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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2020, Vol. 36, No.(3):
Pg. 544-549

Isolation and Identification of Anticancer Apigenin Glycosides Flavonoids from Plantation White Sugar

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http://dx.doi.org/10.13005/ojc/360326

(Received: May 23, 2020; Accepted: June 20, 2020)

ABSTRACT

Apigenin and its derivatives are biological active flavonoids that are useful in a variety of dietary constituents. These flavonoids may exert some influence over the transition from normal to cancerous, and have value as chemopreventive substance. In this study, a new purification method of three apigenin glycosides flavonoids from Indian plantation white sugar extracts was developed. Three unknown sugar flavonoids were isolated from sugar by using resin based column chromatography. After filtration, the colorant solution was adsorbed on to the gel column at a flow rate of 1 mL/3 min and elution was done with water at the same rate. 10 mL fractions were collected which were then chromatographed on cellulose TLC plates. The pure fractions were completely evaporated and investigated for identification. The detected flavonoids were: apigenin-8-C-b-D-glucopyranoside, apigenin 6-C-b-glucopyranoside and apigenin-7-O-b-glucopyranoside. Ultraviolet and nuclear magnetic resonance spectroscopy introduces an additional analytical dimension for the identification of sugar flavonoids.

Keywords: Flavonoid, Sugarcane Plant Extract, Extraction, Resin.

INTRODUCTION

Sugarcane flavonoids receive considerable attention in the literature, because of their biological, chemotaxonomic markers and physiological importance[1-2]. Flavonoids are found in nearly every plant type and are ingested in diets routinely[2]. Flavonoids have frequently found in sugarcane[3-4], cane juice[5-6], molasses[7], and mill syrup[8]. Sugarcane and cane juice contained various phenolics such as quercetin, rutin, morin, and ferulic acids and showed the antibiotic and antioxidant properties.[9-10]

The apigenin flavonoids also occur mainly as C-glycosides in sugarcane, with C-C bonds at the 6 or 8 positions or both in the case of vicenins. Cane sugar by product may contain apigenin as it in case of other mill syrup and molasses. These phenomena developed from another studies dealing with apigenin 5-0-methyl ether in sugarcane flower[11], apigenin 5-0-methyl ether 4'-0-galactoside in peelings[12-13], apigenin 5,7-0- dimethyl ether 4'-01-glucoside and apigenin-6-C-glucoside (Isovitexin) in leaf[14], and apigenin-6-C-glucosyl-7-0-methyl ether, apigenin-6-C-glucosyl-8-C-arabinoside, apigenin-6-C-arabinosyl-8-C glucoside, apigenin-6-C-arabinosyl-8-C glucoside in mill syrup[15-16].

Vitexin, a flavonoid compound found in the sugarcane, possess to have anticancer[17], antioxidant[18], anti-viral[19], anti-inflammatory[20], anti-thyroid, anti-arteriosclerotic[21], antihypertensive[22] and antihepatotoxic properties[23]. Apigenin and its derivatives exist in sugarcane plants and are found at significant concentrations in many spices, fruits, and herbs[24]. In sugarcane,the well-known apigenin glycosides are apigenin-7-0-glucoside, apigenin-8-C-glucoside, and apigenin-6-C-glucoside[25].

Sugarcane flavonoids may interact with protein molecule and be eliminating protein those are broken during the digestion process[26]. Apigenin derived from sugarcane has been used to treat various diseases such as inflammatory, neuralgia, and shingles[27]. An Apigenin derivative has been reported as cancer chemopreventive agents and appears to confer protection against a large variety of cancer as reviewed[28].

These flavonoids suppress cell cycle progression, including those of oral squamous carcinoma, esophageal, gastric, and cancer of organs associate with the gastrointestinal tract[29]. Additional clinical uses include antiviral and antihepatotoxic effects. The antioxidant activity of sugarcane flavonoids leads to the place and sequence of the OH group on the benzenoid ring that inhibits superoxide radicals[30-32].

Rare features of flavonoids in sugar cane to develop flower color for entomophilic pollination. Sugarcane flavonoids (Flavonol, flavonone, chalcones) are mostly water soluble. Some flavonoids were identified in mill syrup, bagasse and sugarcane leaves[33-34].

Many studies have shown that the sugarcane flavonoids possess antioxidant activities. Individual recovery of flavonoids from sugar has not been done yet. Thus, in this study, individual flavonoids components from plantation white sugar were separated by gel permeation technique and characterized by retardation factor, ultraviolet and nuclear magnetic resonance spectroscopy.

EXPERIMENTAL

^{1}H spectra of flavonoids were recorded using JEOL AL 500 MHz spectrometer in DMSO-d_6 containing TMS as internal standard reference. The UV-Vis measurements in the range of 200-800 nm were recorded using the Shimadzu UV-1601 spectrophotometer. Plantation white sugar was supplied by different sugar factories. Analytical grade solvents were used for sample preparation, purchased from Merck (Mumbai, India). For recovering of sugar flavonoids a XAD-4 macroporous adsorption resin (polystyrene resin, 20-60 mesh particle size, pore diameter 40 A0, surface area =725 m^2/g) was used.

Preparation of Plantation white sugar

A 25^0 Bx solution of plantation white sugar was filtered and the pH was adjusted to about 4 with concentrated HCl.

Extraction and Isolation

A glass chromatography column (300×20 mm ID), filled with XAD-4 resin was used for flavonoids adsorption. The column was activated with 4 BV of 5% (v/v) HCl and followed by 4 BV of 5% (v/v) NaOH, and redistilled water to a neutral pH. Initial concentration of plantation white sugar extract was 0.8 mg/mL, pH of sugar solution was 7 (10 bed volume feeding solution; flow rate 2.5 bed volume per hour). For flavonoids recovery a mixture of methanol: ammonia: water (50:5:45) was used. The desorbed solution of colorants was completely evaporated under vacuum. The solid colorants were completely dried over P_2O_5 and weighed. The solid colorant was dissolved in about 100 mL water and 1-2 drops of concentrated HCl were added to precipitate any polymeric colorant[35]. After filtration, the colorant solution was adsorbed on to the gel column at a flow rate of 1 mL/3 min and elution was done with water at the same rate. 10 mL fractions were collected which were then chromatographed on cellulose TLC plates. The pure fractions were completely evaporated and investigated for identification.

RESULTS AND DISCUSSION

The structural characterization of sugar afford three flavonoids (1–3), they are apigenin-8-C-b-D-glucopyranoside (1), apigenin-6-C-b-glucopyranoside(2), and apigenin-7-O-b-

glucopyranoside(3), their structure elucidation was carried out through Rf-values, color reactions (Table 1), and spectral analysis (UV and NMR)[36].

Table 1: Rf values and spot appearance of flavonoids

Compound	Rf value	UV light	UV/NH_3
Apigenin-8-C-b-D-glucopyranoside	0.43 (TBA)	Deep purple	Yellow-green
Apigenin 6-C-b-glucopyranoside	0.57 (TBA)	Deep purple	Yellow-green
Apigenin-7-O-b-glucopyranoside	0.61 (TBA)	Deep purple	Light yellow

Spectral data of the known sugar flavonoids were in good agreement with those previously published[11]. Compounds 1, 2 and 3 were isolated for the first time from the sugar under investigation (Figure 1, 4, 7).

Compound 1(UV spectrum) shows two absorption peaks at 270, 334 nm that matched with that reported for apigenin-8-C-b-D-glucopyranoside (Fig. 2). The data of the analysis for flavonoids characterization of sugar are shown in Table 2.The ^{1}H NMR spectra of compound 1 showed H-3 and H-6 signal at δ_H 6.59 and 6.30 (each 1H). An overlapping complex pattern between 7.7-7.9 (2H) for the C-2' and C-6' proton comprising an up field doublet at 6.87 (J=8.4Hz) for the C-3' and C-5' proton. The ^{1}H NMR spectrum of compound 1 shows signal for 6''-O- methyl group at δ_H 3.4[36] (Figure 3).

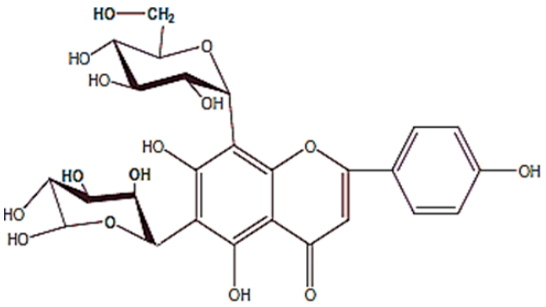

Fig. 1. Chemical structure of compound apigenin-8-C-b-D-glucopyranoside

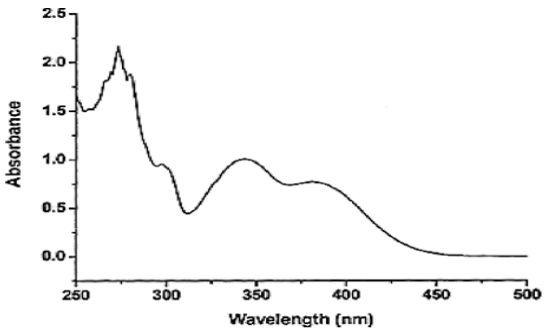

Fig. 2. UV spectrum of apigenin-8-C-b-D-glucopyranoside

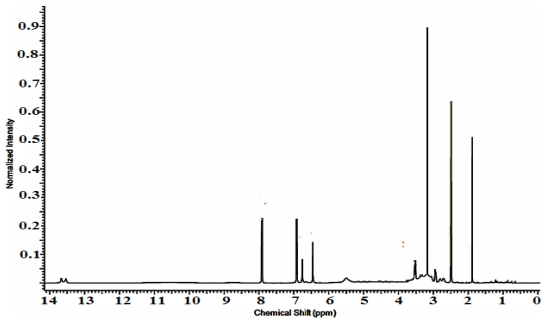

Fig. 3. NMR spectrum of apigenin-8-C-b-D-glucopyranoside

Table 2: The UV Characterization Data of Flavonoids in Plantation White Sugar

Compound	Methyl Acohol	Sodium Methoxide	Aluminum Chloride	Aluminum Chloride /Hydrochloric Acid	Sodium Acetate	Sodium Methoxide/ Hydro Boric Acid
Apigenin-8-C-b-D-glucopyranoside	270	280	265	258sh	275	272
	300sh	330	274	280	300sh	316sh
	334	394	304	305	378	352
			350	344		
			384	382		
Apigenin 6-C-b-glucopyranoside	270	275	265sh	264sh	280	270
	330	330	280	278	300	350
		400	300	300	380	400sh
			350	350		
			375			
Apigenin-7-O-b-glucopyranoside	266	250	273	275	260	265
	332	270	300sh	295	265sh	340
		300sh	326	345	355	
		385	430	380	382	

Compound 2 UV-Vis maxima 270, 270 (shoulder), 330 and was ascribed to apigenin 6-C-b-glucopyranoside (Fig. 5). The ^{1}H NMR spectra of compound 2 indicated the presence of apigenin 6-C-b-glucopyranoside, chemical shift of H-3, and H-8 at δ_H 6.71(1H) and 6.50 (1H, d, J = 2.3). Two aromatic doublet at δ_H 6.89 and 7.86 (each 2H, d, J = 8.4) for C-3' and C-5' proton and one doublet at δ_H 7.86 for C-2'and C-6' proton and a methoxyl group at δ_H 3.88 (Fig. 6). These results allowed us to establish apigenin 6-C-b-glucopyranoside as the structure[36] of compound 2.

Compound 3 shows UV-Vis maxima at 266, 332 (Fig. 8). This compound was tentatively assigned as apigenin-7-O-b-glucopyranoside[11]. The ^{1}H NMR characterization of compound 3 shows an anomeric proton at δ_H 5.08 (1H, d, J=6.85).The compound 3 showed H-3 and H-6 at δ_H 6.41 and 6.61. ^{1}HNMR spectrum of compounds shows doublet at 6.9 (1H, d, J = 2.3Hz) for C-3' and C-5' proton. Doublet at 7.81 (2 H, d, J = 8.4) for 2' and C-6' proton. Shift at δ_H 6.77 for C-8' proton (Fig. 9). The data allowed us to establish apigenin-7-O-β-glucopyranoside as the structure of the compound by comprising of the spectral data with literature value[36].

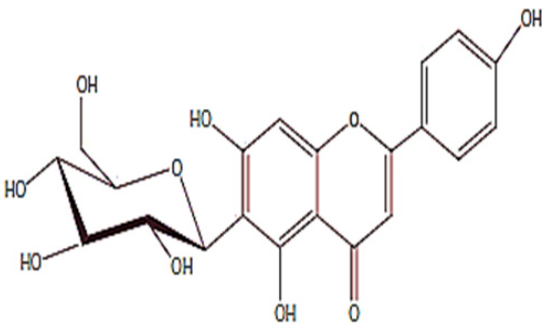

Fig. 4. Chemical structure of compound apigenin-6-C-b-glucopyranoside

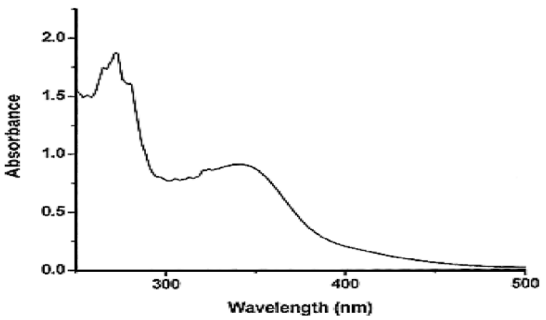

Fig. 5. UV spectrum of apigenin-6-C-b-glucopyranoside

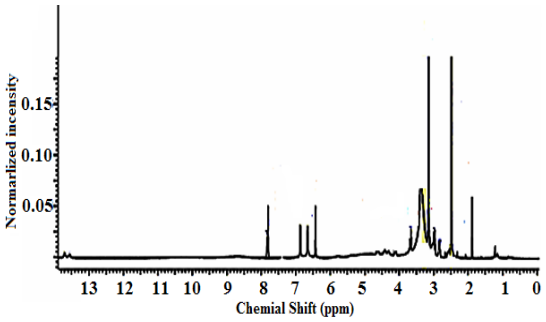

Fig. 6. ^{1}HNMR spectrum of apigenin-6-C-b-glucopyranoside

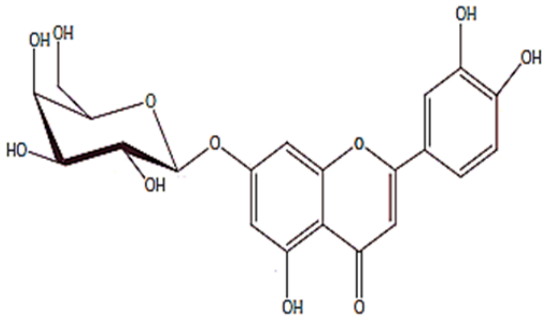

Fig. 7. Chemical structure of compound apigenin-7-O-b-glucopyranoside

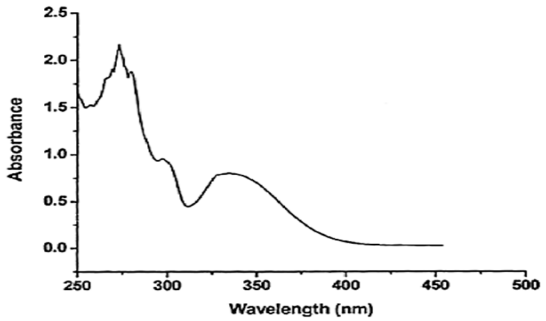

Fig. 8. UV spectrum of apigenin-7-O-b-glucopyranoside

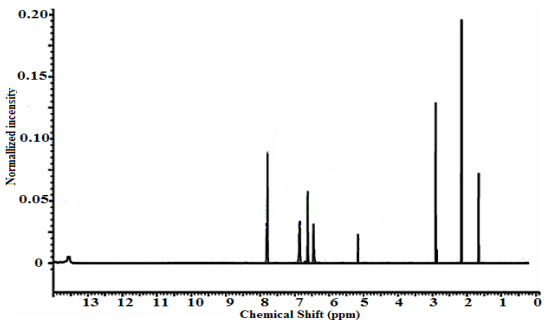

Fig. 9. ^{1}HNMR spectrum of apigenin-7-O-b-glucopyranoside

Various methods have been developed for the identification of apigenin and its glucosides in different plants by spectroscopic and chromatographic

techniques like high-performance thin-layer chromatography[37-38], HPLC[39-42] and UHPLC-DAD[43]. Color concentration and high performance liquid chromatographic (HPLC) methods have been used to measure the approximate levels of major flavonoid colorants in sugar mill and refinery products using apigenin as an internal standard.

ACKNOWLEDGMENT

The author wishes to acknowledge the support of Prof. V. K. Agrawal, Awadhesh Pratap Singh University, Rewa for this work.

Conflict of interest

The author declares no conflict of interest.

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