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Asian Journal of Plant Science and Research, 2016, 6(2):46-51

ISSN : 2249-7412
CODEN (USA): AJPSKY

Screening and characterization of non caloric sweeteners in *Scoparia dulcis* L.

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ABSTRACT

Scopariadulcis Lin. (Scrophulariaceae) is a wasteland herb, introduced from Tropical America. The traditional healers have documented its many promising traditional medicinal uses such as anticancerous, antitumorous, antileukemic and antiviral (including HIV), hypoglycemic, hypotension (lowers blood pressure), cardiotonic, analgesic (pain- relievers), diuretic and sediative property. Main chemicals constituents are scopaduloic acid A and B, scopadiol, scopadulciol, scopadulin, scoparic acid A, B and C and betulinic acid, acatein, amyrin, apigenin, coumaric acid, blulcinol, glutinol, mannitol, scopadiol, dulcioic acid. Triterpene and mannital have been isolated from roots and dulertol from aerial parts. Leaves are used in treatment of fever, cough, bronchitis and dental trouble. Leaves and stems are used for diabetes. The result of the study confirms the sweet taste of the plant as indicated from various literatures and from the Latin binomial 'dulcis' which means sweet taste. The sweet taste was may be because of the presence of β and α methyl glycopyranoside. However, we could not able to identify any glycosides.

Keywords: *Scopariadulcis*, TLC, NMR, GC-MS, β and α methyl glycopyranoside.

INTRODUCTION

Scopariadulcis L. is a small branched glabrous, leafy annual herb with erects or ascending branches. Leaves opposite and 3-notely whorled rhombid elliptic or elliptic lanceolate, obtuse at apex, base tapering, and margin serrate. Flowers pedicels slender, rigid. Calyx lobes 4 oblong. Corolla white, tube very short, capsule globose. Seed minute, many. The whole plant has medicinal significance.

Life without sugar would be unthinkable! Sugar is a vital nutrient from which almost all animal and plants gain the energy to sustain their life. Sugar is by far, the most over used sweeteners in the western world more than ever people are consuming large amounts of sugar as part of their daily diet in desserts, cereals, salad dressings, ketchup relishes, gum, candy and the list goes on.

Alternative sweeteners are highly consumed in America. According to research studies explained by The American Journal of Clinical Nutrition, in 2003-2004, Americans two years or age and older consumed 585g per day of beverages and 375g per day of foods with caloric sweeteners. More than 66% of Americans consumed these beverages with alternative sweeteners and 90.3% of Americans consumed foods with added caloric sweeteners. On the other hand, 10.8% of Americans in 2003-2004 consumed non-caloric alternative sweetener flavored beverages and 5.8% consumed non-caloric alternative sweetener flavored food [1].

Some commonly consumed foods with alternative sweeteners are diet sodas, cereals, and sugar-free desserts such as

ice cream. Alternative sweeteners are found in many products today due to their low or non-caloric characteristics. This can be used as a method of advertisement for dieters or those conscious of their sugar intake. Those with diabetes can greatly benefit from alternative sweeteners that do not affect their blood sugar levels drastically. This aids in maintaining low insulin use in the body and blood sugar levels [2].

Sugar substitutes are used for a number of reasons, including. To assist in weight loss some people choose to limit their food energy intake by replacing high-energy sugar or corn syrup with other sweeteners having little or no food energy. This allows them to eat the same foods they normally would, while allowing them to lose weight and avoid other problems associated with excessive caloric intake. Dental care sugar substitutes are tooth-friendly, as they are not fermented by the microflora of the dental plaque. An example of a sweetener that can benefit dental health is xylitol. Xylitol works to prevent bacteria from adhering to the tooth surface, thus preventing plaque formation and eventually decay. The carbohydrates and sugars consumed usually adhere to the tooth enamel. Bacteria can feed upon this food source allowing them to quickly multiply. As the bacteria feed upon the sugar, they convert it to acid waste that in turn decays the tooth structure. Xylitol cannot be fermented by these bacteria, so the bacteria have difficulty thriving, thus helping to prevent plaque formation [3].

Diabetes mellitus people with diabetes have difficulty regulating their blood sugar levels. By limiting their sugar intake with artificial sweeteners, they can enjoy a varied diet while closely controlling their sugar intake. Also, some sugar substitutes do release energy, but are metabolized more slowly, allowing blood sugar levels to remain more stable over time. Reactive hypoglycemia individuals with reactive hypoglycemia will produce an excess of insulin after quickly absorbing glucose into the bloodstream. This causes their blood glucose levels to fall below the amount needed for proper body and brain function. As a result, like diabetics, they must avoid intake of high-glycemic foods like white bread, and often choose artificial sweeteners as an alternative. Avoiding processed foods individuals may opt to substitute refined white sugar with less-processed sugars, such as fruit juice or maple syrup [4].

Some natural sweeteners include Sorbitol (sweeteness 420), Mannitol (sweeteness 421), Xylitol (sweeteners 967), Lactitol (sweeteness 960), and Isomalt (sweeteness 953) are the most common sweeteners of this type. These are found in berries, fruit, vegetables and mushrooms.

Another important group of non-sugar sweeteners are the polyols which is generally less sweet than sucrose, but have the same bulking properties. Polyols have less available kilo jules than sugar, because slowly absorbed by the body result in minimal effect on blood glucose levels. They are generally acceptable for use in the diets of people with diabetes. However, a fairly larger amount, more than 20- 50g\ day can cause gastrointestinal discomfort and have a laxative effect.

In the present study *Scoparia dulcis* L. was screened for compounds of non caloric sweeteners based on its binomial '*dulcis'* [5] which means sweet taste [6]and characterization of same compound.

MATERIALS AND METHODS

Plant Materials

Healthy plant samples of *Scopariadulcis* were collected from the banks of river Cauvery, Tiruchirappalli, Tamilnadu, India and identified from Botanical Survey of India, Coimbatore. The vegetative parts viz., leaves and stem were screened for the presence of biologically active compound like sugar, amino acid, phenols by suitable methods. Then these vegetative parts were dried in shade at room temperature (31^oC) and ground into powder. They were kept in hot air oven for complete drying 80^oC. These samples were homogenized and extracted with different types of solvent.

Preparation of Plant Extract

A known quantity (100g) of the dried powder from the plant material was taken in a soxhlet apparatus and soaked in methanol and water (4:1 v/v) and closed with cork and kept for seven days at 31^oC room temperature for complete extraction.

After seven days the extract were filtered through Whatmans no.1 filter paper. The filtrate was evaporated at reduced pressure to remove residual solvent and moisture. This thick extract was collected in bottles and kept in refrigerator. This extract was the stock solution.

Column Chromatography
The extract obtained from the soxhlet was vacuum dried and concentrated and the extract was purified by submitting to silica gel chromatography eluting with CH_3Cl_3/ MeOH. (Chloroform (90): Methanol (10).

Conformation Test for Glycosides
5ml of aqueous extract was treated with 2ml of glacial acetic acetic acid containing one drop of $FeCl_2$ solution. This was underplayed with 1ml of Con.H_2SO_4.

A brown ring of interface indicates deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic layer, a greenish ring may form just gradually throughout thin layer indicates the presents of glycocides [7].

Separation of the Plant Constituents by Thin Layer Chromatographic Method
Of the various methods of separating and isolating plant constituent the chromatographic procedure originated by Tswett is one of the most useful techniques for general application and the active constituents were separated by fraction extraction or adsorption or ion exchange or a porous solid by means of following solvents. All finely divided solids have the power to adsorb other substances on their surface to a greater or lesser extent; similarly all substances are capable of being adsorbed. This phenomenon of selective adsorption is the fundamental principle of chromatography. In this, the chromatography methods are used for thin layer chromatography (TLC)[7].

Various bands were eluted using different compounds like Chloroform: Methanol (7:3) and Methanol: Chloroform (3:7).

The Rf value is calculated by using the formula

$$\text{Resonance Front} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

NMR Analysis
The NMR analytical technique provides useful data regarding the type, quantity and arrangement of different atoms in many chemical systems, liquids and solids. NMR is a critical technique for structure determination, often required on short notice. NMR laboratories offer prompt analysis with rapid turnaround[7].

GC-MS Analysis of Plant Extract
The plant extract was analyzed in GC-MS in PPRC laboratory, Thanjavur using GC Clarus equipment.

RESULTS AND DISCUSSION

Naturally occurring non caloric sweeteners were surveyed from various literatures and with the help of the Latin meaning of plant binomials. The following plants were found to possess the non-caloric sweeteners (Table 1).

Among 24 plants listed above [9], *Scoparia dulcis*, *Glyzyrrhiza glabra*, *Saccharum officinarum* are a few plants reviewed. For the present investigation, *Scoparia dulcis* L. was chosen since there is very meager scientific and systematic study on this plant.

Column Chromatography
The extract obtained from the Soxhlet was eluted through the chromatographic column for purification and separation of compound. In this three layers of compounds were eluted with three different colors, such as light green, dark orange and dark green which are collected separately and were further analyzed for the presence of glycosides.

During glycosides confirmation test the dark green and light green fractions of the extract showed negative results and the dark orange fraction showed positive result which were further taken to run thin layer chromatographic plate.

Dark orange extract was eluted in TLC plate using silica gel G as a stationary phase and methanol and chloroform in

the ratio of 7:3 and 3:7 as mobile phase. There were five major and clear bands when the plates were placed in iodine chamber whose Rf value are given. There bands were scrapped and dissolved in methanol. The dissolved compound was filtered using Whatman's No.1 filter paper to remove the Silica Gel G. (Table 2).

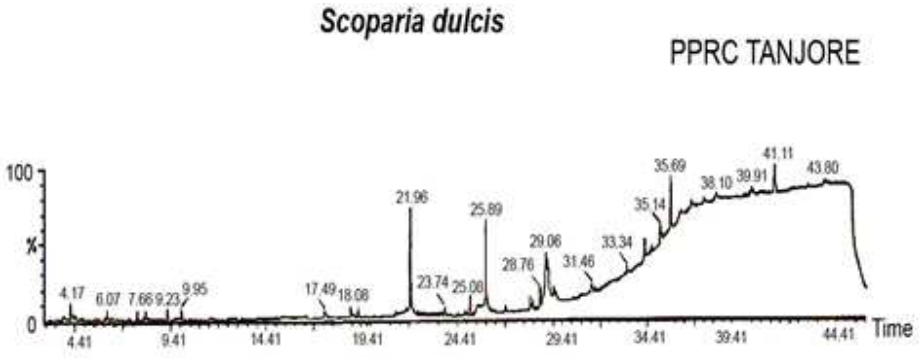

Figure 1- Chromatogram of *Scopariadulcis*analysed by GC-MS

Table 1- List of plants having non caloric sweetener

Sl. No	Name of plants	Family	Reference
1.	*Perillafrutescens* L.	Labiatae	Furukawa *et al*., 1916
2.	*Stevia rebaudiana*	Compositae	Sakaguchi, 1982
3.	*Glycyrrhizaglabra* L.	Leguminosae	Nieman, 1957
4.	*Citrus aurantium* L.	Rutaceae	Horocoitz, 1974
5.	*Citrus paradise*Macf.	Rutaceae	Horocoitz, 1974
6.	*Citrus sinensis* L.	Rutaceae	Horocoitz, 1974
7.	*Citrus limon* L.	Rutaceae	Horocoitz, 1974
8.	*Cymarascolymus* L.	Compositae	Lee, 1976
9.	*Lippiadulcis*Trevir.	Verbenaceae	Douglas, 2002
10.	*Momordicagrosvenori*Swingle	Cucurbitaceae	Douglas, 2002
11.	*Thaumatococcusdanielli*iBenth.	Marantaceae	Zbynekpolesny, 1995
12.	*Dioscorephyllumcumminsii* Diels	Menispermaceae	Zbynekpolesny, 1995
13.	*Siraitiagrosvenori*Swingle	Cucurbitaceae	Kinghorn, 2002
14.	*Baccharisgaudichaudiana* DC.	Compositae	Fulla, 1947
15.	*Pterocaryapaliurus*	Juglandaceae	Kennely, 1995
16.	*Alnus japonica*	Betulaceae	Aoki, 1988
17.	*Tessariadodeneifolia* Hook. &Arn.	Compositae	Shu, 1995
18.	*Hymenoxysturnerik* (Parker)	Compositae	Shu, 1995
19.	*Polypodiumfeii*Bory	Polypodiaceae	Yamada, 1995
20.	*Polypodiumaureum* L.	Polypodiaceae	Hiu*et al*., 1998
21.	*Polypodiumdecumanum* Wild.	Polypodiaceae	Hiu*et al*., 1998
22.	*Polypodiumloriceum* L.	Polypodiaceae	Hiu*et al*., 1998
23.	*Polypodiumlowei*	Polypodiaceae	Hiu*et al*., 1998
24.	*Polypodiumtriseriale*	Polypodiaceae	Hiu*et al*., 1998

Table 2- Solvent combination for TLC separation

Solvent	Band color	Rf value
Chloroform: Methanol (7:3)	Brown (L1) Dark Brown (B2) Orange (O3) Yellow (Y4) Green (T5)	0.37 0.38 0.41 0.49 0.7
Methanol: Chloroform (3:7)	Brown (L1) Dark Brown (B2) Orange (O3) Yellow (Y4) Green (T5)	0.30 0.32 0.40 0.45 0.51

The spectroscopic analysis of *Scopariadulcis* L. reveals the presence of a long chain of unsaturated aldehyde, alcoholic and acidic groups.

The NMR spectrum of this L1 fraction reveals mostly aromatic groups and a sharp singlet at 7.268ppm can be assigned to hydrocarbon or halo substituted gem dimethyl group CH_3- C- Cl. The sharp peak at 1.255ppm may be assigned to methyl group and another sharp peak at 1ppm assigned to methyl group which is 3^o in origin. The GC-MS of L1 fraction reveals to contain high molecular weight of glucopyranoside fraction (Fig. 1) which was observed in the retention time of 17.49 and 18.88 in chromatogram of GC- MS with peak 5.1 and 6.2.

Table 3- Activity of Phytocomponents identified in the methanolic water extract of *Scopariadulcis*

NO	RT	Name of the compound	Molecular Formula	MW	Peak Area%	Nature of compound	Activity***
1	4.40	Phenol	C_6H_6O	94	2.5	Phenolic compound	Analgesic, Anesthetic, Antioxidant, Antiseptic, Antibacterial, Antiviral, Cancer preventive, Fungicide, Rodenticide, Emetic, Vasodilator
2	6.07	Phenol, 4-methyl	C_7H_8O	108	3.6	Phenolic compound	Analgesic, Anesthetic, Antioxidant, Antiseptic, Antibacterial, Antiviral, Cancer preventive, Fungicide, Rodenticide, Emetic, Vasodilator
3	8.07	Phenol, 3,4-dimetyl-methylcarbamate	$C_{10}H_{13}NO_2$	179	3.6	Nitrogen compound	Antimicrobial
4	9.23	Benzaldehyde, 4-methyl	C_8H_8O	120	3.4	Aldehyde	Anesthetic, Antibacterial, Anticancer, Antiseptic, Antitumor, Antispasmodic, Immunostimulat, Insecticide, Insectifuge, Nematicide, Pesticide, Sedative, Termiticide, Tyrosinase inhibitor
5	9.95	Isoquinoline	C_9H_7N	129	3.2	Alkaloid	Antimicrobial
6	17.49	B-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194	5.1	Sugar moiety	No activity reported
7	18.88	α-D- Glucopyranoside, methyl	$C_7H_{14}O_6$	194	6.2	Sugar moiety	No activity reported
8	19.24	Unknown	***	***	3.1	***	***
9	21.98	6-Methoxy-2-benzoxazolinone	$C_8H_7NO_3$	165	33.9	Nitrogen compound	Antimicrobial
10	25.89	n-Hezadecanoic acid	$C_{16}H_{32}O_2$	256	30.0	Palmitic acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic
11	28.76	Phytol	$C_{20}H_{40}O$	296	5.4	Diterpene	Anticancer, Antimicrobial, Antiinflammatory

For B2 fraction of the NMR spectrum shows two sharp singlet at 7.26ppm and 1.25ppm which was assigned to hydrocarbon or halo substituted gem dimethyl group and two multiple at 2.35ppm and 1ppm assigned to methylene group sandwitched between two other metylene group and methyl group of 3^o origin. The GC-MS of B2 fraction reveals to contain predominately benzaldehyde 4- methyl group which is attributed with retention time 9.23 in chromatogram with peak area of 3.4 respectively.

For O3 fraction of the NMR spectrum of this fraction reveals only two singlets at 4.7ppm and 1.7ppm which may be assigned to methyl group and N-H protons. The GC-MS fraction of the sample reveals to contain unsaturated alcohol long chain of hydrocarbon which is with alcoholic and acidic function which may contain phenol, phenyl, 4-methyl and Phenol 3, 4-dimethyl- methyl- carbamate with retention time of 4.40, 6.07 and 8.07 in the chromatogram and peak area of 2.5, 3.6 and 3.6 respectively.

For Y4 fraction of the NMR spectrum reveals a sharp singlet at 4.6ppm which may be assigned to the presence of N-H protons. GC-MS spectrum reveals the fraction might be a compound of long chain unsaturated alcohol (OH) or acids. It may contain 6- methyoxy-2- benzoxazolinone or phenol, 3, 4-dimethyl- methylcarbanate which was nitrogen compounds with retention time 8.07 and 21.98 in the chromatogram peak area of 3.6 and 33.9.

For T5 fraction of the NMR spectrum reveals the presence of sharp single at 7.2ppm, another singlet at 1.6ppm,

multiplet at 1ppm, and triplet at 2.3ppm and low intensity singlet at 3.9, 3.8 and 3.6ppm. The singlet at 7.2ppm is due to N-H proton. The single at 1.6ppm may be assignable to metylene protons. The triplet at 2.3ppm reveals conjugated unsaturation and the presence of CH proton sandwiched between CH groups. This observation might be the reason for intensive colour exhibited by this fraction. The GC-MS of the fraction reveals mainly a mixture of isophenols, isoquinoline, phytol and fraction of glucopyranoside compounds with the retention of 4.40, 9.5, 28.76, 17.49 and 18.88 in chromatogram respectively (Fig. 1). The peak area attributed to 2.5, 3.2, 5.4, 5.1 and 6.2 respectively.

Scoparia dulcis L. has been screened for antimicrobial properties [10].antioxidant properties [11]. sympathemimetic effect [12].This plant also has acetalyated flavones glycosides, a nerve growth factor used to treat nerve disorders[13]. Besides *Scoparia dulcis* have been screened for terpenoids such as Scoparic acid A, Scoparic acid B, Scopadulcic acid A and B, Scopadulciol and Scopaadulin which are responsible for antitumor activity [14],[12],[15],[16],[10].(Table-3).

CONCLUSION

Scopariadulcis L. (leaf and stem) was shade dried, extract with mixture of methanol, water and the extract was eluted in TLC plate. Five clear and broad bands were scrapped and eluted in proton NMR and GC-MS. Eleven major compounds have been identified. The sweet taste was, may be because of the presence of β and α methyl glycopyranoside. Further clinical study is required to test the compound. The plant has high potency as a source of non-caloric sweetner.

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