



EXTRACTION OF FLAVONOIDS OF SEED COAT OF BAUHINIA TOMANTOSA

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ABSTRACT

The potential of Bauhinia tomentosa as a viable source of medicinally important flavonoids like rutin has been investigated four flavonoids, apigenin, quercetin, and rutin, kaempferol-4-methyl ether, have been isolated from seed coat of Bauhinia tomentosa.

Key words: Seed coat of bauhinia tomentosa, Flavonoids, UV, NMR.

INTRODUCTION

Bauhinia tomentosa¹, (leguminosae sub family Caesalpinaceae, Hindi name-Kachnar) Yellow Orchid Tree with dark scabrous bark, found throughout India. The whole plant has known medicinal value^{1,2} used in skin diseases, throat trouble, dysentery and diarrhea, refrigerant, malarial fever etc. Drought tolerant, pretty drooping flower.

Rutin; a well-known natural antioxidant is one of the medicinally important flavonoids found in seedcoat. Rutin can reduce capillary fragility swelling and bruising and has been used in the treatment of venous insufficiency (varicose veins, haemorrhoids, diabetic vascular disease. and diabetic retinopathy) and for improving micro vascular blood flow (pain, tired legs, night cramps and restless legs)^{3,4}. It induces CAMP synthesis⁵, inhibits phospholipase⁶ and super oxidase⁷, inhibits secretion and aggregation of platelets⁵, mild vasoconstrictor and increasing the resistance of small blood vessels through inhibition of COMT⁸, anti-tumor⁹ and analgesic with non-opioid mechanism¹⁰. Rutin has also been used as a colouring agent, food additive in various food preparations and drinks, and for various purposes in cosmetics. However, the necessity of identifying a better and/or cheaper commercial source for rutin and other related flavonoids are still valid. Bauhinia tomentosa

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is a rich source of medicinally important flavonoids including rutin. A large amount of seed of bauhinia is produced Pratap Nursery, Dehradun. We have investigated the potential of seedcoat of bauhinia for being used as an excellent source of flavonoids. We now report on the isolation and identification of four flavonoids from seed coat of bauhinia tomentosa^{11,12}.

EXPERIMENTAL

General NMR 200 MHz (Bruker spectrosopin) and UV-Visible spectrophotometer (Shimadzu, 2100) were used for recording spectra. All chemicals and solvents such as silica gel plate (GF254), methanol, petroleum ether, chloroform, ethyl acetate, n-butanol and rutin standard were purchased from Merck chemical company. The NMR spectra were measured in DMSO-d₆ and recorded at 200.13 MHz for ¹H NMR and 50.32 MHz for ¹³C NMR spectra. Chemical shifts were given in δ values relative to TMS. UV-Visible spectrum of each compound was determined in methanol and after addition of different shift reagents such as AlCl₃, AlCl₃/HCl, MeOH, AcONa and AcONa/H₃BO₄ at 190-500 nm¹²⁻¹⁴.

Plant material

A large amount of seed of Bauhinia tomentosa were obtained from Pratap Nursery Dehradun, India and identified by botany department.

Extraction of flavonoids of seed coat of Bauhinia tomentosa

The seed coat was extracted, repeatedly with using 70% methanol. This procedure was continued till negative cyanidin test. Then the methanol of the extract was evaporated under vacuum at 50°C by rotary evaporator to get an aqueous extract containing flavonoids.

Isolation of flavonoids

The resulted aqueous extract was partitioned using petroleum ether, chloroform, ethyl acetate and n-butanol to obtain respective fractions, which were analysed by TLC and a cyanidin test, was carried out. The ethyl acetate fraction, which contained the highest amounts of flavonoids, was subjected to column chromatography using various mobile phases : ethyl acetate (48 fractions), ethyl acetate-methanol-water (70-15-5) (96 fractions) and methanol-water (85-25) (48 fractions). Flow rate of mobile phase was maintained at 6 drops/min. TLC of CC (column Chromatography) fractions was carried out on silica gel plates using EtOAc-MeOH-H₂O (65-10-15) as a mobile phase. Flavonoid spots were visualised under UV lamp and also using ammonia vapour. Each group of fractions, FI (20-50), FII (55-70) and FIII (83-100) had at least one flavonoid. Main flavonoids of each fraction group were further purified by preparative TLC on silica gel [mobile phases:

EtOAc-MeOH (95-5), EtOAc -MeOH (90-10) and EtOAc-MeOH-H₂O (55-15-4)], which resulted in isolation of three flavonoids (1-3), respectively. Their structures were elucidated by spectroscopy.

RESULTS AND DISCUSSION

UV-Visible spectroscopy

UV-visible and shift reagent data of three flavonoids (1-4) are presented in Table 1.

NMR spectroscopy's

The ¹H NMR ¹³C-NMR data of flavonoids (1-4) were assigned as follows:

Apigenin (1)

¹H NMR (chemical shift δ in ppm, coupling constant J in Hz) 6.81 (1H, s C2-H), 6.21 (1H, d, J = 2.1, C6-H), 6.50 (1H, d, J = 2.1, C8-H), 7.95 (2H, d, J = 8.8, C2', 6'-H), 6.94 (2H, d, J = 8.8, C3', 5'-H), 10.39 (1H, s, C4'-OH), 12.94 (1H, s, C5-OH), 10.80 (1H, s, C7-OH); ¹³C NMR (chemical shift δ in ppm) 165.0 (C-2), 103.7 (C-3), 182.6 (C-4), 158.2 (C-5), 99.7 (C-6), 164.6 (C-7), 94.8 (C-8), 162.3 (C-9), 104.6 (C-10), 122.0 (C-1'), 129.4 (C-2'), 116.8 (C-3'), 162.0 (C-4'), 116.8 (C5'), 129.4 (C-6').

Quercetin (2)

¹H NMR (chemical shift δ in ppm, coupling constant J in Hz) 6.20 (1H, d, J = 2 Hz, C6-H), 6.42 (1H, d, J = 2 Hz, C8-H), 7.69 (1H, d, J = 2.2 Hz, C2'-H), 6.90 (1H, d, J = 8.5 Hz, C5'-H), 7.56 (1H, d, J = 8.5, 2.2 Hz, C6'-H), 9.41 (1H, s, C4'-OH), 9.35 (1H, s, C3'-OH), 9.63 (1H, s, C3-OH), 12.52 (1H, s, C5-OH), 10.82 (1H, s, C7-OH); ¹³C NMR (chemical shift δ in ppm) 147.6 (C-2), 136.6 (C-3), 176.7 (C-4), 157.0 (C-5), 99.0 (C-6), 164.7 (C-7), 94.2 (C-8), 161.6 (C-9), 103.9 (C-10), 122.8 (C-1'), 116.5 (C-2'), 145.9 (C-3'), 148.6 (C-4'), 115.9 (C-5'), 120.8 (C-6').

Rutin (3)

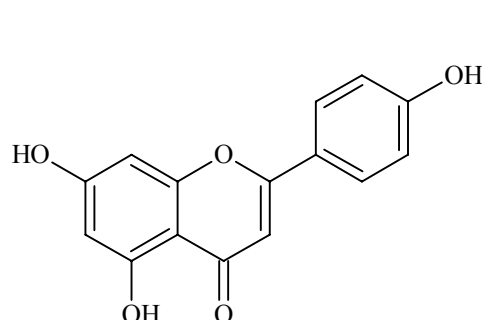
¹H NMR (chemical shift δ in ppm, coupling constant J in Hz) 6.21 (1H, d, J = 2, C6-H), 6.40 (1H, d, J = 2, C8-H), 7.55 (1H, d, J = 2.1, C2'-H), 6.86 (1H, d, J = 9, C5'-H), 7.56 (1H, d, J = 9, 2.1, C6'-H), 9.71 (1H, s, C4'-OH), 9.21 (1H, s, C3'-OH), 12.62 (1H, s, C5-OH), 10.86 (1H, s, C7-OH), 5.35 (1H, d, J = 7.4, H1-G), 5.12 (1H, d, J = 1.9, H1-R), 1.00 (3H, d, J = 6.1, CH3-R); ¹³C NMR (chemical shift δ in ppm) 157.3 (C-2), 134.1 (C-3), 178.2 (C-4), 157.5 (C-5), 99.5 (C-6), 164.9 (C-7), 94.5 (C-8), 162.1 (C-9), 104.8 (C-10), 122.5 (C-1'),

116.1 (C-2'), 145.6 (C-3'), 149.3 (C-4'), 117.1 (C-5'), 122.0 (C-6'), 101.6 (C1-G), 74.9 (C2-G), 77.3 (C3-G), 72.7 (C4-G), 76.7 (C5-G), 67.9 (C6-G), 102.2 (C1-R), 70.8 (C2-R), 71.2 (C3-R), 71.4 (C4-R), 69.1 (C5-R), 18.6 (C6-R) [R and G represent signals from rhamnose and glucose moieties, respectively].

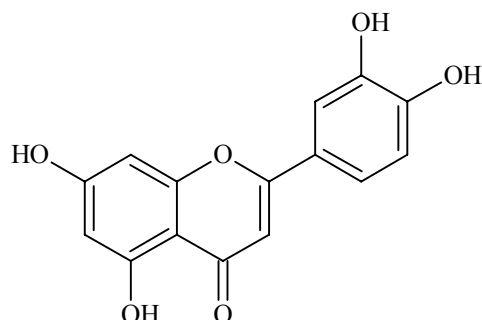
Kaempferol 4-methyl ether (4)

^1H NMR (CDCl_3 , 90MHz): δ ; 6.1 (1H, d, $J = 2.5\text{Hz}$, H-6), 6.4 (1H, d, $J = 2.5\text{ Hz}$, H-8), 8.0 (2H, d, $J = 8.5\text{Hz}$, H-2', 6'), 7.8 (2H, d, $J = 2.5\text{Hz}$, H-3', 5'), 4.2 (3H, s, OCH_3). ^{13}C NMR (DMSO-d_6 , 100MHz) : δ 180.3 (C-4), 164.0 (C-2), 160.6 (C-4'), 158.6 (C-7), 155.2 (C-9), 145.3 (C-5), 126.6 (C-2', 6'), 122.6 (C-1'), 116 (C-3', 5'), 102.8 (C-3), 97.1 (C-8) 96.6 (C-6) 56.2 ($-\text{OCH}_3$).

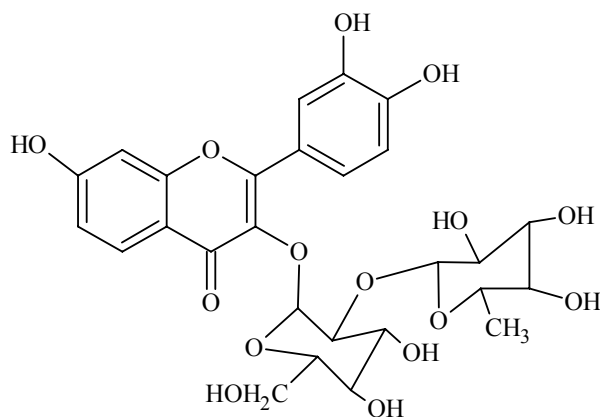
Structure



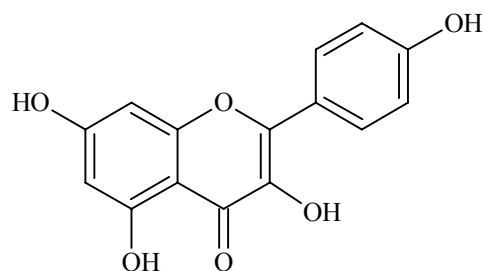
(1)



(2)



(3)



(4)

Table 1: UV-visible absorption peaks of flavonoids (1-4) in MeOH and their shifts in different solvents

Compds.	MeOH	NaOMe	AlCl ₃	AlCl ₃ /HCl	NaOAc	NaOAc/H ₃ PO ₃
1	335	392	381.6	381	381.8	337
	267.4	274.8	346.4	345	274.6	268
	298 (sh)	325.6 (sh)	381.6	299	299.4 (sh)	301 (sh)
			346.4	274.5 (sh)		
2	371		455	426	389 (dec)	386
	301 (sh)	326 (dec)	332	362	327	301
	268 (sh)	247.2	270	302 (sh)	290	260
	255		123	256	262	
3	359	411	430	400	387	378
	299 (sh)	328	203	360	322	292
	267 (sh)	272	475	300	273	260
	257			271		
4	251 (sh)	292	269	268	275	276
	268	341 (sh)	412	412	350 (sh)	352 (sh)
	368	410				

UV spectrum of methanolic solution of apigenin (1) supported the flavonol structure (Table 1). Bathochromic shift of band I in presence of MeOH and its stability after ten minutes relate to 4'-hydroxy, and bathochromic shift of band II about 7 nm is an indication for 7-hydroxyl. Bathochromic shift with AlCl₃ and its stability in the presence of HCl relate to 5-hydroxyl (Mabry et al. 1970). The UV, ¹H NMR and ¹³C NMR data for 1 were in good agreement with that of apigenin (14). This is the first report on the presence of apigenin in the seed coat of *bauhinia tomentosa*.

The UV spectrum of methanolic solution of quercetin (2) exhibited two major absorption bands at 371 nm and 255 nm (Table 1), which confirmed the flavonol structure. Degradation of 2 in presence of MeONa and hypsochromic shifts with AlCl₃/HCl and AcONa/H₃BO₄ supported the presence of 3, 3, 4' trihydroxy system. Bathochromic shifts with AcONa were related to 7-hydroxyl and the bathochromic shift with AlCl₃/HCl to 5-hydroxyl. On the basis of UV, ¹H NMR and ¹³C NMR data, compound 2 was identified as quercetin.

The UV spectrum of compound 3 showed two major absorption bands at 359 nm and 257 nm, which indicated the presence of flavonol structure. Bathochromic shift with MeONa supported the presence of 4'-hydroxyl and with AcONa indicated the 7- hydroxyl functions. The AlCl_3 and AlCl_3/HCl spectrum of 3 showed 5-hydroxyl and ortho dihydroxy in ring B. This fact indicated that the 3-hydroxyl was absent or substituted (Table 1). The ^1H NMR and ^{13}C NMR of 3 revealed the chemical shifts of protons and carbons essentially identical with those reported in the literature for rutin. However, it can be used as an economical source of rutin.

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