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Structural Characterization of Luteolin Glycosides Flavonoids from Indian Plantation White Sugar

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ABSTRACT

Luteolin flavonoid is useful in a variety of dietary constituents and can be used as medicine to protect and suppress the growth of different human cancers. These flavonoids may increase the level of reactive oxygen species, and have value as chemopreventive substances. In this study, a medium grade (L-30) sugar was obtained from a reputed north Indian sugar manufacturer which had turned slightly yellowish due to a long storage period for flavonoids identification. Flavonoids are more stable than other sugar colorants and persist into sugar crystals. A resin-based column chromatography method has been developed for the extraction of three luteolin glycosides flavonoids from Indian plantation white sugar. The fractionation of the colorants was done according to their size by gel permeation chromatography, and finally their isolation and purification by thin-layer chromatography. The detected flavonoids were: luteolin-6-C-β-glucopyranoside, luteolin-7-O-β-glucopyranoside and luteolin-7-O-β-galactopyranoside. Ultraviolet and Nuclear magnetic resonance spectroscopy techniques were used for the structural characterization of flavonoids.

Keywords: Flavonoid, Plantation White Sugar, Extraction, Resin.

INTRODUCTION

The quality of sugar is directly related to its color value. The color incorporated in sugar crystals may originate from the cane plant itself or may be formed during processing. The former comprises colorants such as chlorophyll, carotenes, xanthophylls, anthocyanins, and flavonoids[1-2]. Flavonoids such as tricin, isoorientin, isovitexin, and apigenin glycosides have been identified in sugarcane leaves, liquor, and molasses[3-8]. However, flavonoids have promising applications in food and pharmaceuticals[9-10].

The luteolin group of flavonoids, sugar are joined to the A ring by carbon-carbon bonds at 6 or 8 positions that occur as C-glycosides. Sugar house products may contain luteolin as it does in the case of other mill syrup and molasses. These phenomena developed from other studies dealing with luteolin-6-C-glucoside, iso-orientin-0-rhamnosylglucoside, luteolin-6-C-glucosyl-7-0-glucoside, iso-orientin-tri-0-glucoside and luteolin-6-8-di-C-glycosides in sugarcane leaf[11], and iso-orientin-7-0-methyl ether, iso-orientin7,3'-0-dimethyl ether, 6-methoxyluteolin, orientin-7,3'-0-dimethyl ether and other luteolin derivatives in mill syrup[12-13]. Luteolin, a dietary

flavonoid is possessed to have anti-thyroid, anti-viral, anticancer, and antioxidant[14-15], characteristics found in fruits, herbs[16], sugarcane mill syrup, and bagasse[17-18]. Thus, analysis of flavonoids in sugar and other sugar products commodities like bagasse, mill syrup, and molasses have become essential requirements for the sugar industry.

These flavonoids find their way into the sugar crystal through sugarcane, sugar production process, and enzymatic reaction. No systematic study has been carried out to date to isolate and identify the natural colorants persisting into the granulated direct consumption white sugar made in our country. It is imperative to determine the flavonoids components in sugar samples in India. This paper aims to provide the structural characterization of sugar flavonoids that make it such a useful recipient in the pharmaceutical and sugar industry. The study also includes the application of gel permeation chromatography for the fractionation of sugar flavonoids. Spectral analysis like UV and NMR were used for flavonoids characterization.

EXPERIMENTAL

Chemicals

All solvents like methanol, hydrochloric acid, and ammonia (HPLC grade) were purchased from Merck (Mumbai, India).

Sample Collection

A medium grade (L-30) sugar was obtained in 2019 from a reputed North Indian sugar manufacturer.

Preparation of Solution

Plantation white sugar (25 g) was diluted with 100 mL distilled water to prepare a 25 ^{0}Bx (degree Brix) solution. The solution was filtered and the pH was adjusted to about 4 with concentrated HCl[19].

Extraction and Cleanup

A XAD-4 macroporous adsorption resin (60 mesh particle size, pore diameter 40 A^0, surface area =725 m^2/g) was used for recovering sugar flavonoids. The resin was filled in a chromatographic column preconditioned with hydrochloric acid and sodium hydroxide[20]. The sugar extract was collected into a 100 mL beaker. Dry colorants (20 g) were dissolved with 100 mL distilled water and 1-2 drops of concentrated HCl was added to precipitate polymeric colorant[19]. For flavonoids recovery, a mixture of methanol: ammonia: water (40:4:56) was used. The solution was filtered with Millipore 0.22 µm sterile filter, the solution was then adsorbed onto dextran gel- Sephadex (particle size = 50-150 µ, water regain = 5 mL/g, gel bed volume = 10 mL/g) at a rate of 0.33 mL/min and elution was done with water at the same rate. Three fractions were collected separately for each technique which was then chromatographed on cellulose TLC plate[20]. The fractions were evaporated and investigated for identification.

Analysis

The final extracts were analyzed in DMSO-d_6 containing TMS as the internal standard reference (JEOL AL 500 MHz) ^{1}H and ^{13}C NMR spectrometer. Shimadzu UV-1601 spectrophotometer was used for UV–Vis analysis in the range of 200-800 nm[20].

RESULTS AND DISCUSSION

Flavonoids, the yellow-colored molecules are more stable than other colorants and pass over to the final stages of processing. According to Smith and Paton[1], the group of flavonoids is the most critical to sugar color being responsible for up to 30% (at pH 7) of the raw sugar color. The structural studies of plantation white sugar afford three flavonoids (1–3), they are luteolin-6-C-β-glucopyranoside (1), luteolin-7-O-ß-glucopyranoside (2), and luteolin-7-O-β-galactopyranoside (3). The Rf values, color reaction and spectral analysis (UV and NMR) of 3 analyzed flavonoids[21-23] are shown in Table 1-3. These spectral results confirm that the purification method was successfully employed to isolate pure fractions of different flavonoids and were in good agreement with those previously published[21]. Flavonoids 1, 2, and 3 were characterized for the first time from the sugar under investigation (Figure 1-9).

Table 1: Preliminary identification of flavonoids

Compound	Rf value	UV light	UV/NH_3
luteolin-6-C-β-glucopyranoside	0.43 (TBA)	Deep purple	Yellow-green
luteolin-7-O-β-glucopyranoside	0.16 (TBA)	Deep purple	Light yellow
luteolin-7-O-β-galactopyranoside	0.30 (TBA)	Deep purple	Yellow

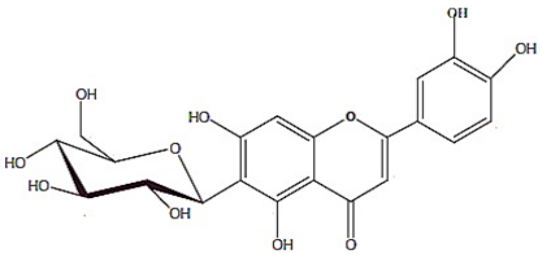
Table 2: The UV-Vis Spectral Data with Different Diagnostic Shift Reagents of Flavonoids in Plantation White Sugar

Compound	Methanol	Sodium Methoxide	Aluminum Chloride	Aluminum Chloride/ Hydrochloric Acid	Sodium Acetate	Sodium Acetate/ Boric Acid
luteolin-6-C-β-glucopyranoside	256	265	276	266sh	275	264
	270sh	275sh	302sh	278	321	370
	350	405	330	365	390	425sh
			423	382		
luteolin-7-O-β-glucopyranoside	254	262	273	275	260	260
	264sh	298sh	300sh	290	265sh	374
	345	392	326	355	403	
			430	387		
luteolin-7-O-β-galactopyranoside	254	265	272	274	260	258
	262sh	300sh	300sh	290	263sh	372
	350	392	325	350	403	
				385		

Table 3: ^{13}C NMR data of luteolin-6-C-β-glucopyranoside, luteolin-7-O-β-glucopyranoside, and luteolin-7-O-β-galactopyranoside

Position	Compound 1	Compound 2	Compound 3
1	-	-	-
2	164.4	164.5	166.9
3	102.8	103.1	104.1
4	182.5	181.9	184.0
5	160. 8	161.1	162.9
6	105.0	99.5	101.1
7	164.6	163.0	164.8
8	98.6	94.7	96.0
9	156.5	157.0	159.0
10	104.4	105.3	107.1
1'	122.4	121.3	123.4
2'	114.4	113.6	114.2
3'	146.3	145.8	147.1
4'	150.1	150.0	151.9
5'	116.1	116.0	119.2
6'	119.8	119.2	116.8
1g	73.8	99.9	101.7
2g	71.2	73.1	74.7
3g	79.2	76.4	71.3
4g	71.2	69.6	77.9
5g	82.4	77.2	78.4
6g	62.1	60.6	62.4

Compound 1 UV-Vis maxima 256, 270 (shoulder), 350 nm and was ascribed to luteolin-6-C-β-glucopyranoside. The ^{1}H NMR spectra of compound 1 indicated the presence of luteolin-6-C-β-glucopyranoside, chemical shift of H-3, and H-8 at δ_H 6.61(1H) and 6.23 (1H, d, J=2.3). Two aromatic doublet at δ_H 6.82 and 7.42 (each 1H, d, J=2.3) for C-2' and C-5' proton and one olefinic proton at δ_H 7.49 for C-6' proton and a methoxyl group at δ_H 3.88 that showed correlation with δ 146.33 (C-3'), 150.14 (C-4'), 160.88 (C-5), and 164.62 (C-7) bearing hydroxyl group respectively (Table 3). Spectral analysis of the known flavonoid allowed us to establish luteolin-6-C-β-glucopyranoside as the structure of compound 1[21-23].



Chemical structure of luteolin-6-C-β-glucopyranoside

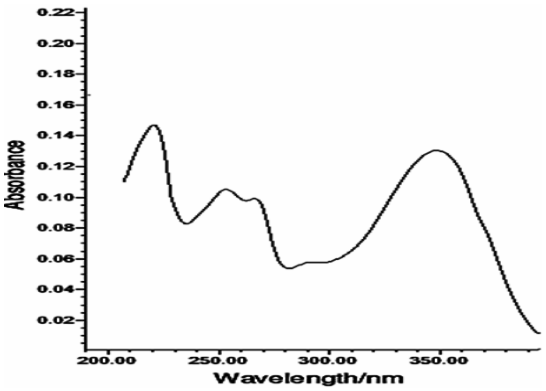


Fig. 1. UV-spectra of luteolin-6-C-β-glucopyranoside

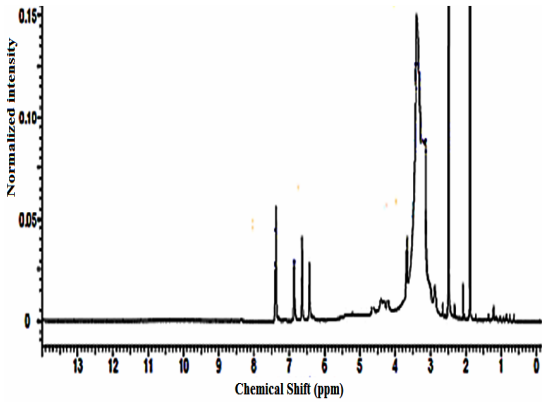


Fig. 2. ^{1}H NMR spectrum of luteolin-6-C-β-glucopyranoside

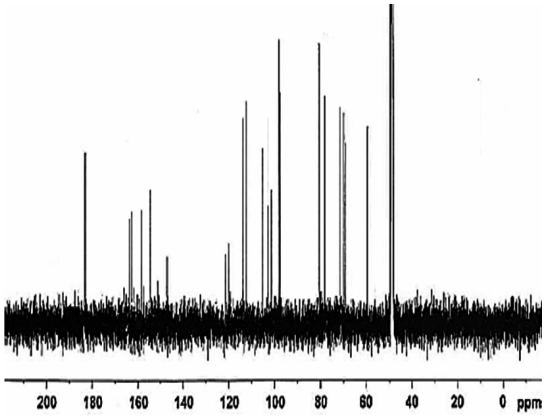
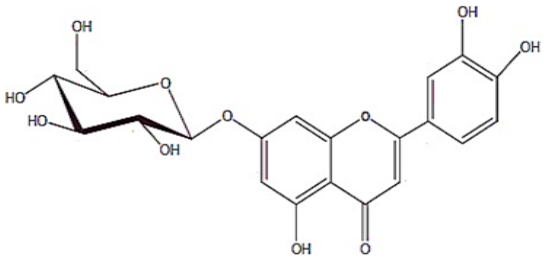


Fig. 3. ^{13}C NMR spectrum of luteolin-6-C-β-glucopyranoside

Compound 2 showed UV-Vis maxima at 254, 264 (shoulder), 345 nm. This compound was tentatively assigned as luteolin-7-O-β-glucopyranoside. The results of the test for flavonoids identification of sugar are shown in Table 2. The ^{1}H NMR spectrum of compound 2 shows an anomeric proton at δ_H 5.08 (1H, d, J=7.65), whereas compound 3 showed one aromatic proton at δ_H 5.5 (1H, d, J=7.65). The compounds 2 and 3 showed H-3 and H-6 at δ_H 6.61 and 6.43. ^{1}H NMR spectrum of compounds shows doublet at 6.9 (1H, d, J=2.3Hz).



Chemical structure of luteolin-7-O-β-glucopyranoside

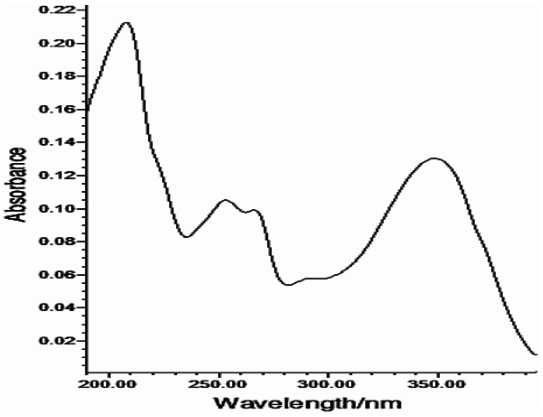


Fig. 4. UV-spectra of luteolin-7-O-β-glucopyranoside

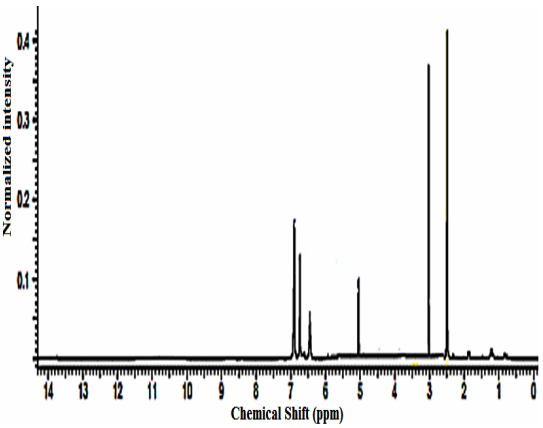


Fig. 5. ^{1}H NMR spectra of luteolin-7-O-β-glucopyranoside

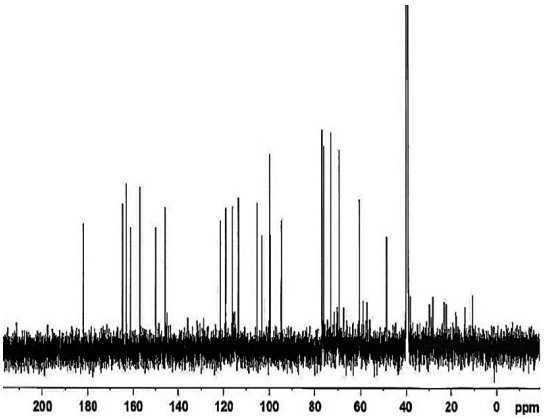
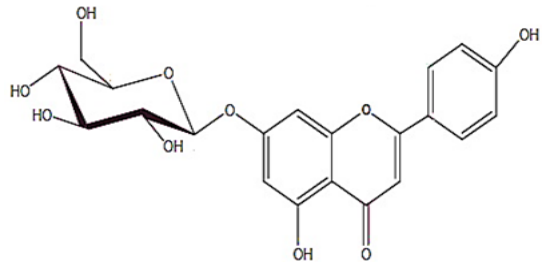


Fig. 6. ^{13}C NMR spectra of luteolin-7-O-β-glucopyranoside

Compound 3 exhibited two peaks at 254 and 350 nm that were matched with the luteolin-7-O-β-galactopyranoside spectrum[21].

The ^{13}C NMR signals of compound 2 and compound 3 exhibited the presence of a ketone carbonyl at δc 181.9 for luteolin-7-O-β-

glucopyranoside, and 184.0 for luteolin-7-O-β-galactopyranoside. Compound 2 showed two olefinic carbons at δc 164.5 and 103.1, whereas compound 3 exhibited signals of two olefinic carbons at δc 166.9 and 104.1. The ^{13}C NMR signals showed the presence of hydroxyl carbon position of compound 2 at 161.1 and 150.0 whereas compound 3 exhibited signals at 162.9 and 151.9 respectively.



Chemical structure of luteolin-7-O-β-galactopyranoside

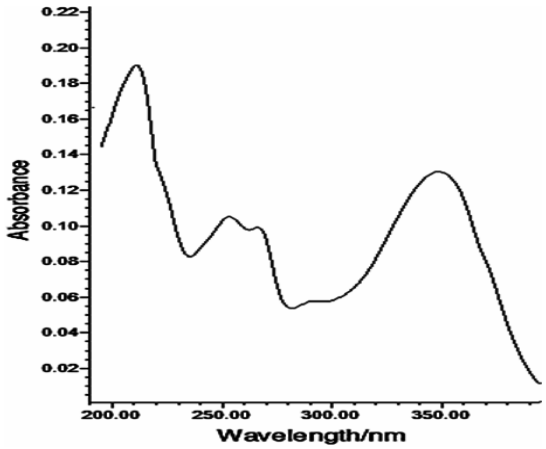


Fig. 7. UV spectra of luteolin-7-O-β-galactopyranoside

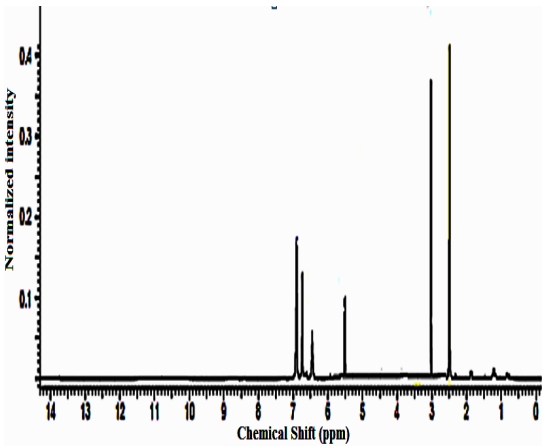


Fig. 8. ^{1}H NMR spectra of luteolin-7-O-β-galactopyranoside

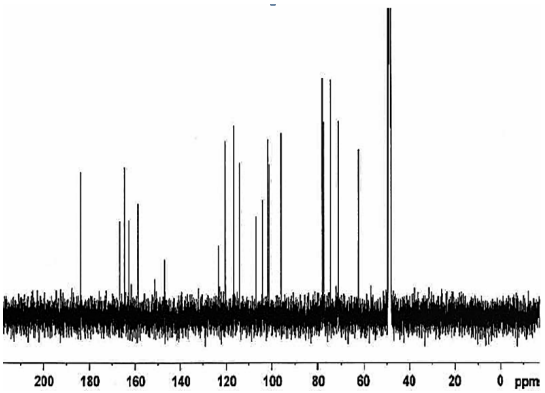


Fig. 9. ^{13}C NMR spectra of luteolin-7-O-β-galactopyranoside

The data suggest that compound 2 had six sugar attachment sites at (δ 99.9, 77.2, 76.4, 73.1, 69.6, and 60.6) revealed the presence of luteolin-7-O-β-glucopyranoside. The ^{13}C NMR chemical shifts of the sugar carbons (δ 101.7, 78.4, 77.9, 74.7, 71.3, and 62.4) showed the presence of luteolin-7-O-β-galactopyranoside (Table 3). The data allowed us to establish luteolin-7-O-β-glucopyranoside and luteolin-7-O-β-galactopyranoside as the structure of the compound by comprising of the spectral data with literature value[21-23].

The presence of flavonoids in sugarcane has become a global phenomenon. Authors have reported the presence of flavones, anthocyanins, and flavonols, along with chalcones and chlorogenic acids in sugarcane and cane juice from India[20,24-25], and abroad[26-27]. Flavonoids mainly flavones enters and accumulates into the sugar crystal through the sugar processing of flavonoids containing commodities (sugarcane, cane juice, molasses, and sugar house products) and may affect sugar quality[20,28].

CONCLUSION

The resin-based gel chromatography method developed for the identification of flavonoids was found to be simple, accurate, and sensitive. Three active flavonoids components from Indian Plantation white sugars were isolated (luteolin-6-C-β-glucopyranoside, luteolin-7-O-ß-glucopyranoside, and luteolin-7-O-β-galactopyranoside). It is concluded that sugarcane and sugar house products extract could potentially be used for food additives and the development of useful natural compounds.

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Conflict of interest

The author declares no conflict of interest.

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