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Isolation and structure elucidation of some of secondary metabolites from *Arbutus pavarii* Pampan growing in east of Libya

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Abstract
Fourteen compounds belong to triterpenoids, flavonoids and phenolic acids classes including Lupeol (1), *β*-amyrin acetate (2), catetchin (3), salicylic acid (4), methyl gallate (5), and gallic acid (6), as well as kaempferole (7), dioctyl phthalate (8), ferulic acid (9), isoquercetin (10), arbutin (11), Quercetin 3-O-β-D-galactopyranoside (12), Kampferol- O- *β*- D- rutinoside (13) and Chlorogenic acid (14) were isolated from the aerial parts of the *Arbutus pavarii* Pampan. using gradient solvent fractionation. The identification of their structures were carried out using spectral methods (UV, 1D-NMR, IR, and ESI-MS).

Keywords: Triterpenoids, flavonoids, organic acids, plant sterols, Libya, *Arbutus pavarii* Pampan

Introduction
Arbutus is a genus of small trees or shrubs with red flaking bark and edible red berries that belongs to the *Ericaceae* which known as the heath family that comprises the flowering plants found commonly in acid and infertile growing conditions. It includes around 4000 different species across 126 various genera, making it one of the most speciose family [1]. The list involves approximately 122 of scientific names of plant species rank for the genus of *Arbutus*. Twelve of the species documented are growing in America. Whereas four species and two hybrids belongs to the Mediterranean region; *Arbutus unedo* and *A. andrachne* (eastern Mediterranean), *Arbutus pavarii* Pampan (coasts of Libya), *Arbutus canariensis* (Canary Islands) [2].
A. pavarii is a Mediterranean perennial shrub widespread in Gebal *Al-Akhdar* ridge in Libya (The Green Mountain) [3,4]. *Arbutus pavarii* Pampan is employed in the production of honey, as a food supplement, and in medicine for the treatment of gastritis, renal infections, as well as an anti-cancer agent [5]. Furthermore, Antihyperglycemic, antihyperlipidmic, cytotoxic and antimicrobial activities have been previously reported [5-8]. Some existing researches described the presence of α-amyrin, lupeol, arbutin, catechin, isoquercitrin, oleanolic acid, myricetin, ferulic and gallic acid as the major compounds in the leaves of the tree [8-9]. The chief identified flavonoids are naringin, kaempferol, and catechin followed by neodiosmin, isovitexin-7-O-glucoside, naringenin-7-O-glucoside, rutin, quercetin, and dihydroquercetin, one anthocyanin; delphinidin-3-O-rutinoside, and five phenolic acids such as gallic, rosmarinic, caffeic, chlorogenic and salicylic acids as well as one carboxylic acid (quinic acid), were also identified [9]. By this time, this report is the first about the isolation of some of these compounds. The purpose of this work is to isolate and characterize the secondary metabolites of *Arbutus pavarii* Pampan.

Materials and Methods
Plant material
Sample of *Arbutus pavarii* Pampan was collected during springtime from *El-Jabal Al Akhdar* area in the east of Libya. The identification and verification of the plant samples was carried in the herbarium of Faculty of Science, Botany department, Benghazi University, Benghazi, Libya.

General experimental procedures
M-Quant Biomolecular spectrophotometer (BioTek Instruments) was used to record the UV (MeOH) spectra. The ^{1}H- and ^{13}C- nuclear magnetic resonance (NMR) spectra were obtained and interpreted from a Varian Mercury plus 100 MHz for the carbon and 400 MHz for the

proton. Chemical shifts were specified in ppm using Me4Si (TMS) as internal standard. MeOH-d4, CDCl3 and DMSO-d6, were used for the nuclear magnetic resonance analyses. A Perkin Elmer FT-IR Spectrum Bx was employed to record the IR spectra. ESI-MS analyses were conducted on a spectrophotometer (Waters 2695 Aliance Micromass ZQ). silica gel 60 (0.063-0.200 mm, Fluka) and Sephadex LH-20 (Pharmacia Fine Chemicals AB Uppsala, Sweden) were applied to conduct chromatographic separations using open column chromatography (CC). Analyses with the thin-layer chromatography were performed on a pre-coated Kieselgel 60 F254 sheets of aluminium. Compounds were detected by ultra-violet fluorescence then sprayed with 1% vanillin-H2SO4 reagent, followed by heating at 105°C for one to two minutes.

Solvent Systems

Identification and confirmation of purity of the fractions and the isolated compounds were done through TLC using a sequence of solvent systems; namely S1: n-Hexane-Ethyl acetate (7:3 v/v), S2: Chloroform-Methanol-Formic acid (95:5:0.2 v/v), S3: Chloroform-Methanol-Formic acid (90:10:0.2 v/v). S4: Chloroform-Methanol-Formic acid (80:20:0.2 v/v), S5: Ethyl acetate – Methanol–H2O- Formic acid (100:16.5:13.5:0.2 v/v) and S6: H2O-Acetic acid (85: 15)**.**

The process of extraction and isolation

Samples of the fresh aerial parts of *Arbutus pavarii Pampan* (1,5 Kg) was chipped into small pieces, grinded then macerated in boiling methanol 90% for 30min (to inactivate enzymes). Extraction of the plant materials was accomplished by the maceration in 70% methanol until full exhaustion. The collected methanolic extract was evaporated under reduced pressure at temperature not exceeding 50 ^{0}C to yield 150g. The residues of each one were immersed in 100ml of distilled H2O and fractionated sequentially with methylene chloride and n-butanol. The solvents were then evaporated under a temperature not exceeding 40^{o}C and reduced pressure. The residues were then weighed and kept to be used in the phytochemical investigation.

The methylene chloride (15gm) soluble fraction was fractionated on VLC, silica gel H (150g, 12cm L× 5cm D) and CC silica gel (5x 55) respectively. A procedure of gradient elution was conducted using n-hexane, n-hexane-methylene chloride, methylene chloride and methylene chloride methanol. Fractions (100ml, each) were collected and monitored on TLC precoated silica gel F_{254} plates using S_1, S_2, S_3 and S_4 as the solvent system for development. The visualization of the spots was carried out by examination under ultra-violet light (365nm) perior and after the exposure to vapors ammonia, as well as, by spraying with variable spraying reagents.

Fractions showing similar chromatographic behavior were pooled together to yield two main collective fractions (I-II).

Fr. I (450 mg) which gives 4 major spots on the TLC with R_f (0.76, 0.65,0.50, 0.42 in S_1) was subjected to silica gel column eluting with CH2Cl2-MeOH (95:5 to 90:10) to yield two subfractions (180 and 120mg respectively). The first fraction was subjected to Silica gel subcolumn and CH2Cl2-EtOAc (70-30) as eluting system to give compound 1 (R_f 0.77 S1 in pure form, 25mg), while the second fraction was exposed to silica gel column eluting with CH2Cl2-EtOAc (1:1) and purified by CC. silica gel, (2x50cm), gradient elution, CH2Cl2-Ethylacetate-CH3OH to yield compound 2 (5mg).

Fr. II (200 mg) which gives 4 spots on the TLC with R_f (0.77, 0.71, 0.44, 0.37 in S4) was subjected to silica gel column with gradient eluting CH2Cl2-MeOH (95:5 to 80:20) compounds 3 and 4 (R_f 0.37 and 0.44 in S1 in pure form 30 mg and 25mg respectively).

The n-butanol fraction (70gm) was fractionated on VLC, silica gel H (150 g, 12cm L× 5cm D). Elution was started with CH_2Cl_2 followed by increasing polarity through 5% additions of EtOAc until 100% then followed by CH_3OH in the same manner. Fractions (100ml, each) were collected and monitored on TLC using S_5 and S_6 as the solvent systems. The visualization of spots was achieved by observation under UV light (254 and 365nm) perior and after the exposure to vapor of ammonia, along with the spraying with aluminum chloride, ferric chloride and NP/PEG. According to their chromatographic pattern, the matching fractions were pooled with each other and joint to give five major fractions (IV-VIII).

Fr IV (10g) was exposed to silica gel column chromatography and eluted through different proportions of CH_2Cl_2: MeOH (90:10→ 80:20). Two fractions were obtained from this separation; the first fraction gave pure compound 5 (R_f 0.85 S5, 50mg), while the other fraction was put through Sephadex LH-20 CC. MeOH was used as an eluting solvent to produce the compound 6 (R_f 0.80 S5, 25 mg).

Fr V (15 g) was subjected to silica gel CC and eluted with different proportions of CH_2Cl_2: MeOH:H2O (90:10:1→ 80:20:2). Two fractions were obtained from this separation and the first fraction was subjected to Sephadex LH-20 CC and MeOH used as eluting solvent to produce two subfractions, the first portion containing one pure spot on the TLC (R_f 0.76 S5) represent compound 7 (30mg) while the second containing two major spots and subjected to subfractionation by using silica gel CC and eluted with different proportions of CH_2Cl_2: MeOH to yield compound 8 (R_f 0.65 S5,30mg).

Fr V (10gm) containing three major spots on TLC **(**R_f 0.76, 0.70 and 0.62 on S5) was subjected to Sephadex LH-20 CC and MeOH as eluting solvent to produce two pure compounds 9 and 10 (45mg and 30mg respectively).

Fr VI (15gm) was fractionated using silica gel CC and CHCl3: MeOH (1:1) was used for the elution. Three spots were identified on the TLC represent three major compounds in the fraction (R_f 0.54, 0.48 and 0.44 on S5), the fraction was subjected to Sephadex LH-20 sub column and MeoH:H_2O (5:5) as eluent to produce three pure compounds 11,12 and 13 (60mg, 30mg and 15mg separately).

Fr VII (3gm) which containing one major and two minor spots on TLC by using S6(R_f 0.40, 0.39 and 0.34) was subjcected to Sephadex LH-20 CC. and MeOH used as eluting solvent to produce one pure compound 14 (R_f, 0.34 and 30mg).

Results and Discussion

The following compounds in the aerial parts of *Arbutus pavarii* pampan were isolated from methylene chloride and n-butanol fractions through an open column chromatograph on silica gel and Sephadex LH-20 with the aid of thin-layer chromatography. The *n*-butanol fraction showed the higher yield of extraction than methylene chloride fraction (70gm and 15gm respectively). Four compounds were isolated from methylene chloride comprising lupeol (1), *β*-amyrin acetate **(**2), catetchin (3) and salicylic acid (4). On the other hand, ten

compounds were isolated from the n-butanol fraction represent methyl gallate (5), gallic acid (6), kaempferol (7), dioctyl phthalate (8), ferulic acid (9), isoquercetin (10), arbutin (11), hyperoside (12), Kaempferol- O- *β*- D-rutinoside (13) and chlorogenic acid (14).

Lupeol (1): white needles crystals, soluble in *n*-hexane and chloroform, positive test for sterols and triterpenes [10], mp 218, dark violet color with ρ-anisaldehyde / H_2SO_4. The ^{1}HNMR spectrum (400 MHz, in $CDCl_3$), 4.61 (1H, s, C-$_{29}H_b$), 4.49 (1H, s, C-$_{29}H_a$), 3.11 (1H, d, J=4Hz, 3.68Hz, C_3-H-axial),1.85 (3H, C_{21}-H), 2.29 (1H, m, C_{19}-H), 1.61 (3H, s, vinylic methyl, C_{30}-CH_3), 1.49 (3H, s, C_{25}-CH_3) 1.18 (3H, s, C_{28}-CH_3), 1.06 (3H, s, C_{23}-CH_3), 0.96 (3H, s, C_{24}-CH_3), 0.89(3H, s, C_{26}-CH_3), 0.80 (3H, s, C_{27}-CH_3), exhibited four tertiary methyl signals of the lupane skeleton at 0.80, 0.89, 0.95, 1.05, as singlets. The C-30 vinylic methyl appeared at δ1.61 ppm (s), α-proton at C-3 appeared at δ 3.11 ppm dd (J=3, 6 Hz). The C-29 olefinic protons H_a, H_b appeared at δ 4.61 and 4.49 pm as broad singlets. Based on interpretation of its spectral data, and by comparison with those published data [10, 11], the compound 1 could be identified as lupeol. According to the literature, lupeol was previously isolated from *Arbutus pavarii* Pampan [9], although being identified in *Arbutus unedo* [12].

β-amyrin acetate (2): white needle crystals, soluble in *n*-hexane and chloroform. The steroidal and/or triterpenoidal nature of the compound was indicated by its response to chemical tests, and by its chromatographic behavior. The IR spectrum showed the presence of an ester carbonyl at 1735cm^{-1} (C=O) and C-H stretching at 2925, 2855cm^{-1}, beside the presence of a characteristic double bond of oleanane type triterpene at 1453cm^{-1}(C=C), 1247cm^{-1} (C-O) and bending at 878. The EI/MS spectrum presented a molecular ion peak [M$^+$] at 468m/z, calculated for the molecular formula $C_{32}H_{52}O_2$. In addition to the characteristic fragmentation peaks with base peak at m/z 218. Fragmentation pattern 218 displays the breakdown $C_{16}H_{25}$ [M+], 203 ([CH3M]+) [13]. The ion abundance at m/z 69, 189, 203, and 218 are matching to ß-amyrin acetate fragmentation [14]. The mass spectrum of the compound 2 is similar to that of ß-amyrin acetate. This compound was obtained in poor yield that hindered its complete identification. However, through allowed spectral data (IR, ESI-MS) and comparing with the authentic could be identified and attributed to be a β-amyrin acetate, which first isolated from *Arbutus* in general.

Catechin (3): was isolated as a white powder. The 1HNMR (400 MHz, DMSO) showed 6.77 (1H, d, J = 1.9 Hz, H-2′), 6.73 (1H, d, J = 8.1 Hz, H-6′), 6.63 (1H, d, J = 8.20 Hz, H-5′), 5.95(1H, d, J = 2.2 Hz, H-8), 5.74 (1H, d, J = 2.2 Hz, H-6), 4.52 (1H, d, J = 7.5 Hz, H-2), 3.84 (1H, m,H-3), 2.68 (1H, dd, J = 16.1, 5.4 Hz, H-4a), 2.55 (1H, dd, J = 20, 8.0 Hz, H-4b). The13CNMR data, showed 156.9 (C-7), 156.62 (C-5), 155.79(C-9), 145.65 (C-3′), 145.29 (C-4′), 131.04 (C-1′), 118.86 (C-6′), 115.52 (C-5′), 114.98 (C-2′), 99.49 (C-10), 95.56 (C-6), 94.28 (C-8), 81.45 (C-2), 66.76 (C-3), 28.32 (C-4). These results were consistent with the reported data for catechin which is previously isolated from *Arbutus pavarii* Pampan [9].

Salicylic acid (4): was isolated as colorless needle crystals. TLC investigations revealed a blue fluorescence in UV light which was intensified upon spraying with AlCl3 while a dark blue color with FeCl3 spray reagent indicating that the compound is a phenolic acid. The ^{1}HNMR 6.89 (dd,1H, *J*=7.48, 7.64 Hz, H-5), 6.94(1H, *d*, *J*=8.72 Hz, H-3), 7.47(1H, *td*, *J*=1.44, 8.6 and 8.36 Hz, H-4), 7.77 (1H, *dd*, *J*=1.24 and 8.2, H-6), 10.17 (br s, COOH). From the data above and by comparing with the previously published data by Khan *et al* (2013) [15] the compound 4 could be defined as: 2-hydroxybenzoic acid (salicylic acid) which is first isolated from Arbutus.

Methyl gallate (5), exhibited a molecular weight of 184 as indicated by EI-MS (70eV) m/z, [M$^+$] = 184, 153 (M$^+$ - OCH_3), 123 (M$^+$ - CO_2Me) corresponding to a molecular formula $C_8H_8O_5$. ^{1}HNMR (300 MHz- D_2O) spectrum showed 6.94 (2H, *s*, H-2, 6), 3.51(3H, *s*, O-CH_3). Based on the previous results, compound 5 could be identified as methyl gallate, it is a first time isolated from the plant.

Gallic acid (6) displayed a molecular weight of 170 indicated by HPLC/DAD/MS2 [M$^+$] = 170, 125 (M$^+$-H-CO_2), corresponding to a molecular formula of $C_7H_6O_5$. The UV spectral data of compound 6 showed one single band at 270 in MeOH. These data were found to be in complete accordance with data reported for gallic acid [16,17].

Kaempferol (7): yellow powder, soluble in methanol, m.p. (276-278 ºC). It exhibited a dark yellow color in UV light at 365nm, which was intensified upon exposure to ammonia vapor [18, 19]. The UV spectral data of the compound 7 confirmed its flavonol structure by the absorption of band I at 368nm in the methanol spectrum, UV λmax on NaOMe275,340 sh,447(free OH on ring A & B), UV λmax on $AlCl_3$/HCl: 275, 306 sh, 347, 419 (free OH at C-3, C-5 & no ortho OH at ring B)and on NaOAc/H_3BO_3 is 264, 297 sh, 377.The ^{1}HNMR spectrum showed 8.04 (2H, d, J=7.06Hz, H-2̀,6̀), 6.92 (2H, d, J=8.7Hz, H-3̀,5̀), 6.44 (1H, d, J=2Hz, H-8), 6.19 (1H, d, J=2Hz, H-6). From the previous data and by comparing with the data in the published literature [18], the structure of the compound number 7 was recognized to be 3, 5, 7, 4'-tetrahydroxyflavone, Kaempferol ($C_{15}H_{10}O_6$).

Dioctyl phthalate (8): obtained as yellowish thick oil showed IR absorption spectrum, 3000-2800cm^{-1} (C-H), 1800-1600 cm^{-1} (C-O), 1600-1580 cm^{-1} (aromatic ring) and 1300-1200 cm^{-1} (C-O). Molecular weight is 390 agreeing to C24H38O4 as a molecular formula. This was further supported by ^{1}HNMR spectra showed the signals for phthalate moiety: δ 7.70 (2H, dd, J=7.5,1.6 Hz, H-2, H-5), 7.67 (2H, dd, J = 7.5, 1.6 Hz, H-3, H-4), and 4.08 (2×2H,m, H-1, H-1'') for O-CH δ, the aliphatic chain was represented as methyl group at 0.84-0.88 ppm, CH2 at 1.37-1.47 ppm and C-H at 1.61-1.62ppm. The analysis of ^{13}CNMR spectral that revealed 12 signals for the carbon atoms present in the molecule including two carbonyl groups at δ 167.43 (2×C=O) and the quaternary carbon at 132.18 (C-1,2) showed phthalate moiety, 132 (C-3,6), 128.64 (C-4,5) for aromatic and[67.84 (C-1', 1''), 38.55 (C-2', 2''), 30.25 (C-3', 3''), 28.82 (-C4', 4''), 22.85 (C-7',7''), 22.85 (C-5', 5''), 14.30 (C-6', 6''), 11.22 (C-8', 8'')] for aliphatic chain. The physical and spectral data (UV, IR, Mass and NMR spectrum) of 8 in the present study were in good agreement with those recorded for dioctyl phthalate which isolated from very few plants viz. *Ferula rutabensis* [20]. In the other study, the author considered this compound as a new class of plant phytotoxins in *Mentha longifolia* [21], and can also be noted it has more anti-inflammatory properties than ß-sitosterol [22]. Dioctyl phthalate also showed antimicrobial activity [23]. This compound was isolated and characterized for the first time from the aerial part extract of *Arbutus pavarii* Pampan.

Ferulic acid 9: showed a blue fluorescence in UV light at λ 365nm and after spraying with NP-PEG indicating that it is a hydroxycinnamic acid derivative.

The UV spectral data of the compound in methanol indicated that it could be hydroxycinnamic acid derivative [24]. ^{1}H-NMR displayed 6.21(1H,d,J=9.44 Hz, H2ˈ),6.76(1H,d, J=8.68 Hz H6),6.94(1H, d,J=1.88 Hz, H3), 7.13(H, dd, J= 8.68 and 1.88 Hz H5), 7.68(1H,d, J=9.44 Hz, H1'), 3.92(s, OCH3). On the basis of the previous discussion and published data [25], the can be identified as Ferulic acid.

Isoquercitrin 10 exhibited a yellow color in visible light and when observed under UV light at 365nm turning to pale green on exposure to ammonia vapor and spraying with $AlCl_3$, indicating that the compound is a quercetin derivative with 3-OH substitution [18]. The UV spectral data of compound dispayed on NaOMe addition 272, 329 sh, 409 (free OH on ring A & B), on addition of NaOAc showed 269, 324 sh, 382 (free OH at C-7 & ring B). The ^{1}HNMR spectrum exhibited, Aglycone: 7.74 (1H, d, J=2.2Hz, H –2̀), 7.49 (1H, d, J=8.7Hz, H–6̀), 6.77 (1H, d, J=8.4 Hz, H-5̀), 6.31(1H, br s, H-8), 6.11 (1H, br s, H-6). Sugar: 5.04 (1H, d, J =7.76, H-1"), 3.2-3.8 (Sugar protons).The ^{13}CNMR spectrum displayed the characteristic 15-carbon resonances of quercetin-3-O-β-D-glycoside. The six resonances of the sugar moiety were typical to those of glucose. From the above spectral data and by comparison to published data [26,27], the compound 10 might be identified as: Quercetin-3-O-*β*-D-glucopyranoside (Isoquercitrin).

The UV spectral data of Arbutin 11 presented one single band at 270 in MeOH. ^{1}HNMR (400 MHz, MeOD) spectrum displayed the characteristic signal of phenolic para substituetions at 6.97with two protons integration assigned to H-2 and H-6 (dd, *J*=6.76,2.2 Hz) and 6.70 with two protons integration assigned to H-3 and H-5 (dd, *J* = 6.76, 2.2 Hz) In addition to the presence of one anomeric protons showed characteristic doublets at δ 4.74 indicated the presence of glucose linked through O-linkage, methyl protons appeared as two doublets H6ˈat 3.6947. The remaining sugar protons were observed in the range δ 3.3207-3.46. ^{13}CNMR spectrum (100 MHz, in MeOD) revealed the presence of signals at δ 102.23 (C-1ˈ) and 61.15 (C-6ˈ) suggesting the presence of a glucoside moiety, the signals of para substitutions at 118.01 (C-3, 5) and 115.26 (C-2,6) After comparing the resulting spectral data with the previous literature, the compound was confirmed to be arbutin [28-30].

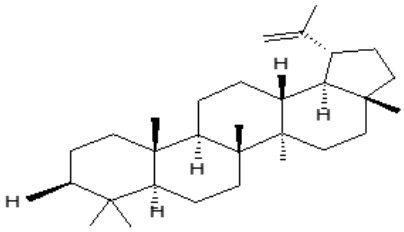
Hyperoside 12: UV spectral data in MeOH and in the presence of shift reagents suggested to be a flavonol structure type (band I and II at 356 and 257 nm, respectively) [18,19].^{1}H-NMR (400 MHz, CD3OD),displayed a spectrum typical to that of 3-OH substituted quercetin, which showed 7.52 (1H, dd, J=1.92- 8.04 MHz, H-6ˈ), 7.66 (1H, d, J=2.08 Hz, H-2ˈ), 6.85(1H, d, J=7.96 MHz, H-5ˈ), 6.39 (1H, d, J=1.84 MHz, H-6), 6.19 (1H, d, J=1.92 MHz, H-8), 5.35 (1H, d, J=7.72 MHz, H-1ˈˈ anomeric sugar proton), 3.23-3.65 (sugar protons). ^{13}CNMR Spectral data (400 MHz, DMSO) showed 157.46 (C-2), 134.38 (C-3), 178.15 (C-4), 164.67 (C-5), 98.53 (C-6), 164.67 (C-7), 93.35 (C-8), 161.57 (C-9), 104.33(C-10), 121.49 (C-1ˈ), 114.67 (C-2ˈ), 145.32 (C-3ˈ), 149 (C-4ˈ), 115.65 (C-5ˈ), 124.45 (C-6ˈ),102.90 (C-1ˈˈ),73.64(C-3ˈˈ),68.61(C-4ˈˈ),76.27(C-5ˈˈ), 60.57(C-6ˈˈ). The sugar moiety signals were matching the published data of galactose. Identification was confirmed with anomeric carbon at δ102.90ppm and by comparing with published data [18]. Reviewing the available literature, Quercetin 3-O-β-D-galactopyranoside (hyperoside) was earlier isolated from genus *Arbutus* [26, 27].

UV spectral data of the compound 13 in MeOH and in the presence of shift reagents are in accordance with 3, 7-disubstituted flavonol [18,30]. Band I and II appeared at 352 and 265 nm, respectively. with NaOMe, 278, 340 sh, 445 (free OH on ring A & B), with NaOAc 273, 359, 387 (OH at C-7), $AlCl_3$ / HCl: 270, 301 sh, 346, 419 (free OH at C-3, C-5 & no *ortho* OH at ring B). The 1HNMR spectrum of compound 14 showed 7.98 (2H, d, J=7.2, H-2′/6′), 6.77(2H, d, J=8, H-3′/5′), 6.32 (1H, d, J=1.8 MHz, H-8), 6.12 (1H, d, J=1.8 MHz, H-6), 5.27 (1H, d, J=1.8 MHz, H-1″), 4.32 (1H, s, H-1‴), 3.26-4.18 (m, remaining sugar protons), 0.9 (3H, d, J=6.16 MHz CH3-6‴). These data indicated a rutnosyl moiety [32, 33]. From the above chromatographic, spectroscopic results, compound 13 could be identified as Kaempferol-3- O- β- D-rutinoside.

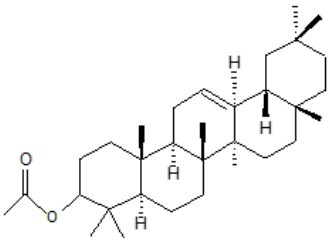
Chlorogenic acid (14) appeared blue in U.V light at 365nm, the fluorescence turns into yellow on exposure to ammonia vapor or spraying with AlCl3 indicating the presence of phenolic acid. The UV spectral data of compound 15 in methanol had a UV absorbance maximum at 324 nm and a shoulder at 240nm, indicating a phenolic acid. The 1HNMR spectrum the interpretation of the characteristic signals revealed the presence of caffeic acid moiety by two trans-olefinic protons at δ 6.14and 7.48ppm with large coupling constant 15.92 Hz, assigned to H8ˈ and H7ˈ, respectively. In addition, an ABX system represented by H2ˈ, H6ˈ and H5ˈ, appeared as a doublet (J=1.88 Hz) at 6.95ppm, a doublet (J=8.4 Hz) at 6.84 and doublet doublet (J=1.84 and 8.2) at δ 6.66 respectively. On other hand, protons of quinic acid moiety could be observed as follows, mulitplet at δ 2.55 ppm integrated as two protons and assigned to H-2 ax and H-2 eq as well as another multiplet at 1.93ppm were assigned to H-6ax and H-6 eq besides, two multiplets at δ 4.05and 3.59 ppm were assigned for H-4 and H-5. The downfield shift of H-3, appearing at 5.23 ppm indicated acylation of C-3 hydroxyl group of quinic acid by the caffeoyl moiety, compound 14 could be identified as 3-caffeolquinic acid (chlorogenic acid). It is previously isolated from the genus Arbutus [26, 27].

Conclusion

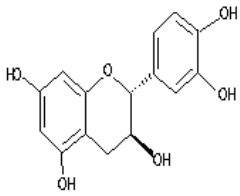
Phenols, triterpenes, flavonoids and flavonoid glycosides are the main classes of phytochemicals identified in the aerial parts of *Arbutus pavarii* Pampan species. According to its chemical and biological profiles, arbutus species can be used in some food products in the Mediterranean area. However, further studies are required to analyze different parts of Arbutus extracts and determine the pharmacokinetics and pharmacodynamics together with the techniques of isolation and identification of bioactive compounds. After identifying these parameters, it will be possible to set up the most potentially active samples for the clinical studies.



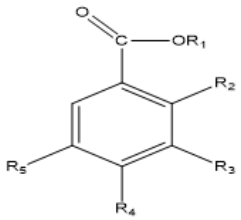
Lupeol **1**



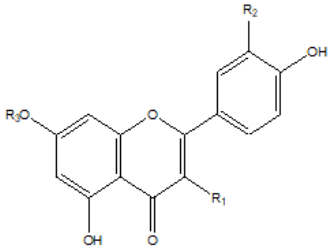
β-amyrin acetate **2**



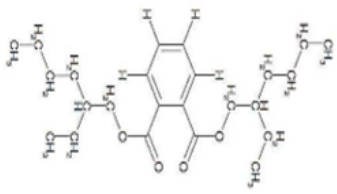
Catetchin **3**



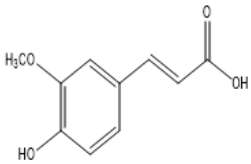
No	Name	R_1	R_2	R_3	R_4	R_5
4	Salicylic acid	H	OH	H	H	H
5	Methyl gallate	H	H	OH	OH	OH
6	Gallic acid	CH3	H	OH	OH	H



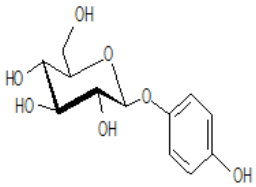
No	Name	R_1	R_2	R_3
7	Kmpfearol	OH	H	H
10	Kampferol-3- O- β- D- rutinoside.	O-*β*-D-Glc-Rha	H	H
12	Isoquercetin	O-*β*-D-glucopyranoside	OH	H
13	hyperoside	O-β-D-galactopyranoside	OH	H



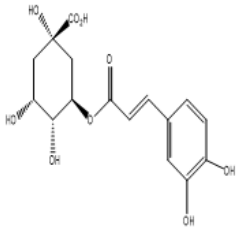
Dioctyl phthalate **8**



Ferulic acid **9**



Arbutin **11**



Chlorogenic acid **14**

Fig 1: The structures of compounds 1-14

References

1. Axelrod DI. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. Annals of the Missouri Botanical Garden 1975; 62(2):280-334. doi: 10.2307/2395199.
2. Torres JA, Valle F, Pinto C, Garcia-Fuentes A, Salazar C, Cano E. *Arbutus unedo* L. communities in Southern Iberian peninsula mountains. Plant Ecology 2002;160(2):207-223.
3. Jafri SM, El-Gadi A. (Eds). Flora of Libya. Tripoli: Botany department, Faculty of Science, Al Fatteh University 1977-1993.
4. Hegazy AK, Al-Rowaily SL, Faisal M, Alatar AA, ElBana MI, Assaeed AM. Nutritive value and antioxidant activity of some edible wild fruits in the Middle East. Journal of Medicinal Plants Research 2013;7:938-946.
5. Hamad HH, Mariam IHH, Gonaid H, Mojahidul I. Comparative phytochemical and antimicrobial investigation of some plants growing in Al-Jabal Al-Akhdar. Journal of Natural Product and Plant Resources 2011;1:15-23.
6. Elmhdwi MF, El Tumi SG. Effect of methanolic extract of Arbutus pavarii leaves as an antioxidant in the treatment of hypercholesterolemia in albino rats.

International Journal of Chemistry and Pharmaceutical Sciences 2014;2:1046-1052.
7. Ezzat M, El-Hawary SS, El Shabrawy S, AER, AA El-Shibani F. Evaluation of the phenolic and flavonoid contents, antimicrobial and cytotoxic activities of some plants growing in Al Jabal Al-Akhdar in Libya., International Journal of Pharmacognosy and Phytochemical Research 2016;8(7):1083-1087.
8. El-Shibani F. A Pharmacognostical study of Arbutus pavarii Pampan. Family Ericaceae and Sarcopoterium spinosum L. Family Rosaceae Growing in Libya. PhD diss., Cairo University, Cairo 2017.
9. Zuriati Zakaria, Ikram M Said, Fakhri Elabar. Chemical constituents of The leaves of Arbutus Pavarii; School of chemical sciences and food technology 1999.
10. Jamal A, Yaacob W, Din LB. "A chemical study on Phyllanthus reticulatus." J Phys Sci 2008;19(2):45-50.
11. Jain P, Bari S. "Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem of Wrightia tinctoria." Asian journal of plant sciences 2010;9(3):163-167.
12. Carcache-Blanco EJ, Cuendet M, Park EJ, Su BN, Rivero-Cruz JF, Farnsworth NR *et al*. "Potential cancer chemopreventive agents from *Arbutus unedo*." Natural product research 2006;20(04):327-334.
13. Kvangarsnes SK. "Phytochemical Observations on European Mistletoe (*Viscum album* L.) 2009.
14. Liu Y, Cai Y, Zhao Z, Wang J, Li J, Xin W *et al*. "Cloning and Functional Analysis of a. BETA.-Amyrin Synthase Gene Associated with Oleanolic Acid Biosynthesis in *Gentiana straminea* MAXIM." Biological and Pharmaceutical Bulletin 2009;32(5):818-824.
15. Khan H, Saeed M, Mehmood MH, Rehman NU, Muhammad N, Haq IU *et al*, "Studies on tracheorelaxant and anti-inflammatory activities of rhizomes of *Polygonatum verticillatum*." BMC complementary and alternative medicine 2013;13(1):1.
16. Sakar MK, Berkman MZ. "Flavonoids of Arbutus andrachine leaves." Doga-Tr. J. of Pharmacy 1992;2:17-23.
17. Mena P, Calani L, Dall'Asta C, Galaverna G, García-Viguera C, Bruni R *et al*. "Rapid and comprehensive evaluation of (poly) phenolic compounds in pomegranate (*Punica granatum* L.) juice by UHPLC-MSn." Molecules 2012;17(12):14821-14840.
18. Mabry TJ, Markham KR, Thomas MB. The ultraviolet spectra of flavones and flavonols, Springer 1970.
19. Markham KR. "Techniques of Flavonoid Identification." Academic press, Biological Technique series, Academic Press Inc. London, New York 1982.
20. Abdel-Sattar E, El-Mekkawy S. "New sulphide derivative from *Ferula rutabensis*." Natural product research 2009;23(9):861-865.
21. Al-Mutabagani L, Saleem MS, Al-Hazimi H. "Further highly oxygenated flavonoids from *Mentha longifolia*." Journal of Saudi Chemical Society 2004;8(3):541-546.
22. Mavar-Manga H, Haddad M, Pieters L, Baccelli C, Penge A, Quetin-Leclercq J. "Anti-inflammatory compounds from leaves and root bark of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg." Journal of ethnopharmacology 2008;115(1): 25-29.
23. Sastry V, Rao G. "Dioctyl phthalate, and antibacterial compound from the marine brown alga-*Sargassum wightii*." Journal of applied Phycology 1995;7(2):185-186.
24. Biskup I, Golonka I, Gamian A, Sroka Z. "Antioxidant activity of selected phenols estimated by ABTS and FRAP methods." Postepy Hig. Med. Dosw 2013;67:958-963.
25. Fiamegos YC, Nanos CG, Vervoort J, Stalikas CD. "Analytical procedure for the in-vial derivatization—extraction of phenolic acids and flavonoids in methanolic and aqueous plant extracts followed by gas chromatography with mass-selective detection." Journal of Chromatography A 2004;1041(1):11-18.
26. Maleš Ž, Plazibat M, Bilušić Vundać V, Žuntar I. "Qualitative and quantitative analysis of flavonoids of the strawberry 2006.
27. Maleš Ž, Šarić D, Bojić M. "Quantitative determination of flavonoids and chlorogenic acid in the leaves of *Arbutus unedo* L. using thin layer chromatography." Journal of analytical methods in chemistry 2013.
28. Wen-Yun G, Bao-De W, Yi-Ming L, Shan-Hao J, Da-Yuan Z. "A new alkaloid and arbutin from the whole plant of *Huperzia serrata*." Chinese Journal of Chemistry 2000;18(4):614-616.
29. Huang S, Zhu Y, Pan YJ, Wu SH. "Synthesis of arbutin by two-step reaction from glucose." Journal of Zhejiang University Science 2004;5(12):1509-1511.
30. Elshibani F, El Hawary S, Abd El Rahaman El Shabrawy S, Ezzat MA, Elremali N, Eltaboni F. Determination of Arbutin Content and In-vitro Assessment of Anti-hyperpigmentation Activity of Microemulsion Prepared from Methanolic Extract of the Aerial Part of Arbutus Pavarii Pampan Growing in East of Libya, International Journal of Innovative Research in Science, Engineering and Technology 2020; 9(3):1086-1093.
31. Geissman TA, Ollis W, Domínguez X, Deverall B, Dunn A, Arditti J, Guzmán M *et al*. The chemistry of flavonoid compounds, Centro Regional de Ayuda Técnica, México, DF (México) 1962.
32. Harborne JB. "Flavonoid sulphates: a new class of sulphur compounds in higher plants." Phytochemistry 1975;14(5, 6):1147-1155.
33. Silverstein R, Webster F. "^{13}C NMR spectrometry." Spectrometric identification of organic compounds 1998, 217-249.