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Chemical Studies on Flowers of *Michelia champaca*

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The flowers of *Michelia champaca* have been shown to contain the flavonoid quercetin and an unidentified flavonoid glycoside besides β-sitosterol, unsaturated aliphatic ketones, and hydrocarbons. The isolation of quercetin forms the first report of the occurrence of this flavonoid in the genus *Michelia*.

Michelia (Magnoliaceae) is a genus of shrubs and trees distributed in Southeast Asia and China. Major constituents isolated from the genus *Michelia* are essential oils[1-5], alkaloids[3,6-8] (magnoline[3], magnolamine[3] and liriodenine[6-8]), sesquiterpene lactones[6-9] and tannins[3]. Aerial parts of *Michelia sphaerantha* have been reported to contain a sesquiterpene sphaelactone A and other constituents sinapaldehyde, syringaresinol, syringaldehyde, 3,3'-dimethoxy-4,4'-dihydroxy-9,9'-monoepoxylignan, nonacosane, hexadecanoic acid and β-sitosterol[10] whereas *M. yunnanensis* has yielded a new sesquiterpene, 12,13-diacetoxyl-1,4,6,11-eudesmanetetrol[11-12]. *Michelia champaca* Linn. is an evergreen plant occurring wild in the Eastern sub-Himalayan tract and lower hills up to 3000 ft., Assam, Myanmar, Western Ghats and South India[5,14]. In India it is cultivated in gardens and near temples for its fragrant flowers and handsome foliage. Its volatile oil is highly esteemed in perfumery[3] and is useful in cephalalgia, ophthalmia, gout and rheumatism[3,5]. Fruits and flowers of *M. champaca* are attributed several useful properties[3,5,14]. Literature survey on this plant revealed the leaves of the plant to contain parthenolide[15]. However, no systematic chemical study on the flowers of *M. champaca* has been carried out. It was therefore thought to undertake a thorough chemical investigation on the *M. champaca*. The present communication reports the isolation of flavonoid and other non-nitrogenous constituents from the flowers of *M. champaca*.

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MATERIALS AND METHODS

Melting points are uncorrected. UV spectra were recorded on a Perkin-Elmer Model Lambda 15 spectrometer; IR spectra were recorded on a Perkin-Elmer Model 882; and ^1H and ^{13}C NMR spectra on a 300 MHz Bruker AC 300 F spectrometer with TMS as internal standard using $CDCl_3$ and DMSO-d_6 as solvents. Mass spectra were recorded on a VG Micromass 7070 F mass spectrometer. Column chromatography was carried out using silica gel (60-120 mesh) and TLC was done on silica gel G (E. Merck), which was activated at 110° for 30 min. The flowers of *M. champaca* were collected locally from the trees growing within the Panjab University Campus.

Extraction and isolation:

The flowers were dried in shade and converted to moderately coarse powder. The dried powder (500 g) was Soxhlet extracted with petroleum ether (60-80°) for 30 h. Removal of the solvent yielded 7 g of crude product. The extractive was chromatographed over silica gel eluting with stepwise increase of ethyl acetate in petroleum ether followed by pure ethyl acetate and mixtures of ethyl acetate and methanol. The fractions obtained were monitored by TLC using toluene:ethyl acetate (80:2) as the solvent system and fractions found to be identical were combined. Six compounds were isolated.

Compound A, which was obtained as a waxy residue gave negative Liebermann-Burchard reaction. Its IR spectrum in KBr showed stretchings at 2940, 2850, 1450, 750 cm^{-1}; the ^1H NMR ($CDCl_3$) gave signals at δ 0.88, 1.25. Com-

pound B also a waxy residue gave negative Liebermann-Burchard reaction. Its IR (KBr) showed stretchings at 2920, 2840, 750 cm^{-1} and the ^1H NMR ($CDCl_3$) gave signals at δ 0.88, 1.25. Compound C again a waxy residue gave brownish colouration on Liebermann-Burchard reaction. Its IR (KBr) showed stretchings at 2930, 2850, 1710, 1640, 1460, 1410, 750 cm^{-1} and the ^1H NMR ($CDCl_3$) gave signals at δ 0.88, 1.25, 1.63, 2.04, 2.34, 5.34. Compound D, a waxy residue, gave brownish colouration on Liebermann-Burchard reaction. Its IR (KBr) showed stretchings at 2920, 2840, 1720, 1460, 1410, 720 cm^{-1} and the ^1H NMR ($CDCl_3$) gave signals at δ 0.86, 1.26, 1.63, 2.04, 2.33, 5.34. The compound E which was obtained as a white crystalline solid, m p 133-135°, gave R_f value 0.58 in $CHCl_3$:MeOH (9:1). A green colouration was obtained on Liebermann-Burchard reaction. The IR (KBr) spectra showed stretchings at 3430, 1660, 1050, 980 cm^{-1} and the ^1H NMR ($CDCl_3$) gave signals at δ 0.68, 1.03, 5.08 and 5.34.

Isolation of flavonoids:

The marc obtained after extraction with petroleum ether (60-80°) was further extracted in a Soxhlet with ethanol for 28 h. The extract was concentrated under vacuum to a syrupy consistency to which near boiling water (500 ml) was added and the mixture was stirred thoroughly. Mixture was cooled and filtered, the filtrate washed with solvent ether (4x250 ml) followed by extraction with ethyl acetate (5x250 ml). The combined ethyl acetate extract was dried (Na_2SO_4), filtered and solvent removed under vacuum. A dark brown residue (10 g) was obtained. The residue gave positive HCl-Mg test giving pink colour. TLC (silica gel G) using EtOAc:HCOOH:H_2O (10:2:3) revealed 3 spots. The residue was chromatographed over silica gel (60-120 mesh, Merck) and eluted sequentially with $CHCl_3$, $CHCl_3$:EtOAc, EtOAc, and EtOAc:MeOH, gradually increasing the polarity; elution being monitored by TLC. The fractions obtained with $CHCl_3$:EtOAc and EtOAc were further purified by preparative TLC using EtOAc:HCOOH:H_2O (10:2:3) to give flavonoid A as light yellow residue which crystallised from aqueous ethanol (0.021 g). The elution from EtOAc:MeOH (95:25) from the column gave a brownish sticky residue (0.032 g) was designated as flavonoid B.

Flavonoid A as light yellow crystals, melted at 314-320° and gave positive test for flavonoids; R_f 0.69 in EtOAc:HCOOH:H_2O (10:2:3); UV (MeOH) (nm): 370; 255; IR (KBr): 3370, 1665, 1610, 1150, 1360, 1200 cm^{-1}, EIMS m/z : 302 [M^+], 153. For acetylation of flavonoid A, 8 mg were taken in acetic anhydride (2 ml) and pyridine (1 ml) and heated on a water bath for 2 h. The usual workup gave precipitates which were crystallised from aqueous ethanol as white needles (5 mg), m p 195-198°. Flavonoid B, a brownish residue, gave positive tests for flavonoids and a positive Molisch test for sugars; R_f 0.50 in EtOAc:HCOOH:H_2O (10:2:3); UV (MeOH) (nm): 340, 275; IR (KBr): 3400, 1660, 1610, 1500, 1360, 1210cm^{-1}, ^1H NMR (DMSO-d_6): δ 6.80, 7.02, 7.25, 7.5, 8.46, 8.56, 9.06, 9.39; ^{13}C NMR (DMSO-d_6): δ (ppm) 113.38, 115.5, 120.14, 124.05, 125.72, 127.87, 128.60, 129.16, 129.68, 143.20, 144.65, 144.98, 145.39, 148.26, 165.59 (tentatively assigned for flavonoid part), and 34.90, 38.65, 39.20, 40.04, 40.31, 60.64, 68.32, 70.31, 71.82, 74.35, 100.77, 102.38 (could be due to sugar moieties).

RESULTS AND DISCUSSION

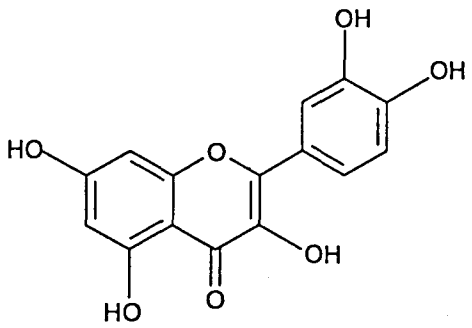
Petroleum ether extract of the dried flowers on chromatographic resolution on a silica gel column (elution being monitored by TLC) yielded six fractions, which were designated as compound A, B, C, D, E and F. Compounds A and B, which were waxy residues that gave negative Liebermann-Burchard reaction and were identified to be *n*-alkane hydrocarbons by IR and ^1H NMR spectra. The IR spectrum of compound A showed bands at 2940 and 2850 cm^{-1} due to aliphatic C-H stretching; a band at 1450 cm^{-1} due to C-H bending of methylenes; and a band at 750 cm^{-1} due to $-CH_2-$ rocking. It's ^1H NMR showed methyl signal at δ 0.88 as triplet, methylenes [-$(CH_2)_x$-] were indicated by the signal at δ1.25. Likewise the IR spectrum of compound B showed bands 2920, 2840 cm^{-1} and at 750 cm^{-1}; and the ^1H NMR showed signals at δ 0.88 and 1.25. The compound C, also a waxy residue, produced a brownish colouration on Liebermann-Burchard reaction which, however, was not characteristic of sterols or triterpenes. The infrared spectrum of compound C showed bands at 2930 and 2850 cm^{-1} due to aliphatic C-H stretching. A strong band at 1710 cm^{-1} was indicated of keto carbonyl stretching. The band at 1640 cm^{-1} was due to non-conjugated C=C stretching. The ^1H NMR showed a triplet at δ 0.88 for methyl and a signal 1.25 for methylene protons. Signal due to methylene protons adjacent to carbonyl appeared at δ 2.04; while the protons of methylene groups flanking C=C appeared at δ 2.34. Signal at δ 5.34 was indicative of double bond protons. The data suggested that the compound C was an unsaturated aliphatic ketone. Compound D was found to be identical to compound C. It also gave brownish colouration on Liebermann-Burchard reaction. The infrared spectrum showed bands at 2920, 2840, 1720, 1460 and 720 cm^{-1}. The ^1H NMR signals appeared at δ 0.88, 1.26, 1.63, 2.04, 2.33 and 5.34.

The compound E which could be crystallized from methanol to give a crystalline solid, m.p. 133-135° (Lit[16] for β-sitosterol 136-137°) gave a green colour on Liebermann-Burchard reaction indicative of sterols. The IR spectrum showed bands at 3430 cm^{-1} for associated O-H stretching and at 1660 cm^{-1} for nonconjugated C=C stretching. The band at 980 cm^{-1} was indicative of *trans* disubstituted double bond of stigmasterol. The ^1H NMR spectrum showed a multiplet at δ 5.34 for vinylic proton and also at δ 5.08 for *trans* disubstituted double bond protons of stigmasterol. Singlets for C-18 and C-19 methyl groups appeared at δ 0.68 and 1.03, respectively. The spectra indicated compond E to be β-sitosterol. The natural β-sitosterol is commonly associated with stigmasterol having the *trans* 22-ene system.

Extraction of the petroleum ether-extracted dried marc of the flowers with ethanol followed by partitioning with ethyl acetate gave residue when responded positively to the tests for flavonoids. The residue on column chromatography and preparative TLC on silica gel afforded a crystalline, m p 314-320°(decomp) (Lit[17] for quercetin 316-318°) and a non-crystalline flavonoidal components, designated as flavonoid A and flavonoid B, respectively.

The UV of the flavonoid A in methanol showed absorption at 370 and 255 nm; due to the characteristic band I and II, respectively, of flavonols. The IR showed a band at 3370 cm^{-1} for O-H stretching. A band at 1665 cm^{-1} was due to C=O stretching of flavonoids. Benzenoid absorption bands appeared at 1610 and 1540 cm^{-1}. A band at 1360 cm^{-1} was due to in-plane phenolic O-H band, and at 1200 cm^{-1} was due to phenolic C-O stretching. The mass spectrum of the fraction showed a molecular ion peak at m/e 302, corresponding to the molecule formula $C_{15}H_{10}O_7$; and a characteristic fragment ion (a) appeared at m/e 153. Acetylation of the flavonoid with acetic anhydride/pyridine gave an acetyl derivative, m.p. 195-198° (Lit[17] for quercetin pentaacetate, m.p. 200°). On the basis of the data flavonoid A was characterised as quercetin (1). It was confirmed by co-TLC with an authentic sample of quercetin. The isolation of quercetin forms the first report of its occurrence in the genus *Michelia*.

The flavonoid B which responded positively to both HCl-Mg and Molich's tests was suggested by the qualitative test to be a flavonoid glycoside. It gave absorption bands in UV spectra at 340 and 275 nm, characteristic of flavonoid skeleton. The infrared spectrum showed bands at 3400 cm^{-1} due to O-H stretching and at 1660 cm^{-1} due to C=O of flavonoid. Bands at 1610 and 1500 cm^{-1} were due to benzenoid absorption. Bands at 1360 cm^{-1} and 1200 cm^{-1} were due to in-plane phenolic O-H bond and phenolic C-O stretch, respectively. The ^1H NMR showed aromatic protons at signals δ 7.50, 7.25, 7.02 and at 6.8. The signals at δ 8.46, 8.56, 9.06 and 9.39 could be attributed to hydroxyl protons of flavonoid portion. The ^{13}C NMR spectrum indicated the presence of 27 carbons suggesting that the flavonoid glycoside has two sugar units. The ^{13}C signals at δ (ppm) 113.38, 115.5, 121.14, 124.05, 125.72, 127.87, 128.60, 129.16, 129.68, 143.2, 144.65, 144.98, 145.39, 148.26 and 165.9 could be tentatively assigned for the flavonoid part, while the values at δ (ppm) 34.90, 38.65, 39.20, 40.04, 40.31, 60.64, 68.32, 70.31, 71.82, 74.35, 100.77 and 102.38 could be due to sugar moieties. No definite structure could be assigned to the isolated flavonoid glycoside, and further work is warranted.



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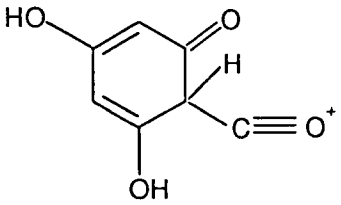

1 a m/z = 153

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