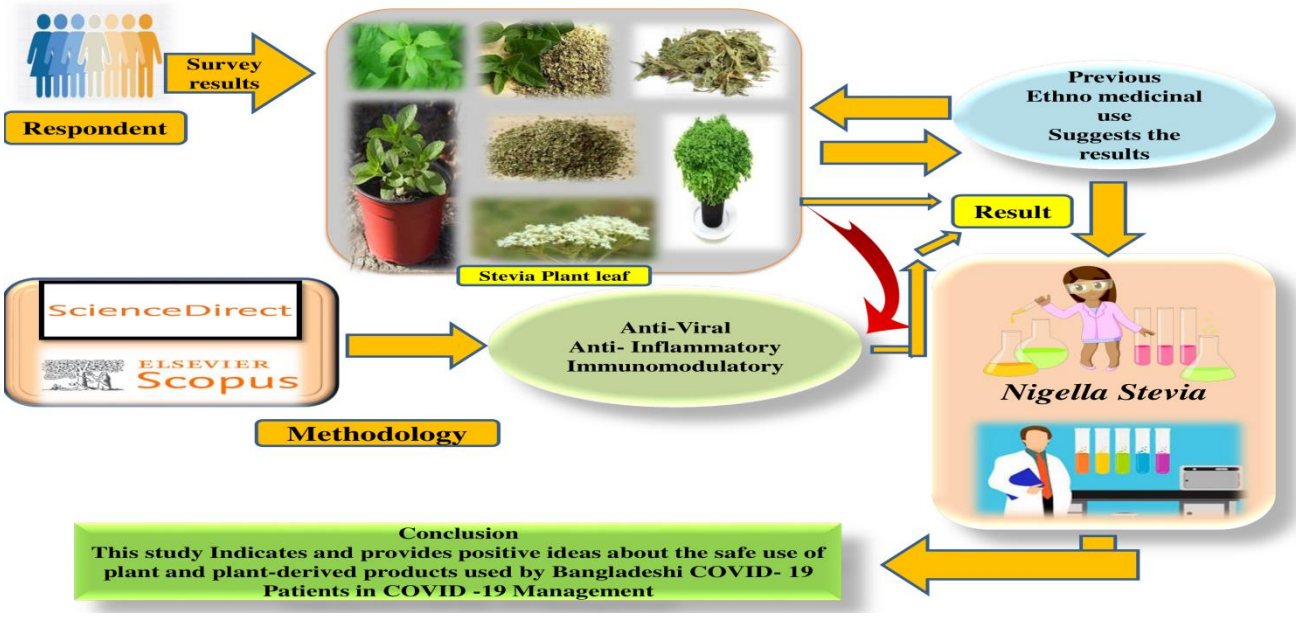
HPLC : Using HPLC, the main medication, any reaction impurities, any potential synthesis intermediates, and any degrades are separated and measured. For both quantitative and qualitative pharmaceutical product analysis, it is the most accurate analytical method. It has a small sample size, moderate analytical conditions, easy fractionation, higher mobile phase pressure, fast analysis, controlled flow rate, and excellent resolution. A porous column is used to inject the sample under high pressure, and the solute adsorption mechanism is used to separate the mixture according to the solute's inclination towards the point of no movement. [11,12] .

Fig. Analysis of Stevioside Stevia Leaf Extract



HPLC OF STEVIA- Commercial samples with variable steviol glycoside concentrations and dried S. rebaudiana Bertoni leaves from Paraguay were used in the study. The leaves were A halogen lamp with a moisture balance type XM-121T (Cobos, the city of Barcelona, Spain) at 104 degrees Fahrenheit was used to assess the moisture content after drying the material at ambient temperatures to a level of 6% to 7%. Each specimen was considered to have reached an equilibrium mass when, following a total of 181 seconds, the loss of water was below 0.2%. [11] .

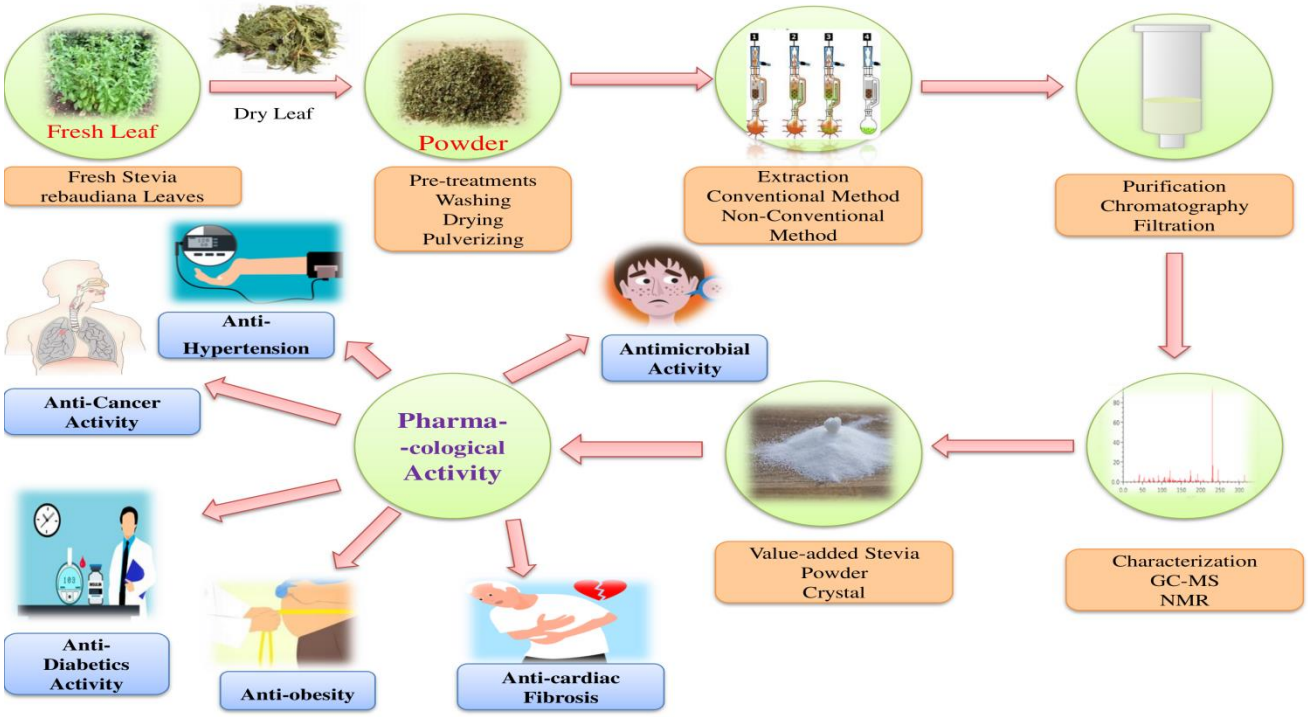
Fig. Application



HPLC STATUS- For liquid chromatography, In an isocratic setup, an The Philips 100 HPLC equipment was utilised, and its UV light detectors was adjusted to 212 nm.v. A Luna C's18(2) was employed in the process of separation. The phase that moved included acetone and sodium buffered phosphate (10 mmol/l) at a steady flow velocity of 1 millilitre per minute. (32:68). For the chromatographic analysis, Clarity software, edition 2.7.3.498 (2010), was used. The amount injected of the sample was twentieth litres.

Stevia Rebaudionica Extract - In Mexico, Bonita and Criolla II, two kinds of S. rebaudiana,had their leaves collected and sun-dried. Three extracts were made in thirty minutes using water at 100°C and 0.5 g of powdered leaves that had been weighed. The leaf extracts were separated into their aqueous phases by centrifuging the extracts for 10 minutes at 2500g and 10°C. The aqueous phases were moved to a 25 mL volumetric bottle after being filtered with a membrane filter with a diameter of 0.45 mHPLC .[12]

Fig. Activity with Extraction, Isolation Process



ACCURACY- An outstanding recovery rate of 92.2-104.4% was obtained from the triple recovery experiment using spiked samples. Although there aren't many studies comparing the precision of By applying the exact same methodology to measure minority glycosides, as According to Chester et al. (2012), Jaworska and colleagues (2012), Wöelwer-Rieck et al. (2010), and a molecule known as (95.7-106%) or rebaudioside A's (93-108%), the recovery rates of steviolbioside, dulcoside A, rebaudioside C, or rebaudioside B are comparable to each other.[11,12].

INTRADAY PRECISION-Three glycoside concentrations were analysed using HPLC, and the The range of RSD values was 2.12 to 7.99%. The afternoon correctness was also evaluated using the amount of retention time.Rebaudioside C had an average retention duration of 9.5 minutes, dulcoside A of 10.28 minutes, rebaudioside B of 20.73 minutes, and steviolbioside of 22.29 minutes.

Everyday Accuracy - Considering a 9% RSD for peak area and a 2.5 percent Rf for duration of retention, there was intermediate or interday precision. While the peak area RSD is marginally higher than that of rebaudioside A and stevioside, 10% is frequently thought to be sufficient. The conditions described permit The detection of rebaudioside D, rebaudioside A, and steroids[11] .

FINAL SUMMARY- The suggested H.P.L.C , technique maintains high selectivity, sensitivity, and accuracy while making primary steviol glycoside measurement easier. It is advised to use the following extraction conditions: Bake at 100°C for 30 minutes, taking care not to crush or stir the leaves. By using micro- and ultrafiltration to clarify the extracts, a reasonable It's possible to eliminate a portion of the main steviol derivatives. The proposed technique is a good way to rapidly and simply demonstrate because rebaudioside and the two primary glycosides known as steviol and a substance called An—are less harmful to human health and the environment than earlier methods that have been documented in the literature.

HPTLC- Numerous substances, such as nutraceuticals, traditional Chinese medicine, conventional western medications, herbal and botanic food supplements, and Ayurveda (Indian) medicine, have been demonstrated to respond well to HPTLC in terms of separation, qualitative analysis, and quantitative analysis. medications. Its features, which compared to other data-driven methodologies in

in both the overall cost & the analytical time. These benefits involve the capacity to evaluate crude material with several components, thespray-on technique's capacity to apply many different samples and a series of standards, the selection of solvents for HPTLC expansion, the capacity to process samples and standards many ubiquitous targeted detection techniques, similarly on identical plate, and and the ability to record in situ spectra sequentially.Additionally, high-performance thin-can help to significantly minimise disposal problems and the risk of exposure to hazardous organic wastes, which would lessen environmental contamination.[13]

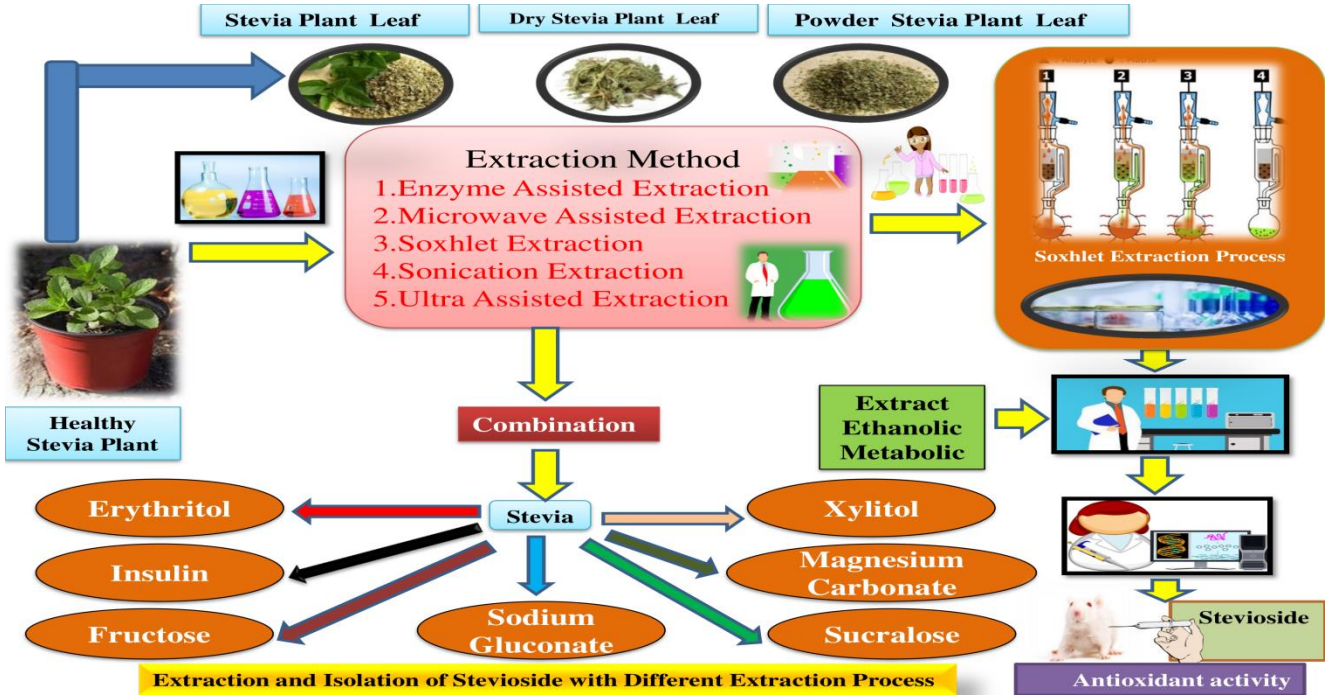
Isolation of steviol glycosides- MeOH-H2O 80:20 (v/v) was used to extract S. One pound of rebaudiana leaf powder was air-dried for a full day at the ambient temperature. After drying, the percolations were divided using butanol, ethyl acetate, hexane, and methanol. Each fraction was desiccated using anhydrous Na2SO4 and then concentrated at 50±5 °C under reduced pressure to provide extracts in butanol (151.1 g), the ethyl acetate fraction (10.4 g), hexane (31.0 g), and chloroform (11.0 g). Four

portions (i-iv) were obtained by column chromatography of the the extract of butanol (150.2 g) over a silicone gel utilising a rate-controlled elution of CHCI3:MeOH having various concentrations of methane hydrocarbon (05%, ten percent, fifteen percent, and 20%) in chloroform. Rechromatography was performed on fraction (iii) using silica gel and a gradient precipitation of 5- twentieth percent MeOH in chloroform. On silica gel, fragment (iv) was re-chromatographed utilising pure corticosteroids The gradient extraction of 6–31% MeOH in formaldehyde produced 10 g with a melting point of 196–200° C and 400 mg of rebaudioside-A with a melting temperature of 241-243° C. 100% pure steviolbioside in one hundred milligrammes with a maharashtra of 188–192° C[14]

HPTLC technique- A coated beforehand silicon gel HPTLC 60 F254 plates with a layer thickness of 0.20 mm was analysed using a Camag HPTLC machine. ADC was pre-saturated for For thirty minutes at ambient temperature with a relative saturation of 50±5% and a mobile phase consisting of 20 ml of ethyl acetate-ethanol water (v/v/v, 81:21:12). Standards and samples were positioned in relation to the screen in 6 mm broad bands, with a gap of 6 millimetre between each band and 10 mm from the side and bottom, using a computerised Ctc sampling (an ATS4) with a N2 gas flow. 150 nm/s was the application speed. Then, using an air drier in a hardwood chamber with enough ventilation, the plates containing TLC were dried in the air current. Sulfuric acid, ethanol, and acetic anhydride (01:01:10, v/v/v) sprayed to thAfter that, bands of interest were heated with a Camag high-performance liquid plate burner for twenty seconds at 111° C. After 19 moments, the dish was measured assessed using a 511 nm reflection-absorption mode of operation, a slit length of 6 mm x 0.3 millimetre, a two hundred millimetres per second of scanned speed and one hundred feet per step of measuring resolution. The Reprostar and a Camag recording apparatus were used to record and store the thin-layer chromatograms in 3. (Jaitak V, Agarwal AP, and others, 2008), and so forth[14]

AWARENESS- Using LOD and LOQ, the technique's sensitivity was evaluated. Having a Pearson correlation coefficient of 0.998-0.999, the standard solutions for stevioside and rebaudioside-A (n = 6) were found to be between 0.5 and 3 g/spot and between one and six g/spot, respectively. In accordance with ICH recommendations, the quantification and detection limits were determined by the use of a calibration curve and verified through experimentationAs stated by Jaitak V. & Agarwal [14].

DETAILS - The the baseline and the sample solutions were assessed in order to determine the procedure's specificity. By contrasting the superimposed spectra of the spotted bands and their RF values with standards, The material, steviolbioside, or rebaudioside-A groups in the specimens revealed verified. [14] .



RELIABILITY - The preciseness of the analytical method was evaluated using the recuperation experiment. This was placing one of the samples that had been previously analysed into a stock solution that had predetermined amounts of the standard of reference constituents in it. The preanalyzed sample's proportion of three glycosides was used to determine the dilution of the specified standards. Three concentration ranges were examined: medium, high, and low.

FINAL HPTLC –In this work , steviolbioside, stevioside, and rebaudioside-A—which are often used as sugar substitutes—were quantified in the species S. plant leaves utilising an HPTLC method. Comparing to existing analytical approaches, the method for measuring quantitatively and simultaneous testing of steviol glycosides is straightforward, affordable, environmentally friendly, and easily customisable. After testing, it was discovered that the procedure were repeatable, linear, accurate, and selective inside the specified parameters. The technique will be effective in controlling the quality of synthesis of steviol glycosides in the leaves of the rebaudiana tree.

HPTLC Chemical Substance- The purity of more than 98 percent about rebaudioside A, steviolbioside, steviol, & stevioside. Ammonium formate, ammonia chlorine dioxide, and ammonia acetate. LC-MS grade acetonitrile and methanol Water.

EXTRACTION AND PURIFICATION BY SPE- A 500m (35 micron) filter was used to filter two hundred grammes of dried Stevia leaves that had been heated to 105°C for two hours and then finely ground into a powder. To maximiseextraction in order conditions, the granulation was separated into separate pieces and sterilised for ten minutes in 25 millilitres of alcohol. The combination was centrifuged for five minutes at 1,500 g, and the liquid that was produced was then transferred into a 25 ml beaker. Methanol was used to build up the volume of each extract, which was then mixed with

alcohol and agitated for a minute at 4500 g. The extract made of methanol was put onto a 3 ml HLB a tool like O 100mg SPE cartridge that had been pre-activated with 3 ml of methanol after being diluted with 2 ml of water. 5. ml of water washed. Three millilitres of 70% methanol in water were used to elute the steviol-glycosides from the cartridge, and methanol was used to adjust the volume to ten millilitres. The outcome was a serially Before adding 2:l to the UHPLC-MS, the resultant water was progressively decreased and agitated for thirty seconds at 4000grams. 20 ml of water was added to 1 g of dry Truvia® powder after it had been sonicated for 10 minutes with 15 ml of water. Solution dilution, 0.22µm filtration, and 2:1 injection into the UHPLCMS apparatus. 17]

SPECIFICITY- Using a genuine reference, co-chromatography was utilised to validate the peak identities of SV, Ra, Sb, and ST, while molecular weight determination analysis was utilised to identify Rc, Du, and ST-glucosides. Ions that are associated with [M+Cl35] or [M+Cl37] were followed in the quantitative analysis of steviol-glycosides. ACCURATE Three assay runs over the course of five days were conducted to confirm The assay's accuracy within and between dayson spiked samples. Identification and peak purity were confirmed by UHPLCMS(MS). By figuring out one's retention and quantity deviations from average time, the accuracy was confirmed.

CONCLUSION- The everlasting herb of the same name is a member of the Asteraceae family of plants. highly valued for its steviol glycosides (SGs) and sweetness. Stevia gets its sweetness via its secondary metabolites, or SGs. Their synthesis is carried out via the SG biosynthetic pathway in the leaves. Most of the enzyme-coding alleles in the above pathway have been cloned. Stevia is the product's distinguishing feature. Among the main metabolites of a number of SGs are stevioside and rebaudioside A. Research has shown that microbial cells and enzymes may also manufacture stevioside. These are non-toxic, antibacterial, non-mutagenic, and have no observable side effects when used. The accuracy, dependability, and consistency of the results generated by an analytical procedure are ensured by validation. As a result, these methods are required to ensure that only the best products are released onto the market. These techniques were Steviol glycol derivatives in the stems and leaves taken from various sites were shown to be quantitatively observable and reproducible, and they will function as an inspection indication to track the industrial manufacture of a compound called and its related substances all throughout various phases of its processing.

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