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Phytochemical investigation and antimicrobial activity of *Acacia senegal* root heartwood

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ABSTRACT

The column chromatographic separation of the pet. ether, dichloromethane and ethyl acetate solubles of the ethanolic extract of the root heartwood of *Acacia senegal*, Mimosaceae led to the isolation of four waxy components (**1-4**), three steroids (**7, 8, 11**), three triterpenoids (**5**, **6**, **9**), a new quinic acid diester (**10**), and a cyclohexitol (**12**). Compound eicosanyl 3-O-feruloylquinate (**10**) has been isolated from the nature for the first time while 3α-hydroxyeuph-25-ene (**5**) and α-amyrin (**6**) have been isolated for the first time from this plant. Their structures were unambiguously determined by IR, ^{1}H and ^{13}C NMR spectroscopy as well as by mass spectrometry and by comparison with literature data. The ethanolic extract and all the solubles were screened for antimicrobial activity. The ethanolic extract exhibited moderate activity against the selected bacteria. However, the dichloromethane solubles exhibited significant activity against *E. coli* (ATCC 25922) and *S. aureus* (MTCC 740) (AI = 0.96). Moreover, the ethanolic extract and the dichloromethane and ethyl acetate solubles showed antifungal action against *C. albicans* (ATCC 4718) (AI ˜ 0.6). This is the first report of phytochemical investigation and antimicrobial activity of *A. senegal* root heartwood.

Keywords: *Acacia senegal*, Mimosaceae, quinic acid diester, steroids, triterpenoids, antimicrobial activity.

INTRODUCTION

Acacia, a genus of shrubs and trees belongs to the family Mimosaceae of the superfamily Fabaceae (Leguminoseae) which is the third largest amongst the angiosperms with approximately 650 genera and more than 18,000 species.[1] This is the second largest genus in the Fabaceae, comprising of more than 1200 species spread around the tropical to warm-temperate regions of both hemispheres, of which ˜ 20 are found in India,[2] most of which are medicinally useful.[3]

The selected plant, *Acacia senegal* (L.) Willd., known by the common names Gum Acacia or Gum Arabic tree is a small thorny xerophytic tree. It is native to semi-desert regions of Sub-Saharan Africa, as well as Oman, Pakistan and north-western India. The flowers are used for infection of eyes.[4] The demulcent, emollient gum is used internally in inflammation of intestinal mucosa and externally to cover inflamed surfaces as burns, sore nipples and nodular leprosy. It is used for its antitussive, astringent and expectorant properties in cold, cough, diarrhea, dysentery, gonorrhea, sore throat, typhoid and urinary tract infections.[3]

Several aspects of the phytochemistry of *A. senegal* have been reported previously. These include the isolation of triterpenoids, flavonoids, aliphatic alcohols and alkaloids from its fruits,[5] flowers,[6] leaves,[6-8] heartwood[6] and stem bark.[5,6] The gum obtained from the stem and branches is a rich source of polysaccharides.[9] Previous reports on biological activities revealed that the ethanolic extract of the stem bark exhibits antispasmodic and anti-inflammatory activities, although it was devoid of antibacterial, antifungal, antiviral, antifertility, hypoglycemic and diuretic activities.[10]

To the best of our knowledge, no phytochemical work on the roots of this plant has been reported till now. In continuation to our interest to isolate and identify the bioactives from arid zone plants,[11-13] the present study to investigate the chemistry alongwith the antimicrobial activity of the root heartwood of *A. senegal* was undertaken. The investigation of the chemistry of root bark is underway in our laboratory and will be reported in due course.

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MATERIALS AND METHODS

General

IR spectra were recorded on a Shimadzu FT IR - 8400S spectrometer using KBr pellets. The UV spectra were taken in ethanol (95%) using Shimadzu model Pharma Spec-1700 automatic recording spectrophotometer and Beckmann model DU. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using TMS as an internal standard on Jeol AL spectrometer at 300 MHz & 75 MHz respectively. Mass spectra were recorded on Waters Xevo Q-TOF spectrometer. Melting points were recorded in soft glass capillaries in Toshniwal apparatus and are uncorrected. Thin layer chromatograms were conducted on Merck silica gel G plates and in the column chromatographic fractionation, Merck silica gel (60-120 mesh) was used. For antibacterial assay, the test bacteria were procured from IMTECH, Chandigarh, India while for antifungal assay, the test fungi were obtained from IARI, New Delhi, India.

Plant material

The roots of *A. senegal* were collected from the dry hills of Triveni, about 60 km away from Jaipur, Rajasthan, India in August, 2011 during daytime and were positively identified by Prof. S. C. Jain, Department of Botany, University of Rajasthan, Jaipur, where a voucher specimen is deposited (Herbarium Sheet No. RUBL 20162).

Extraction and isolation of constituents

Air dried powdered root heartwood of *A. senegal* (2.8 kg) were extracted with ethanol (95%) on a steam bath thrice. The ethanolic extract was concentrated using rotary evaporator to a dark brown solid (130 g). This was re-extracted with pet. ether, dichloromethane and ethyl acetate, which on concentration afforded dark brown semi-solids (10.6 g, 13.5 g and 5.4 g respec

tively). The pet. ether and dichloromethane fractions, exhibiting a similar TLC profile, were mixed together and chromatographed over a column of silica gel (45 x 4 cm). Elution with solvents of increasing polarity viz. pet. ether, dichloromethane, ethyl acetate and methanol afforded fourteen fractions (Fraction A - N). Fraction B (eluent pet. ether : dichloromethane 9 : 1) afforded 0.224 g of **1** on recrystallisation (pet. ether). Fraction D with eluent pet. ether : dichloromethane 3 : 1 upon recrystallisation (methanol) yielded compound **2** (0.192 g). In the thin layer chromatogram of fraction E, two spots were clearly visible. When this was crystallised with pet. ether, the crystallised compound **3** (0.059 g) exhibited a single spot in TLC. Again, the filterate was recrystallised with ethyl acetate to afford compound **4** (0.052 g). The TLC of fraction F (eluent pet. ether : dichloromethane 3 : 1) exhibited two spots, so it was subjected to rechromatography (neutral alumina), which on elution with pet. ether : dichloromethane 9 : 1 yielded compounds **5** (0.121 g) and **6** (0.061 g) with pet. ether : dichloromethane 4 : 1. Fraction H (eluent pet. ether : dichloromethane 3 : 1 and 1 : 1) on crystallisation with chloroform/methanol yielded white needles of compound **7** (0.213 g). Compound **8** (0.162 g) was obtained as white needles from fraction I (eluent pet. ether : dichloromethane 1 : 1) on recrystallisation (chloroform/methanol). Fraction J (eluent pet. ether : dichloromethane 1 : 1) after removal of solvent gave a white solid which on crystallisation from pet. ether/chloroform gave white granules of compound **9** (0.194 g). Fraction L eluted with dichloromethane : ethyl acetate 4 : 1 gave light brown solid on removal of solvent which afforded white granules of compound **10** (0.503 g) on crystallisation with chloroform/acetone. The light-brown semi-solid obtained on evaporation of solvent from fraction N (eluent dichloromethane : ethyl acetate 1 : 4) furnished compound **11** (0.192 g) on crystallisation from chloroform/methanol. Fractions A, C, G, K and M did not yield any crystallisable compound. Similarly, the ethyl acetate fraction was also chromatographed over a column of silica gel (25 x 2.5 cm). This column, when eluted with solvents of increasing polarity, viz. pet. ether, dichloromethane, ethyl acetate and methanol, afforded five fractions (Fraction O - S). The light brown solid obtained after the removal of solvent from fraction R (eluent dichloromethane : ethyl acetate 3 : 2) yielded white granules of compound **11** (0.096 g) on crystallisation with methanol. A brown solid was obtained on removal of solvent from fraction S (eluent dichloromethane : ethyl acetate 1 : 4), which on crystallization with methanol yielded compound **12** (0.128 g) in the form of white powder. We could not crystallise compounds from fractions O, P and Q.

Ceryl cerotate (1 ; Fig. 1): $C_{52}H_{104}O_2$, mp 80-82°C. IR (u_{max}) cm^{-1}(KBr): 1735, 1140, 730 and 720. ^1H NMR ($CDCl_3$, 300 MHz): 4.05 (2H, t), 2.29 (2H, t), 1.58 (46H, br s), 1.25 (48H, br s), 0.88 (6H, t).

Eicosanoic acid (2 ; Fig. 1): $C_{20}H_{40}O_2$, mp 70-72°C. MS m/z 312 (M^+), IR (u_{max}) cm^{-1}(KBr): 3300-2650, 1745, 1300, 730 and 720. ^1H NMR ($CDCl_3$, 300 MHz): 2.25 (2H, t), 1.25 (34H, br s), 0.88 (3H, t).

Tetracosanol (3 ; Fig. 1): $C_{24}H_{50}O$, mp 72-74°C. MS m/z 354 (M^+), IR (u_{max}) cm^{-1}(KBr): 3320, 1060, 725 and 720. ^1H NMR ($CDCl_3$, 300 MHz): 3.85 (2H, t), 1.22 (44H, br s), 0.90 (3H, t).

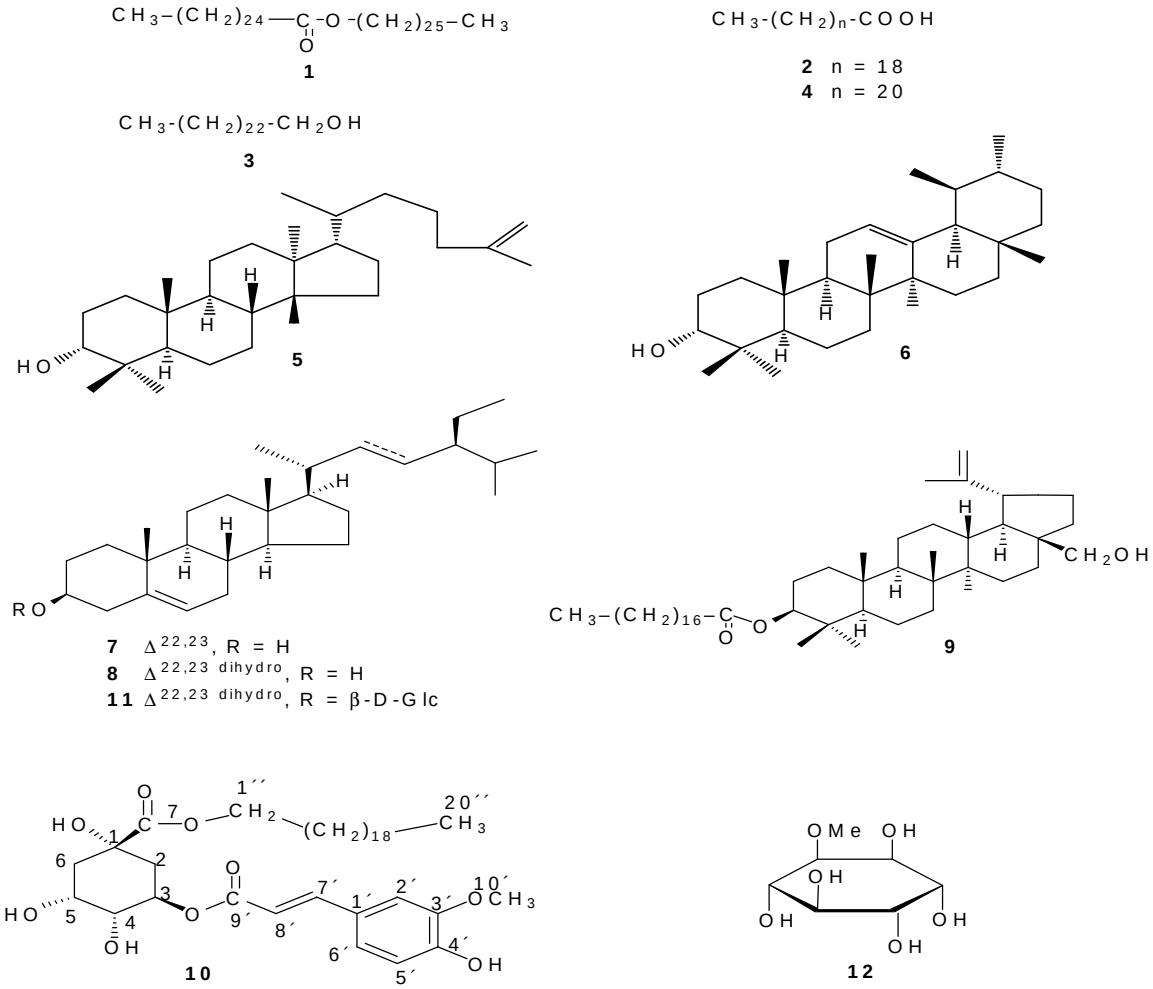

Figure 1. Phytochemicals from *Acacia Senegal* root heartwood.

Docosanoic acid (4 ; Fig. 1): $C_{22}H_{44}O_2$, mp 78-80°C. MS m/z 340 (M^+), IR (u_{max}) cm^{-1}(KBr): 3300-2650, 1730, 1300, 730 and 720. ^1H NMR ($CDCl_3$, 300 MHz): 2.23 (2H, t), 1.23 (38H, br s), 0.88 (3H, t).

3a**-Hydroxyeuph-25-ene (5 ; Fig. 1)**: $C_{30}H_{52}O$, mp 170-172°C. MS m/z 428 (M^+), IR (u_{max}) cm^{-1}(KBr): 3490, 1640, 1520, 1380, 1350, 1100 and 880. ^1H NMR ($CDCl_3$, 300 MHz): 4.56, 4.68 (2H, br s), 3.24 (1H, m), 2.36 (2H, m), 1.98 (1H, m), 1.62 (3H, s), 1.08 (3H, s), 1.02 (3H, s), 0.96 (3H, s), 0.82 (3H, s), 0.78 (3H, s), 0.76 (3H, s).

a**-Amyrin (6 ; Fig. 1)**: $C_{30}H_{50}O$, mp 183-185°C. MS m/z 426 (M^+), IR (v_{max}) cm^{-1}(KBr): 3400, 1640 and 1120. ^1H NMR ($CDCl_3$, 300 MHz): 5.17 (1H, dd), 3.53 (1H, dd), 2.15-1.21 (24H, m), 1.08-0.74 (8×CH_3, s,d).

Stigmasterol (7 ; Fig. 1): $C_{29}H_{48}O$, mp 166-167°C. MS m/z 412 (M^+), IR (u_{max}) cm^{-1}(KBr): 3400, 1620 and 1050. ^1H NMR ($CDCl_3$, 300 MHz): 5.36 (1H, t), 5.11 (1H, dd), 5.04 (1H, dd), 3.53 (1H, m), 2.29-1.25 (26H, m), 1.16-0.68 (6×CH_3, s,d).

ß-Sitosterol (8 ; Fig. 1): $C_{29}H_{50}O$, mp 135-136°C. MS m/z 414 (M^+), IR (u_{max}) cm^{-1}(KBr): 3400, 1640 and 1090. ^1H NMR ($CDCl_3$, 300 MHz): 5.27, (1H, t), 3.48 (1H, m), 2.0-1.19 (30 H, m), 1.16-0.70 (6×CH_3, s,d).

Betulin-3-O-stearate (9 ; Fig. 1): $C_{48}H_{84}O_3$, mp 58-60°C. IR (u_{max}) cm^{-1}(KBr): 3030, 1730, 1660, 880, 730 and 720. ^1H NMR ($CDCl_3$, 300 MHz): 4.69, 4.58 (2H, d), 4.24 (1H, dd), 3.82-3.64 (2H, d), 2.35 (1H, m), 1.68 (3H, s), 2.04-1.32 (24H, m,s), 1.25 (30H, br s), 1.03 (3H, s), 0.96 (3H, s), 0.90 (3H, s), 0.88 (3H, t), 0.82 (3H, s), 0.76 (3H, s).

Eicosanyl 3-O-feruloyl-quinate (10 ; Fig. 1): $C_{37}H_{60}O_9$, mp 83-85°C. EIMS m/z 648 (M^+), IR (u_{max}) cm^{-1}(KBr): 3550 (broad), 1740, 1710, 1640, 1595, 1590, 1500, 1180, 960, 730 and 720. ^1H NMR ($CDCl_3$, 300 MHz): 7.58 (1H, d), 7.36 (1H, s), 7.04 (1H, d), 6.93 (1H, d), 6.32 (1H, d), 5.18 (1H, ddd), 4.59 (1H, m), 4.18 (2H, t) 3.93 (3H, s), 3.67 (1H, dd), 2.35 (1H, ddd), 2.18 (1H, dt), 2.06 (1H, dd), 1.87 (1H, dd), 1.25 (36H, br s), 0.88 (3H, t). ^{13}C NMR ($CDCl_3$, 75 MHz): 174.38 (C-7), 168.18 (C-9'), 149.65 (C-4'), 147.12 (C-3'), 144.65 (C-7'), 127.08 (C-1'), 123.06 (C-6'), 116.95 (C-8'), 115.72 (C-5'), 110.66 (C-2'), 76.60 (C-1'), 70.23 (C-4), 69.38 (C-3), 69.07 (C-5), 55.94 (OMe), 63.27 (C-1"), 38.86 (C-2), 37.79 (C-6), 34.16-22.70 (C-2" – C-19"), 14.14 (C-20").

ß-Sitosterol-ß-D-glucoside (11 ; Fig. 1): $C_{35}H_{60}O_6$, mp 278-280°C. IR (u_{max}) cm^{-1} (KBr): 3400 (broad), 1640 and 1120. ^1H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz): 5.42 (1H, t), 4.49 (1H, dd), 3.98-3.33 (6H, m), 1.76-0.71 (48H, m).

D-Pinitol (12 ; Fig. 1): $C_7H_{14}O_6$, mp 185-186°C. IR (u_{max}) cm^{-1}(KBr): 3410, 3310, 1130 and 1075. ^1H NMR ($CDCl_3$, 300 MHz): 3.92 (2H, q), 3.80-3.76 (2H, ddd), 3.65 (3H, s), 3.62 (1H, t), 3.35(m), 3.29 (1H, t).

Antibacterial and Antifungal tests

Antibacterial assays were conducted with *Bacilllus subtilis* (ATCC 6633), *Enterobacter aerogenes* (ATCC 13048) , *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25668), *Raoultella planticola* (MTCC 530) and *Staphylococcus aureus* (MTCC 740). The cultures of test bacteria were grown and maintained on Nutrient Broth Medium (NBM) at 27°C for 48 hr.

The antifungal assays were conducted with *Aspergillus flavus* (ATCC 16870), *Aspergillus niger* (ATCC 322), *Candida albicans* (ATCC 4718), *Penicillium chrysogenum* (ATCC 5476) and *Trichophyton rubrum* (ATCC 2327). The test fungi were cultured on Sabouraud Dextrose Broth (SDB) at 37°C for 48 hr.

Stock cultures were maintained at 4°C on slops of nutrient agar. Active cultures for experiments were prepared by transferring an inoculating loop of cultures from the stock cultures to test tubes of NB Medium for bacteria and SD Broth for fungi which were incubated without agitation of 24 hr at 37°C and 25°C respectively. The Agar-well diffusion method[14] was used to screen the antimicrobial activity. The plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min. Thereafter, 40 µl (in case of bacteria) and 80 µl (in case of fungi) suspension was spread uniformly with the help of a sterile glass spreader and dried for 5 min. The wells (6 mm diameter) were punched in the plates using a sterile stainless steel borer. The test extracts and control (gentamycin for bacteria and ketoconozole for fungi) was loaded in 6 mm well and the test sample was allowed to diffuse for 30 min. The plates were kept for incubation at 37°C for 24 hr in case of bacteria and 36 hr in fungi. At the end of incubation, inhibition zones formed around the well were measured with transparent scale in millimeters. The experiments were performed in triplicate and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.[15]

RESULTS AND DISCUSSION

Identification of the isolated compounds

Compound **1**, mp 80-82°C[11] was obtained as white solid. The elemental analysis and mass spectral analysis established its molecular formula $C_{52}H_{104}O_2$. The infrared spectrum showed important peaks at 1735 (C=O stretching), 1140 (O=C–O stretching), 730 and 720 cm^{-1} [doublet, $–(CH_2)_n–$ bending, n>4] indicating it to be a long chain saturated aliphatic ester. The ^1H NMR spectrum exhibited two triplets at δ 4.05 and 2.29 due to CH_2O and CH_2CO groups respectively indicating it to be an ester. A broad singlet at d 1.25 corresponding to long chain methylene groups and a triplet at δ 0.88 for the two terminal methyl groups were also observed. The compound on hydrolysis with 5% alcoholic potassium hydroxide gave an alcohol and an acid which were identified as ceryl alcohol[16] and cerotic acid[17] respectively by the spectroscopic studies. Based on the above chemical and spectral studies, Compound **1** was characterized as ceryl cerotate.

Compound **2**, mp 70-72°C[18] was isolated as white granules. Its molecular weight and mass studies were in agreement with molecular formula $C_{20}H_{40}O_2$. The infrared spectrum showed important peaks at 3300-2650 (broad, O–H stretching of COOH group), 1745 (C=O stretching), 1300 (O–C=O stretching), 730 and 720 cm^{-1} [doublet, $–(CH_2)_n–$ deformation, n>4] indicating it to be a long chain fatty acid. Its mass spectrum closely resembled to that of eicosanoic acid. It was further confirmed by preparation of its methyl ester, m.p. 55-56°C.

Compound **3**, mp 72-74°C[17] was isolated as white shiny powder. The elemental analysis and mass spectral data established its molecular formula as $C_{24}H_{50}O$. The important peaks observed in the infrared spectrum were at 3320 (broad, O–H stretching), 1060 (C–O stretching), 725 and 720 cm^{-1} [doublet $–(CH_2)_n–$ bending, n>4]. Based on its spectral studies, it was identified as tetracosanol.

Compound **4**, mp 78-80°C[17] was isolated as white granules. Its molecular weight and elemental analysis were in agreement with the molecular formula $C_{22}H_{44}O_2$. The infrared spectrum showed important peaks at 3300-2650 (broad, O–H stretching of COOH group), 1730 (C=O stretching), 1300 (C–

O stretching), 730 and 720 cm^{-1} [doublet, $-(CH_2)_n-$ deformation, n>4] indicating it to be a long chain fatty acid. Its analytical and spectral data indicated it to be docosanoic acid.

Compound **5**, mp 170-172°C was isolated as white powder. The elemental analysis and mass spectral studies indicated its molecular formula as $C_{30}H_{52}O$. The important peaks observed in the infrared spectrum were at 3490 (broad, O–H stretching), 1640 and 880 (C=CH_2 stretching), 1380 and 1350 (gem dimethyl stretching) and 1100 cm^{-1} (C–O stretching). In ^{1}H NMR spectrum, appearance of signals due to five quaternary methyls at δ 0.76, 0.78, 0.82, 0.96 and 1.02, a vinylic methyl at δ 1.62 and a secondary methyl at δ 1.08 were indicative of a tetracylic triterpene skeleton.[19] Two broad singlets at δ 4.56 and 4.68 each integrating for one proton, corresponded to the protons of an olefinic methylene group which in conjunction with a broad singlet at δ 1.62 suggested the existence of a isopropylidene group.[20] Two multiplets at δ 3.18 and 2.42 were assigned to a methine proton with an axially oriented C-3 –OH group and for C-24 protons respectively while the remaining methylene and methine protons appeared as multiplet in the region δ 1.13-1.95. From the above evidences, the structure of Compound **5** was established as 3α-hydroxyeuph-25-ene. It is a very rarely occurring tetracyclic triterpenoid with only one report available in literature indicating its isolation from the stem of *Butea monosperma*.[21]

Compound **6**, mp 183-185°C was isolated as white needles. Its molecular formula was found to be $C_{30}H_{50}O$. The IR, ^{1}H NMR and mass spectral data of compound **6** were in close agreement to that of α-amyrin.[22]

Compound **7**, mp 166-167°C was isolated as white needles. The elemental analysis and mass spectral studies indicated its molecular formula as $C_{29}H_{48}O$. Its IR, ^{1}H NMR and mass spectra closely resembled to those of stigmasterol.[23]

Compound **8**, mp 135-136°C was isolated as white needles. The elemental analysis and mass spectral studies indicated its molecular formula as $C_{29}H_{50}O$. Its IR, ^{1}H NMR and mass spectra were in close agreement to those of ß-sitosterol,[23] a very commonly occurring phytosteroid.

Compound **9**, mp 58-60°C was isolated as white granules. The elemental analysis and molecular weight determination by EI-MS established its molecular formula to be $C_{48}H_{84}O_3$. Its IR and ^{1}H NMR spectral data indicated it to be a long chain ester of betulin.[22] On the basis of mass spectral data, it was identified as betulin-3-O-stearate.[24]

Compound **10**, mp 83-85°C, isolated as white granules was assigned the molecular formula $C_{37}H_{60}O_9$ based on its elemental analysis and EI-MS. Its infrared spectrum showed the absorption at 3550 (broad OH stretching), 1740 and 1710 (C=O stretching), 1640 (C=C stretching), 1595, 1590, 1500 aromatic C=C, 1180 (O=C–O stretching), 960 (*trans* C=C), 730 and 720 cm^{-1} [doublet, $-(CH_2)_n-$bending, n>4]. The UV absorption at 336 nm indicated the presence of an unsaturated carbonyl chromophore conjugated with an aromatic moiety. In the ^{1}H NMR spectrum, the peaks at δ 5.18, 4.59 and 3.67 for three oxymethines and at δ 2.06/2.18, 1.87/2.35 for two pairs of sp^3 methylenes indicated the presence of quinic acid moiety.[25] This was further supported by ^{13}C NMR spectral analysis which showed peaks at δ 70.23, 69.38 and 69.07 corresponding to three oxymethines, δ 38.86 and 37.79 to two sp^3 methylenes, δ 76.60 to an oxygenated quarternary carbon and d 174.38 to a carboxylic carbon. The presence of a pair of downfield doublets at δ 7.58 and 6.32 ppm with coupling constant J = 15.9 Hz clearly indicated the presence of *trans*-olefinic protons. In addition, one ABX spin system signals (7.36, 6.93 and 7.04) in the aromatic region originating from 1,3, 4-trisubstituted benzene ring and a methoxy protons singlet at δ 3.93 ppm revealed the presence of ferulic acid moiety. Further, a triplet at δ 4.18, a broad singlet at δ 1.25 and a triplet at δ 0.88 ppm suggested the presence of a long chain ester moiety in the compound. It was ascertained from DEPT experiment that no tertiary carbon (except C=O and aromatic carbons) was present, so the possibility of any branching in the long chain was ruled out. The ^{1}H and ^{13}C NMR spectral data of our compound **10** were approximately similar to that of hycanidic ester-1[26] except for the molecular mass of our compound which was quite high. This indicated the presence of a long chain (IR, mass) with twenty carbon atoms which was further supported by the MS data where a strong peak at m/z 281 for eicosanyl side chain is obtained. The above spectral data led to the identification of compound **10** as eicosanyl 3-O-feruloyl-quinate.

Compound **11**, mp 278-280°C was isolated as white granules. The elemental analysis and molecular weight determination by mass spectrometry established its molecular formula to be $C_{35}H_{60}O_6$. The positive Molisch's test indicated its glycosidic nature. Its ^{1}H NMR and mass spectra were in close agreement to those of ß-sitosterol-ß-D-glucoside.[27]

Compound **12**, mp 185-186°C was isolated as white powder. Its analysis agreed with the molecular formula $C_7H_{14}O_6$. On the basis of IR and ^{1}H NMR spectral data, compound **12** was identified as the monomethyl ether of inositol, i.e. D-pinitol.[28]

Evaluation of the antibacterial and antifungal activity

The ethanolic extract and the pet. ether, dichloromethane and ethyl acetate solubles were tested against two Gram-positive and four Gram-negative bacteria, as well as five fungi.

The results of the antibacterial activity presented in Table 1 indicated that the ethanolic extract of root heartwood of *A. senegal* exhibited only moderate activity against the selected bacteria. However, the dichloromethane solubles were found to be very effective against *E. coli* and *S. aureus* (AI = 0.96 in both cases). *S. aureus* is one of the most common and versatile human pathogens, responsible for various infectious diseases such as skin and soft-tissue infections, osteomyelitis, endocarditis, meningitis, and even severe sepsis[29] whereas *E. coli* is responsible for acute prostatitis in infertile patients.[30] The ethyl acetate solubles also demonstrated moderate activity against *R. planticola*.

Table 1. Antibacterial activity of *A. senegal* root heartwood.

S. No.	Test Microbes		Nature of extract / fraction			
			Ethanol	Pet. ether	Dichloromethane	Ethyl acetate
1	*B. subtilis*	+IZ	12.00 ±0.57	10.66 ± 0.66	12.33 ± 0.33	12.66 ± 0.88
		*AI	0.54	0.48	0.56	0.57
2	*E. aerogenes*	IZ	10.00 ± 0.00	11.33 ± 0.33	11.33 ± 0.33	10.33 ± 0.33
		AI	0.71	0.8	0.8	0.73
3	*E. coli*	IZ	9.6 ± 0.33	16.00 ± 0.57	18.33 ± 0.91	16.66 ± 0.33
		AI	0.5	0.84	0.96	0.85
4	*P. aeruginosa*	IZ	10.00 ± 0.00	12.00 ± 0.57	12.33 ± 0.33	11.33 ± 0.66
		AI	0.5	0.6	0.61	0.56
5	*S. aureus*	IZ	10.00 ± 0.00	18.33 ± 0.33	20.33 ± 0.33	16.66 ± 0.33
		AI	0.47	0.87	0.96	0.79
6	*R. planticola*	IZ	12.33 ± 0.33	11.66 ± 0.33	13.66 ± 0.33	18.66 ± 0.66
		AI	0.59	0.53	0.62	0.89

+IZ = Inhibition zone (in mm) including the diameter of well (6 mm, mean ± standard deviation);

****AI = Activity index = Inhibition zone of sample/Inhibition zone of standard; Standard : Gentamycin.***

From the results of antifungal activity presented in Table 2, it is evident that the ethanolic extract and the dichloromethane and ethyl acetate solubles were active against *C. albicans* (AI ~ 0.6 in all the cases). Also, all the extracts exhibited moderate effect against *T. rubrum* (AI ~0.53).

Table 2. Antifungal activity of A. senegal (root heartwood).

S. No.	Test Microbes		Nature of extract / fraction			
			Ethanol	Pet. ether	Dichloromethane	Ethyl acetate
1	*A. flavus*	IZ	10.00 ± 0.00	-	11.00 ± 1.15	11.00 ± 0.00
		AI	0.37	-	0.4	0.4
2	*A. niger*	IZ	10.33 ± 0.33	-	-	10.33 ± 0.33
		AI	0.38	-	-	0.38
3	*C. albicans*	IZ	13.33 ± 0.33	12.33 ± 0.33	13.66 ± 0.66	14.66 ± 0.33
		AI	0.6	0.56	0.62	0.66
4	*P. chrysogenum*	IZ	10.33 ± 0.33	11.33 ± 0.33	13.66 ± 0.33	16.00 ± 1.73
		AI	0.35	0.39	0.47	0.55
5	*T. rubrum*	IZ	10.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.81	11.33 ± 0.66
		AI	0.47	0.57	0.57	0.53

Standard : Ketoconazole; (-) = No activity.

CONCLUSIONS

This paper describes the phytochemical screening of *A. senegal* root heartwood alongwith the evaluation of its antimicrobial activity. Eicosanyl 3-O-feruloyl-quinate **10** has been isolated for the first time from nature while 3α-hydroxyeuph-25-ene **5** and α-amyrin **6** have been isolated for the first time from this species. The dichloromethane solubles were found to be very active against two bacteria *E. coli* and *S. aureus* whereas in case of antifungal activity, the ethanolic extract and the dichloromethane and ethyl acetate solubles exhibited significant activity against *C. albicans*.

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