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Short Communication

Structural Characterization of Chlorogenic Acid and Myricetin Flavonoids from Indian Plantation White Sugars

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Flavonoids are used as multifunctional natural antioxidants in food, feed, pharmaceutical, cosmetics, or nutraceutical industries. Natural colorants, such as chlorogenic acid are antioxidants widespread in the plant kingdom [1] and well represented in sugarcane. Various methods have been used to extract and isolate chlorogenic acid from cane juice [2]. Chlorogenic acids, a phenolic compound found in the sugarcane, possess to have anticancer, antioxidant, anti-viral, anti-inflammatory, anti-thyroid, anti-arteriosclerotic, antihypertensive and antihepatotoxic properties [3-6].

Myricetin and chlorogenic acid are a key ingredient in many foods and is used as a food additive as a result of its antioxidant activity and ability to protect lipids against oxidative damage. Available literature portrays the compound as a wonder nutraceutical and there is no doubt that the molecule holds the potential to protect against life-threatening diseases, including antibacterial activity and cancer [7]. Separation and identification of flavonoids myricetin are fruitful due to its various biological and pharmacological activities such as anti-carcinogenic, antiviral activities, cyto-protective capacity and therapeutic benefit of cardiovascular diseases [8-11]. Myricetin reported strong scavenging activity against DPPH radicals and activity against a variety of DNA and RNA polymerases. Myricetin also proves activity against skin cancer, anti-proliferative activity against human cell, and inhibits UV B induced activation [12-14].

Many studies have shown that 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.

^{1}H spectra of flavonoids were recorded using JEOL AL 500 MHz spectrometer in DMSO-d_6 containing TMS as an 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 A°, surface area =725 m^2/g) was used.

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Preparation of plantation white sugar

A 25°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 a 4-bed volume of 5% (v/v) HCl and followed by a 4-bed volume of 5% (v/v) NaOH, and redistilled water to a neutral pH. The initial concentration of plantation white sugar extract was 0.8mg/ml, the pH of the 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. After filtration, the colorant solution was adsorbed on to the gel column at a flow rate of 1ml/3min, 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.

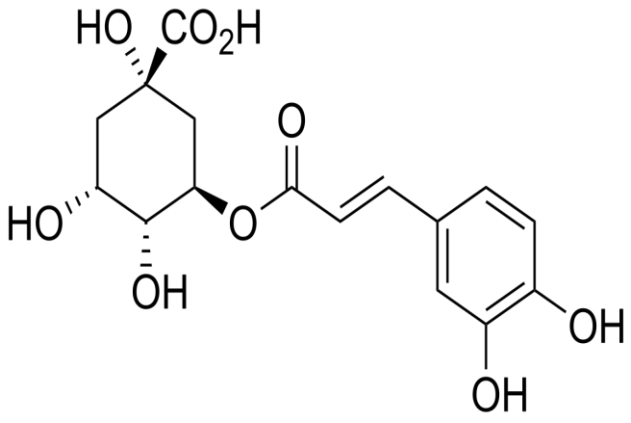
304 mg of pure light-yellow compound (m.p.168°C) resulted from first fraction. The isolated compound was elucidated by, Rf (Table 1), UV (Table 2), ^{1}H NMR, as well as comparison of the data with those reported in the literature [15].

Table 1 Rf values and spot appearance of flavonoids

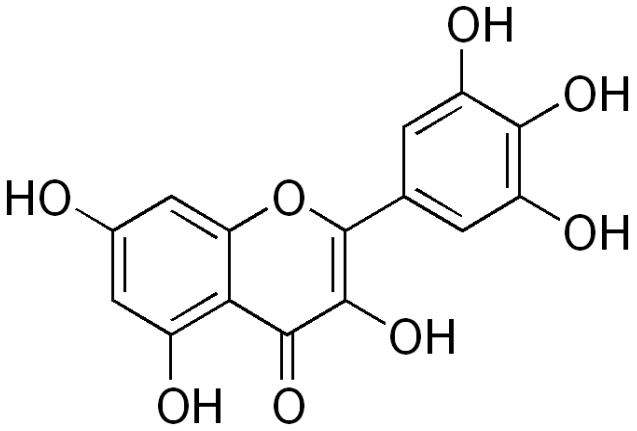
Compound	R_f value	UV light	UV/ NH_3
Chlorogenic acid	0.43 (TBA)	Deep purple	Yellow-green
Myricetin	0.57 (TBA)	Deep purple	Yellow-green

Table 2 The UV spectral data of flavonoids in plantation white sugar

Compound	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
Chlorogenic acid	270	280	265	258sh	275	272
	300sh	330	274	280	300sh	316sh
	334	394	304	305	378	352
			350	344		
			384	382		
Myricetin	270	275	265sh	264sh	280	270
	330	330	280	278	300	350
		400	300	300	380	400sh
			350	350		
			375			



Structure of chlorogenic acid



Structure of myricetin

Fig 1 shows the UV-Vis absorption spectra of chlorogenic acid at wavelength 200 to 400 nm. The compound has two absorption peaks at 217 (Band II) and 324 (Band I), with shoulder peak at 240 nm. Fig 2 is an expansion of the working region of the spectrum.

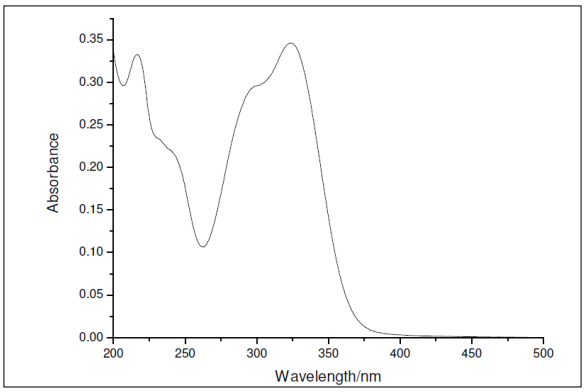


Fig 1 UV spectra of Chlorogenic acid

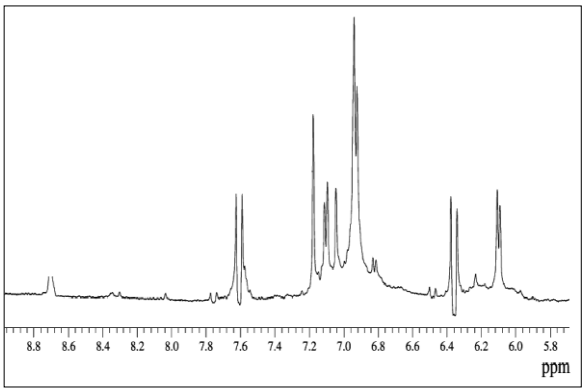


Fig 2 NMR spectrum of Chlorogenic acid

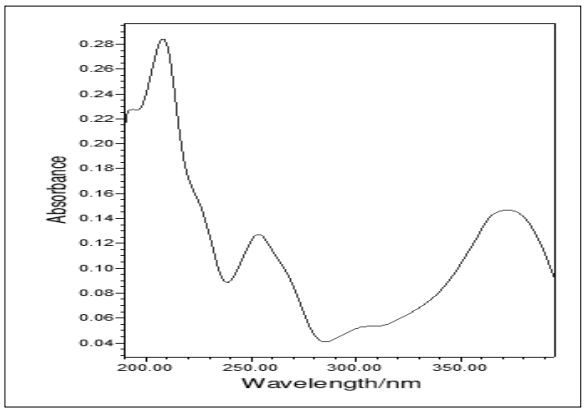


Fig 3 UV spectra of myricetin

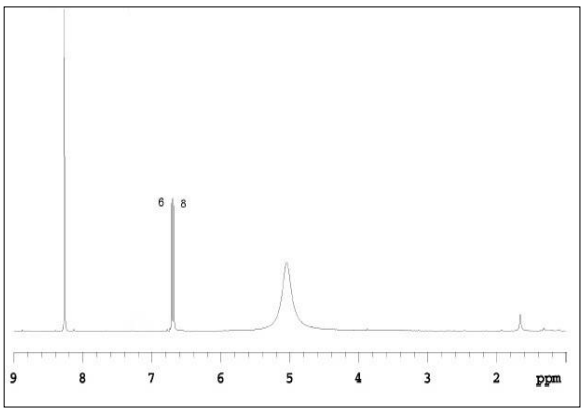


Fig 4 NMR spectrum of myricetin

The signals of chlorogenic acid are indicated on the spectrum and correspond to chemical shifts of 7.64, 7.20, 7.13, 6.96, and 6.38 ppm [15]. The 7.20 ppm signal was selected for measurement because it is strong enough and appears alone; hence, its intensity was used for calibration. The 6.96 ppm signal is inadequate since it is overlapped by many phenolic signals, for example, the 6.93 ppm signal of epicatechin, also present in cane juices. The 6.92 ppm signal is assigned to "polyphenols". The ^{1}H NMR characterization revealed two doublets (each 1 H) at 6.92 and 6.73, suggesting they are ortho coupled. Broad singlet peak at 7.01 for 1 H, proving the presence of tri substituted aromatic ring. A peak at 7.56 (H-7') and 6.23 (H-8') confirming ethylene moiety in the compound.

The second fraction resulted in 105 mg of compound resulted in the pale-yellow supernatant upon co-paper chromatography (TBA) with an authentic sample of myricetin was revealed to be myricetin (R_f=0.29)[12]. The UV spectrum of the above-purified sample was recorded with a double beam UV spectrophotometer (Fig 3). λ max (methanol) values were 254 nm (the band I) and 374 nm (band II) which were identical with that reported for myricetin. Final confirmation came from the NMR spectrum (Fig 4) [15]. Myricetin revealed doublet at 6.37, J=1.8 (each 1H) and 6.18, J= 2.4 for C-8' and C-6' proton. The compound shows a single peak at 7.24 (2H) confirming the position of H-2' and H-6' proton in the molecule. The ^{1}H NMR and UV spectral data were in agreement with the literature [15].

SUMMARY

Chlorogenic acid and myricetin are natural flavonoids found in many medicinal plants, is well known for its rich pharmacological activities. This paper describes the flavonoids composition of the plantation white sugar and the method of isolation thereof. A simple method for the simultaneous determination of chlorogenic acids and myricetin content in plantation white sugar has been reported. Two 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 1ml/3min, 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. Flavonoids structures were elucidated by Rf value, UV and NMR spectroscopic techniques, and chromatography. This is the first report of chlorogenic acid and myricetin as cane sugar pigments that can be used as natural antioxidants in food or non-food products.

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Conflict of interest: The author declares no conflict of interest.

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