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Secondary Metabolites from Seed Extracts of *Syzygium Cumini* (L.)

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Abstract: *Four compounds were isolated from the pet-ether and carbon tetrachloride soluble fractions of a methanol extract of seeds of Syzygium cumini. The structures of the isolated compounds were elucidated as 7-hydroxycalamenene (1), methyl-β-orsellinate (2), β-sitosterol (3) and oleanolic acid (4) through extensive spectroscopic studies, including high-field NMR analyses. This report appears to be the first to identify 7-hydroxycalamenene (1) in S. cumini and the Myrtaceae family, although it has been reported in cultured cells of the liverwort Heteroscyphus planus. This is also the first report of the isolation of compounds 2-4 from this plant species.*

Keywords: *Syzygium cumini*, *Myrtaceae*, 7-hydroxycalamenene, methyl-β-orsellinate

1. INTRODUCTION

Syzygium cumini L. (Bengali name - Jam; Family - *Myrtaceae*) is a large evergreen tree, reaching approximately 30 m in height, and is found throughout the Indian subcontinent.[1] *S. cumini* is a medicinal plant, various parts of which have been pharmacologically proven to possess hypoglycaemic, antibacterial and anti-HIV activities. The bark of the tree is employed in folk medicine for treatment of inflammation.[2]

Previously, flavonol glycosides have been isolated from the roots of this plant.[3,4] Acylated flavonol glycosides, including mearnsetin (3-*O*-(4″-*O*-acetyl)-α-L-rhamnopyranoside) and myricetin (3-*O*-(4″-*O*-acetyl-2″-*O*-galloyl)-α-L-rhamnopyranoside), have been isolated from the leaves of *S. cumini*.[5] The seeds of the tree have also been reported as a rich source of polyphenols, gallic acid and ellagic acid derivatives, corillagin and related ellagitannins, 3,6-hexahydroxydiphenoyl-glucose, 4,6-hexahydroxydiphenoyl-glucose, 1-galloyl glucose, 3-galloyl glucose and quercetin.[6] This paper is a phytochemical study of other secondary metabolites of the seeds of *S. cumini*.

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2. EXPERIMENTAL

2.1 General Experimental Procedures

The ^{1}H NMR spectra were recorded with a Bruker AMX-400 (400 MHz) instrument and the ^{13}C NMR spectra were obtained with the same instrument at 125 MHz in $CDCl_3$. The δ values for the ^{1}H and ^{13}C data were referenced to the residual nondeuterated solvent signals.

2.2 Plant Material

The seeds of *S. cumini* were collected from a local market in Dhaka in March 2008. A voucher specimen for this collection has been maintained in the Bangladesh National Herbarium, Dhaka, Bangladesh (accession no. DACB-32926).

2.3 Extraction and Isolation

The powdered seed (1,000 g) was soaked in 2.0 l of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator, and a portion (5 g) of the concentrated methanol extract was fractionated with the modified Kupchan partitioning protocol[7] into pet-ether (0.55 g), carbon tetrachloride (0.65 g), dichloromethane (0.50 g) and aqueous (2.4 g) soluble materials.

The pet-ether and carbon tetrachloride soluble fractions were separately chromatographed over silica gel (Kiesel gel 60H, mesh 70–230), and the columns were eluted with pet-ether followed by mixtures of pet-ether and ethyl acetate in order of increasing polarities. Compound 1 was isolated as a yellowish amorphous mass from the column fractions of the pet-ether soluble materials eluted with 10% ethyl acetate pet-ether. Fractions eluted with 15–20% ethyl acetate in pet-ether, upon re-chromatography over silica gel (PF_{254}), provided compounds methyl-β-orsellinate (2) and β-sitosterol (3). A similar column chromatographic separation of the carbon tetrachloride soluble materials eluted with 30% ethyl acetate in pet-ether yielded compound 4.

2.4 Compounds Isolated

7-Hydroxycalamenene (1) (5 mg, 0.009% yield): yellowish and amorphous; MS m/z: 219.1 [M^+], $C_{15}H_{22}O$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (1H, s, H-5), 6.64 (1H, s, H-8), 2.75 (1H, m, H-1), 2.66 (1H, m, H-4), 2.20 (3H, s, H_3-15), 1.96 (1H, m, H-2β), 1.82 (1H, m, H-3α), 1.58 (1H, m, H-3β), 1.33 (1H,

m, H-2α), 1.23 (3H, d, J = 6.8 Hz, H_3-14), 0.97 (3H, d, J = 6.8 Hz, H_3-13), and 0.70 (3H, d, J = 6.8 Hz, H_3-12). ^{13}C NMR spectroscopic data (125 MHz, 400 MHz, $CDCl_3$): δ 32.62 (C-1), 30.82 (C-2), 21.59 (C-3), 43.08 (C-4), 130.50 (C-5), 120.56 (C-6), 151.37 (C-7), 113.01 (C-8), 142.32 (C-9), 132.24 (C-10), 31.86 (C-11), 17.23 (C-12), 21.19 (C-13), 22.24 (C-14) and 15.51 (C-15).

Methyl-β-orsellinate (2) (5 mg, 0.009% yield): white amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.94 (1H, s, OH-2), 6.01 (1H, s, H-5), 4.21 (1H, s, OH-4), 3.64 (3H, s, -$COOCH_3$), 2.22 (3H, s, H_3-6),) and 2.15 (3H, s, H_3-3).

β-sitosterol (3) (10 mg, 0.018% yield): colourless crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (1H, d, J = 5.2 Hz, H-6), 3.51 (1H, m, H-3), 1.0 (3H, s, H_3-19), 0.92 (3H, d, J = 6.4 Hz, H_3-21), 0.85 (3H, d, J = 7.0 Hz, H_3-29), 0.83 (3H, d, J = 7.0 Hz, H_3-26), 0.81 (3H, d, J = 7.0 Hz, H_3-27) and 0.67 (3H, s, H_3-18).

Oleanolic acid (4) (6 mg, 0.009% yield): amorphous white powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.29 (1H, t, J = 6.4 Hz, H-12), 3.20 (1H, dd, J =10.8, 4.4 Hz, H-3), 2.83 (1H, dd, J =13.6, 4.4 Hz, H-18), 1.13 (3H, s, H_3-21), 0.98 (3H, s, H_3-23), 0.91 (3H, s, H_3-30), 0.90 (3H, s, H_3-25, 29), 0.77 (3H, s, H_3-26) and 0.76 (3H, s, H_3-24).

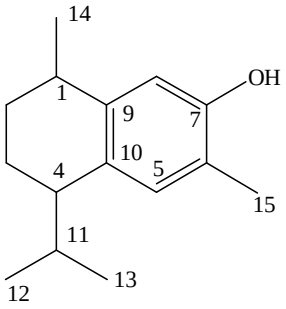
3. RESULTS AND DISCUSSION

A total of four compounds were isolated from the pet-ether and carbon tetrachloride soluble fractions of a methanol extract of seeds of *S. cumini* through repeated chromatographic separation and purification over silica gel. The structures of the isolated compounds were solved through mass and extensive NMR data analyses.

The ^{1}H NMR spectrum of compound 1 revealed the presence of a three-proton singlet at δ 2.20 for a methyl group on an aromatic ring, two doublets (J = 6.8) each of three-proton intensity at δ 0.70 and at δ 0.97 for an isopropyl group, another methyl group at δ 1.23 (3H d, J = 6.8) and two isolated aromatic singlets at δ 6.64 and 6.93 (each 1H s). The methyl group resonances at δ 0.70, 0.97, 1.23, and 2.20 were attributed to H_3-12, H_3-13, H_3-14 and H_3-15 (Me_2-11, Me-1 and Me-6), respectively. The ^{1}H NMR spectrum also displayed a broad singlet at δ 4.49, which suggested the presence of an aromatic hydroxyl group.

The ^{13}C NMR spectrum of compound 1 displayed fifteen carbon resonances, confirming its sesquiterpene nature. Thus, it showed signals for four methyls (δ 0.70, 0.97, 1.23, and 2.20), two aliphatic methylenes (δ 1.33, 1.96, 1.82 and 1.58), two aromatic methines (δ 6.64, 6.93) and four quaternary carbons.

The assignments of the ^{13}C NMR chemical shifts were performed through comparison with literature values.[8]



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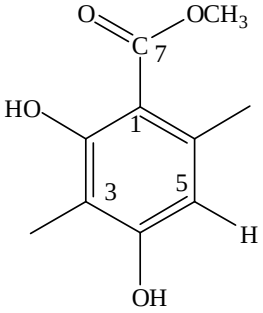

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On this basis, compound 1 was characterised as 7-hydroxycalamenene, the identity of which was further confirmed through the comparison of its spectral data with published values.[8] This is the first report of its occurrence in *S. cumini* and the *Myrtaceae* family, although it has previously been found in cultured cells of the liverwort *Heteroscyphus planus*.[8]

The ^{1}H NMR spectrum of compound 2 revealed a one-proton singlet at δ 6.01 assignable to the aromatic proton H-5 and a three-proton singlet at δ 3.64, which was characteristic of a carboxy methyl (–$COOCH_3$) group. The resonances at δ 4.21 and δ 12.94 were indicative of two OH groups. The downfield shift at δ 12.94 for one of the OH group functionalities suggested intramolecular hydrogen bonding; therefore, it must be adjacent to a carbonyl group. The ^{1}H NMR spectrum further showed two three-proton singlets at δ 2.22 and 2.15 attributable to the methyl groups at C-6 and C-3 of the aromatic ring, respectively. Thus, this compound was identified as methyl-β-orsellinate (2). Its identity was further substantiated through the comparison with published data.[9]

Compounds 3 and 4 were readily characterised as β-sitosterol and oleanolic acid through direct comparison of their ^{1}H NMR spectra with those previously acquired in our laboratory samples and with co-TLC with authentic samples.

4. CONCLUSION

Phytochemical investigatons of the crude methanol extract of *S. cumini* led to the isolation of four metabolite: 7-hydroxycalamenene (1), methyl-β-orsellinate (2), β-sitosterol (3) and oleanolic acid (4). Further biological study is required to explore their potential therapeutic activities.

5. ACKNOWLEDGEMENTS

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