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Flavonoid Content and Antiobesity Activity of Leaves of *Myrtus communis*

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The flavonoid content, as well as, the antiobesity activity of leaves of *Myrtus communis* (*Myrtaceae*), growing in Egypt, were investigated. Five flavonoids *e.g.*, 5,8-dihydroxy-6,7,4' trimethoxyflavone, quercetin-3-*O*-neohesperidoside, quercetine-3-O-galactoside and *trans*-1',5'-5-(5-carboxymethyl-2-oxocyclopentyl)-3Z-pentenyl-(6-O-galloyl) glucopyranoside and 3-methoxy myricetin 7-O-α-L-rhamnopyranoside were isolated. Their structure elucidation were performed on the basis of different spectroscopic techniques including UV, ^{1}H NMR, ^{13}C NMR and mass spectrometry. Methanolic extracts of *Myrtus communis* were evaluated for the antiobesity efficacy in high fat diet induced male wistar albino obese rats. The body weight of high fat diet induced obese rats was reduced by 32 % when administered with sibutramine (standard antiobesity drug, 5 mg/kg body weight), while it was reduced by 21 % and 24 % respectively by 200 mg and 400 mg of the methanolic extract of *Murtus communis*/kg body weight.

Key Words: Antiobesity, Flavonoid, *Myrtus communis*.

INTRODUCTION

Myrtus communis Linn. or common myrtle (family: myrtaceae) is one of the important drugs being used in Unani system of medicine since ancient Greece period[1]. It is recognized as *Aas* and its berries are known by the name of *Habb-ul-Aas*[1,2]. *Myrtus communis* Linn. (family: myrtaceae) is an aromatic evergreen perennial shrub or small tree, 1.8-2.4 m in height with small foliage and deep fissured bark[1-3]. It is native to southern Europe, north Africa and west Asia. It is distributed in south America, north western Himalaya and Australia and widespread in the Mediterranean region. It is also cultivated in gardens especially north-west Indian region for its fragrant flowers[2-4]. Berries are used as antiseptic, astringent[5], carminative[2,4-6], emmenagogue[2,4], demulcent, dessicant, analgesic, hair tonic, haemostatic[6-8], antiemetic, lithotripsic[9], cardiotonic, diuretic[4,10,11], anti-inflammatory[12], stomachic, brain tonic[4,10], haemostatic, nephroprotective, antidote[12], antidiaphoretic[11] and antidiabetic[13]. Various pharmacological actions of leaves are astringent, antiseptic[2], hypoglycaemic, laxative[13], analgesic[4,11], haemostatic[11], hair tonic[9,14] and stimulant[2]. Root is reported to have antibacterial property[15]. It is traditionally used as an antiseptic, disinfectant drug and hypoglycemic agent[16]. The flavonoidal content of *Myrtus communis* was previously investigated, the aim of this study is to isolate new flavonoids for the first time from its leaves as well as investigate a new pharmacological activity for the methanolic extract of the leaves.

EXPERIMENTAL

The fresh leaves of *Myrtus communis* were collected from Garden of Al- Orman, Giza, Egypt and was kindly identified by Mrs. Treeze, specialist in plant taxonomy on September 2009.

Acid hydrolysis: Few milligrams of each of the separated compounds were dissolved in MeOH-H_2O (1:1) and hydrolyzed under reflux with 2 N HCl (2 h, 100 ºC). The hydrolyzate mixture after neutralization with dilute solution of $NaHCO_3$ was then extracted with ethyl acetate in separating funnel to separate the aglycone in the organic phase, while the sugar being in the aqueous phase[17]. Hydrolyzates were compared by PC to authentic aglycone and sugar samples. PC of the aglycones was sprayed with Naturstoff reagent while PC of sugars was sprayed with aniline hydrogen phthalate reagent. The sugars were identified after comparison with authentic sample.

^{1}H and ^{13}C NMR spectra were recorded in deutromethanol (CD_3OD) on a Varian Mercury VXR 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) chemical shifts (ppm) were related to that of the solvent. Mass spectrum was recorded on a GC/MS. QP 1000 Ex. Shimadzu mass spectrometer at 70 e.v. Ultraviolet absorption spectrum was recorded on a Perkin-Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed on a Perkin- Elmer 1710 infrared fourier transformation spectrometer. Column chromatography was performed using silica gel (Merck 7749).

Experimental animals: The male healthy wistar albino rats weighing 150-160 g were used in this study. The animals were maintained in well ventilated rooms with 12 h light and dark cycle in polypropylene cages. All animals were acclimatized to the laboratory conditions one week prior to the initiation of the study.

Composition of normal and high fat diet[18]: Composition of normal feed is 27 % whole wheat, 25 % yellow corn, 15 % barley, 15 % milk powder, 1 % bone meal, 1 % calcium chloride, 1 % sodium chloride, 15 % coconut oil and one multivitamin capsule. The high fat diet contains 23 % whole wheat, 23 % yellow corn, 11 % barley, 17 % milk powder, 1 % bone meal, 1 % calcium chloride, 1 % sodium chloride, 11 % coconut oil, 11 % butter and one multivitamin capsule.

Experimental design[18-20]: The obtained male healthy wistar albino rats were divided in to five groups, each group containing six animals. Group 1 was fed with normal diet (ND) and remaining groups fed with high fat diet (HFD) for 6 weeks.

The experimental design was as follows:

Group 1: (normal diet)

Group 2: (high fat diet control)

Group 3: high fat diet + standard drug

Group 4: high fat diet + 200 mg *Myrtus communis* methanolic extract/kg body weight (extract 1).

Group 5: high fat diet + 400 mg *Myrtus communis* methanolic extract/kg body weight (extract 2).

Test drug preparation for antiobesity efficacy evaluation[18,21-23]: The methanolic extract of *Myrtus communis* and standard Sibutramine are soluble in water, so distilled water was used as a media to dissolve. All the drug concentrations were prepared freshly, just before administration. All the test drugs including the standard were given by oral route.

Body weight: The body weight of rats (g) was recorded every week for 48 days for each group, just before dosing, by using a precision balance of 10 mg sensitivity.

Extraction and isolation of flavonoids: The air dried powdered leaves (1 Kg) were extracted with chloroform, ethyl acetate and methanol at room temperature, the ethyl acetate extract was filtered and then evapourated under reduced pressure to dryness, a brownish mass (54 g) obtained and subjected to column chromatography on silica gel, the column was successively eluted with hexane, chloroform, ethyl acetate, methanol and their mixtures of increasing polarity, elution with $CHCl_3$: Methanol (8:2) afforded a yellow powder (0.9 g). Elution with chloroform: methanol (5:5) was further separated using column chromatography on Sephadex LH-20 (20 g in column 1.6 i.d. × 40 cm) with methanol as eluent. The eluates were combined on the basis of similar TLC profile in $CHCl_3$:CH_3OH: H_2O 60:40:5 to afford eight fractions. Further purification of fraction no. 4 and 5 was performed on 15 g Sephadex LH-20 with methanol as eluent in column 1.6 i.d. × 40 cm to yield compound no. 2. Fraction no. 6 purified by preparative TLC with ethyl acetate:formic acid:acetic acid: water 100:11:11:27 to yield compound no. 3. The methanolic extract was filtered and the solvent evaporated under reduced pressure, residue was taken in 10 mL of 50 % aqueous methanol and subjected to column chromatography on silica gel and successively eluted using petroleum ether, petroleum ether: chloroform mixtures (10-90 %), chloroform, chloroform, chloroform: methanol mixtures (10-90 %), then methanol. Similar fractions were collected together and monitored by TLC using chloroform: acetone: methanol: acetic acid 2: 12: 2: 0.05, v/v. Further purification were carried out by different chromatographic techniques VLC, PC, Sephadex column, preparative PC afforded compounds no. 4 and 5. The structure identification of all pure isolated compounds was carried out by UV, ^{1}H NMR and ^{13}C NMR and mass spectral data.

RESULTS AND DISCUSSION

Product I: m.p.: 170 ºC-172 ºC. UV: (MeOH) λ_{max}: 272 and 334 nm. IR: (KBr, ν_{max}, cm^{-1}): 3488, 1632, 1591, 1498, 1039, 811. ^{1}H NMR: (300 Hz, $CDCl_3$) δ: 12.66 (1H, s, 5-OH), 8.57 (1H, s, 8-OH), 3.90- 4.01 (9H, s, 3x -OCH_3), 7.86 (2H, d, J = 8.6 Hz, H-2', H-6'), 6.98 (2H, d, *J* = 8.6 Hz, H-3', H-5'), 6.55 (1H, s, H-3). ^{13}C NMR: (100 Hz, $CDCl_3$) δ: 162.7 (C-2), 101.8 (C-3), 183.4 (C-4), 151.4 (C-5), 129.9 (C-6), 158.5 (C-7), 128.3 (C-8), 139.9 (C-9), 108.6 (C-10), 125.4 (C-1'), 129.2 (C-2'/6'), 113.9 (C-3'/5'), 161.5 (C-4'), 56.4 (6 -OCH_3), 56.1 (7-OCH_3), 55.5 (4' -OCH_3). MS: (*m/z*) 344 $[M+H]^+$, 329 $[M-CH_3]^+$, 316 $[M-CO]^+$, 314 $[M-2xCH_3]^+$, 301 $[M-CO-CH_3]^+$, 197 [r.D.A. cleavage], 169 $[197-CO]^+$, 153 $[169-CH_3]^+$, 141 $[169-CO]^+$, 135 [fragmented ion peak], 126$[141-CH_3]^+$, 113 $[141-CO]^+$ and 107 $[135-CH_3]^+$.

The compound was isolated from the EtOAc extract by eluting the column with chloroform: methanol (8:2) mixture. The compound showed a positive ferric chloride and Shinoda test for flavonoids, indicating that the compound may be a flavonoid[24,25]. These results also suggested that the compound is a flavonoid derivative with a free hydroxyl group at C-5[24]. In its mass spectrum, the molecular ion peak of the compound at *m/z* 344 $[M+H]^+$ corresponded to the molecular formula $C_{18}H_{16}O_7$. The UV spectrum exhibited absorption maxima at 272 and 334, suggesting that the compound belongs to the flavone family, unsubstituted at the 3-position[26]. The IR spectra of the compound showed absorption bands for hydroxyl group (3488 cm^{-1}), chelated α,β-unsaturated carbonyl attached with aromatic nucleus (1632, 1591, 1448 cm^{-1}), methoxy group (1039 cm^{-1}) and *p*-substituted benzene ring (811 cm^{-1}) functionalities[27]. The ^{1}H NMR spectrum of the compound exhibited a signal at δ 12.66 (1H, s), attributed to a chelated hydroxyl group. Further, a signal observed at δ 8.57 (1H, s) was due to a phenolic hydroxyl group. The ^{1}H NMR displayed one singlet at δ 6.55 that could be assigned to an H-3 proton[26]. The three singlets were observed in the range of δ 3.90-δ 4.01 (9H, s) assigned to the three methoxy groups. The ^{1}H NMR also demonstrated two protons doublets at δ 7.86 (2H, d, *J* = 8.6 Hz), 6.98 (2H, d, *J* = 8.6 Hz), assignable to H-2'/H-6' and H-3'/H-5' protons[27]. The appearance of two doublets and their coupling constant values are further in agreement with the methoxy group at C-4'. The mass spectrum of the compound showed important mass peaks at *m/z* 344 $[M+H]^+$, 329 $[M-CH_3]^+$, 316 $[M-CO]^+$, 314 $[M-2XCH_3]^+$, 301 $[M-CO-CH_3]^+$, 197 [r. D. A. cleavage], 169 $[197-CO]^+$, 153 $[169-CH_3]^+$, 141 $[169-CO]^+$, 135 [fragmented ion peak], 126 $[141-CH3^+]$, 113 $[141-CO]^+$ and 108 $[135-CH_3]^+$. The MS fragmentation pattern clearly indicated that two methoxy and two hydroxyl groups

were attached to the ring-A, while the remaining methoxy group was linked with the ring-B at C-4'[28]. The UV spectrum of the compound, in the presence of aluminium chloride, remained unchanged upon the addition of hydrochloric acid, which confirmed the presence of a hydroxyl function at C-5 and one of the methoxy groups at C-4'[26]. The mass fragmentation pattern also confirmed that the C-6 position was blocked by a methoxy group. The compound gave a negative Gibb's test, indicating the other hydroxyl group was at C-8 and, consequently, the remaining methoxy group must be at the C-7 position[29-31]. In view of these spectral data, the compound was identified as 5,8-dihydroxy-6,7,4'-trimethoxyflavone. This structure was further confirmed by ^{13}C NMR spectral studies. The ^{13}C NMR spectrum of the compound showed a total of 16 signals for 18 carbons. A signal was observed at δ 183.4 and was allocated to C-4. Signals observed at δ 56.4, 56.1 and 55.5 were ascribed to 3 methoxy groups at C-6, C-7 and C-4'. An additional 2 signals were observed resonating at δ 129.2 and δ 113.9 attributed to C-2'/C-6' and C- 3'/C-5', respectively[32]. All these spectral data were in good concurrence with those reported in the literature[33-35].

Compound II: Compound II was isolated as yellow amorphous powder which had purple colour under UV light changed to yellow upon exposure to ammonia vapour or spraying with $AlCl_3$. It yielded two sugar moieties (glucose and rhamnose) upon complete acid hydrolysis in the aqueous phase and quercetin in the organic phase. UV λ_{max} (nm): (MeOH): 257, 270sh, 299sh, 361, (NaOMe): 273, 324, 408, ($AlCl_3$): 271, 302sh, 428, ($AlCl_3$/HCl): 267, 304, 363sh, 400, NaOAc)): 272, 325, 398, (NaOAc/H_3BO_3): 263, 301, 384. ^1H NMR (DMSO-d_6): (ppm) = 7.57(2H, m, H-2`, 6`), 6.84 (1H, d, *J* = 8.5 Hz, H-5`), 6.39 (1H, d, *J* = 2.5 Hz, H-8),), 6.20 (1H, d, *J* = 2.5 Hz, H-6), 5.33 (1H, d, *J* = 7.2 Hz, H-1``), 4.37 (1H, s, H-1```), 3-3.9 (sugar protons), 1.11 (3H, d, *J* = 6 Hz, H-6```).

^{13}C NMR (DMSO-d_6): 177.9 (C-4), 164.8 (C-7), 161.6 (C-5), 156.9 (C-2), 156.6 (C-9), 148.8 (C-4'), 146.1 (C-3'), 133.8 (C-3), 121.3 (C-6'), 121.1 (C-1'), 116.9 (C-5'), 115.8 (C-2'), 104.5 (C 10), 101.5 (C-1'"), 101.3 (C-1"), 99.1 (C-6), 94.3 (C-8), 77.4 (C-3"), 75.9 (C-5"), 73.7 (C-2"), 72.3 (C-4'"), 71.1 (C-4"), 70.9 (C-2'"), 70.7 (C-3'"), 70.4 (C-4"), 68.7 (C-5'"), 67.1 (C-6"), 18.2 (C-6'"). The bathochromic and hypsochromic shifts were in a good agreement with quercetin 3-*O*-glycoside structure. Free 4'-OH group was clear from the bathochromic shift in Band I (47 nm) with the increase of its intensity upon addition of NaOMe. On addition of NaOAc, a bathochromic shift (16 nm) in Band II occurred due to free 7-OH. The bathochromic shift (67 nm) in Band I upon the addition of $AlCl_3$ indicated the substitution in position 3. The presence of *ortho*-dihydroxy groups in B ring was deduced from the hypsochromic shift in Band I in $AlCl_3$/HCl spectrum (28 nm) relative to that in case of addition of $AlCl_3$ (36). The presence of glucose and rhamnose was deduced from the ^1H NMR and ^{13}C NMR spectra. In the aliphatic region of ^{13}C NMR 12 carbon resonances assigned for a rutinoside moiety among which the most downfield signals at δ 101.3 and 101.5 assigned for the two anomeric carbons C-1" and C-1'", respectively. The downfield shift of C-6" signal was due to rhamnoglycosylation. The anomeric protone signals at δ 5.33 (d, *J* = 7.2) and 4.37 (s) together with a doubet at δ 1.11 (3H, d, *J* = 6 Hz) accounted for a β-D-rutinoside moiety. Comparing the reported data with those reported in literatures[36-43] revealed that compound II was quercetin-3-*O*-α-L-rhamnopyranosyl-(1```,6``)-*O*-β-D-glucopyranoside or quercetin-3-*O*-β- neohesperidoside.

Product III: Compound **3** showed an [M-H]-at *m/z* 539, corresponding to the m.f. $C_{25}H_{32}O_{13}$. The IR spectrum revealed absorption bands due to hydroxyl (3600-3000 cm^{-1}), carboxylic hydroxyl (3000-2500 cm^{-1}), ketone (1715 cm^{-1}), carboxylic carbonyl (1710 cm^{-1}) and aromatic ester (1701 cm^{-1}) functions and an aromatic ring (1614 and 1520 cm^{-1}). The presence of a galloyl group was supported by a two proton singlet at δ 7.07 in the ^1H NMR spectrum and five carbon signals (δ 168.3, 146.5, 139.8, 121.4 and 110.2). The ^1H and ^{13}C NMR spectra indicated the presence of α-glucopyranose moiety. The 6"-methylene protons (δ 4.52 and 4.40) showed correlation with the carbonyl carbon (δ 168.3) of the galloyl group, indicating the acylation of the C-6" α-hydroxy function. The NMR data indicated that the compound had a 5-(5-carboxymethyl-2-oxocyclopentyl)-3*Z*-pentenol moiety. In the ^1H NMR spectrum, H-1' and H-5' resonated at δ 1.91 and 2.24, respectively. Resonances for H-1' and H-5' of the 1',5'-*cis* isomer were reported at δ 2.35-2.45 and 2.80, 10-13 whereas the trans isomer showed chemical shifts of H-1' and H-5' at δ 2.20 and 2.30, respectively. Thus it possesses 1',5'-*trans* relative configuration. Comparing these data with literature[27,36,41,42] revealed that compound III was characterized as *trans*-1',5'-5-(5-carboxymethyl-2-oxocyclopentyl)-3*Z*-pentenyl-(6-*O*-galloyl) glucopyranoside.

Product IV: Yellow amorphous powder, m.p. 220-222 ºC, UV (λ_{max} nm) MeOH: 255, 267sh, 303sh, 357, NaOMe/MeOH: 273, 329, 414, $AlCl_3$/MeOH: 268, 299sh, 305sh, 359, 399, $AlCl_3$/HCl/MeOH: 268, 298sh, 305sh, 359, 400, NaOAc/MeOH: 271, 322, 404, NaOAc/ H_3BO_3/MeOH: 257, 267sh, 290, 361.

MS *m/z*: 594, 577, 286, 285, 257. ^1H NMR (300 MHz, d_6-DMSO, γ (ppm)): 7.90 (H 2', 6', 2H, d, *J* = 8.7 Hz), 6.80 (3', 5', 2H, d, *J* = 8.7 Hz), 5.91 (8, 1H, d, *J* = 2.1 Hz), 5.75 (6, 1H, d, *J* = 2.1 Hz), 5.12 (glc. H-1", 1H, d, *J* = 7.5 Hz), 4.40 (rha. H-1'", 1H, d, *J* = 1.5 Hz), 3.66-3.07 (rha.glc., 10H, m), 0.98 (rha. CH3, 3H, d, *J* = 6.3 Hz). ^{13}C NMR (75 MHz, d_6-DMSO, γ (ppm)): 133.00 (C-3), 98.00 (C-6), 93.00 (C-8), 104.00 (C-10), 120.62 (C-1'), 130.59 (C-2 '), 115.26 (C-3'), 115.26 (C-5'), 130.59 (C-6'), 102.72 (C-1"), 74.28 (C-2"), 76.62 (C-3"), 69.85 (C-4"), 75.63 (C-5"), 66.93 (C-6"), 101.08 (C-19"), 70.42 (C-2'"), 70.42 (C-3 '"), 72.08 (C-4'"), 68.45 (C-5'") and 17.88 (C-6'").

Compound **IV** was identified as kaempferol-3-*O*-α-L-rhamnopyranosyl (1'"→6")-*O*-α-d-glucopyranoside. This evidence was supported by UV analysis with different diagnostic shift reagents. UV absorption spectrum in methanol exhibited two absorption maxima at 357 nm (band-I, cinnamoyl system) and 255 nm (band-II, benzoyl system), which indicated that it was a flavonol-3-*O*-glycoside. The sodium methoxide/methanol solution spectrum showed a bathochromic shift of 57 nm in band-I with increase in intensity and slow degeneration indicating the presence of 3, 49-dihydroxy flavone. Band-II appeared two peaks, designated IIa and IIb, with IIa being the peak at longer wavelength. Flavonols exhibited two absorption peaks, one maximum at 273 nm with a shoulder at 329 nm.

This indicated the presence of 3, 4'-hydroxy groups. MS spectra of the compound exhibited a molecular ion at *m/z* 593 and a fragment ion peak at 285 which were ascribed to $[M-H]^-$ and $[M-H-146-162]^-$, suggesting the presence of two glycosyl moieties, hexose and deoxyhexose (glucose and rhamnose). The fragment ion peak of the aglycone at *m/z* 285 (24.6 %) is in accordance with the molecular formula $C_{15}H_9O_6$ of *tetra*-hydroxy substitution pattern. MS mass spectral data are in agreement with those reported for kaempferol[43-45]. Hence, the fragmentation pathway undergoes the Retro-Diels Alder reaction giving rise to ring-A fragment at *m/z* 153 (59.32 %) and 152, However, the hydrogen transfer ion at *m/z* 153 is much more intense than that of the normal fragment ion at *m/z* 152 which indicated to the presence of 5,7-dihydroxy group. Furthermore, loss of CO directly from the molecular ion [M-H-146-162]-CO was also shown, leading to the phenyl-benzofuran fragment ion at *m/z* 257, which further fragments giving rise to the benzoyl ion at *m/z* 121 and then *m/z* 93. ^{1}H NMR spectrum showed the expected signals of a 1,4-disubstituted B ring protons as two *ortho*-coupled resonances at γ (ppm) 7.9 (2H, d, *J* = 8.7 Hz) and γ 6.80 (2H, d, *J* = 8.7 Hz) for H-2', H-6' and H-3', H-5', respectively. The presence of a 5,7-dihydroxy A-ring was deduced from the typical two *meta*-coupled resonances of H-8 and H-6 at σ 5.91 (1H, d, *J* = 2.1 Hz) and σ 5.72 (1H, d, *J* = 2.1 Hz), respectively[46-48]. The upfield shift of H-8 and H-6 resonances, relative to their normal positions 6.4 and 6.2 ppm, respectively, was attributed to salt effect of an inorganic salt contamination from the plant extract[46]. The presence of a β-glucopyranosyl moiety directly attached to the aglycone was detected from the relatively downfield β-anomeric proton resonance at δ 5.12 (1H, d, *J* = 7.5 Hz). The terminal attachment of α-rhamnopyranosyl moiety to C-6" on glucose was evidenced from the resonance of the anomeric proton H-1"' at δ 4.40 (1H, d, *J* = 1.5 Hz) and CH_3-resonance at δ 0.98 (3H, d, *J* = 6.3 Hz). In addition, the value of δ at 3.66-3.07(m, 10H) was assigned to the remaining rhamnosyl-glucosyl moiety, 10 protons. Comparison of the ^{13}C NMR spectral data of compound **IV** with its aglycone, kaempferol, showed an up-field shift of 2.6 ppm for C-3 signal which confirm the position of glycosylation at C-3[43]. The (1"→6")-*O*-glycosidic linkage of the rhamnosyl on the glucoside moiety was evidenced from the fact that the C-6" signal at 66.93 ppm was shifted downfield (6.33 ppm). This is related to the 60.60 ppm chemical shift of the corresponding carbon atom (C-6"') of the terminal glucose[44]. Moreover, the C-2"' signal of terminal rhamnose at 70.42 ppm was shifted up-field (1.18 ppm) of the corresponding carbon atom C-2"' (71.60 ppm) of rhamnose directly attached with position-3[45]. The configuration of the anomeric center of the rhamnopyranosyl moiety was determined to be alpha due to the presence of anomeric carbon signal at 101.08 ppm in its ^{13}C NMR spectrum. Also, it is confirmed from small coupling constants (*J* = 1.5 Hz) for the anomeric proton signal of the rhamnosyl moiety in ^{1}H NMR spectrum[27]. The sugar moiety was identified as glucose and rhamnose after partial and complete hydrolysis of this compound and comparison with authentic references.

Product V: The pure compound was obtained as a yellow amorphous powder and gave positive reaction with diphenyl-boryloxyethyl amine test on TLC suggesting that it is a flavonoid compound. The mass spectrum of the compound indicated a molecular ion peak at *m/z* 478 which demonstrated that its molecular formula is $C_{22}H_{22}O_{12}$. Acid hydrolysis of this compound afforded L-rhamnose as a sole sugar on TLC by direct comparison with authentic samples. The presence of -L-rhamnose was confirmed by the ^{1}H NMR spectrum due to the appearance of only one anomeric proton signal at δ 5.32 (1H, d, *J* = 1.2 Hz) and methyl group signal at δ 0.96 (3H, d, *J* = 6.3Hz C-6`), as well as the carbon signals in the ^{13}C NMR spectrum at δ 99.88 and δ 17.7 ppm for C-1" and C-6" of rhamnose respectively. The aglycone was clearly deduced as 3-methoxy myrycetin by the ^{1}H and ^{13}C NMR spectral data as follows: the presence of a pair of meta coupled doublets of one proton each at δ 6.2 (*J* = 2.1 Hz) and δ 6.36 (*J* = 2.1 Hz) assigned to H-6 and H-8 protons of ring A and a signal of two protons at δ 6.95 (2H, s) ascribed to H-2` and H-6` of B ring. In addition to three protons signals at δ 3.6 ppm (OCH_3, s). The methyl etherification at C-3 of the aglycone moiety was established by the characteristic downfield shift of the resonance of this carbon to δ 141.85 ppm in the ^{13}C NMR spectrum as well as by comparing the carbon chemical shifts with previously reported.

The recognizable upfield shifts of C-6 and C-8 compared with previously reported for these positions[47-50] indicating that the C-7 position of this compound must be occupied with the sugar moiety (rhamnose). The position of the rhamnose sugar was established by comparing the ^{13}C NMR signals of ring A with that previously reported researchers[51-53]. On the basis of the above findings, compound **V** was elucidated to be 3-methoxy myricetin 7-*O*-α-L-rhamnopyranoside.

Body weight increased significantly in rats fed on high fat diet compared with controls (fed on normal diet) (Table-1). Treatment with Sibutramine 5 mg/kg body weight reduced body weight of high fat diet fed obese rats by 32 % while 200 mg and 400 mg/kg body weight of methanol extract of *Myrtus communis* caused a reduction of 21 % 24 %, respectively in body weight during the treatment period (Table-1).

TABLE-1
BODY WEIGHTS OF RATS FED ON NORMAL DIET (ND) AND HIGH FAT DIETS (HFD) AND THE EFFECT OF SIBUTRAMINE (SBT) AND METHANOLIC EXTRACT OF *MYRTUS COMMUNIS* ON BODY WEIGHT OF RATS

	Animal groups				
Body weights	ND	HFD	SBT+HFD	Extract 1+HFD	Extract 2+HFD
Initial body weight (g)	150 ± 4.5	153 ± 3.2	152 ± 0.5	156 ± 1.5	154 ± 5
Final body weight (g)	170 ± 0.78	260 ± 6	185 ± 3	203 ± 0.84	190 ± 0.98

*Values represent mean ± SEM of six rats; ND = Normal diet: HFD = High fat diet: SBT + HFD = Sibutramine + High fat diet; MEBP1 + HFD = Methanol extract of *Bauhinia purpurea* (200 mg/kg) + High fat diet; MEBP2 + HFD = Methanol extract of *Bauhinia purpurea* (400 mg/kg) + High fat diet

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