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α-Glucosidase Inhibitory Constituents from *Ficus bengalensis*

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ABSTRACT
Chromatographic purification of the methanolic extract of the aerial roots of *Ficus bengalensis* resulted the purifiaction of 3*β*-acetoxyurs-9(11),12-diene (**1**), a new triterpene together with lupeol (**2**), lupeol acetate (**3**), conrauidienol (**4**), *β*-sitosterol (**5**), alpinum isoflavone (**6**), methyl 4-hydroxybenzoate (**7**), 4-hydroxymellein (**8**), 4-hydroxybenzoic acid (**9**), *p*-coumaric acid (**10**), oleanolic acid (**11**) and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**12**). These compounds (**1-12**) were characterized by using 1D- (^{1}H, ^{13}C) and 2D-NMR (HMQC, HMBC, COSY, NOESY) spectroscopy and mass spectrometry (EI-MS, HR-EI-MS, FAB-MS, HR-FAB-MS), and in comparison with the data reported for related compounds in literature. These isolates (**1-12**) showed inhibitory activity against enzyme *α*-glucosidase with IC_{50} values ranging between 26.3-297.7 µM.

Key words: *Ficus bengalensis,* Methanolic extract, Secondary metabolites, *α*-Glucosidase inhibition.

1. INTRODUCTION
The Genus *Ficus* belongs to the plant family Moraceae consists of 800 species and most of which are native to the old world tropics[1]. *Ficus bengalensis* is a large evergreen tree distributed throughout subcontinent[2]. In English, it is known by the name "Banyan" because the Banias (Hindu merchants) used to assemble their business under this tree. Various species of the genus *Ficus* are used in local medicine as astringents, carminatives, stomachaches, vermonicides, hypotensives, antihelmintics and anti-dysentery drugs[3]. The aerial roots of *F. bengalensis* are used for the healing of wounds formed during urine flow, dysentery, diarrhea, conjunctivitis, scabies, diabetes, styptic, useful in syphilis, biliousness and inflammation of liver[4]. The aqueous extract of its aerial roots is used to increase immunity against various diseases in Ayurvedic system of medicine[5] to heal skin cracks and its young twigs are used as tooth brushes[6]. Its stem latex is used as aphrodisiac, tonic, vulnerary, maturant, lessens inflammations and is useful in piles, nose-diseases and gonorrhea in Unani system of medicine[7]. Its bark is used to decrease cholesterol levels[8]. Various extracts of bark of *F. bengalensis* possess anti-allergic and anti-stressive potential against asthma[9]. This plant contains phenolics which are used for preventing cardiovascular diseases, neurodegenerative diseases and cancer[10]. The various fruit extracts of *F. bengalensis* exhibited antitumor activity[11]. Previously, we reported the cholinesterase inhibitory constituents from the aerial roots of *F. bengalensis*[12]. Now, we are reporting the purification, characterization and *α*-glucosidase inhibition of the isolated compounds (**1-12**) from this source.

2. RESULTS AND DISCUSSION
The methanolic extract of aerial roots of *F. bengalensis* was divided into *n*-hexane, ethyl acetate and *n*-butanol soluble fractions. The chromatographic purification of the ethyl acetate soluble fraction resulted in the purification of twelve compounds (**1-12**) which were characterized by using spectroscopic techniques such as UV, IR 1D- (^{1}H, ^{13}C) and 2D-NMR (HSQC, HMBC, COSY, NOESY), and mass spectrometric techniques (EI-MS, HR-EI-MS, FAB-MS, HR-FAB-MS).

Compound (**1**) was purified as colorless amorphous powder. Its IR spectrum showed peaks for ester C=O (1735 cm^{-1}) and C=C (1650 cm^{-1}). The HR-EI-MS showed molecular ion peak at *m/z* 466.3800 corresponding to the molecular formula $C_{32}H_{50}O_2$. The ^{1}H-NMR spectrum of **1** (Table 1) showed two olefinic doublets at *δ* 5.59 (1H, d, *J* = 5.8 Hz) and 5.45 (1H, d, *J* = 5.8 Hz) coupled together in COSY spectrum and an oxygenated methine at *δ* 4.47 (1H, dd, *J* = 11.1, 5.2 Hz). In addition, six tertiary methyls were observed at *δ* 1.22, 1.17, 0.90, 0.89, 0.86, and 0.84 (3H each, s) and two secondary methyls at *δ* 0.92 (3H, d, *J* = 6.4 Hz) and 0.78 (3H, d, *J* = 6.0 Hz). It also showed the signal for acetyl group at *δ* 2.05 (3H, s). The above data was consistent with ursane type triterpene with two double bonds and an acetyl group[13]. The ^{13}C-NMR spectrum (BB and DEPT) of **1** (Table 1) revealed the presence of 32 carbon signals for nine methyl, eight methylene, seven methine and eight quaternary carbon atoms. The downfield signals at *δ* 170.3 could be assigned to ester carbonyl while the signals at *δ* 154.1, 141.3, 122.9 and 115.5 confirming the presence of two double bonds and an oxymethine at *δ* 80.5. The presence of double bonds at alternate position in ring C was confirmed by HMBC correlations in which Me-25 & 26 (*δ* 1.22, 1.17) correlated with C-9 (*δ* 154.1) and Me-27 & 28 (*δ* 0.89, 0.84) with C-13 (*δ* 141.3), respectively and characteristic EI-MS fragments at *m/z* 313 and 255 due to cleavage of ring B and D indicating the presence of cisoid diene at C-9(11),12 of a pentacyclic triterpene[14]. The presence of acetyl group at C-3 was confirmed due to its downfield NMR shifts (δ_H 4.67; δ_C 80.5) and HMBC correlation of H-3 (*δ* 4.67) with ester carbonyl at *δ*170.3. The stereochemistry at C-3 was determined by ^{1}H-NMR

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spectrum through coupling constant in which the larger J = 11.1, 5.2 Hz values confirming it as axial and α-orientation and the NOESY correlation between H-3 (δ 4.67) with Me-23 (δ 0.90). Based on above evidences **1** could be 3β-acetoxyurs-9(11),12-diene, which is reported synthetically[14,15] but isolated for the first time from any natural source.

Table-1: ^{1}H- and ^{13}C-NMR spectral data and HMBC correlations of **1** ($CDCl_3$; 400, 100 MHz)

Position	δ_H, (J in Hz)	δ_C	HMBC (H→C)
1	1.32, m 1.27, m	37.4	C-2, C-3, C-5, C-10, C-25
2	2.30, m 1.71, m	24.2	C-3, C-4, C-10
3	4.66, dd (11.1, 5.2)	80.5	C-1, C-4, C-23,C-1′
4	-	38.5	-
5	0.86, m	51.1	C-3, C-7, C-23, C-24, C-25
6	1.60, m	18.2	C-4,C-8, C-10
7	1.44, m	31.9	C-5, C-9, C-26
8	-	40.6	-
9	-	154.1	-
10	-	37.8	-
11	5.59, d (5.8)	115.5	C-8, C-10, C-13,
12	5.45, d (5.8)	122.9	C-9, C-14, C-18
13	-	141.3	-
14	-	43.0	-
15	1.84, m 1.10, m	28.6	C-13, C-17, C-27
16	1.43, m 1.30, m	41.4	C-14, C-18, C-28
17	-	32.9	-
18	1.49, d (6.5)	57.2	C-12, C-14, C-20, C-22, C-29,
19	1.26, m	39.4	C-13, C-17, C-21, C-29, C-30
20	0.89, m	38.9	C-18, C-22, C-29
21	1.34, m 1.18, m	30.8	C-17, C-19, C-30
22	1.26, m 1.44, m	41.3	C-18, C-20, C-28
23	0.90, s	27.6	C-3, C-4, C-5, C-24
24	0.86, s	16.1	C-3, C-4, C-5, C-24
25	1.22, s	18.6	C-1, C-5, C-9, C-10
26	1.17, s	22.9	C-7, C-8, C-9, C-14
27	0.89, s	17.7	C-8, C-13, C-14, C-15
28	0.84, s	28.6	C-16, C-17, C-18, C-22
29	0.78, d (6.0)	17.3	C-18, C-19, C-20
30	0.92, d (6.4)	19.3	C-19, C-20, C-21
CH_3CO	2.05, s	170.3, 21.2	C=O

Compound **2** was isolated as white amorphous solid and its molecular formula $C_{30}H_{50}O$ was established on the basis of HR-EI-MS by a peak at *m/z* 426.3851. Its ^{1}H-NMR spectrum displayed seven methyl singlets at δ 1.70, 1.03, 0.97, 0.94, 0.83, 0.81 and 0.76 (3H each, s) and two broad singlets (1H each) at δ 4.69 and 4.57 is an indication of lupeol type triterpene[16]. It also showed an oxymethine at δ 3.19 (1H, dd, J = 11.6, 4.8 Hz, H-3). The larger coupling constant indicates the axial and α-orientation of H-3. The ^{13}C-NMR spectra (BB and DEPT) displayed 30 carbon signals for seven methyl, eleven methylene, six methine and six quaternary carbons. The downfield signals at δ 151.1, 109.7 and 79.3 assigned to double bond and an oxymethine. The above data was completely overlapped with the data reported for lupol[17].

The colorless amorphous solid compound **3** having molecular formula $C_{32}H_{52}O_2$. The ^{1}H-NMR spectrum of **2** was almost similar to that for **1** except with the additional signal for acetyl group at δ 2.01 (3H, s) and downfield shift of oxymethine at δ 4.44 (1H, dd, J = 10.8, 5.8 Hz) indicated the attachment of acetyl group at C-3 position. The ^{13}C-NMR spectra (BB and DEPT) of **3** disclosed altogether 32 carbon signals for eight methyl, eleven methylene, six methine and seven quaternary carbons. The signal at δ 171.0 and 21.3 was due to acetyl group. The remaining signals were almost similar to those for **2**. This data was in complete agreement with the data reported for lupeol acetate[18].

Compound **4** having the molecular formula $C_{32}H_{50}O_3$ was established through HR-EI-MS by the molecular ion peak at *m/z* 482.3748. The ^{1}H-NMR spectrum of **3** displayed a pair of olefinic doublets at δ 6.50 (1H, d, J = 5.4 Hz) and 5.45 (1H, d, J = 5.4 Hz) two oxymethines at δ 4.53 (1H, dd, J = 12.0, 3.6 Hz, H-3) and 3.91 (1H, dd, J = 11.4,

4.2 Hz, H-1). The larger coupling constants of these oxymethines indicated both of these are axial and *α* in orientation. The same spectrum also displayed six methyl singlets at *δ* 1.25, 1.14, 0.87, 0.86, 0.85, 0.82 (3H each, s) and a pair of doublets resonating at *δ* 0.90 and 0.78 (3H each, d, *J* = 6.0 Hz), indicating **4** an ursane type triterpene[19]. The same spectrum also showed a singlet at *δ* 2.04 (3H, s). The ^{13}C-NMR spectra of **4** (BB and DEPT) displayed 32 carbon signals including nine methyl, seven methylene, eight methine and eight quaternary carbon atoms. The signal resonating at *δ* 170.8 and 21.2 were due to the presence of acetyl group while the signals resonating at *δ* 152.0, 141.6, 123.3, 117.6, were attributed to two double bonds and signals at *δ* 77.2 and 75.6 were due to the presence of two oxymethines, respectively. This data was in complete agreement with the data already published for conrauidienol[13].

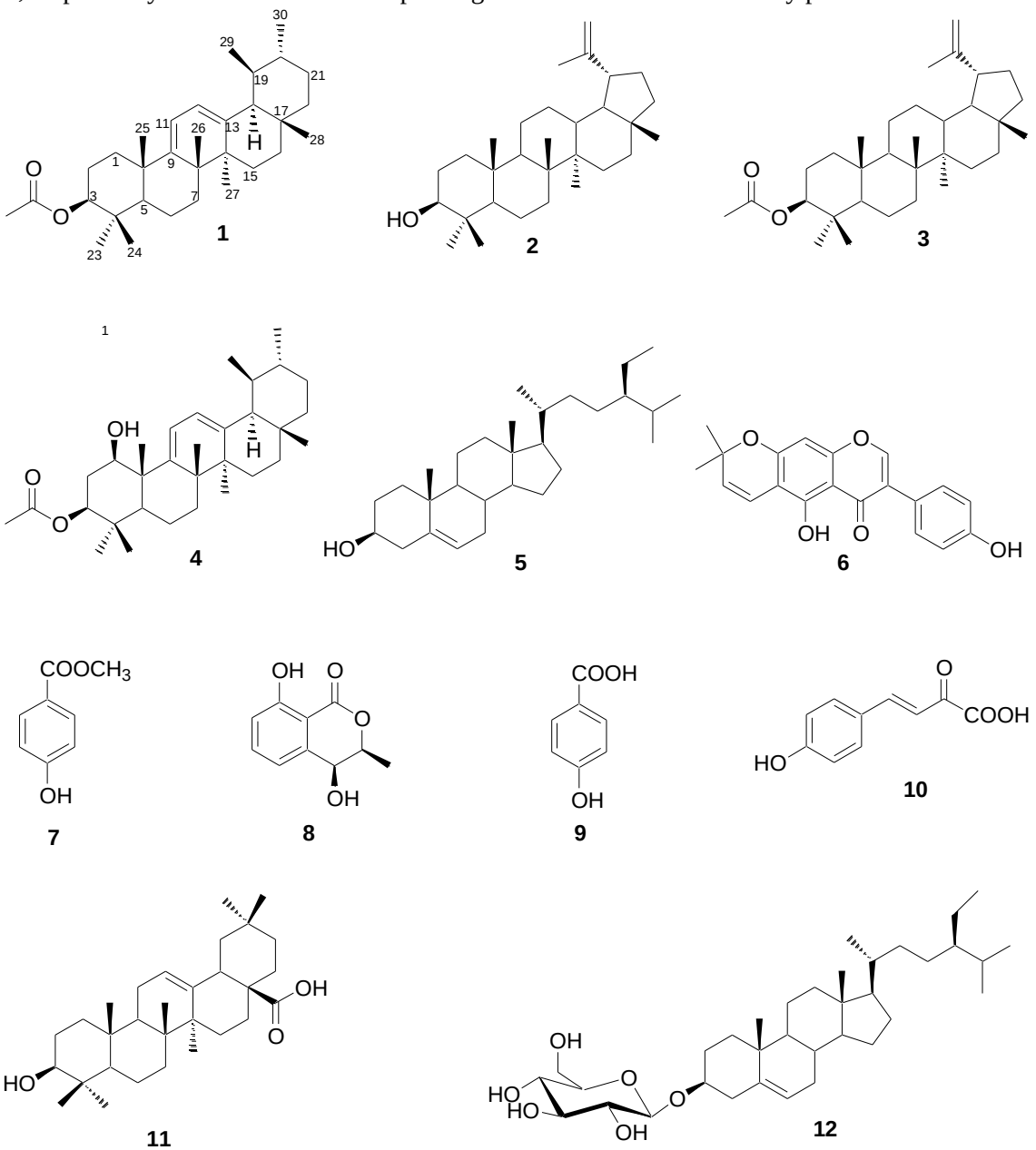

Fig.1: Structures of compounds (**1-12**) isolated from *F. bengalensis*

Compound **5** having molecular $C_{29}H_{50}O$ deduced through HR-EI-MS at *m/z* 414.3851. The ^{1}H-NMR spectrum of **5** displayed six methyl signals: two tertiary methyl resonating at *δ* 1.00, 0.68, (3H each, s), three secondary at *δ* 0.92, 0.83, 0.80 (3H each, d, *J* = 6.2, 6.0 and 6.0 Hz, respectively) and a primary at *δ* 0.86 (3H, t, *J* = 6.0 Hz) and two downfield signals at *δ* 5.13 (1H, br s, H-5) and 3.42 (1H, m, H-3). The ^{13}C-NMR spectra (BB and DEPT) of the **5** disclosed total 29 carbon signals for six methyl, eleven methylene, nine methine and three quaternary carbon atoms. The signals at *δ* 140.7 and 121.9 were due to presence of double bond and a signal at *δ* 71.6 was due an oxygenated methine. All the physical and spectral data completely matched with the data reported for *β*-sitosterol[20,21].

Compound **6** was isolated as white amorphous solid. The molecular formula $C_{20}H_{16}O_5$ was established by HR-EI-MS by a peak at *m/z* 336.0987. The ^{1}H-NMR spectrum of **5** displayed a pair of doublets at *δ* 6.72 (1H, d, *J* = 10.6 Hz) and 5.62 (1H, d, *J* = 10.6 Hz). A sharp singlet at *δ* 7.81 (1H, s) was the characteristic of C-2 of isoflavone nucleus[22]. An A^2B^2 system type doublets at *δ* 7.40 (2H, d, *J* = 7.5 Hz) and 6.74 (2H, d, *J* = 7.5 Hz) were due to the

presence of *p*-substituted benzene. A singlet at δ 1.47 (6H, s) showing the presence of two magnetically equivalent methyls. The ^{13}C-NMR spectra (BB and DEPT) of **6** showed eighteen signals for twenty carbons, for two methyl, eight methine and ten quaternary carbon atoms at δ 180.0, 159.0, 157.1, 155.7, 152.4, 141.2, 130.3, 128.1, 127.9, 123.4, 123.0, 115.5, 115.4, 94.8, 77.2 and 28.3. This data was in complete agreement with the data reported for alpinum isoflavone[23].

Compound **7** having the molecular formula $C_8H_8O_3$ was based on HR-EI-MS measurements at *m/z* 152.0462. Its ^{1}H-NMR spectrum showed two aromatic doublets at δ 7.96 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.8 Hz) and a singlet at δ 3.90 (3H, s). The ^{13}C-NMR spectra (BB and DEPT) of **7** showed total six signals for eight carbons with one methyl, four methine and three quaternary carbons. The downfield signals at δ 167.3 and 163.6 were due to an ester carbonyl and an oxygenated aromatic quaternary carbons. The signal appeared at δ 52.3 was due to the presence of methoxy group. This data were in complete agreement with the data reported for the methyl 4-hydroxybenzoate[25].

The molecular formula $C_{10}H_{10}O_4$ of **8** was established through HR-EI-MS due to a molecular ion peak at *m/z* 194.0568. The ^{1}H-NMR spectrum displayed a pair of doublets at δ 7.03 (1H, d, J = 7.45 Hz), 6.96 (1H, d, J = 7.5 Hz) and a triplet at δ 7.50 (1H, t, J = 6.0 Hz) in the aromatic region indicating the presence of *ortho*-trisubstituted benzene ring. It also showed two oxygenated methines at δ 4.50 (1H, m), 4.52 (1H, d, J = 6.0 Hz) and a methyl at δ 1.44 (3H, d, J = 6.5 Hz). The ^{13}C-NMR spectra of **8** (BB and DEPT) displayed 10 carbon signals for one methyl, five methine and four quaternary carbons. This data was in complete agreement with that reported for 4-hydroxymellein[25].

Compound **9** was obtained as crystalline solid having molecular formula $C_7H_6O_3$. The ^{1}H-NMR of **9** was similar to that for **7** except the absence of methoxy group. The ^{13}C-NMR spectrum of **9** showed five carbon signals for seven carbons with similar resonance like that for **7** except the missing of methoxy signals. This information completely matched with values reported for 4-hydroxybenzoic acid[26].

Compound **10** was obtained as white amorphous powder. Its molecular formula $C_9H_8O_3$ was determined by HR-EI-MS through a molecular ion peak at *m/z* 164.0461. Its ^{1}H-NMR spectrum showed four doublets at δ 7.57 (1H, d, J = 16.0 Hz), 7.43 (2H, d, J = 8.5 Hz), 6.79 (2H, d, J = 8.5 Hz) and 6.27 (1H, d, J = 16.0 Hz). The larger coupling constants (16.0 Hz) of two doublets is a clear indication for the presence of a *trans* double bond. The ^{13}C-NMR spectrum of **10** showed seven signals for nine carbon atoms. The signal at δ 172.0 was due to the presence of conjugated carboxyl acid where as four methines at δ 146.2, 130.2, 116.9 and 162.0 indicated the presence of *p*-substituted cinnamic acid. The above data completely overlapped with the data reported for *p*-coumaric acid[27].

Compound **11** was obtained as white powder. The molecular formula $C_{30}H_{48}O_3$ was established by HR-EI-MS through its molecular ion peak at *m/z* 456.3593. The ^{1}H-NMR spectrum of **11** showed a tertiary methine at δ 5.49 (1H, t, J = 3.5 Hz), an oxymethine at δ 3.44 (1H, dd, J = 9.8, 4.2 Hz) and seven tertiary methyls at δ 1.30, 1.24, 1.04, 1.02, 1.01, 0.97 and 0.93. The ^{13}C-NMR spectra of **11** showed 30 carbon signals, for seven methyls, ten methylenes, five methines and eight quaternary carbons. The signals resonating at δ 144.8 and 122.6 were attributed to double bond where as the signal at δ 78.2 was due to the presence of oxygenated methine. The above spectral data resembles completely with the data reported for the oleanolic acid[28,29].

The molecular formula $C_{35}H_{61}O_6$ compound **12** was established by HR-FAB-MS through a molecular ion peak at *m/z* 577.4455 $[M+H]^+$. The ^{1}H-NMR spectrum displayed same signals as observed for **5** with the additional signals for glucose moiety whose anomeric proton was observd at δ 4.38 (1H, d, J = 6.5 Hz) with multiplets observed between δ 3.01-3.73. The ^{13}C-NMR spectrum of **12** displayed 35 carbon signals for six methyl, twelve methylene, fourteen methine and three quaternary carbon atoms. The signals at δ 140.7 and 121.9 were due to the presence of double bond.

The signal for sugar moiety were appeared at δ 101.0, 74.4, 79.1, 70.1, 78.2 and 61.9. The downfield shift of C-3 (δ 76.2) is an indication for the glycosidation at this position. This data was in complete agreement with that reported for β-sitosterol 3-*O*-β-D-glucopyranoside[30].

Table 2: α-Glucosidase inhibition of compounds **1-12**

Compound	Percentage Inhibition	IC_{50} (□M)
1	95.36 ± 0.04	169.97 ± 0.07
2	98.74 ± 0.03	51.29 ± 0.003
3	99.09 ± 0.01	26.31 ± 0.003
4	97.16 ± 0.02	288.2 ± 0.003
5	42.37 ± 0.04	277.7 ± 0.003
6	97.12 ± 0.09	75.89 ± 0.008
7	47.21 ± 0.03	277.7 ± 0.003
8	87.68 ± 0.02	202.82 ± 0.09
9	41.12 ± 0.04	297.7 ± 0.002
10	99.59 ± 0.05	54.15 ± 0.005
11	98.35 ± 0.04	231.33 ± 0.05
12	87.15 ± 0.09	258.71 ± 0.07
Acarbose	92.23 ± 0.14	38.25 ± 0.12

All the samples were dissolved in methanol and experiments were performed in triplicate (mean±sem, n=3).

2.1 *α-Glucosidase Inhibition Studies of compounds 1-12*

The compounds **1-12** were subjected for *α*-glucosidase inhibitory activity and the results are shown in Table 2. Compound **3** with IC_{50} value 26.3 µM showed promising activity followed by compounds **2, 10** & **6** with IC_{50} value 51.2, 54.1 & 75.8 µM, respectively.

3. EXPERIMENTAL

3.1 *General experimental procedures*

Melting points were recorded by using apparatus of Buchi 434. UV spectra were measuerd (in methanol) on spectrophotometer of Schimadazu UV-240. Optical rotations were measured by polarimeter (JASCO DIP-360). IR spectra were performed on IR spectrometer (IR-460 Shimadzu). ^{1}H- and ^{13}C-NMR, HMQC, COSY and HMBC spectra were recorded using spectrometer of Bruker, operating at 500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR, respectively. Chemical shift values (*δ*) are reported in ppm and the coupling constants (*J*) measured in Hz. Mass spectra (EI-MS, HR-EI-MS) were measured on mass (JMS HX 110) instrument and HR-FAB-MS were recorded on mass spectrometers (JMS-DA 500) and unit is shown in *m/z*. Thin layer chromatography (TLC) were carried out by using aluminum sheets coated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E. Merck) and column chromatography (CC) using silica gel (230-400 mesh). TLC plates were visualized under UV at 254 and 366 nm and sprayed with ceric sulfate solution (1% in 10% H_2SO_4) with heating.

3.2 *Plant material*

The aerial roots of *F. bengalensis* was collected from Sahiwal District (Pakistan) in July 2010 and was identified by Dr. Muhammad Arshad (late), Plant Taxonomist, Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, where a plant specimen is deposited (0046-FB/CIDS/10).

3.3 *Extraction and isolation*

The shade dried ground aerial roots was extracted in metanol. The methanolic extract was evaporated on rotary evaporator to obtain black gummy material (750 g) which suspended in water and extracted with *n*-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate fraction (50 g) was subjected to the column chromatography over flash silica gel and eluted with dichloromethane (DCM), DCM-ethyl acetate, ethyl acetate, ethyl acetate-methanol, and methanol in increasing order of polarity to get eight sub-frations. These sub-fractions were further purified by gradient elution using *n*-hexane and DCM (10% DCM in *n*-hexane) to afford **1,** (12% DCM in *n*-hexane), lupol (**2**), (15% DCM in *n*-hexane), lupeol acetate (**3**), (20% DCM in *n*-hexane), conrauidienol (**4**), (20% DCM in *n*-hexane), *β*-sitosterol (**5**), (28% DCM in *n*-hexane), alpinum isoflavone (**6**), (35% DCM in *n*-hexane), methyl 4-hydroxybenzoate (**7**), (50% DCM in *n*-hexane), 4-hydroxymellein (**8**), (45% DCM in *n*-hexane), 4-hydroxybenzoic acid (**9**), (55% DCM in *n*-hexane), *p*-coumaric acid (**10),** (70% DCM in *n*-hexane) oleanolic acid (**11**), (1% MeOH in DCM) and *β*-sitosteryl 3-*O*-*β*-D-glucopyranoside (**12**).

3.4 *Chracterization of isolated compounds (1-12)*

3.4.1 *3β-acetoxyurs-9(11),12-diene (1)*

Colorless amorphous powder (20 mg); $[\alpha]_D^{24}$ + 24.7 (*c* 0.16, MeOH); IR (KBr, ν_{max}, cm^{-1}): 2966, 1735, 1650; ^{1}H- and ^{13}C-NMR spectral data, see Table 1; HR-EI-MS: *m/z* 466.3800 $[M]^+$ (calcd for $C_{32}H_{50}O_2$, 466.3810).

3.4.2 *Lupeol (2)*

White amorphous solid (12mg); m.p 213 ˚C; $[\alpha]_D^{24}$ + 25.7 (*c* = 0.70 in $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}): 3326, 2931, 1631, 1450, 1377, 1035, 874; ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 4.69 (br s, 1H, H-29a), 4.57 (br s, 1H, H-29b), 3.19 (dd, *J* = 11.6, 4.8 Hz, 1H, H-3), 2.39 (dd, *J* = 9.6, 4.0 Hz, 1H, H-19), 1.70 (s, 3H, H-30), 1.03 (s, 3H, H-28), 0.97 (s, 3H, H-27), 0.94 (s, 3H, H-26), 0.83 (s, 3H, H-25), 0.81 (s, 3H, H-24), 0.76 (s, 3H, H-23); ^{13}C-NMR ($CDCl_3$, 100 MHz, *δ*/ppm): *δ* 151.1 (C-20), 109.7 (C-29), 79.3 (C-3), 55.6 (C-5), 50.7 (C-9), 48.8 (C-19), 48.3 (C-18), 43.4 (C-17), 43.2 (C-14), 41.2 (C-8), 40.4 (C-22), 39.2 (C-4), 39.1 (C-1), 38.4 (C-13), 37.5 (C-10), 35.9 (C-16), 34.6 (C-7), 30.2 (C-21), 28.4 (C-23), 27.9 (C-2), 27.8 (C-15), 25.5 (C-12), 21.3 (C-11), 19.8 (C-30), 18.7 (C-6), 18.4 (C-28), 16.5 (C-25), 16.3 (C-26), 15.8 (C-24), 14.9 (C-27); HR-EI-MS *m/z*: 426.3851 (calcd for $C_{30}H_{50}O$, 426.3861).

3.4.3 *Lupeol acetate (3)*

Colorless amorphous solid (14 mg); IR IR (KBr, ν_{max}, cm^{-1}): 2942, 2866, 1735, 1451, 1379, 1243, 1027 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 4.69 (br s, 1H, H-29a), 4.57 (br s, 1H, H-29b), 4.44 (dd, *J* = 10.8, 5.8 Hz, 1H, H-3), 2.01 (s, 3H, OAc), 1.70 (s, 3H, H-30), 1.03 (s, 3H, H-28) 0.94 (s, 3H, H-26), 0.91 (s, 3H, H-27), 0.81 (s, 3H, H-23,24), 0.83 (s, 3H, H-25), 0.76 (s, 3H, H-23); ^{13}C NMR ($CDCl_3$, 100 MHz, *δ*/ppm): 171.0 (OAc), 150.9 (C-20), 109.3 (C-29), 80.9 (C-3), 55.3 (C-5), 50.3 (C-9), 48.2 (C-19), 48.0 (C-18), 42.9 (C-17), 42.8 (C-14), 40.8 (C-8), 39.9 (C-22), 38.3 (C-1), 38.0 (C-13), 37.3 (C-4), 37.0 (C-10), 35.5 (C-16), 34.7 (C-7), 29.1 (C-21), 28.2 (C-23), 27.4 (C-15), 25.0

(C-12), 23.2 (C-2), 21.3 (OAc), 20.9 (C-11), 19.0 (C-30), 18.1 (C-6), 17.9 (C-28), 16.4 (C-26), 16.1 (C-25), 15.9 (C-24), 14.5 (C-27); HR-EI-MS at *m/z* 442.3801 (calcd for $C_{32}H_{52}O_2$, 442.3811)

3.4.4 *Conrauidienol (4)*

White amorphous powder; $[\alpha]_D^{24}$ + 12.4 (*c* 0.0014, acetone); UV (MeOH, λ_{max}, log ε, nm): 198 (3.4), 203 (3.45), 215 (3.36), 220 (3.32); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 6.50 (d, *J* = 5.4 Hz, 1H, H-11), 5.45 (d, *J* = 5.4 Hz, 1H, H-12), 4.53 (dd, *J* = 12.0, 3.6 Hz, 1H, H-3), 3.91 (dd, *J* = 11.4, 4.2 Hz, 1H, H-1), 2.04 (s, 3H, OAc), 1.25 (s, 3H, H-25), 1.14 (s, 3H, H-26), 0.90 (d, *J* = 6.0 Hz, 3H, H-30), 0.87 (s, 3H, H-27), 0.86 (s, 3H, H-24), 0.85 (s, 3H, H-23), 0.82 (s, 3H, H-28), 0.78 (d, *J* = 6.0 Hz, 3H, H-29), ^{13}C-NMR ($CDCl_3$, 125 MHz, δ/ppm): 170.8 (OAc), 152.0 (C-9), 141.6 (C-13), 123.3 (C-12), 117.6 (C-11), 77.2 (C-3), 76.5 (C-1), 57.2 (C-18), 48.7 (C-5), 44,5 (C-10), 43.1 (C-8), 41.2 (C-16), 40.7 (C-14), 39.3 (C-20), 39.0 (C-19), 37.9 (C-4), 34.4 (C-2), 33.6 (C-17), 31.1(C-21), 30.9 (C-7), 28.6 (C-28), 28.2 (C-22), 27.6 (C-23), 26.1 (C-15), 22.9 (C-26), 21.5 (C-30), 21.2 (OAc), 18.6 (C-25), 18.2 (C-6), 17.7 (C-27), 17.3 (C-29), 16.1 (C-24); HR-EI-MS *m/z*: 482.3748 (calcd for $C_{32}H_{50}O_3$, 482.3759).

3.4.5 *β-Sitosterol (5)*

White crystalline solid (20 mg); m.p 135˚C; IR (KBr, ν_{max}, cm^{-1}): 3446, 3050, 1650; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 5.13 (m, 1H, H-5), 3.42 (m, 1H, H-3), 1.00 (s, 3H, H-19), 0.92 (d, *J* = 6.2 Hz, 3H, H-21), 0.86 (t, *J* = 7.0 Hz, 3H, H-29), 0.83 (d, *J* = 6.0 Hz, 3H, H-26), 0.80 (d, *J* = 6.0 Hz, 3H, H-27), 0.68 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ/ppm): 140.7 (C-5), 121.9 (C-6), 71.6 (C-3), 56.6 (C-14), 55.9 (C-17), 51.3 (C-9), 48.9 (C-24), 42.8 (C-4), 42.2 (C-13), 40.3 (C-12), 36.9 (C-1), 36.6 (C-10), 36.1 (C-20), 34.4 (C-22), 32.7 (C-7), 32.2 (C-16), 32.0 (C-8), 31.4 (C-2), 28.9 (C-23), 26.2 (C-25), 25.5 (C-15), 23.2 (C-28), 21.1 (C-11), 19.8 (C-27), 19.6 (C-19), 19.0 (C-21), 18.7 (C-26), 12.1 (C-29), 11.9 (C-18); HR-EI-MS *m/z* 414.3851 (calcd for $C_{29}H_{50}O$, 414.3862).

3.4.6 *Alpinum isoflavone (6)*

White amorphous solid (10 mg); m.p 213-214˚C; UV (MeOH, λ_{max}, log ε, nm): 284 (4.7); IR (KBr, ν_{max}, cm^{-1}): 3570, 3300, 1650; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.81 (s, 1H, H-2), 6.32 (s, 1H, H-8), 7.40 (d, *J* = 7.5 Hz, 2H, H-2,6), 6.74 (d, *J* = 7.5 Hz, 2H, H-3,5), 6.72 (d, *J* = 10.0 Hz, 1H, H-4″), 5.62 (d, *J* = 10.0 Hz, 1H, H-3″), 1.47 (s, 6H, H-9,10); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ/ppm): 180.0 (C-4), 159.0 (C-5), 157.1 (C-8a), 155.7 (C-4′), 152.4 (C-2), 141.2 (C-6), 130.3 (C-2′,5′), 128.1 (C-3″), 127.9 (C-3), 123.4 (C-1′), 123.0 (C-7), 115.5 (C-4″), 115.4 (C-3′,5′), 94.8 (C-8), 77.2 (C-2″), 28.3 (Me-9,10); HR-EI-MS *m/z*: 336.0987 (calcd for $C_{20}H_{16}O_5$, 336.0998).

3.4.7 *Methyl 4-hydroxybenzoate (7)*

Crystalline solid (13 mg); m.p 128˚C; UV (MeOH, λ_{max}, log ε, nm): 220 (3.80), 308 (3.91); IR (KBr, ν_{max}, cm^{-1}): 3515, 1696; ^{1}H-NMR (CD_3OD, 400 MHz, δ/ppm): 7.96 (d, *J* = 8.8 Hz, 2H, H-2,6), 6.86 (d, *J* = 8.8 Hz, 2H, H-3,5), 3.90 (s, 3H, OCH_3); ^{13}C-NMR (CD_3OD, 100 MHz, δ/ppm): 167.3 (C-7), 163.6, (C-4), 132.7, (C-2,6), 122.6 (C-1), 116.1, (C-3,5), 52.3 (OCH_3); HR-EI-MS *m/z* 152.0462 (calcd for $C_8H_8O_3$, 152.0473).

3.4.8 *4-Hydroxymellein (8)*

White amporphous solid (20 mg); m.p 118-119˚C; UV (MeOH, λ_{max}, log ε, nm): 246 (2.9), 314 (3.6); IR (KBr, ν_{max}, cm^{-1}): 3300, 1680; ^{1}H-NMR ($CDCl_3$, 400 MHz, δ/ppm): 7.03 (d, *J* = 7.5 Hz, 1H, H-7), 6.96 (d, *J* = 7.5 Hz, 1H, H-5), 7.53 (t, *J* = 7.5 Hz, 1H, H-6), 4.52 (d, *J* = 6.0 Hz, 1H, H-4), 4.50 (m, 1H, H-3), 1.44 (d, *J* = 6.5 Hz, 3H, C-9); ^{13}C-NMR ($CDCl_3$, 100 MHz, δ/ppm): 168.5 (C-1), 161.9 (C-8), 141.1 (C-4a), 136.8 (C-6), 117.7 (C-7), 116.2 (C-5), 106.5 (C-1a), 79.9 (C-4), 69.0 (C-3), 17.8 (C-9); HR-EI-MS *m/z* 194.0568 (calcd for $C_{10}H_{10}O_4$, 194.0579).

3.4.9 *4-Hydroxybenzoic acid (9)*

Crystalline solid (8 mg); m.p 213-214˚C; UV (MeOH, λ_{max}, log ε, nm): 222 (3.80), 310 (3.89); IR (KBr, ν_{max}, cm^{-1}): 3515, 3335-2730, 1710; ^{1}H-NMR (CD_3OD, 400 MHz, δ/ppm): 7.86 (d, *J* = 8.4 Hz, 2H, H-2,6), 6.80 (d, *J* = 8.4 Hz, 2H, H-3,5); ^{13}C-NMR (CD_3OD, 100 MHz, δ/ppm): 170.9 (C-7), 163.3 (C-4), 132.9 (C-2,6), 122.9 (C-1), 116.0 (C-3,5); HR-EI-MS *m/z*: 138.0307 (calcd for $C_7H_6O_3$, 138.0317).

3.4.10 *p-Coumaric acid (10)*

White powder (14 mg); m.p 211-213˚C; UV (MeOH, λ_{max}, log ε, nm): 290 (4.4), 306 (4.6) nm; IR (KBr, ν_{max}, cm^{-1}): 3400, 3350-2250, 1685, 1625, 1425, 1380; ^{1}H-NMR (CD_3OD, 400 MHz, δ/ppm): 7.57 (d, *J* = 16.0 Hz, 1H, H-1′), 7.43 (d, *J* = 8.5 Hz, 2H, H-2,6), 6.79 (d, *J* = 8.5 Hz, 2H, H-3,5), 6.27 (d, *J* = 16.0 Hz, 1H, H-2′); ^{13}C-NMR (CD_3OD, 100 MHz, δ/ppm): 172.0 (C-7), 162.0 (C-4), 146.2 (C-7), 130.2 (C-2,6), 127.5 (C-1), 116.9 (C-3,5), 116.0 (C-8); HR-EI-MS *m/z*: 164.0461 (calcd. for $C_9H_8O_3$, 164.0473).

3.4.11 *Oleanolic acid (11)*

White powder (15 mg); m.p 305-306˚C; $[\alpha]_D^{25}$ + 78.9˚ (*c* 0.015, $CHCl_3$); IR

(KBr, ν_{max}, cm^{-1}): 3410-2650, 1710, 1660, 820; ^{1}H-NMR ($CDCl_3$, 400 MHz, *δ*/ppm): 5.49 (t, *J* = 3.5 Hz, 1H, H-12), 3.44 (dd, *J* = 9.8, 4.2 Hz, 1H, H-3), 1.30 (s, 3H, H-27), 1.24 (s, 3H, H-23), 1.04 (s, 3H, H-26), 1.02 (s, 3H, H-24), 1.01 (s, 3H, H-30), 0.97 (s, 3H, H-29), 0.93 (s, 3H, H-25); ^{13}C-NMR ($CDCl_3$, 100 MHz, *δ*/ppm): 180.0 (C-28), 144.8 (C-13), 122.6 (C-12), 78.2 (C-3), 55.9 (C-5), 48.2 (C-9), 46.7 (C-17), 42.2 (C-19), 41.7 (C-14), 40.7 (C-18), 39.2 (C-8), 38.7 (C-4), 38.5 (C-1), 37.2 (C-10), 33.8 (C-21), 32.9 (C-29), 32.5 (C-7), 32.4 (C-22), 30.6 (C-20), 28.2 (C-23), 27.7 (C-15), 27.8 (C-2), 25.9 (C-27), 23.5 (C-30), 23.4 (C-11), 23.3 (C-30), 23.2 (C-16), 18.3 (C-6), 17.20 (C-26), 15.5 (C-24), 15.3 (C-25); HR-EI-MS *m/z* 456.3593 (calcd for $C_{30}H_{48}O_3$, 456.3603).

3.4.12 *β-Sitosterol 3-O-β-D-glucopyranoside (12)*

Colorless amorphous powder (25mg); $[\alpha]_D^{25}$: 14.5˚, (*c* 0.003, MeOH); IR (KBr, ν_{max}, cm^{-1}): 3452. 3044, 1646, 1618, 1559, 1550; ^{1}H-NMR ($CDCl_3$+ CD_3OD, 400 MHz, *δ*/ppm): 5.13 (br s, 1H, H-5), 4.38 (d, *J* = 6.5 Hz, 1H, H-1′), 3.01 (m, 1H, H-2′), 3.32 (m, 1H, H-3′), 3.2 (m, 1H, H-4′), 3.39 (m, 1H, H-5′′), 3.73, 3.65 (br s, 2H, H-6′), 3.45 (m, 1H, H-3), 1.00 (s, 3H, H-19), 0.92 (d, *J* = 6.2 Hz, 3H, H-21), 0.86 (t, *J* = 7.0 Hz, 3H, H-29), 0.83 (d, *J* = 6.0 Hz, 3H, H-26), 0.80 (d, *J* = 6.0 Hz, 3H, H-27), 0.68 (s, 3H, H-18); ^{13}C-NMR ($CDCl_3$+CD_3OD, 100 MHz, *δ*/ppm): 140.7 (C-5), 121.9 (C-6), 101.0 (C-1′), 78.2 (C-5′), 76.2 (C-3), 74.4 (C-2′), 79.1 (C-3′), 70.1 (C-4′), 61.9 (C-6′), 56.6 (C-14), 55.9 (C-17), 51.3 (C-9), 48.9 (C-24), 42.8 (C-4), 42.2 (C-13), 40.3 (C-12), 36.9 (C-1), 36.6 (C-10), 36.1 (C-20), 34.4 (C-22), 32.7 (C-7), 32.2 (C-16), 32.0 (C-8), 31.4 (C-2), 28.9 (C-23), 26.2 (C-25), 25.5 (C-15), 23.2 (C-28), 21.1 (C-11), 19.8 (C-27), 19.6 (C-19), 19.0 (C-21), 18.7 (C-26), 12.1 (C-29), 11.9 (C-18); HR-FAB-MS *m/z* 577.4455 $[M+H]^+$ (calcd for $C_{35}H_{61}O_6$, 577.4468).

3.4.13 *α-Glucosidase Inhibition Assay*

The method used to perform *α*-glucosidase inhibition assay was similar but with slight modifications as done by Pierre *et al*[31]. 100 µL of total volume of reaction mixture having 70 µL (50 mM) phosphate buffer, pH 6.8, 10 µL (0.5 mM) test compound, continued with the addition of 10 µL (0.0234 units, Sigma Inc.) enzyme. These reagents were mixed, preincubated for 10 min at 37˚C and pre-read at 400 nm. This was further initiated by adding 10 µL (0.5 mM) substrate (*p*-nitrophenyl glucopyranoside, Sigma Inc.). The incubation was done for 30 min at 37˚C, yellow color showed absorbance due to the formation of *p*-nitrophenol which was measured at 400 nm using Synergy HT (BioTek, USA) using 96-well microplate reader. For positive control, acarbose was used. The inhibition percentage was calculated by using the equation below

Inhibition (%) = (abs of control – abs of test / abs of control) × 100

IC_{50} values were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

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5. REFERENCES

1. Manoj, A., Urmila, A., Bhagyashri, W., Meenakshi, V., Akshaya, W., Kishore, N. G., *Int. J. Green Pharm*. **(2008)**, 2, 170, http://dx.doi.org/10.4103/0973-8258.42737.
2. Sharma, A., Kumari, M., Jagannadham, M. V., *J. Agric. Food Chem*. **(2009)**, 57, 11120, http://dx.doi.org/10.1021/jf902279u.
3. Trivedi, C. P., Shinde, S., Sharma, R. C., *Indian J. Med. Res*. **(1969)**, 57, 1070.
4. Mukherjee, P. K., Saha, K., Murugesan, T., Mandal, S. C., Pal, M., Saha, B. P., *J. Ethnopharmacol.* **(1998)**, 60, 85, http://dx.doi.org/10.1016/S0378-8741(97)00130-X.
5. Khan, T., Tatke, P., Gabhe, S. Y., *Indian J. Pharm. Sci*. **(2008)**, 70, 287, http://dx.doi.org/10.4103/0250-474X.42970.
6. Muthu, C., Ayyanar, M., Raja, N., Ignacimuthu, S., *J. Ethnobiol. Ethnomed*. **(2006)**, 2, 43, http://dx.doi.org/10.1186/1746-4269-2-43.
7. Varanasi, S., Pasu, N. A., *Bull. Indian Ins. His. Med*. **(2007)**, 37, 117.
8. Shukla, R., Gupta, S., Gambhir, J. K., Prabhu, K. M., Murthy, P. S., *J. Ethnopharmacol*. **(2004)**, 92, 47, http://dx.doi.org/10.1016/j.jep.2004.01.020.
9. Taur, D. J., Nirmal, S. A., Patil, R. Y., Kharya, M. D., *Nat. Prod. Res*. **(2007)**, 21, 1266, http://dx.doi.org/10.1080/14786410701757330.
10. Sirisha, N., Sreenivasulu, M., Sangeeta, K., Chetty, C. M., *Int. J. Pharm. Tech. Res.* **(2010)**, 3, 2174.
11. Mousa, O., Vuorela, P., Kiviranta, J., Wahab, S. A., Hiltohen, R., Vuorela, H., *J. Ethnopharmacol*. **(1994)**, 41, 71-76, http://dx.doi.org/10.1016/0378-8741(94)90060-4.

12. Riaz, N., Naveed, M. A., Saleem, M., Jabeen, B., Ashraf, M., Ejaz, S. A., Jabbar, A., Ahmed, I., *J. Asian Nat. Prod. Res*. **(2012)**, 14, 1149, http://dx.doi.org/10.1080/10286020.2012.733702.
13. Rodrigue, T. K., Kapche, G. D. W. F., Dzoyemc, J. P., Simod, I. K., Ambassaa, P., Sandjoa, L. P., Abegaze, B. M., Ngadjui, B. T., *Helv. Chim. Acta* **(2011)**, 94, 3321.
14. Matsunaga, S., Reiko Tanaka, R., Akagi, M., *Phytochemistry* **(1988)**, 27, 535, http://dx.doi.org/10.1016/0031-9422(88)83136-4.
15. Ito, K., Lai, J., *Chem. Pharm. Bull*. **(1979)**, 27, 2248, http://dx.doi.org/10.1248/cpb.27.2248.
16. Tanaka, R., Masuda, K., Matsunaga, S., *Phytochemistry* **(1993)**, 32, 472, http://dx.doi.org/10.1016/S0031-9422(00)95021-0.
17. Fotie, J., Bohle, D. S., Leimanis, M. L., Georges, E., Rukunga, G., Nkengfack, A. E., *J. Nat. Prod.* **(2006)**, 69, 62, http://dx.doi.org/10.1021/np050315y.
18. Prachayasittikul, S., Saraban, P., Cherdtrakulkiat, R., Ruchirawat, S., Prachayasittikul, V., *Lec. Exp. Clin. Sci. J*. **(2010)**, 9, 1.
19. Ahmed, Z., Ali, D., Malik, A., *Mag. Reson. Chem*. **(2006)**, 44, 717, http://dx.doi.org/10.1002/mrc.1803.
20. Rubinstein, I., Goad, L. J., Clague, A. D. H., Mulheirn, L. J., *Phytochemistry* **(1976)**, 15, 195, http://dx.doi.org/10.1016/S0031-9422(00)89083-4.
21. Hollaud, H. L., Diakow P. R. P., Taylor, G. J., *Can. J.Chem*. **(1978)**, 56, 3121, http://dx.doi.org/10.1139/v78-510.
22. Markham, K. R., "Techniques of flavonoid identification", Academic press, New York, USA, **(1982)**, 3, 36.
23. Olivares, E. M., Lwande, W., Monache, W. F. D., Bettolo, G. B. M., *Phytochemistry* **(1982)**, 21, 1763, http://dx.doi.org/10.1016/S0031-9422(82)85056-5.
24. Kannathasan, K., Senthilkumar, A., Venkatesalu, V., *Acta Tropica* **(2011)**, 120, 115, http://dx.doi.org/10.1016/j.actatropica.2011.07.001.
25. Camarda, L., Merlini, L., Nasini, G., *Phytochemistry* **(1976)**, 15, 537, http://dx.doi.org/10.1016/S0031-9422(00)88966-9.
26. Falodun, A., Ali, S., Quadir, I. M., Choudhary, M. I., *J. Med. Plants Res.* **(2008)**, 2, 365.
27. Dosumu, O. O., Idowu, P. A., Onocha, P. A., Ekundayo, O., *Exp. Clin. Sci. J*. **(2010)**, 9, 173.
28. Ageta, H., Ageta, T., *Chem. Pharm. Bull*. **(1984)**, 32, 369, http://dx.doi.org/10.1248/cpb.32.369.
29. Seebacher, W., Simic, N., Weis, R., Saf, R., O. Kunert, O., *Mag. Reson. Chem*. **(2003)**, 41, 636, http://dx.doi.org/10.1002/mrc.1214.
30. Mizushina, Y., Nakanishi, R., Kuriyama, I., Kamiya, K., Satake, T., Shimazaki, N., Koiwai, O., Uchiyama, Y., Yonezawa, Y., Takemura, M., Sakaguchi, K., Hiromi Yoshida, H., *J. Steroid Biochem. Mol. Biol*. **(2006)**, 99, 100, http://dx.doi.org/10.1016/j.jsbmb.2005.12.007.
31. Pierre, C., Roland, R., Tremblay, Dube, J. Y., *Clin. Chem*. **(1978)**, 24, 208.