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Triterpene Saponins from *Wisteria floribunda* "macrobotrys" and "rosea"

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Five oleanane-type saponins were isolated from two cultivars of *Wisteria floribunda* (Willd.) DC. (Fabaceae): From the roots of *Wisteria floribunda* "macrobotrys", one new oleanane derivative elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid, and two known glycosides, and from the roots of *Wisteria floribunda* "rosea", two known ones. Their structures were elucidated by a detailed 600 MHz NMR analysis including 1D and 2D NMR (^{1}H, ^{13}C, COSY, TOCSY, ROESY, HSQC, HMBC) experiments and mass spectrometry. Chemotaxonomic conclusions were proposed.

Keywords: 2D NMR, Oleanane-type glycosides, *Wisteria floribunda "*macrobotrys", *Wisteria floribunda "*rosea", Fabaceae.

Wisteria Nutt. (also spelled *Wistaria* Spreng. [1]) is a genus of woody vines of the Fabaceae family, very appreciated in horticulture for a garden use. From a phytochemical point of view, triterpene saponins were previously isolated from different species as *W. brachybotrys* Sieb. & Zucc., and *W. frutescens* (L.) Poir. [2-6]. As part as our phytochemical study of the *Wisteria* genus, two cultivars of *W. floribunda* (Willd.) DC., "rosea" and "macrobotrys", were studied. *W. floribunda,* also called the "Japanese *Wisteria*", occurs in Japan and Korea [7,8]. Ten flavonoids were previously isolated and identified from the flowers [9]. The cultivar "rosea" differs mainly by its pink flowers contrary to "macrobotrys" with huge purple and white flowers grapes, which can measure until one meter. In this paper, we report about the isolation and characterization of five saponins from these horticultural species. Their structures were elucidated by a detailed 600 MHz NMR analysis including 1D and 2D NMR (^{1}H, ^{13}C, COSY, TOCSY, ROESY, HSQC, HMBC) experiments and mass spectrometry. From the roots of *W. floribunda* "macrobotrys", a previously undescribed saponin was isolated and its structure was elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid (**1**), together with two known ones previously isolated from *W. frutescens,* 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid (**2**) and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22,28-*O*-diacetylolean-12-ene-3β,16β,22β,28-tetrol (**3**) [2]. From the roots of *W. floribunda "*rosea", two known saponins were identified as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-olean-12-ene-3β,22β,24-triol (**4**)

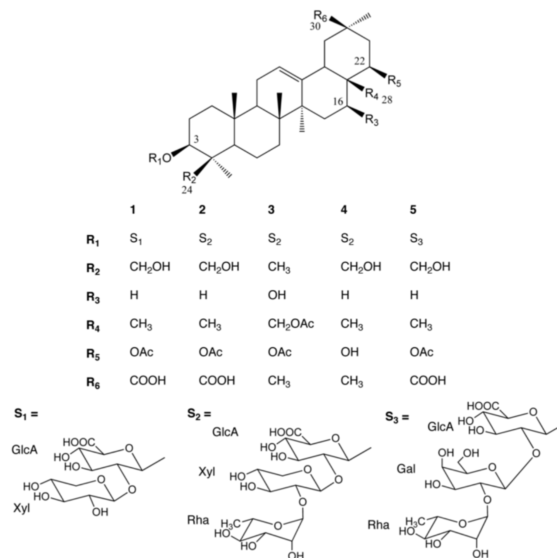

Figure 1: Structure of compounds **1-5**.

(Astragaloside VIII) [10], and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid (**5**) (Millettiasaponin A) [11].

Compound **1** was isolated from an aqueous-ethanolic extract of the roots of *W. floribunda* "macrobotrys" by various solid/liquid chromatographic methods: Vacuum Liquid Chromatography (VLC)

Table 1: ^{13}C NMR and ^{1}H NMR data of compound **1** in Pyridine-d_5 (δ in ppm).

Position	δ_C	δ_H (J in Hz)
Aglycon		
1	38.8	0.91 (m), 1.47 (m)
2	26.2	1.94 (m), 2.49 (m)
3	89.9	3.46
4	44.0	-
5	56.2	0.88 (br d, J = 10.5)
6	18.7	1.43 (m), 1.66 (m)
7	32.8	1.26 (m), 1.45 (m)
8	39.9	-
9	47.5	1.58 (d, J = 14.0, 6.8)
10	36.4	-
11	23.8	1.78 m, 1.82 m
12	123.3	5.46 (br t, J = 3.0)
13	144.0	-
14	41.7	-
15	26.1	1.78 (m), 1.90 (m)
16	25.9	0.98 (m), 1.76 (m)
17	36.0	-
18	44.0	2.82 (dd, J = 12.8, 3.4)
19	41.5	1.79 (dd), J = 13.5, 3.4), 2.22 (dd, J = 13.5, 12.8)
20	40.6	-
21	35.0	1.74 (dd, J = 13.6, 3.0), 2.69 (brd, J = 13.6)
22	77.8	4.75 (br s)
23	22.6	1.36 (s)
24	62.7	3.48 (d, J = 11.2), 4.25 (d, J = 11.2)
25	15.4	0.82 (s)
26	16.7	0.91 (s)
27	26.2	1.26 (s)
28	21.2	0.93 (s)
29	29.7	1.27 (s)
30	179.2	-
Acetyl at C-22		
CO	170.2	-
CH_3	21.1	2.07 (s)
GlcA-1	104.3	4.77 d (7.6)
2	81.1	4.00 dd (8.4, 7.6)
3	78.2	4.11 dd (8.4, 8.0)
4	73.4	3.97
5	75.0	3.98
6	174.7	-
Xyl-1	105.2	5.21 d (6.9)
2	75.4	3.84
3	78.0	3.83
4	70.4	4.02
5	66.8	3.47 (dd, J = 12.0, 10.0), 4.22 (dd, J = 12.0, 5.6)

Overlapped proton signals are reported without designated multiplicity.

on normal and reverse phase RP-18 silica gel, Medium Pressure Liquid Chromatography (MPLC), size exclusion Column Chromatography on Sephadex LH-20 (CC), and High Performance Liquid Chromatography (HPLC).

The HR-ESIMS (positive-ion mode) of **1** established its molecular formula as $C_{43}H_{66}O_{16}$ with a pseudo-molecular ion peak at *m/z* 861.4268 $[M + Na]^+$ (calcd 861.4249). Its ESIMS (negative-ion mode) displayed a quasi-molecular ion peak at *m/z* 837 $[M - H]^-$, indicating a molecular weight of 838.

The ^{1}H NMR spectrum of the aglycon part displayed signals assignable to six angular methyl groups as singlets at δ_H 0.82 (H_3-25), 0.91 (H_3-26), 0.93 (H_3-28), 1.26 (H_3-27), 1.27 (H_3-29), 1.36 (H_3-23), one olefinic proton at δ_H 5.46 (br t, J = 3,0 Hz) (H-12), two oxygen-bearing methine protons at δ_H 3.46 (H-3) and 4.75 (br s) (H-22), and one primary alcoholic function at δ_H 3.48 (d, J = 11.2 Hz), 4.25 (d, J = 11.2 Hz) (H_2-24) (Table 1). A signal in the ^{13}C NMR spectrum at δ_C 179.2 suggested a carbonyl function of a free carboxylic acid group. Another one at δ_C 170.2 suggested a

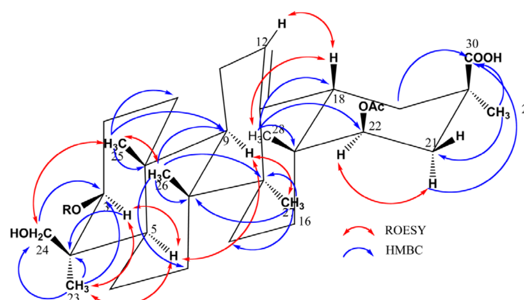

Figure 2: Key HMBC and ROESY correlations for the aglycon of **1**.

carbonyl function of an acetyl group, which was confirmed by a HMBC cross-peak at δ_H 2.07 (s)/δ_C 170.2, and a HSQC correlation at δ_H 2.07 (s)/δ_C 21.1. The HMBC correlation between an angular methyl group at δ_H 1.36 (s, H_3-23) and δ_C 89.9 (C-3), allowed the location of the first secondary alcoholic function at C-3.

The second one was located at C-22, by observation of a HMBC correlation at δ_H 0.93 (s, H_3-28)/δ_C 77.8 (C-22). The deshielded chemical shift of H-22 at δ_H 4.75 suggested an acetylation at the C-22 position. In the HMBC spectrum, a cross-peak between δ_H 1.36 (H_3-23) and δ_C 62.7 (C-24) allowed the location of the primary alcoholic function at C-24 position. More correlations were observed between δ_H 1.27 (s, H_3-29), 1.74 (dd, J = 13.6, 3.0, H-21), and 1.79 (dd, J = 13.5, 3.4, H-19), and δ_C 179.2, to find the location of the free carboxylic group at the C-30 position. The lack of ROESY correlations between H_3-30β_{ax} and H-18β_{ax} and H_3-28β_{eq} suggested the location of the carboxylic acid group at the C-30 position. The configuration of C-3 was determined by the interactions observed in the ROESY spectrum between H-3 α-axial and H_3-23 α-equatorial. Moreover, the multiplicity of the H-22 at δ_H 4.75 as a (br s), confirmed the α-equatorial orientation of the H-22. The structure of the aglycon of **1** was thus recognized to be the 22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid, previously described in *W. frutescens* [2], *W. brachybotrys* [5], and *Millettia speciosa* Champ. ex Benth. [11] (Table 1, Figures 1 and 2).

In the osidic region, the HSQC spectrum of **1** displayed two anomeric signals at δ_H 4.77 (d, J = 7.6 Hz)/δ_C 104.3 and δ_H 5.21 (d, J = 6.9 Hz)/δ_C 105.2. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the ^{1}H-^{1}H COSY, TOCSY, HSQC, and HMBC experiments. The monosaccharides obtained by acid hydrolysis of **1** were identified by comparison on TLC with authentic samples as glucuronic acid (GlcA) and xylose (Xyl). The absolute configurations of the sugars were determined to be D for GlcA and Xyl by GC analysis according to a method previously described [12]. The relatively large $^3J_{H\text{-}1,H\text{-}2}$ value of the GlcA and Xyl (7.6, 6.9 Hz) in their pyranose form indicated a β anomeric orientation for GlcA and Xyl. Units of one β-D-glucuronopyranosyl and one β-D-xylopyranosyl were thus identified (Table 1). The 3-*O*-heterosidic linkage was suggested by a HMBC cross-peak δ_H 4.77 (d, J = 7.6 Hz, GlcA-1)/δ_C 89.9 (C-3), and a ROESY cross-peak at δ_H 4.77 (GlcA-1)/δ_H 3.46 (H-3). The HMBC correlations at δ_H 5.21 (d, J = 6.9 Hz, Xyl-1)/δ_C 81.1 (GlcA-2) and the ROESY correlation at δ_H 5.21 (Xyl-1)/δ_H 4.00 (dd, J = 8.4, 7.6, GlcA-2) proved a β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl sequence (Table 1, Figure 1).

On the basis of the above results, the structure of **1** was elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid (**1**).

A literature survey of other *Wisteria* species [2-6, 13,14] and other genera of the Fabaceae [15] such as *Astragalus* L. [16-18], *Glycine* Willd. [19] or *Medicago* L. [20] for example, showed that monodesmosidic oleanane-type saponins with glucuronic acid linked at the C-3 position are very common to this family. More specifically, the sequence 3-*O*-β-D-glucuronopyranosyl-22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid is found in saponins isolated from *Wisteria* [2,5], *Derris* Wall. [21], and *Millettia* Wight & Arn. [11] genera, which belong to the subfamily Faboideae. This may represent a chemotaxonomic marker of this subfamily.

Experimental

General experimental procedures: Optical rotation values were recorded on a AA-10R automatic polarimeter (Optical Activity LTD). The 1D and 2D spectra (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, TOCSY, ROESY, HSQC and HMBC) were performed using a Varian VNMR-S 600 MHz spectrometer equipped with 3 mm triple resonance inverse and 3 mm dual broadband probeheads. Spectra are recorded in pyridine-d_5. Solvent signals were used as internal standard (pyridine-d_5: δ_H = 7.21, δ_C = 123.5 ppm), and all spectra were recorded at T = 35°C. The carbon type (CH_3, CH_2, CH) was determined by DEPT and coupling constants (*J*) were measured in Hz. HRESIMS (positive-ion mode) were carried out on a Bruker micrOTOF spectrometer and ESIMS (negative-ion mode) on a Finnigan LCQ Deca. A R.E.U.S. ultrasonic apparatus was used for the extraction (US frequency 24 KHz, Power 200 W). Compound isolations were carried out using column chromatography (CC) on Sephadex LH-20 (550 mm x 20 mm, GE Healthcare Bio-Sciences AB), and vacuum liquid chromatography (VLC) on reversed-phase RP-18 silica gel (75-200 µm, Silicycle) and silica gel 60 (Merck, 60-200 µm). Medium-pressure liquid chromatography (MPLC) was performed on silica gel 60 (15-40 µm, Merck) with a Gilson M 305 pump (25 SC head pump, M 805 manometric module), a Büchi glass column (460 mm x 25 mm and 460 mm x 15 mm), and a Büchi precolumn (110 mm x 15 mm). MPLC was performed on reversed-phase RP-18 silica gel (75-200 µm, Silicycle) with a Gilson Pump Manager C-605, having two pumps (2x Büchi pump modul C-601). HPLC was performed on a 1260 Agilent instrument, equipped with a degasser, a quaternary pump, an autosampler, and an UV detector at 210 nm. Chromatographic separation for analytical part was carried out on a C18 column (250 mm × 4.6 mm id, 5 µm; Phenomenex LUNA) at room temperature and protected by a guard column. The mobile phase constituted of (A) 0.01% (*v*/*v*) aqueous trifluoroacetic acid and (B) acetonitrile delivered at 1 ml/min according to the gradient 1: 0-20 min 20% B - 55% B, and the gradient 2: 0-20 min 20% B - 80% B. The injection volume was 10 µl at the concentration of 1 mg/ml. Semi preparative part: Chromatographic separation was carried out on a C-18 column (250 mm x 10 mm id, 5 µm; Phenomenex LUNA) at room temperature and protected by a guard column. The gradient was the same at 3 ml/min. The injection volume was 0.3 ml at the concentration of 10 mg/ml. Thin-layer chromatography (TLC, Silicycle) and high-performance thin-layer chromatography (HPTLC, Merck) were carried out on precoated silica gel plates $60F_{254}$, solvent system $CHCl_3/MeOH/H_2O$ (60:32:7, 60:35:8 and 60:35:10 lower phase). The spray reagent for saponins was vanillin reagent (1% vanillin in $EtOH/H_2SO_4$, 50:1).

Plant material: The two plants were provided in 2015 from Botanic® (Quétigny, France). Voucher specimens, N°20151001 for *W. floribunda* "macrobotrys" and N°20151002 for *W. floribunda* "rosea", were deposited in the herbarium of the Laboratory of Pharmacognosy, Université de Bourgogne Franche-Comté, Dijon, France.

Extraction and isolation of saponins from *W. floribunda* "macrobotrys": The dried powdered roots (122 g) were extracted by an ultrasonic method three times with EtOH-H_2O (75/35, 3 x 1 L) for 1 h. After evaporation of the solvent under vacuum, the resulting extract (23.4 g) was submitted to VLC (RP-18 silica gel, H_2O, MeOH/H_2O 50:50 and MeOH). The fraction eluted with 50:50 MeOH/H_2O (3g) was fractionated by CC (Sephadex LH-20, MeOH) to give four fractions (F1-F4). F1 (810 mg), rich in saponins, was submitted to a VLC on silica gel 60 ($CHCl_3/MeOH/H_2O$ 70:30:5; 60:32:7; 64:40:8). Ten fractions (f1-f10) were obtained. Fraction f4 (139.3mg) was submitted to a MPLC (silicagel 60, $CHCl_3/MeOH/H_2O$ 70:30:5), yielding seven fractions. One of these (61mg) was submitted to successive semi-preparative HPLC (H_2O/ACN, gradient 1, 20 min) to obtain saponin **2** (12.2 mg). Remaining fractions were collected together according to their TLC profile, and were submitted to MPLC (silicagel 60, $CHCl_3/MeOH/H_2O$ 65:35:10, lower phase) to give saponin **1** (9.5mg), and successive HPLC (H_2O/ACN, gradient 2, 20 min) to give saponin **3** (10.0 mg).

Extraction and isolation of saponins from *W. floribunda* "rosea": The dried powdered roots (79 g) were extracted by an ultrasonic method three times with EtOH-H_2O (75/35, 3 x 1 L) for 1 h. After evaporation of the solvent under vacuum, the resulting extract (4.1 g) was submitted to a VLC on silica gel 60 ($CHCl_3/MeOH/H_2O$ 65:35:10, lower phase) to obtained sixteen fractions (F1-F16). F3 and F8 were submitted to MPLC (silicagel 60, $CHCl_3/MeOH/H_2O$ 65:35:10, lower phase) to give several fractions. Remaining fractions were collected together according to their TLC profile, and were submitted to successive MPLC on normal phase silicagel ($CHCl_3/MeOH/H_2O$ 65:35:10, lower phase) and reversed-phase RP-18 silicagel (MeOH/H_2O 30:70 to 45:55) to give saponins **4** (7.5 mg) and **5** (8.7mg).

Acid hydrolysis and GC analysis: Each compound (3 mg) was hydrolyzed with 2N aq. CF_3COOH (5 mL) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 x 5 mL), the aq. layer was repeatedly evaporated to dryness with MeOH until neutral, and then analyzed by TLC over silica gel ($CHCl_3/MeOH/H_2O$ 8:5:1) by comparison with authentic samples. Furthermore, the residue of sugars was dissolved in anhydrous pyridine (100 µL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60 °C for 1 h, then 150 µL of HMDS-TMCS (hexamethyldisilazane/trimethylchlorosilane 3:1) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under a N_2 stream. The residue was partitioned between *n*-hexane and H_2O (0.1 mL each), and the hexane layer (1 µL) was analyzed by GC [12]. The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich).

3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-acetyl-3β,22β,24-trihydroxyolean-12-en-30-oic acid (1)
White amorphous powder.
$[\alpha]_D^{25}$: – 28.0 (*c* 0.60, MeOH)
^{1}H and ^{13}C NMR (600 and 150 MHz, pyridine-d_5): Table 1
HR-ESIMS (positive-ion mode): *m/z* 861.4268 $[M + Na]^+$ (calcd $C_{43}H_{66}NaO_{16}$ 861.4249); ESIMS (negative-ion mode) *m/z* 837 $[M - H]^-$.

References

[1] http://www.tropicos.org/Name/40026522

[2] Champy A-S, Mitaine-Offer A-C, Miyamoto T, Tanaka C, Papini A-M, Lacaille-Dubois M-A. (**2017**) Structural analysis of oleanane-type saponins from the roots of *Wisteria frutescens*. *Magnetic Resonance in Chemistry*, ***55***, 595-600.

[3] Konoshima T, Kozuka M, Haruna M, Ito K, Kimura T. (**1989**) Studies on the constituents of Leguminous plants. XI. The structure of new triterpenoids from *Wistaria brachybotrys* SIEB. *et* ZUCC. *Chemical and Pharmaceutical Bulletin*, ***37***, 1550-1553.

[4] Konoshima T, Kozuka M, Haruna M, Ito K, Kimura T, Tokuda H. (**1989**) Studies on the constituents of Leguminous plants. XII. The structure of new triterpenoids from *Wistaria brachybotrys* SIEB. *et* ZUCC. *Chemical and Pharmaceutical Bulletin*, ***37***, 2731-2735.

[5] Konoshima T, Kozuka M, Haruna M, Ito K. (**1991**) Constituents of Leguminous plants. XIII. New triterpenoid saponins from *Wistaria brachybotrys*. *Journal of Natural Products*, ***54***, 830-836.

[6] Kinjo J, Fujishima Y, Saino K, Tian R-H, Nohara T. (**1995**) Five new triterpene glycosides from *Wisteria brachybotrys* (Leguminosae). *Chemical and Pharmaceutical Bulletin*, ***43***, 636-640.

[7] Jiang Y, Chen X, Lin H, Wang F, Chen F. (**2011**) Floral scent in *Wisteria*: chemical composition, emission pattern, and regulation. *Journal of the American Society for Horticultural Science*, ***136***, 307-314.

[8] Li J, Jiang J-H, Fu C-X, Tang S-Q. (**2014**) Molecular systematic and biogeography of *Wisteria* inferred from nucleotides sequences of nuclear and plastid genes. *Journal of Systematics and Evolution*, ***52***, 40-50.

[9] Ono M, Iwashina T. (**2015**) Quantitative flavonoid variation accompanied by change of flower colors in *Edgeworthia chrysantha, Pittosporum tobira* and *Wisteria floribunda*. *Natural Product communications*, ***10***, 413-416.

[10] Benchadi W, Haba H, Lavaud C, Harakat D, Benkhaled M. (**2013**) Secondary metabolites of *Astragalus cruciatus* Link. and their chemotaxonomic significance. *Records of Natural Products*, ***7***, 105-113.

[11] Uchiyama T, Furukawa M, Isobe S, Makino M, Akiyama T, Koyama T, Fujimoto Y. (**2003**) New oleanane-type triterpene saponins from *Millettia speciosa*. *Heterocycles*, ***60***, 655-661.

[12] Hara S, Okabe H, Mihashi K. (**1987**) Gas-liquid chromatographic separation of aldose enantiomers as trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates. *Chemical and Pharmaceutical Bulletin*, ***35***, 501-506.

[13] Tai B H, Trung T N, Nhiem N X, Ha D T, Phuong T T, Thu N B, Luong H V, Bae K H, Kim Y H. (**2011**) Chemical components from the fruit peels of *Wisteria floribunda* and their effects on rat aortic vascular smooth muscle cells. *Bulletin of the Korean Chemical Society*, ***32***, 2079-2082.

[14] Mohamed M A, Hamed M M, Abdou A M, Ahmed W S, Saad A M. (**2011**) Antioxidant and cytotoxic constituents from *Wisteria sinensis*. *Molecules*, ***16***, 4020-4030.

[15] Birjatinder S, Joga S, Singh MV. (**2014**) Ethno medicinal, pharmacological properties and chemistry of Fabaceae family. *Journal of Medical Pharmaceutical and Allied Sciences*, ***02***, 24-28.

[16] Kinjo J, Udayama M, Okawa M, Nohara T. (**1999**) Study of structure-hepatoprotective relationships of oleanene-type triterpenoidal glucuronides obtained from several fabaceous plants on rat primary hepatocyte cultures. *Biological and Pharmaceutical Bulletin*, ***22***, 203-206.

[17] Abbas F, Zayed R. (**2005**) Bioactive saponins from *Astragalus suberi* L. growing in Yemen. *Zeitschrift für Naturforschung*, ***60c***, 813-820.

[18] Ionkova I, Shkondrov A, Krasteva I, Ionkov T. (**2014**) Recent progress in phytochemistry, pharmacology and biotechnology of *Astragalus* saponins. *Phytochemistry Reviews*, ***13***, 343-374.

[19] Tantry M A, Khan I A. (**2013**) Saponins from *Glycine max* Merrill (soybean). *Fitoterapia*, ***87***, 49-56.

[20] Tava A, Scotti C, Avato P. (**2011**) Biosynthesis of saponins in the genus *Medicago*. *Phytochemistry Reviews*, ***10***, 459-469.

[21] Zhang H-X, Sun G, Gu J-L, Du Z-Z. (**2017**) New sweet-tasting oleanane-type triterpenoid saponins from “Tugancao” (*Derris eriocarpa* How). *Journal of Agricultural and Food Chemistry*, ***65***, 2357-2363.