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RESEARCH ARTICLE

MEDICINAL PROSPECTIVE AND FLORAL BIOLOGY OF CANDY LEAF (*Stevia rebaudiana* Bertoni)

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Abstract

Stevia is one of the most important genus of the tribe Eupatoriae of family Asteraceae. Out of 230 species of the genus, *Stevia rebaudiana* Bertoni is one of them produce sweet steviol glycoside. Flowers are arranged in head with five tubular florets surrounded by five involucrate bract. Plant is major source of high potency sweetener which produces sweet taste but has no calorific value and at the same time aerial parts of *S. rebaudiana* were used to treat diabetes. It is used as antitumor, anticancerous, antihypertensive, antihyperglycemic, anti-diarrheal, anti-oxidant, prevents dental caries and having enzyme inhibitory activities. It also enhances flavor. Chemical and biological properties are studied by some scientists. Several countries including India have started its commercial cultivation. In 2006, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) announced a temporary Accepted Daily Intake (ADI) of stevioside of up to 5.0 mg/kg body weight (BW).

INTRODUCTION

The genus *Stevia* belongs to the family Asteraceae, tribe Eupatoriae and comprises about 150-300 species of perennial herb and shrubs, growing mostly at altitudes of 500-3000 metres in semidry mountainous terrain (Robinson and King, 1977). The species can also grow in grassland, scrub forests and sub-alpine areas. *Stevia rebaudiana* is entirely a New World species, its distribution ranges from Southern United States and Northeastern Paraguay to Southeastern Brazil through Mexico, Central America, The Southern American Andes and the Brazilian highlands (Fig 1). The plant yields a sweet aqueous extract containing various glycosides and therefore has been used for centuries by the indigenous Guarani natives as a traditional sweetener, added to herbal teas and other beverages. It is of immense value due to its adaptability to wide climatic range, the high-sweet content, and its significant agricultural impact in countries such as Japan, China, Taiwan, Korea, Mexico, USA, Thailand, Malaysia, Indonesia, Australia, and Russia (Brandle and Rosa, 1992; Chen & Chang, 1978; Sumida 1968) and efforts were initiated in India recently. *Stevia* is self-incompatible (Chalapathi et.al, 1997b, Miyagawa et.al. 1986) and probably insect pollinated plant (Oddone, 1997). The flowers are small and white (Dwivedi 1999) with purple throat. The pollen can be highly allergenic. The tiny white florets are perfect, borne in small corymbs of 2-6 florets. Corymbs are arranged in loose panicles (Goetternmoeller and Ching 1999). Seeds are contained in slender achenes cypsella about 4 mm in length. The sweetness in Stevia is attributed to the presence of glycosides which are water soluble (Duke and Decelie, 1993) and about 300 times as sweet as cane sugar (Metivier and Viana, 1979b). Stevioside is a white amorphous powder present in leaf and stem tissue (Kinghorn and Soejarto, 1985). Stevioside is used for treating hypertension and hyperglycemia. Stevioside and related compounds are also reported to possess anti-tumor

activity. Versatile bioactive properties of stevioside provoked scientists to undertake synthesis of several stevioside analogues i.e. chemically modified structures (viz. sulphopropyl and sodio-sulphopropyl esters) to improve its bioactive properties such as organoleptic activity. The objectives of this paper are to study systematic literature and floral biology, nutritional, pharmacological and therapeutic applications of *S. rebaudiana* and its related compounds. Present study was carried out to study the floral biology and medicinal uses of sweet herb *S. rebaudiana.*

The vernacular names of the species in various languages are: **Hindi-***Meethi patti, Madhu parni, Meethi Tulsi;* **Sanskrit-** *Madhu patra, Bhoomisarkara, Sarkara chada*; **English-***Candy leaf, Sweet leaf, Sugar leaf, Sweet honey, Sweet herb of Paraguae;***Tamil-***Seeni tulsi;* **Marathi-***Oil-kiryata.*

CULTIVATION:

Stevia is a natural herb native of Paraguay, cultivated as a cash crop in number of countries. In India cultivation of *Stevia* as a crop is still restricted to the research level. The climatic requirement for the plant is semi humid subtropical like other vegetable crops. The crop could be transplanted in February or March and seed collected in the late summer. Flowering under these conditions should occur between 54-104th days of plantation. Seed viability and yield are affected by growing condition during pollination. Vegetative propagation is possible. For raising crops, pot culture experiments are conduct in Botanical garden of Udai Pratap College, Varanasi located between 24^0 56' and 25^0 35'N latitude and 82^0 14' and 83^0 12' E of longitude. The experimental soil was alluvial in nature having pH 8.2, organic carbon 0.6%, available N, P and K 256, 180 and 210 kg ha^{-1} respectively. The experiment consisted of 3 levels of Nitrogen (N_1 = 150 kg ha^{-1}, N_2 = 250 kg ha^{-1}, N_3 = 350 kg ha^{-1}) 3 levels of phosphorus (P_1 = 100 kg ha^{-1}, P_2 = 150 kg ha^{-1}, P_3 = 200 kg ha^{-1}) and 2 levels of potassium (K_1 = 100 kg ha^{-1}, K_2 = 150 kg ha^{-1}). The nutrients N, P and K were applied through Urea, Di-ammonium phosphate (DAP), and Muriate of Potash (MOP) respectively. Experiment was conducted by adopting completely randomized design with 4 replicates. The pots were placed in green house and plants were allowed to grow for a period of 60 days. The growth parameters were recorded periodically at 30, 45, and 60 Days after Planting (DAP). Full doses of P and K and 50% of N were applied two week after planting. Remaining 50% N was applied in three equal splits at 30, 45 and 60 days after planting (DAP). The Flowers was collected at appropriate time and floristic study was by using Olympus Stereo Zoom MSZ – TR microscope and measurement of floral parts was done by using Magnus Pro 4.2 MIPS Software.

CHEMICAL CONSTITUTION

Chemically Stevioside is the chemical a diterpene glycoside ($C_{38}H_{60}O_{18}$) and as an active component in *Stevia* leaves. It is responsible for the edulcorant properties. Structurally, ***stevioside (13-[2-Oβ-D-glucopyranosyl-α-glucopyranosyl) oxy] kaur-16-en- 19-oic-acid β-D- glucopyranosyl ester)*** is a glycoside with a glucosyl and a sophorosyl residue attached to the aglycone steviol, which has a cyclopentanon–hydrophenanthrene skeleton. In addition to stevioside, several related sweet compounds such as steviobioside, rebaudioside A, B, C, D, E, F and ducoside A were isolated from *S. rebaudiana* leaf. All other isolated diterpenoid glycosides possess an ent-kaurene diterpene steviol skeleton ***(ent-13-hydroxy kaur- 16-en-19-oic acid)*** but differ in the residues of carbohydrate at position C-13 and C-19 Stevioside and its specification were shown in Fig 2.

FLORAL BIOLOGY

***Stevia rebaudiana* Bertoni** (Fig 3)

Habit: Perennial herb with filiform roots, 30-50 cm tall, erect, slender and weak-pubescent, lower stem woody.

Stem: Produces secondary shoots (suckers) from its base, dies off and is renewed annually.

Leaves: Simple, opposite, subsessile, 22-25 long, blades subcoriaceous narrowly elliptic to oblanceolate to linear oblong, wide, apex obtuse to subacute, base cuneate, margine entire often toothed (crenate to serrate) on upper half, entire on the lower half, three primary veins arise from the leaf base, raised and prominent on the blades lower side somewhat immersed on the upper side, secondary venation reticulate, somewhat immersed.

Inflorescence: Capitulum or head with involucres arranged in loose corymbose panicles. Peduncle 1-4 mm, borne at terminal ends of branches.

Flower: As long as the pedicel, light green on the lower half, yellowish on the upper half in fresh state, phyllaries 5, finely hairy, green when fresh, linear to subulate, 3.5–7.0 mm long acute to rounded at apex, 5 disc florets in each capitulum about 10-16 mm long exerted above the involucres, corolla actinomorphic white, corolla tube slender equal in length 12-16 mm longer than the pappus awns 3-6 mm long, greenish below, dirty white to purplish above, covered with very fine hairs on the inside, almost glabrous outside, the lobes ovate-lanceolate, unequal, white with purplish throat, obtuse to subacute 0.5-1.0 mm long, ciliate bifid style twice the length of the corolla lobes (21-23 mm long), divergent and usually recurved, densely covered by clear-brownish glands and very fine short hairs, achenes subglabrous, 2.5-3.0 mm long, but finely barbed along the rides, pappus awns straw colored of 9-17 subequal (4-6 mm long) somewhat rigid and finely barbed awns, base of awns at an acute angle and pointing upwords. Stamen syngenesius 10 -11mm long, pollen grain 389-514 micrometer in diameter.

Fruit is a five ribbed spindle shaped achene with persistent pappus (cypsela). Seed 3.5-4.0 mm long.

Flowering: The crop could be transplanted in February or March. Flowering occurs between 54-104th day of transplantation, depending upon the day length.
Fruiting: Seed production in the Northern hemisphere is in Feb-March and seed collected in late summer.
Distribution: *Stevia rebaudiana* is native to Paraguay and probably endemic to this country. Bertoni (1905) gave a distribution range from Amambay South to the Monday River approximately $22^0 30'$ to 25^0 $30'$ South latitudes and from 55^0 to 57^0 West longitudes within 200-700 m altitudinal zones with an average annual temperature of 25^0C and average rainfall of about 1357 mm year^{-1}. *Stevia* has been introduced as a crop into a number of countries in the world. So far it is under cultivation in such American and Asian countries as Paraguay, Mexico, Central America, China and Malaysia. Several parts of **India** such as Himanchal Pradesh, Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, West Bengal, Karnatka and Tamilnadu also cultivate.

PHARMACOLOGICAL ACTIVATES:
Stevia is used in many parts of the world as a non- calorie sweetener (Madan et.al., 2010). As Stevia leaf powder without processing is highly safe to use, calorie free and moreover around 200-300 times sweeter than cane sugar.
Metabolism of glycoside: Studies on the absorption and metabolism of glycosides in rats showed that stevioside is not readily absorbed from the upper small intestine due to its high molecular weight. However, stevioside degraded by bacteria of the colon, resulting in free steviol part of which is absorbed by the colon and transported to the liver and parts is excreted in faeces. Liver converted steviol into glucuronide which is excreted from body through urine (Geuns et.al., 2007).
Anti hyperglycemic activity: Steviol glycosides after ingestion do not induce glycemic response and act as natural zero calorie or low calorie sweetener to diabetics and others on carbohydrate controlled diets. The leaves extracts of *S.rebaudiana* has been used traditionally in the treatment of diabetes. (Jeppensen et.al. 2002) reported the antihyperglycaemic, insulinotropic and glucagonostatic effects of stevioside in type-2 diabetic Gotokakizaki (GK) rats as well as in normal wister rats. Stevioside was found to suppress significantly the glucose response and increase the insulin response during the glucose tolerance test (IVGT) in GK rats, but in normal wister rats stevioside was found to enhance insulin levels above basal during same test, without altering the blood glucose response. So, the investigator concluded that stevioside being antihyperglycaemic, insulinotropic and glucagonostatic may have the potential of becoming a new antidiabetic drug for use in type-2 diabetes.
Obesity: Obesity is due to accumulation of fat in the body. It is most common nutrition disorder. Obesity and overweight is major risk factor associated wide number of health problem including hypertension hyperlipidemia, diabetes, surgical risk, pulmonary and renal problems, pregnancy complications and certain type of cancer. Increased consumption of sugar leads to several disorder including obesity. Therefore, it is used in weight management strategy is to substitute sugar with low calorie sweeteners (Stephen et.al.2010). Leaves of *Stevia* contain zero calorie stevioside and rebauioside that are not metabolized to produce energy and taste 300 times sweeter than sucrose (Soejarto et.al, 1982; Megeji et.al, 2005). High doses of steviol by ingestion showed reduction in body weight as experiment in rats (Curry and Robertis, 2008).
Antihypertensive: 95% pure stevioside demonstrated a significant hypotensive rats without any adverse effect on heart rate or serum catecholamine levels (Chan et.al.1998). In another study by the same group with stevioside was administrated at a dose of 250 mg thrice a day for 1 to 60 year hypotensive volunteers (Chan et.al 2000). The study revealed that after 3 months the pressure decreased significantly and the effect was also found to be persisting. No significant adverse effect was recorded hence the investigator concluded that stevioside is a well tolerated and effective compound that may be considered as an alternative therapy for patients with hypertension.
Antitumor: The inhibitory effect of stevioside on tumor promotion by TPA in two-stage carcinogenesis in mouse skin; four steviol glycosides *viz* stevioside, rebaudiosides A & C and dulcoside A isolated from *Stevia rebaudiana* were found to exhibit strong inhibitory activity against TPA-induced inflammation in mice. The ID50 (50% inhibitory dose) values of these compounds for TPA-induced inflammation were determined as 54.1, 92.2, 92.5, and 291.6 mg/ear, respectively for the test compound. Inhibited the TPA-induced inflammation in a dose-dependent manner. The inhibitory effects of these compounds were compared with antitumor–promoting agent quercetin and anti-inflammatory drugs indomethacin and hydrocortisone; rebaudiosides A and C and dulcoside A were found to be similar in activity to hydrocortisone, and stevioside was found to be more effective than indomethacin (Yasukawa et. al., 2002).
Anticancerous: *Stevia* has anti-tumorous and cytotoxic properties (Kaushik et.al., 2010). Studies have demonstrated the inhibitory effects of *Stevia* leaf extracts and their polyphenolic constituents on tumor promotion and initiation. Stevioside, the *Stevia* leaf aglycones, steviol and isosteviol, and their metabolites have been reported to inhibit tumor promotion by blocking Epstein-Barr virus early antigen (EBV-EA) induction (Akihisa et.al., 2004) as well as by reducing tumor formation in the two-stage mouse skin carcinogenesis model following sequential exposure to 7,12-

dimethylbenz[a] anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Konoshima and Takasaki, 2002; Yasukawa et al., 2002; Takasaki et al., 2009). The hydrolysis product of stevioside, isosteviol, potently inhibits DNA replication and human cancer cell growth *in vitro* (with LD50 values of 84 to 167 μMol) (Mizushina et.al., 2005). The toxicity of rebaudioside-A was studied by bacterial reverse mutation test (Ames test) using standard *Salmonella typhimurium* as well as *Escherichia coli*, there was no statistically significant increase in the number of relevant colonies exposed to rebaudioside-A at concentrations up to 5000μg/plate.

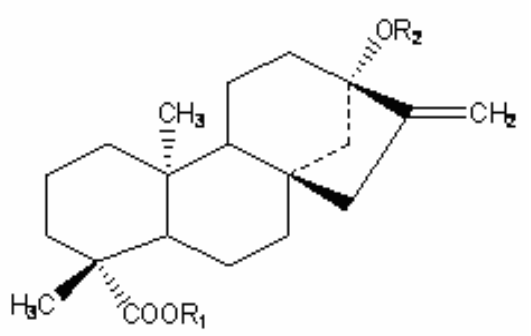
Dental caries: Dental caries means tooth decay or cavities is a breakdown of teeth due to activities of bacteria and dietary sugars particularly sucrose. Several microorganisms are capable of fermenting dietary carbohydrate. *Streptococcus mutans* is the most prevalent followed by *Lactobacillus casein* and *Streptococcus sanguis*. Regular consumption of nutritive sweeteners provides energy in the form of carbohydrate, causes cavities which encourage the growth of harmful bacteria in the mouth contributing to plaque formation and gingivitis. There is a requirement to substitute sucrose with natural sweetener which should be nutritionally appropriate and not being detrimental to the overall general health of the individual (Matsukubo and Takazoe, 2010). *Stevia*, as a non nutritive sweetener are zero- or low-calorie alternatives to nutritive sweeteners, bacteriocidal properties benefit oral health. *Stevia* is a natural sucrose substitute with high nutritional value beneficial in the battle against dental caries. Extract of *Stevia* leaves and its major secondary metabolites, steviol, isosteviol, stevioside and rebaudio-side A, B, C and E are noncariogenic and have been found to inhibit glucan induced aggregation of cariogenic organism, Thus *Stevia* have potential of providing oral health benefits (Wu et al., 1998; Grenby, 1991). Studies suggested that development of dental caries in rat pups are triggered in presence of sucrose solution while it is not with stevioside (Das et.al., 1992).

Safety and doses: Stevioside does not appear to be carcinogenic. The toxicology and safety of stevioside used as a sweetener were studied by different investigator (Soejarto et.al.1982). Stevioside is absorbed and degraded to steviol, which undergo further metabolism. Other studies indicate that none of the digestive enzyme from gastro-intestinal tract of different animals and human are able to degrade stevioside into steviol. Stevioside was metabolized to steviol by the bacterial flora of the caecum and no any adverse effect or toxicity associated with stevioside consumption. Stevioside was evaluated for safety by the 51st meeting of the **JECFA (Joint FAO/WHO Expert Committee on Food Additives)** in 1998. The JECFA considered the toxicity data of steviol glycosides in 1999 but was unable to recommend on **ADI (Acceptable Daily Intake)** due to insufficient data, including a lack of human metabolism studies, lack of information on the purities of the product and mutagenicity studies. Later on, by 2004, JECFA set a temporary ADI OF 2mg/kg b.w/day for *stevia* at that time and requested extensive additional information to be submitted by 2007 on the effects of steviol glycoside in humans including special population such as people with diabetics or hypertension

Conclusion

Stevia rebaudiana Bertoni is an herb from South America that has found a great potential of use, primarily in the production of natural sweetener with emphasized sweetness. Due to its chemical structure and health-promoting phytochemical components, *Stevia* is suitable for the extraction and production of functional food ingredients. Also, *Stevia* is a good source of carbohydrates, proteins, dietary fibers, minerals, and amino acids. *Stevia* leaves contain diterpene glycosides that are low calorie sweeteners, with Stevioside being there most abundant. Sweet diterpene glycosides are non-fermentative and non-toxic compounds that contribute to the flavor of a product in which they are used. Diterpene glycosides do not have mutagenic and carcinogenic properties. *Stevia* has been consumed for centuries as a natural low calorie sweetener without any adverse effects on human health which is contrary to artificial sweeteners, which are determined by low caloric characteristics and a large percentage of carcinogenicity. *Stevia* for these reasons has a much greater advantage over other sweeteners (artificial sweeteners and sucrose) as an ingredient for the food industry and it is suitable as a replacement for sucrose in beverages, drinks and baked products. Besides the sweet content, owing to its secondary plant compounds (phytochemicals), *Stevia* has antihyperglycemic, anti-inflammatory, anticancer, diuretic and immunomodulating effects.

Fig.1 Native place of *Stevia*: Paraguay



Compounds Name	**R_1**	**R_2**
Stevioside	*β-Glc*	*β-Glc-β-Glc(2→1)*
Steviolbioside	*H*	*β-Glc-β-Glc(2→1)*
Rebaudioside A	*β-Glc*	*β-Glc-β-Glc(2→1)* | *β-Glc(3→1)*
Rebaudioside B	*H*	*β-Glc-β-Glc(2→1)* | *β-Glc(3→1)*
Rebaudioside C	*β-Glc*	*β-Glc-α-Rha(2→1)* | *β-Glc(3→1)*
Rebaudioside D	*β-Glc-β-Glc(2→1)*	*β-Glc-β-Glc(2→1)* | *β-Glc(3→1)*
Rebaudioside E	*β-Glc-β-Glc(2→1)*	*β-Glc-β-Glc(2→1)*
Rebaudioside F	*β-Glc*	*β-Glc-β-Xyl(2→1)* | *β-Glc(3→1)*
Ducloside A	*β-Glc*	*β-Glc-α-Rha(2→1)*

Glc = β – D – glucopyransosyl; rha = α – L – rhamnopyranosyl

Figer 2. Structures of stevioside and related compounds. In rebaudioside D and E R1 is composed of 2 β-Glc-β-Glc(2→1). In rebaudioside A, B, C, D, E and F in group R_2 an additional sugar moiety in added on carbon 3 of the first β-Glc .In rebaudioside F one β-Glc is substituted for by -β- Xyl. Glc and Rha represent, respectively, glucose and rhamnose sugar moieties.

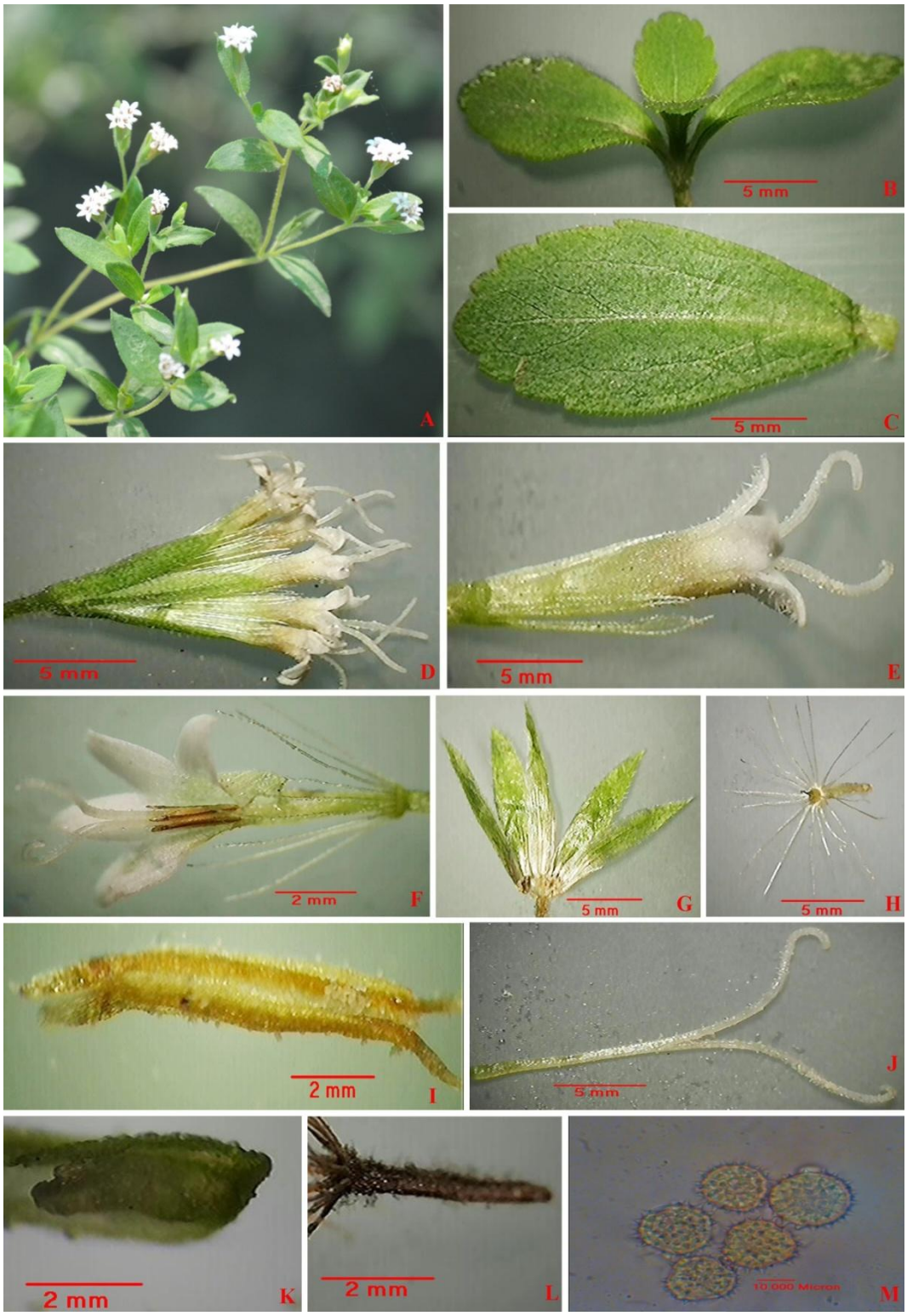


Fig. 3 Floral biology of *S. rebudiana.* A. Flowering twig; B. Leaf arrangement; C. Single leaf D. Inflorescence E. Florets, F. L.S of Flower; G. Calyx; H. Pappus; I. Single Stamen; J. bifid Stigma K. Ovary; L. Seed; M. Pollen grains.

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References:
Akihisa, T., Hamasaki, Y., Tokuda, H., Ukiya, M., Kimura, Y. and Nishino, H. (2004): Microbial transformation of isosteviol and inhibitory effects on Epstein-Barr virus activation of the transformation products. J. Nat. Prod., 67:407-410.
Brandle, J.E. and Rosa, N. (1992): Heritability for yield, leaf: stem ratio and stevioside content estimated from a landrace cultivar of *Stevia rebaudiana*, Can. J. Plant Set., 72: 1263.
Chalapathi, M.V., Thimmegowda S., Sridhara S., Parama, V. R. and Prasad, T.G. (1997b): Natural non-calorie sweetener stevia (*Stevia rebaudiana* Bertoni) a future crop of India. Crop Res., 14: 347-350.
Chan, P., Tomlinson, B,. Chen, Y.J., Liu, J.C., Hsieh, M.H. and Cheng, J.T. (2000): A double-blind placebo-controlled study of the effectiveness and tolerability of oral stevioside in human hypertension. Br. J. Clin. Pharmacol. 50: 215-220.
Chan, P., Xu, D.Y., Liu, J.C., Chen, Y.J., Tomlinson, B., Huang, W.P. and Cheng, J.T. (1998): The effect of stevioside on blood pressure and plasma catecholamines in spontaneously hypertensive rats. Life Sci., 63:1679-1684
Chen, K., Chang, T. R., Chen, S. T. (1978): Studies on the cultivation of stevia and seasonal variation of stevioside. China Gartenbau., 24: 34-42.
Curry, L.L., Roberts, A. (2008): Subchronic toxicity of rebaudioside A. Food Chem. Toxicol., 46:S11–S20.
Das, S., Das, A.K., Murphy, R.A., Punwani, I.C., Nasution, M.P., Kinghorn, A.D., (1992): Evaluation of the cariogenic potential of the intense natural sweeteners stevioside and rebaudioside A. Caries Res., 26:363–366.
Duke, J.A., Decellier, J.C. (1993): *Stevia rebaudiana* (Bert.). In: Duke J, editor., CRC handbook of alternative cash crops., London: CRC Press. pp. 422–424.
Dwivedi, R. S. (1999:Unnurtured and untapped supersweet nonsacchariferous plant species in India. Current Sci. (Bangalore), 76: 1454 – 1461.
Geuns, J.M., Buyse, J., Vankeirsbilck, A., Temme. E.H. (2007): Metabolism of stevioside by healthy subjects. Exp. Biol. Med., 232:164-173.
Goettemoeller, J., Ching, A. (1999): Seed germination in *Stevia rebaudiana*. Perspectives on new crops and new users. J Janick (Ed.), ASHS Press. Alexandria, VA.
Grenby, T.H. (1991): Update on low-calorie sweeteners to benefit dental health. International Dental Journal., 41: 217–24.
Jeppesen, P.B., Gregersen, S., Alstrupp, K.K., Hermansen, K. (2002): Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic goto-Kakizaki (GK) rats. Phytomedicine., 9: 9-14.
Kaushik, R., Narayanan, P., Vasudevan, V., Muthukumaran, G., Antony, U. (2010): Nutrient composition of cultivated Stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. J. Food Sci. Tech., 47:27-33.
Kinghorn, A., Soejarto, D. (1985): Current status of stevioside as a sweetening agent for human use, In: Economic and medicinal plant research by Wagner H., Hikino H., Farnsworth N., (Eds.), Academic Press, London, 1: 1-52.
Konoshima, T., Takasaki, M. (2002): Cancer-chemopreventive effects of natural sweetners and related compounds. Pure Appl. Chem., 74:1309-1316.
Madan, S., Ahmad, S., Singh G.N., Kohli, K., Kumar, Y., Singh, R. and Garg, M. (2010): Stevia rebaudiana (Berr.) Bertoni- A review. Ind. J. of nat. pro. and reso., 1(3): 267-286.
Matsukubo, T., Takazoe, I., (2010): Sucrose substitutes and their role in caries prevention, Int. Dent. J., 56:119-130.
Megeji, N.W., Kumar, J.K., Singh, V., Kaul, V.K., Ahuja, P.S. (2005): Introducing Stevia rebaudiana, a natural zero-calorie sweetener. Curr. Sci., 88: 801-80.
Metivier, J. and Viana, A. M. (1979b): Determination of microgram quantities of stevioside from leaves of *Stevia rebaudiana* Bert. by two-dimensional thin layer chromatography. J. Exp. Bot., 30: 805-810.
Miyagawa, H., Fujikowa, N., Kohda, H., Yamasaki, K., Taniguchi, K., Tanak, R. (1986): Studies on the tissue culture of *Stevia rebaudiana* and its components: (II). Induction of shoot primordia. Planta Med., 4: 321-324.
Mizushina, Y., Akihisa, T., Ukiya, M., Hamasaki, Y., Murakami, N.C., Kuriyama, I., Takeuchi, T., Sugawara, F., Yoshida, H. (2005): Structural analysis of isosteviol and related compounds as DNA polymerase and DNA topoisomerase inhibitors. Life Sci., 77:2127-2140.
Oddone, B. (1997): ‘‘How to Grow Stevia. Technical Manual.’’ Guarani Botanicals, Pawtucket, CT.

Robinson, H. and King, R.M. (1977): Eupatorieae—systematic review. In The Biology and Chemistry of the Compositae, V.H.Heywood, J.B.Harborne and B.L.Turner (Eds), Academic Press, New York, 1, 437-485.
Soejarto, D.D., Kinghorn, A.D., and Farnsworth, N.R. (1982): Potential sweetening agents of plant origin. III. Organoleptic evaluation of stevia leaf herbarium samples for sweetness. J. Nat. Prod., 45: 590-599.
Stephen, D.A., Corby, K.M., Hongmei, H., Sandra, C., William, T.C., Paula, G. Donald, A.W. (2010): Effects of Stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. Appetite, 55:37–43.
Sumida, T. (1968): Reports on *Stevia rebaudiana* Bertoni M. introduced from Brazil as a new sweetness resource in Japan. Misc. Pub. Hokkaido Natl. Exp. Sta., 2: 69-83.
Takasaki, M., Konoshima, T., Kozuka, M., Tokunda, H., Takayasu, J. (2009): Cancer preventive agents. Part 8: Chemopreventive effects of stevioside and related compounds. Bio. org. Med. Chem., 17: 600-605.
Wu, C.D., Johnson, S.A., Sriakantha, R., Kinghorn, A.D. (1998): Intense natural sweetener and their effect on cariogenic bacteria. J. Dental. Res., 77:283.
Yasukawa, K., Kitanaka, S., Seo, S. (2002): Inhibitory effect of stevioside on tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two stage carcinogenesis in mouse skin. Biol. Pharm. Bull., 25:1488-90.