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Research Article

NATURAL CHEMICAL COMPOUNDS FROM STEM BARK OF *ACACIA NILOTICA*: ISOLATION AND CHARACTERIZATION

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ABSTRACT

Acacia is a genus of about 800 species of deciduous shrubs and trees and occupies the largest area of Rajasthan, Gujarat and the Deccan. About 25 species are of Indian origin. Some species of *Acacia* genus are commonly used in dying industries. Different parts of the plant *Acacia nilotica* are used in traditional medicine system due to their bioactive compounds. Hexacosanol (A), Lupa-12,20(29)-diene-3-one (B), Triacontanol (C) Stigmasterol (D) and Quercetin (E) natural compounds were isolated from methanolic extract of *Acacia nilotica* stem bark and characterized on the basis of different type of spectroscopic techniques *i.e* ^{1}H NMR, ^{13}C NMR, IR and MS.

Keywords: Phytochemical, Fabaceae, Mimosoidae, *Acacia nilotica,* stem bark, Biological Activity.

INTRODUCTION

Acacia Genus belongs to the sub-family Mimosoidae of the family Fabaceae. *Acacia* is a genus of about 800 species of deciduous shrubs and trees and occupies the largest area of Rajasthan, Gujarat and the Deccan. About 25 species are of Indian origin. Some of its species used in traditional medicine system[1]. The gum of many species of *Acacia* is used as a demulcent. Many species of *Acacia* are used to cure rabies disease[2]. The bark in most cases is a powerful astringent and tonic. Catechin, dicatechin, epicatechin, epigallocatechin, gallic acid, leucocyanidin gallate and quercetin were isolated from the bark of *Acacia arabica*[3,4]. The bark of this plant is used to cure cough, dysentery and leucoderma, while its leaves are found to have a curative action in the diseases of the eyes[3]. Leaves of *A. catechu* afforded 1-acacatechol, dl-acacatechol and 1-isoacacatechol[5,6]. Acetone extract of its heartwood yielded epicatechol[7] whereas water extract gave catechin and dicatechin derivatives[8]. The bark of *A. catechu* is reported to strengthen the teeth. The leaves of *A. farneciana* boiled in water are used for the treatment of gonorrhea[9] and decoction of the bark of *A. leucophloea* has antipyretic and anthelmintic action. α-Spinasterol and lupeol were separated from the benzene extract of bark of *A. pennata*. The juice of the bark acts as an antidote for snake poison. The leaves of *A. pinnate* are used to cure the bleeding gums[10]. Some species of *Acacia* genus are commonly used in dying industries. Wood of *A. catechu* contains catechin phytochemical and gives reddish brown color when used to dying cotton, silk and in calico printing[11]. Wool fabric has been dyed with an aqueous extract from the bark of *A. pennata* containing tannin as the major colourant[10]. Bark, pods and leaves of the *A. arabica* produce black, brown and khaki color on textiles while gum of this plant is used in calico printing, sizing material for cotton and silk for fixing paint and white wash. The pods give fast buff color to leathers. A fast brown color from the pods is used for manufacture of ink[12].

Acacia nilotica is commonly known as babul or kikar in Rajasthan. It is small or medium sized tree with gloomy coloured bark. Bark of this plant has antimicrobial activity and play role for hair wash. Its powder is used as tooth powder[13]. In ayurvedic medicine, the bark, leaves, pods and flowers of this plant are used against bleeding piles, cancer, cold, congestion, cough, diarrhea, dysentery, fever, gall bladder, hemorrhoid, leprosy, leucoderma, ophthalmia, sclerosis, tuberculosis, small pox and menstrual problems[14]. It was also considered as a remedy for treating premature ejaculation[14]. It has been observed that different parts of the plant are flourishing in tannins, stearic acid, vitamin-C, carotene, crude protein, crude fiber, arabin, calcium, magnesium and selenium[15]. This plant contain different types of bioactive components such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-diglucoside, glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives[16]. Traditionally *A. nilotica* pods are being used as a dye source for colouring the winnowing bamboo items and door panels[17] in some states of India. Pod of *A. nilotica* has rich source of phytochemical pyrogallol and pyrocatechol and good source for dyeing silk and other fibers in eco-friendly way with different mordants and mordant concentrations[17]. In view of our interest in dye yielding plants we have selected bark of *Acacia nilotica* for phytochemical study.

MATERIALS & METHODS

General experimental procedures

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. Qualitative TLC was conducted on aluminium sheet Kieselgel 60 F_{254} (E. Merck). Silica gel (E. Merck, 60-120 mesh, 550 gm) used for column (1.5m × 4.0cm) chromatography. The IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer with KBr pellets. The ^{1}H and ^{13}C NMR spectra were collected on JEOL FX 400 FT

NMR spectrometer in $CDCl_3$ at 400.4 MHz and 75.45 MHz frequencies for ^1H and ^{13}C NMR respectively using TMS as internal standard (From MNIT, Jaipur). FAB mass spectra were recorded on JEOL SX 102 /DA-6000 mass spectrometer using Argon /Xenon as FAB gas.

Plant Material

The plant material (stem bark), *Acacia nilotica* was collected from Jaipur District of Rajasthan (India) and the authenticity was confirmed by Incharge of Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen was submitted to the Herbarium of the University (voucher no. RUBL-225174).

Extraction and Isolation of the Constituents

The shade dried plant material (Stem Bark of *Acacia nilotica*, 1.5 kg) was powdered and extracted with methanol in a 5.0 liter round bottom flask for 72 hrs on water bath. The extract was filtered and solvent was removed by distillation under reduced pressure where a semi-solid dark brown mass (27g) was obtained. The solvent free extract was chromatographed over silica gel column. The column was eluted with different solvents in order of increasing polarity where following compounds (A to E) were isolated, purified and characterized.

Isolation of compound A as Hexacosanol: This compound was isolated when column was eluted with petroleum ether and crystallized with ethyl acetate as white microcrystals, m.p. 79-80°C. It did not respond to TNM test and showed no absorption in UV and visible regions. MS (m/z) 382 [M^+], 364, 321, 308, 307, 293, 279, 265, 251 etc. Molecular formula calculated as $C_{26}H_{54}O$. IR (cm^{-1}, KBr) 3280 (broad, O–H stretching), 2910, 2830, 1480, 1370, 1067 (C–O stretching), 730, 715. ^1H NMR (δ ppm, CDCl3) 0.89 (t, 3H, *J = 6.2, 6.9Hz*, C-26, -CH_3), 2.32 (t, 2H, *J = 7.3, 7.6Hz*, C-1, -CH_2-), 2.88 (br, s, 1H, C-1, -OH), 1.61 (m, 2H, C-2, -CH_2-), 1.24 (br, s, 46H, C-3 to C-25,).

Isolation of compounds B as Lupa-12,20(29)-diene-3-one: When column was eluted with petroleum ether and benzene in the ratio of 3:1 fraction no. 2 was obtained and compound B and C was separated through TLC (n-hexane : benzene :: 2 : 3). After removal of solvent colourless solid was obtained and its melting point was observed 194-195°C. MS (m/z) 422(M^+) Molecular formula calculated as $C_{30}H_{46}O$. IR (cm^{-1}, KBr) 1749 (>C=O str.), 1615 [C=C at C-20(29)], 1635 [C=C at C-12 (13)]. ^1H NMR (δ ppm, $CDCl_3$) 0.78 (s, 3H, C-24), 0.82 (s, 6H, C-25, C-27), 1.12 (s, 9H, C-23, C-26, C-28), 1.64 (s, 3H, C-20), 4.68, 4.69 (d, 2H, C-30), 2.20-2.53 (m, 2H, C-2), 1.17-1.57 (remaining 18 protons), 5.03 (br, s, 1H, C-12), 1.70 (d, 2H, C-2).

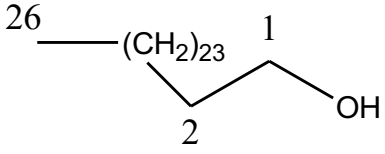
Isolation of compounds C as Triacontanol: It was isolated with compound B when elution of column by petroleum ether with benzene in the ratio 3:1 and they were separated by PTLC by using n-hexane : benzene (2:3) as mobile phase. Melting point was found to be 85-86°C. It did not respond to TNM test and showed no absorption in UV and visible regions. Mass (m/z) 438 [M^+], 420, 405, 392, 391, 377, 363, 349, 335 etc. Molecular formula calculated as $C_{30}H_{62}O$. IR (cm^{-1}, KBr) 3430 (broad, O–H stretching), 2945, 2870, 1475, 1380, 1065 (C–O stretching), 735 and 730. ^1H NMR (δ ppm, CDCl3) 0.82 (t, 3H, *J = 6.1, 6.7Hz*, C-30, -CH_3), 2.25 (t, 2H, *J = 7.5, 7.8Hz*, C-1, -CH_2-), 2.90 (br, s, 1H, C-1, -OH), 1.65 (m, 2H, C-2, -CH_2-), 1.17 (br, s, 46H, C-3 to C-29).

Isolation of compound D as Stigmasterol: Removal of solvent afforded yellow solid, by eluting the column with petroleum ether and benzene (1:1) compound D was obtained (m.p. 167-168°C). It gave positive Liebermann-Burchard sterol and TNM test for unsaturation. Mass (m/z) 412 [M^+], 399, 384, 369, 314, 302, 273, 255 etc. Molecular formula calculated as $C_{29}H_{48}O$. IR (cm^{-1}, KBr) 3410-3215 (OH), 1467 (-CH=CH-bending), 1378, 1362, 1265, 1055, 965, 810. ^1H NMR (δ ppm, CDCl3) 5.32 (t, C-6), 5.07 (dd, *J = 16.0, 10.0 Hz*, C-22), 5.17 (dd, *J = 16.0, 10.0 Hz*, C-23), 3.48 (m, H-3α), 0.83 (t, *J = 7.0 Hz*, C-29 methyl), 0.98 (d, *J = 7.0 Hz*, C-21 methyl), 1.12 (s, C-27 methyl), 0.92 (s, C-19 methyl), 0.74 (s, C-18 methyl).

Isolation of compound E as Quercetin: Compound E was isolated by eluting the column with Ethyl acetate. The solvent was removed and the product obtained was crystallized with methanol and acetone in the ratio of 1:1 as light yellow needles, m.p. 315-316°C. MS (m/z) 302 (M^+), 284, 152, 105, 95 etc. Molecular formula calculated as $C_{15}H_{10}O_7$. IR (KBr, cm^{-1}) 3460 (O-H stretching), 3015 (aromatic C–H stretching), 1593, 1520 (aromatic C-C stretching), 910, 825, 790. ^1H NMR (δ ppm, $CDCl_3$) 12.30 (s, OH), 10.25 (s, OH), 8.86 (s, OH), 8.85 (s, OH), 8.30 (s, OH), 7.73 (m, 2H, C-2′, 6′), 7.02 (d, 1H, 8.4, C-5′), 6.30 (d, 1H, 2.5, C-6, 8). ^{13}C NMR (δ ppm, $CDCl_3$) 156.92 (C-2), 134.86 (C-3), 178.28 (C-4), 160.82 (C-5), 99.23 (C-6), 164.75 (C-7), 93.50 (C-8), 156.10 (C-9), 104.76 (C-10), 125.91 (C-1′), 114.55 (C-2′), 143.35 (C-3′), 147.60 (C-4′), 115.34 (C-5′), 120.83 (C-6′).

RESULT & DISCUSSION

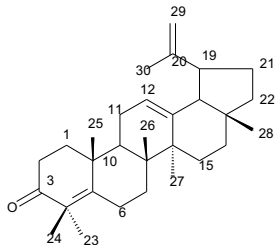
Characterization of compound A as Hexacosanol: The elemental analysis and mass spectral data established its molecular formula as $C_{26}H_{54}O$. Its saturated nature was confirmed by negative TNM test. In the mass spectrum, the molecular ion peak appeared at m/z 382. A peak at m/z 364 ($M^+ - H_2O$) and the intense peak at m/z 31 ($CH_2{=}O^+H$) confirmed its primary alcoholic nature. The significant peaks at low masses found were a set of alcohol type fragments (m/z 45, 59, 73 etc) resulting from cleavage of C-C bonds successively removed from oxygen atom. It also contained the sets of alkane type (m/z 29, 43, 57 etc) and olefin type (m/z 41, 42, 55, 56 etc) fragments, as expected for a primary alcohol. The base peak appeared at m/z 336 and other peaks appeared at an interval of 14 mass units by successive loss of –CH_2– groups. The important peaks observed in the IR spectrum were at 3280 (broad, O–H stretching), 1067 (C–O stretching), 730 and 715 cm^{-1} [doublet –$(CH_2)_n$– bending, n > 4]. The ^1H NMR spectrum (δ ppm, $CDCl_3$) showed a triplet for methyl group at 0.89 (t, 3H, *J = 6.2, 6.9 Hz*, C-26) and a broad signal with side bands integrating for forty six protons of methylene groups were accounted at 1.24 (br, s, 46H, C-3 to C-25). A triplet for two protons at 2.32 (t, 2H, *J = 7.3, 7.6 Hz*, C-1) was observed along with a broad singlet at 2.88 for one proton of hydroxyl group. A multiplate for two protons was observed at 1.61 for C-2 protons. On the basis of above data, Compound A appeared to be hexacosanol (Reported[18] m.p. 79-81˚C)



Compound A

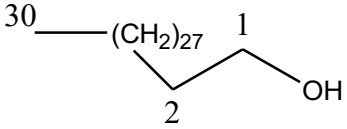
Characterization of compounds B as Lupa-12,20(29)-diene-3-one

In the mass spectrum of compound B, prominent molecular ion peak was appeared at m/z 422 [M^+]. The molecular formula of compound was assigned as $C_{30}H_{46}O$ by calculating protons and carbon atoms in the ^1H NMR and ^{13}C NMR spectrum respectively. The IR spectrum (cm^{-1}, KBr) showed characteristic absorption at 1749 suggested the presence of carbonyl group. The presence of >C=C< at C-20(29) and C-12(13) were confirmed by characteristic absorptions at 1615 and 1635 respectively. In the ^1H NMR spectrum (**δ** ppm, $CDCl_3$) three signals were observed at 0.78 (s, 3H), 0.82 (s, 6H) and 1.12 (s, 9H), were characterized for six tertiary methyl groups. Presence of a singlet at 1.64 confirmed the attachment of methyl group to the olefinic carbon (C-20). A pair of broad singlet at 4.68 and 4.69 for one proton each was attributed for the vinylic protons. A multiplet between 2.20-2.53 was assigned for two protons at C-2 position. A singlet observed at 5.03 for one olefinic proton present at C-12 position. A doublet was observed at 1.70 for two protons at position C-7. A complicated pattern was observed at 1.17-1.57 for eighteen protons. On the basis of above data and observation compound B was characterized as lupa-12,20(29)-diene-3-one (m.p. 194-195°C). The spectral data were compared with reported literature values[19].



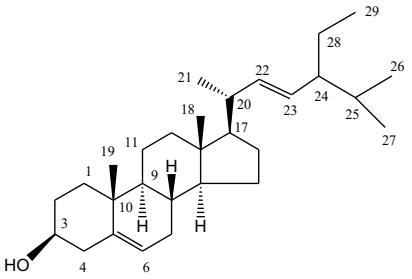
Compound B

Characterization of Compound C as Triacontanol: The elemental analysis and mass spectral data established its molecular formula as $C_{30}H_{62}O$. Its saturated nature was confirmed by negative TNM test. In the mass spectrum, the molecular ion peak appeared at m/z 438. Other characteristic peaks for a long chain primary alcohol were also observed. The important peaks observed in the infrared spectrum were at 3430 (broad, O–H stretching), 1065 (C–O stretching), 735 and 730 cm^{-1} [doublet – $(CH_2)_n$– bending, n > 4]. The ^1H NMR spectrum (δ ppm, $CDCl_3$) showed a triplet for methyl group at 0.82 (*t*, 3H, *J = 6.1, 6.7 Hz*, C-30) and a broad signal with side bands integrating for fifty four protons of methylene groups were accounted at 1.17 (br, *s*, 46H, C-3 to C-29). A triplet for two protons at 2.25 (*t*, 2H, *J = 7.5, 7.8 Hz*, C-1) was observed along with a broad singlet at 2.90 for one proton of hydroxyl group. A multiplate for two protons was observed at 1.65 for C-2 protons. These data were in complete agreement with those reported for triacontanol. (Reported[20], m.p. 85-86°C).



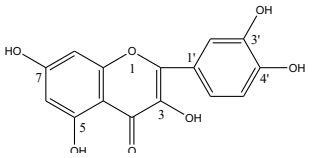
Compound C

Characterization of compounds D as Stigmasterol: Compound D was isolated as shining needles, m.p. 167°C. It gave positive TNM test for unsaturation. IR (cm^{-1}, KBr) spectrum displayed characteristic absorptions at 3410-3215 (OH stretching) and 1467 (-CH=CH- bending). It was analyzed for molecular formula $C_{29}H_{48}O$ [M^+, 412]. The ^1H NMR (**δ** ppm, $CDCl_3$) spectrum showed a pair of double doublets at 5.07 (*J = 16.0, 10.0 Hz*) and 5.17 (*J = 16.0, 10.0 Hz*) which were explainable to olefinic proton at C-22 and C-23 in the side chain. Large *J* values for these signals indicated the trans orientation of corresponding protons. A broad triplet at **δ** 5.32 was observed and accounted for C-6 olefinic proton. A multiplet at 3.48 corresponded to C-3 hydroxy methine proton. The singlets at 0.74 (C-18), 0.92 (C-19), 1.12 (C-27), a doublet centered at 0.98 (C-21), and a triplet centered at 0.83 (C-29) for methyl groups in the compound D was found similar to those stigmasterol. The chemical shifts for C-18 and C-19 methyl protons were in close agreement with those reported for Δ^5 sterols[21] From the above spectral data, the compound 'D' was characterized as stigmasterol and was confirmed by Co-TLC and Co-m.p. with authentic sample[22].



Compound D

Characterization of compound E as Quercetin: The molecular formula of the compound E was established with the help of mass spectral studies at 302 [M^+] as $C_{15}H_{10}O_7$. In the IR spectrum (KBr, cm^{-1}), a broad peak at 3460 indicated the presence of hydroxyl group. The aromatic C-H stretching was observed at 3015 along with the aromatic C=C stretching at 1593 and 1520. The ^1H NMR spectrum (δ ppm, $CDCl_3$) displayed a set of doublets at 6.29 (*J = 2.5 Hz*) for the meta-coupled protons at C-6 and C-8 positions respectively. A doublet observed at 7.04 (*J = 8.4 Hz*) was assigned to the proton at C-5′ position. The protons at C-2′ and C-6′ positions appeared at 7.72 as overlapping doublet (*J = 2.5 Hz*) and quartet (*J = 2.5, 8.4 Hz*) respectively. Five singlets observed at 12.26, 10.30, 8.90, 8.87 and 8.27 were assigned to five hydroxyl groups attached to C-5, C-3, C-7, C-3′ and C-4′ positions respectively. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) showed absorption at 178.28 (C-4) which indicated the presence of one carbonyl group and these values were assigned on the basis of reported values[23-25]. The carbon atom C-3, C-5, C-7, C-3' and C-4' those were attached to hydroxyl group showed absorptions at 134.86, 160.82, 164.75, 143.35 and 147.60 respectively. Other absorptions observed at 156.92 (C-2), 99.23 (C-6), 93.50 (C-8), 156.10 (C-9), 104.76 (C-10), 125.91 (C-1'), 114.55 (C-2'), 115.34 (C-5') and 120.83 (C-6'). From the above evidences, compound E was characterized as Quercetin.



Compound E

CONCLUSION

So many species of *Acacia* genus are commonly used in traditional medicine system as well as in dying industries. All parts of *Acacia nilotica* are rich source of phytochemicals. These natural compounds can be used to synthesis medicines and in dying industries. In this paper we have isolated five different natural compounds from stem bark and all have medicinal values. This paper is useful to researchers who are working in the field of natural products chemistry and medicinal chemistry.

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