***Synadenium grantii* Hook f.: HPLC/QTOF-MS/MS TENTATIVE IDENTIFICATION OF THE PHYTOCONSTITUENTS, ANTIOXIDANT, ANTIMICROBIAL AND ANTIBIOFILM EVALUATION OF THE AERIAL PARTS**

Asmaa S. Abd Elkarim^{1,✉}, Amal H. Ahmed², Hanan A.A. Taie³, Abdelbaset M. Elgamel⁴, Mohammed Abu-Elghait⁵ and Samah Shabana⁶

¹Chemistry of Tanning Materials and Leather Technology Department, National Research Centre, 33 El Bohouth st. (former EL Tahrir st.)-Dokki-Giza-Egypt-P.O.12622

²Pharmacognosy Department, Faculty of Pharmacy, Al-azhar University, 11884 Nasr City, Cairo, Egypt

³Plant Biochemistry Department, Division of Agriculture and Biological Researches, National Research Centre, 33 El-Bohouth St. (Former El-Tahrir St.), Dokki 12622, Giza, Egypt

⁴Chemistry of Natural and Microbial Products Department, National Research Centre, 33 El Bohouth st. (former EL Tahrir st.)-Dokki-Giza-Egypt-P.O.12622

⁵Department of Botany and Microbiology, Faculty of Science (Boys), Al-azhar University, 11884 Nasr City, Cairo, Egypt

⁶Faculty of Pharmaceutical Sciences and Drug Manufacturing, Misr University for Science and Technology, MUST, 6th October City, Egypt

✉Corresponding Author: asmaa_nrc@yahoo.com

ABSTRACT

A rapid and simple effective technique HPLC/QTOF-MS/MS was developed to identify the constituents in *Synadenium grantii*. This study aims to investigate antioxidant, antimicrobial and antibiofilm of aqueous methanol extract. Adequate conditions for extraction were employed and LC-MS/MS analysis was optimized in positive ion mode using full scan MS measurements and MS/MS fragmentations. The high concentration of *S. grantii* extract exhibited a promising radical scavenging activity with all the tested antioxidant assays. It found to be 82.40±0.24, 75.69±0.30 and 90.27±0.16% using DPPH, Ion metal chelating and ABTS radical scavenging activity respectively, while it found to be 1.41±0.02 Reducing power capacity and 2787± 27.1µmolTrolox/100 g D.W. (Ferric reducing power). The antioxidant activity of the extract was found to be as near as that of the synthetic BHA. Biofilm is a specific polymeric matrix produced by several bacterial species to make it more resistant against the immune system and diverse antibiotics. A total of 84 different metabolites were tentatively identified. The fragmentation characteristics are determined based on the exact mass information and *R_f* values. It was found that flavonols, flavones, Isoflavone, chlorogenic acids, amino acids, stilbene and anthocyanins constitute mainly the phytoconstituents of the aerial parts. The biological examination exhibited excellent antioxidant activity using all tested assays and high antibiofilm activity against *S. aureus* with a reduction value 97.7% at 0.5 mg/ml without affecting bacterial growth which makes these compounds a promising agent for antimicrobial and antibiofilm against bacterial pathogens.

Keywords: *Synadenium*, LC/QTOF-MS/MS, Phytoconstituents, Antioxidant, Antimicrobial, Antibiofilm.

RASĀYAN J. Chem., Vol. 14, No.2, 2021

INTRODUCTION

Synadenium grantii Hook f. is a member of the Euphorbiaceae family, which includes over 300 genera and 8900 species, several of which are used in traditional medicine to treat diseases such as peptic ulcers, cancer and other health issues. Among them, *Synadenium*, is a small euphorbiaceous genus that contains nineteen species. This genus was found to be rich in various classes of bioactive constituents of flavonoids, saponins and diterpenes.¹ To avoid tedious and long time-consuming classical methods of

chromatographic separation for most major known compounds, hyphenation of HPLC with high-resolution QTOF-MS/MS has successfully enabled us for rapid identification of a large number of minor constituents. This technique proves accurately the exact mass, formulae and differentiates between the isomeric compounds following their different R_f -values, MS/MS fragmentation trends and molecular formulae.² There is a growing tendency in comparing the phytochemical constituents isolated from plants and their pharmacological actions.³ This due to the effective pharmacological activities, economic viability and low toxicity, the therapeutic properties of plants have been examined in the light of recent systematic developments throughout the world. According to pharmacological studies, herbal extracts contain important nutrients as well as a variety of bioactive compounds with antioxidant properties, such as flavonoids, phenolics, sterols, alkaloids, carotenoids, and glucosinolates.^{4,5} The present study deals with the examination of the antioxidant, antimicrobial and antibiofilm of the aqueous methanol extract (AME). Biofilms are a specific structure produced by a wide range of bacterial species. It has a special interest in the medical field due to the antibiotic-resistant characteristic gained from these special formulations and the effect it on the medical devices and human direct contact substances especially catheter surfaces which make it difficult to removed.^{6,7} Natural products compound especially plant originated products area promise sources for combating this phenomenon.⁸ The antimicrobial and antibiofilm activities of (AME) against Gram-positive and Gram-negative bacterial strains were investigated in this research to exploring its specific biological activities, which may introduce new therapeutic agents against bacterial problems.

EXPERIMENTAL

Plant-based materials

The aerial parts of *S. grantii* were collected in November 2017 from National Research Centre (NRC) garden (Dokki, Cairo, Egypt). The plant was classified as a species by Dr. M. EL-Gibaly, Lecturer of Taxonomy and Consultant for Central Administration of Plantation and Environment. Avoucher sample (No: A79), where it was held was deposited at the chemistry of tanning materials and leather technology department. National Research Centre (NRC), Dokki, Cairo, Egypt.

Chemicals

Acetonitrile (Fisher, optima®, LC/MS-grade; Fair Lawn, NJ, USA), DI-water further purified by Milli-Q Plus water purification system (Millipore Ltd., Bedford, MA, USA) and methanol HPLC (Merck, EMSURE®, analytical grade; Darmstadt, Germany).

2,2-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferri-cyanide, Trolox, ferrozine, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid, potassium ferri-cyanide, butylated hydroxytoluene (BHT), FeCl_2 and FeCl_3 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals used including the solvents were of analytical grade.

Equipment and Conditions

Fragmentation analyses were performed using (Triple TOF 5600+). The mobile phases consisted of DI-Water contains 0.1%FA (formic acid), ammonium format buffer pH=8 containing 1% methanol and 100 % acetonitrile, in-Line filter disks Pre column (0.5 μm x 3.0 mm, Phenomenex), Xbridge C18 column (3.5 μm , 2.1x50 mm, waters) and the column temperature was maintained at 40°C, flow rate 0.3 ml/min and the scan type (Information Dependent Acquisition (IDA)).

Extraction Methods

The air-dried aerial parts of *S. grantii* (1.75kg) were crushed and extracted with 70% (Aq) CH_3OH by soaking at room temperature (Macération). A rotary evaporator was used to evaporate the solvent under vacuum at 50°C. Then the residue (dry) was sequentially defatted and desalted by CHCl_3 and $\text{C}_2\text{H}_5\text{OH}$, respectively, by warming under reflux conditions. Thereafter, the residue was taken in methanol, affording a methanol-soluble portion (MSP) that yielded methanol extract of 150g.

Sample Preparation

The mobile phase working solution (MP-WS) is made up of 50: 25: 25 v/v DI-Water-Methanol-Acetonitrile. To a 50 mg weighted sample, add 1 ml MP-WS, vortex for 2 minutes, then ultra-sonicate for 10 minutes before centrifuging for 5 minutes at 10000 rpm. An amount of 20 μ l stock (50/ 1000 μ l) was diluted in 1000 liter reconstitution solvent. Finally, a concentration of 1 μ g/ μ l was injected. Inject 25 l μ l of total extract and 25 μ l of MP-WS as a blank sample in positive mode.

Data Processing

Master View was used for feature peaks extraction from the total ion chromatogram (TIC) based on features that should have Signal-to-Noise greater than 5 (Non-targeted analysis). Features intensities of the sample-to-blank should be greater than 5. Marker View was used for feature annotation and removing isotopic peaks. MasterView was used again to identify peaks based on their fragments using the Build-in database and online database; RESPECT and MONA (Mass Bank of North America).

Investigation of Antioxidant Activity

Following a previously reported protocol.^{9, 10} DPPH, ABTS percentage, FRAP and reducing power values of the studied extract and compounds were calculated.¹¹ Metal chelating activity assay was determined according to the previous protocol.¹²

Antimicrobial Activity and MIC Determination

The antimicrobial activity was calculated using microtiterplate (MTP) method in this analysis and inhibitory concentration at minimum (MIC) of (AM) extract against Gram-positive and negative bacteria in addition to Candida ATCC strains as the following; *S.aureus* ATCC 29213, *Bacillus subtilis* ATTC605, *P.aeruginosa* 9027, *E. coli* ATCC8739 and *C. albicans* ATCC 10213 according to the Clinical and Laboratory Standards Institute (CLSI) (2019).¹³ Briefly, (AM) extract was diluted in DMSO 5% (final concentration), gradient concentrations of the compound (6.0 - 0.3 mg/ml w/v) were added to the MTP wells before adding 200 μ l of 1: 100 v/v of overnight cultures of the *S. aureus* and *P. aeruginosa* in TSB inoculum size 5×10^5 CFU/ml and incubated with shaking 100 rpm in 37°C for 24 h. after incubation, plates were read in ELIZA reader (Tecan Elx800, USA) at 620 nm for detecting the growth turbidity. The MIC was calculated as the lowest concentration at which the compound prevented the growth of test species using TSB media as a negative monitor. All tests were performed in triplicate.

Assay for Biofilm Inhibition

The ability of (AME) to inhibit or prevent bacterial biofilm formation was assessed using a microtiter plate assay against established biofilm generating strains *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 9027. Sub-lethal concentrations of (AME) (4.0–0.3 mg/ml) were loaded in flat bottom MTP containing Tryptic Soy broth media (TSB) with 1% glucose. *S. aureus* and *P. aeruginosa* overnight cultures were diluted 1: 100 in TSB to produce 1×10^5 CFU/ml, and both plates were incubated at 37°C for 48 hours. The planktonic cells were transferred without disrupting the biofilm after the incubation time, and cell growth was measured at 620 nm using an ELIZA reader (Tecan Elx800, USA). To remove excess planktonic cells, the plates were washed three times with sterilized phosphate-buffered saline (PBS) pH 7.2, and then biofilm fixed with 200 μ l of 95 percent methanol for 10 minutes. The plates were incubated for 15 min. at room temperature after adding crystal violet (0.1 % w/v). Then, the crystal violet was removed, and the wells were gently washed with sterile distilled water to remove the excess of the stain. Finally, an inverted microscope (Olympus Ck40 Japan) x40 was used to analyze and photograph the adhered biofilm bounded crystal violet, which was then eluted in acetic acid (30%) and the absorbance measured at 540 nm using an ELIZA reader (Tecan, Elx800- USA).¹³ As a solvent monitor, wells containing a volume of 5% DMSO were used. The results of the treated wells were compared to the untreated control wells. Any of the experiments were completed three times.¹⁴

RESULTS AND DISCUSSION

Chemical Composition of Methanol-soluble Portion (MSP)

LC-MS/MS analysis of (MSP) prepared from *S. grantii* aerial parts enabled us to identify about 84 compounds belonging to different types of natural products. Depending on the structural information

obtained from HPLC/QTOF-MS/MS data, particularly $[M+H]^+$ ions, total ion current chromatogram (TIC) (Fig.-1). A total of 84 components were unambiguously identified including 27 flavonoids of 14 flavonol and 13 flavone C- and/or O-hexosides nature (Fig.-2). In addition to 6 phenolic acids, 23 amino acid derivatives, 6 anthocyanin, one isoflavone, 2 terbin, 9 Hydrolysable tannins derivatives and 10 organic compounds derivatives were tentatively identified based on their fragments (Table-1). Most of them were reported in *S. grantii* for the first time.

Fig.-1: LC-QTOF-MS/MS Total Ion Chromatogram (TIC) of AM Extract of Aerial Parts from *S. grantii* in Positive Mode.

	Peak no.	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈
	29	H	H	H	OH	H	H	H	H
	37	H	OH	C-hexose-	OH	H	H	OH	H
	38	C-Hexoside	OH	C-Hexoside	OH	H	H	OH	
	39	H	OGlu	OGlu	OH	H	H	OH	H
	42	H	OH	H	OH	O-Rutinoside	OH	OH	H
	43	H	OH	H	OH	OH	H	OGlu	OH
	44	H	OH	H	OH	OGlu	OH	OH	H
	45	H	OH	H	OH	OGal	OH	OH	H
	48	H	OH	H	OH	O-Glucronide	OH	OH	H
	50	C-hexoside	OH	H	OH	H	OH	OH	H
	51	H	OH	OGlu	OH	H	OH	OH	H
	52,53	H	OH	H	OH	ORham or Rham	H	OH	H
	54	H	ORham	H	OH	OH	H	OH	H
	55,56	H	OH	H	OH	OGlu or Glu	H	OH	H
	57	H	OH	H	OH	H	OH	OGl	H
	58	H	OGlu	H	OH	H	OH	OH	H
	60	H	H	OH	H	OH	OH	OH	H
	61	H	OH	H	OH	OH	H	OH	H
	62	H	OH	H	OH	H	OH	OH	H
	66	H	OGL-GLU	H	OH	H	OH	OCH ₃	H
	67,68	H	OH	H	OH	ORham or α-L-Rham	OH	OH	H

Sufftuticoside(B)
R₁=galloyl, R₂=H
(77)

Sufftuticoside(D),
R₁=galloyl, R₂=H

Fig.-2: Chemical Compositions of the Major Compounds Discovered

Phenolic Acids

The HPLC/QTOF-MS² analysis (in positive ion mode) revealed the presence of six isomers of mono caffeoylquinic acid like-structures (31-36), including chlorogenic acid (3-*O-E*-caffeoylquinic acid), depending on a molecular ion peak at m/z 355.10299 $[M+H]^+$. This group of positional isomers was further proved through the detection of the characteristic fragment positive ions at m/z 193 $[M+H\text{-caffeoyl}]^+$ or $[quinic+H]^+$, and 163 $[M+H\text{-quinoyl}]^+$ or $[caffeic+H]^+$, together with a third positive ion at m/z 137 amu. According to the matching of such information with the library database, these compounds were tentatively identified as chlorogenic acid, 1-caffeoylquinic acid, 1-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid.¹⁵

Flavonoids

The flavonoids were tentatively identified by MS/MS pattern (Table 1). The systematic fragmentation of aglycones and their derivatives by LC-MS/MS experiments resulted in key aglycone fragments at m/z 179 and 151 for quercetin; 257 and 243 for kaempferol; 241 and 175 for luteolin; 225, 201 and 149 for apigenin.¹⁶ Also, the fragments corresponding to the loss of 162 and 146 amu were indicative of the hexosyl (e.g. glucosyl or galactosyl), and the deoxyhexosyl (e.g. rhamnosyl), the most common sugars found in the case of flavonoids, respectively. Moreover, the presence of the di-*O*-glycoside flavonoides was concluded according to their typical fragment due to the loss of 308 amu, e.g. rutinose or neohesperidose.¹⁷

Flavones Glycosides

The molecular ion i.e. $[M+H]^+$ at m/z 595.16598 produced fragment ions at m/z 541 (loss of 44 amu, $\cdot C-CH_2-OH$ from hexose), 475.0000 (loss of 120 amu from hexose moiety), 355.15847 (loss of 240 amu from di hexoside), 325.15847 (loss of 270 amu, aglycone), 271.15847 (loss of di hexose), 309.15847 (loss of 286, saponarin aglycone), 449.103652 (loss of 146 amu) corresponding to the C-glycoside fragmentation pattern so peak (37) was tentatively identified as apigenin di-*C*-hexoside. Peaks (38, 39) were assigned as di-*C*, *C* hexosyl-Apigenin and saponarin, respectively.

Two derivatives of luteolin hexosyl were proposed for compounds (50, 51) at m/z 449.10801, $[M+H]^+$. In the MS/MS spectra, the loss of hexose moieties gave a fragment ion at m/z 353.10056, 287.10056, which corresponds to luteolin. These compounds have been characterized as luteolin-*C*-hexosyl and homoorientin (Isoorientin).

Peak 66 produced a molecular anion at m/z 609.18197 and diosmetin aglycon, MS² fragment at m/z 301.0719 ($M-308$, neohesperidose) and 286.048652 which pointed out the presence of diosmetin 7-*O*-neohesperidoside (neodiosmin).¹⁸

Flavonols Glycosides

The components exhibit peaks (41, 42) both of them had $[M+H]^+$ at m/z 611.16034 which resulted in a fragment at m/z 465.104052 (loss of a rhamnose moiety, 147 amu), 303.0497 (loss of a hexose moiety,

162amu and rhamnose), 274.043952(direct loss of CO), 285.041752 (loss of OH) after loss of two hexoses moieties. In both cases, MS² spectrum of m/z 303 yielded quercetin. Peak (41) is tentatively identified as quercetin-3-O-β-glucopyranosyl-7-O-α-rhamnopyranoside while peak (42) was identified as quercetin-3-O-rutinoside (rutin) with MS fragments at 465.0998 and 303.742952 obtained due to loss of 308amu (a rutinosyl unit).^{19, 20}

Peaks (43-45) had [M+H]⁺ at m/z 465.10307 and characteristic fragmentation at m/z 303 (loss of 162amu). This loss of 162 indicated attachment of the sugar moiety (hexose) to the aglycone part indicating that it was present in glycosidic form and not as an aglycone. The characteristic fragments observed with m/z 275 [M+H-(glucose+CO)], 257, [M+H-(glucose+CO+H₂O)], 229 [M+H-(glucose+2CO+H₂O)], 165[M+H-(glucose+3CO+3H₂O)] and 153 corresponding to [M + H - (glucose - H₂O - C₈H₆O₃)]⁺ were similar to the results reported by ¹² in their study on Phenolic compounds. These compounds were assigned as speriaeoside, quercetin-3-O-β-glucopyranoside (Isoquercitrin), and quercetin-3-O-β-D-galactoside (hyperoside).²¹

Compound 48 was identified as quercetin 3-O-glucuronide with a pseudomolecular ion at m/z 479.08283 [M+H]⁺ and fragment ion 303.0497 after the loss of a glucuronide unit (176 amu).²⁰

The peaks (52- 54) have identical mass at m/z 433.11541 with fragments at m/z 287.120202 (loss 147 amu, rhamnose unit), were identified as kaempferol-3-O-rhamnose, rhamnosyl-kaempferol and kaempferol 7-O-rhamnoside.²²

A precursor ions of m/z 449.10805, 449.1077 at R_t of 4.31 and 4.71 min, gave fragment ions at m/z 303.047, 287.0525, 270.10056, 245.046852, 229.048, 181.014252, 165, 153.115112, 121.0277 corresponding to (Quercetin) fragments due to loss of rhamnosyl moiety (147 amu), compounds (63, 64, 67 and 68,), are suggested as quercetin-3- rhamnoside, quercetin-3-O-rhamnose, quercitrin and quercetin-3-O-α-L-rhamnopyranoside.^{21, 23, 24}

Peaks (55-58) gave the same MS base peak at m/z 449.10825, showed fragments at m/z 287.115112 with the same retention time which corresponds to the characteristic loss of O-hexoside were tentatively assigned as kaempferol 3-O-glucoside, kaempferol 3-glucoside, luteolin-4'-O-glucoside, luteolin-7-O-glucoside and maritimenin.^{25,26}

Flavanone

In the positive LC-MS/MS mode, peak (47) exhibited protonated ions at m/z 325.14386 and produced MS² ions at m/z 283.137452[M+H- (CH₃ -CH-CH₃)], 255.138452[M+H - (C₅H₁₀)], 238.091052 [M+H - (C₅H₁₀+ OH)], 221.079652 [M+H - (C₅H₁₀+ 2OH)], 165.0164[M+H- (C₅H₁₀+ 2OH+2CO)] and 137.023752 [M+H-(C₅H₁₀+ 2OH+3CO)] in the QTOF-MS analysis. This compound was assigned as glabranine based on literature data for hydroxyl flavanon. The exact mass of the peak (47) was m/z 325.14386 as stated in (table 1). These measurements were made as a result of the [M+H]⁺ and the [M-C₅H₈O₄+H]⁺ ions.

Aglycons

Peaks (60-62) showing a [M+H]⁺ ion at m/z 287.05539 and MS² ions at m/z 231.062292, 153.019952, 153.019952 corresponding to the aglycone fragmentation pattern, so were tentatively proposed as fisetin, kaempferol and luteolin.²⁷ Peak (29) was identified to be primuliten (7-hydroxy flavones) based on the parent ion at m/z 239.07192 similar to apigenine aglycon at m/z 271 by loss of 32amu) and the fragment ions obtained at m/z 103.0547, 115.0606 by the expulsion of (C₇H₄O₃· radical, M-136) from the base molecular ion.²⁸

Isoflavone

Single peak (40) of ipriflavone with protonated molecular ion [M+H]⁺ at m/z m/z 281.11585. Fragmentation of protonated ipriflavone leads to product ions m/z (1 137.0211 37, 165.0686, 102.045, 105.0321) due to cleavage of C-ring, loss of isopropyl group and H₂O, respectively (Table-1).²⁹

Anthocyanins Derivatives

One peak with retention time 0.73min and formula C₂₃H₂₅O₁₂ exhibited molecular ion at m/z 494.14094 was assigned to methoxylated anthocyanin glycosides. The product ions at m/z 374.1346 due to loss of

120 amu from glucose moiety, 331.1346, (M-162 of glucose), 181.1346 and 121.1346 resulted from cleavage of C-ring, were characteristic fragments for malvidin 3-glucoside, Peak (24).

Peak (71) produced $m/z = 611.3249$ and MS^2 fragments 449, 287, resulted in the loss of 3264amu for two glucose units was characterized as cyanidin-3, 5-O-diglucoside. Two isomers (75, 76) at $m/z = 611.28587$, 595.28978 and MS^2 fragments at 287 due to loss of 324amu (sophorosyl unit) and 308 amu (rutinosyl unit) were identified as cyanidin-3-O-sophoroside and cyanidin-3-O-rutinoside.¹⁷

Peak (80) with $m/z = 615.3256$ and fragment at 301 was identified as 7-O-Methyl-cyanidin-3-O-(2"-galloyl).³⁰ Peak (83) was identified as proanthocyanidin dimer, $[M+H]^+ 579.33614$ and fragments at 427, 409, 291, loss of 152amu (By using the retro Diels-Alder reaction, you can create a unique fragmentation pathway.).³¹

Amino Acids

Aliphatic Amino Acid

Four isomers of 2-Aminobutyric acid and three derivatives of glycine ($C_4H_9NO_2$) at m/z 104.07079 were observed, peaks (2-8, 22), as shown by the appearance of product ions at m/z 58.0658 (loss of $CO+H_2O$), 57.06333, 87.0447 (loss of NH_3) and corresponded to N-methyl-DL-alanine, N,N-dimethylglycine, N,N-dimethylglycine hydrochloride, L- α -amino-n-butyric acid, L-2-aminobutyric acid, 2-aminoisobutyric acid and L-2-aminobutyric acid.

The QTOF-MS/MS analysis revealed the presence of seven isomers in the ESI + modes with ions at m/z 120.06603, 120.06566, respectively. The appearance of fragment ion at m/z 102.0552, (M- H_2O), and product ions at m/z 74.05824, [M-($CO+H_2O$)] corresponded to homoserine isomer in peaks (9-14, 20) were assigned as D- β -homoserine, L- β -homoserine, L-allo-threonine, D- β -homoserine [(S)-3-Amino-4-hydroxy-butyric acid (-)], L-homoserine and L- β -homoserine [(R)-3-Amino-4-hydroxy-butyric acid (+)].

The compound (R_t 0.65 min) with the molecular formula $C_4H_7N_1O_4$ and having the precursor ion at m/z 134.04499 in the ESI+ mode, was tentatively proposed as L-aspartic acid Peak(15) which showed product ions at m/z 115.03751, 116.03751, 117.052062 (loss of H_2O).³²

The compounds (25) and (26) possessed a molecular ion at m/z 146.11736 were characterized as L- β -homoleucine-HCl and L- β -homoisoleucine hydrochloride due to the characteristic product ions at 86.0961, 69.0711, 55.0559, 86.0969, 83.11028.

Aromatic Amino Acids

The ions m/z 352.16039 $[M+H]^+$ and 220.118052 $[M+H-ribose\ C_5H_8O_4]^+$ are characteristic of trans-zeatin-riboside, compound (27). A component (16) with molecular ion $[M+H]^+$ at m/z 152.05747 was proposed to be guanine with fragment ions at m/z 135.029852 (loss of NH_3), 111.0206, 110 (loss of 42amu, -HNCNH), 68.024552 [loss of 84amu, $NH_3^+(-HCNCCO)$].

Adenosine and 2'-deoxyguanosine monohydrate both had identical formula $C_{10}H_{13}N_5O_4$ with different (R_t , 0.86, 0.71min) were suggested for compounds (18, 23), m/z 268.10440/268.10445, $[M+H]^+$. In the MS/MS spectra, [M-132 (pentose) +H]⁺ gave a fragment ion at m/z 136.09675, which corresponds to aglycone of adenosine and 119.035 $[M+H-C_5H_{10}O_3]^+$. A product ion at 150.09675 formed guanosine radical which characteristic of 2'-deoxyguanosine monohydrate.³³

The QTOF-MS/MS analysis showed the presence of molecular ion at m/z 310.11343 in the ESI + mode with product ions at m/z 292.10598, (M- H_2O), 274.10598, (M-2 H_2O), 232.10598, (M- $H_2O+C_2H_4NO$) revealed that peak (21) was tentatively proposed as N-acetylneuraminic acid.

There was a dominant neutral loss of 17amu, which could be due to the loss of NH_3 with fragment ion at m/z 149.093532 and 120.07898, (M- $CO+H_2O$) in positive ionization mode. Compound (28) with a precursor ion $[M+H]^+$ at m/z 166.08616 and with formula $C_9H_{11}NO_2$ had been assigned as phenylalanine.³²

Stilbenes

Two stilbenes derivatives were tentatively identified in *S. grantii*. The molecular ion at m/z 391.13934, fragmentation of the parent ion at m/z 231.1010213, 287.1119799, 269.1014152, 147.0646358 were produced from cleavage of glucoside and successive losses of $[M+HCOO]^+$ which corresponded to the molecular formulae of $C_{20}H_{22}O_8$. These fragments were fitted to the resveratrol 3-O-glucoside, peak

(19).³⁴ Compound (17) had a molecular ion at m/z 275.09285 and MS/MS spectra that showed product ions at m/z 153.017452, 121.064552 were formed from cleavage of bond between methylene and carbonyl group $O=C-CH_2$, 125.093652, (M-149, $C_9H_9O_2^+$), 229.085352, (M-CO). The compound was characterized as Phloretin.

Hydrolysable Tannins Derivatives

Two peaks (71, 73) showed precursor ions at m/z 547.20978 and 623.32565 were tentatively identified as dihydroxybenzoic-digallate and galloyl-valoneic acid bilactone.³⁵ Four isomers of gallic acid derivatives (74, 79, 81, 82) with molecular formula $C_{21}H_{14}O_{13}$, $C_{20}H_{20}O_{14}$, $C_{27}H_{22}O_{17}$ and $C_{27}H_{24}O_{18}$ exhibited molecular ions at m/z 475.24853, 485.25135, 619.27619 and 637.25234 with fragment ions at 323, 425, 303, 153333, 171, 485, 467 were assigned as Trigallic acid, Trigalloyllevoglucosan, di and tri galloyl hexoside.^{16,36} Peak (84) was detected as galloyl-HHDP-glucose (corilagin isomer with molecular ion m/z 635.28597 produced MS² fragments at 483 by loss of galloyl unit and 303 (loss of galloylglucose) as the main fragment.¹⁷

Table-1: Tentative Identification of the Phytoconstituents from the Methanol Extract of *S. grantii* aerial parts by (LC-QTOF-MS/MS) in Positive Mode

Peak	Formulae	Rt (min)	LC-MS [M+H] ⁺	QTOF-MS-MS	Tentative Assignment	Intensity
1	C ₁₅ H ₁₆ O ₅	0.53	277.10608	208, 193, 207, 163,	Capensine	5841
2	C ₄ H ₉ NO ₂	0.57	104.07079	58, 57	N-Methyl-DL-Alanine	3498
3	C ₄ H ₉ N ₁ O ₂	0.57	104.07079	58	N,N-Dimethylglycine	3498
4	C ₄ H ₉ N ₁ O ₂	0.57	104.07079	58, 57	N,N-Dimethylglycine hydrochloride	3498
5	C ₄ H ₉ N ₁ O ₂	0.57	104.07079	58, 87	L- α -Amino-n-butyric acid	3498
6	C ₄ H ₉ NO ₂	0.57	104.07079	58, 57	L-2-Aminobutyric acid	2755
7	C ₄ H ₉ NO ₂	0.57	104.07079	58, 57	2-Aminoisobutyric acid	3498
8	C ₄ H ₉ NO ₂	0.57	104.07079	58, 57	D-L-2-Aminobutyric acid	3498
9	C ₄ H ₉ NO ₃	0.61	120.06603	102, 60	D- β -Homoserine	2382
10	C ₄ H ₉ NO ₃	0.61	120.06603	102, 60, 84	L- β -Homoserine	2382
11	C ₄ H ₉ NO ₃	0.61	120.06603	102, 74, 84, 56	L-allo-threonine	2382
12	C ₄ H ₉ NO ₃	0.62	120.06603	102, 85, 76, 74	D- β -Homoserine [(S)-3-Amino-4-hydroxy-butyric acid (-)]	2382
13	C ₄ H ₉ NO ₃	0.63	120.06566	102, 74, 92, 73, 56	L-Homoserine	2755
14	C ₄ H ₉ NO ₃	0.61	120.06566	102, 85	L- β -Homoserine [(R)-3-Amino-4-hydroxy-butyric acid (+)]	2755
15	C ₄ H ₇ N ₁ O ₄	0.65	134.04499	115, 117, 116	L-Aspartic acid	1917
16	C ₅ H ₅ N ₅ O	0.65	152.05747	135, 110, 111, 68	Guanine	2534
17	C ₁₅ H ₁₄ O ₅	0.66	275.09285	169, 153, 121, 125, 235, 231, 229	Phloretin	1255
18	C ₁₀ H ₁₃ N ₅ O ₄	0.68	268.10440	136, 135, 119, 118, 116, 107	Adenosin	3699

19	C ₂₀ H ₂₂ O ₈	0.69	391.13934	231, 287, 269ni, 265ni, 147,	Resveratrol 3-O-glucoside	1561
20	C ₄ H ₉ NO ₃	0.69	120.06603	74, 102, 84	L-Threonine	2368
21	C ₁₁ H ₁₉ NO ₉	0.7	310.11343	292,274, 232, 169, 103	N-Acetylneuraminic acid	1955
22	C ₄ H ₉ NO ₂	0.71	104.07061	87, 69	γ-Amino-n-butyric acid	3565
23	C ₁₀ H ₁₃ N ₅ O ₄	0.71	268.10445	150	2'-Deoxyguanosine monohydrate	3699
24	C ₂₃ H ₂₅ O ₁₂	0.73	494.14094	374, 331, 181, 121	Malvidin 3-glucoside	883
25	C ₇ H ₁₅ NO ₂	0.76	146.11736	86, 69, 55	L-β-Homoleucine-HCl	1613
26	C ₇ H ₁₅ NO ₂	0.76	146.11736	86, 68, 55, 83	L-β-Homoisoleucine hydrochloride	1613
27	C ₁₅ H ₂₁ N ₅ O ₅	0.79	352.16039	220, 136, 333 251, 202	Trans-Zeatin-riboside	1271
28	C ₉ H ₁₁ NO ₂	0.8	166.08616	149, 120, 118, 105	L-(-)-Phenylalanine	130007
29	C ₁₅ H ₁₀ O ₃	0.99	239.07192	103, 115	Primuliten	1421
30	C ₁₉ H ₁₂ O ₇	1.21	353.06511	165, 310, 254, 208, 147	Daphnoretin	1293
31	C ₁₆ H ₁₈ O ₉	1.23	355.10299	193, 163, 137	Chlorogenic acid	52946
32	C ₁₆ H ₁₈ O ₉	1.23	355.10299	193	1-Caffeoylquinic acids	52946
33	C ₁₆ H ₁₈ O ₉	1.23	355.10299	193	1-O-Caffeoylquinic acids	52946
34	C ₁₆ H ₁₈ O ₉	1.23	355.10299	193	3-Caffeoylquinic acids	52946
35	C ₁₆ H ₁₈ O ₉	1.23	355.10299	193	4-Caffeoylquinic acids	52946
36	C ₁₆ H ₁₈ O ₉	1.23	355.10299	193, 162, 192, 116	5-Caffeoylquinic acids	52946
37	C ₂₇ H ₃₀ O ₁₅	1.93	595.16598	541, 475, 355, 325	Apigenin-di-C-hexoside	839
38	C ₂₇ H ₃₀ O ₁₅	1.93	595.16598	457, 439, 409, 307, 295,	Di-C,C hexosyl-Apigenin	835
39	C ₂₇ H ₃₀ O ₁₅	1.94	595.16598	449, 336, 309, 282, 271,	Saponarin	839
40	C ₁₈ H ₁₆ O ₃	3.16	281.11585	165, 137,, 105, 102	Ipriflavone	1649
41	C ₂₇ H ₃₀ O ₁₆	3.49	611.16034	303, 465, 247, 153	Quercetin-3-O-β-glucopyranosyl-7-O-α-rhamnopyranoside	9795
42	C ₂₇ H ₃₀ O ₁₆	3.49	611.16034	302, 465, 322, 285, 181, 153, 272,	Rutin	9795
43	C ₂₁ H ₂₀ O ₁₂	3.63	465.10307	303, 302, 257, 201, 165, 153	Speriaeoside	20911

44	C ₂₁ H ₂₀ O ₁₂	3.63	465.10307	303, 285, 272, 245, 229, 165, 153, 109, 273, 229	Quercetin-3-O-β-glucopyranoside	20911
45	C ₂₁ H ₂₀ O ₁₂	3.63	465.10307	303, 302, 297, 257, 245, 229, 201, 165, 153	Quercetin-3-O-β-D-galactoside (Hyperoside)	20911
46	C ₁₇ H ₂₂ N ₂ O ₁₀ S ₂	3.63	479.08085	465, 301, 293, 261	4-Methoxy-3-indolylmethyl glucosinolate	599
47	C ₂₀ H ₂₀ O ₄	3.64	325.14386	221, 165, 135, 283, 269, 240, 222, 189	Glabranine	1199
48	C ₂₁ H ₁₈ O ₁₃	3.71	479.08283	303, 302, 299, 257, 229, 201, 165, 153, 109,	Quercetin-3-Glucuronide	631
49	C ₁₉ H ₂₂ O ₆	3.76	347.14937	239, 227, 213, 143	Gibberelin A3	624
50	C ₂₁ H ₂₀ O ₁₁	3.84	449.10801	353, 287	Luteolin –C-hexosyl	136475
51	C ₂₁ H ₂₀ O ₁₁	3.84	449.10801	353, 412, 368 330, 328	Homoorientin (Isoorientin)	136475
52	C ₂₁ H ₂₀ O ₁₀	3.92	433.11541	287, 229	Kaempferol-3-O-rhamnose	708
53	C ₂₁ H ₂₀ O ₁₀	3.92	433.11541	287	Rhamnosyl-kaempferol	708
54	C ₂₁ H ₂₀ O ₁₀	3.92	433.11541	287	Kaempferol 7-O-rhamnoside	137258
55	C ₂₁ H ₂₀ O ₁₁	4.02	449.10825	287, 286, 258, 153, 165	kaempferol-3-O-glucoside	135868
56	C ₂₁ H ₂₀ O ₁₁	4.02	449.10825	287, 286, 284, 258, 185, 165, 229, 257	kaempferol-3-glucoside	135868
57	C ₂₁ H ₂₀ O ₁₁	4.02	449.10825	287, 284, 201, 177	luteolin-4'-O-glucoside	135868
58	C ₂₁ H ₂₀ O ₁₁	4.02	449.10825	287, 284 258, 201	luteolin-7-O-glucoside	135868
59	C ₂₁ H ₂₀ O ₁₁	4.02	449.10825	287, 284, 241, 185, 152, 135, 123, 107, 105	Maritimein	135868
60	C ₁₅ H ₁₀ O ₆	4.26	287.05534	231, 165, 258, 197	Fisetin	36814
61	C ₁₅ H ₁₀ O ₆	4.26	287.05539	231, 229 153, 165,	Kaempferol	36727
62	C ₁₅ H ₁₀ O ₆	4.26	287.05539	153	Luteolin	36727
63	C ₂₁ H ₂₀ O ₁₁	4.31	449.10805	303, 287, 270,	Quercetin-3-rhamnoside	136374

				165,229, 147, 153, 121		
64	C ₂₁ H ₂₀ O ₁₁	4.31	449.10805	303, 302, 153	Quercetin-3-O-rhamnose	136374
65	C ₂₄ H ₂₂ N ₄ O	4.61	383.18691	261, 351, 281, 145, 132	1-(2,3-dibenzimidazolyl-2-propyl)-2-methoxybenzene	1142
66	C ₂₈ H ₃₂ O ₁₅	4.63	609.18197	301, 286	Neodiosmin	720
67	C ₂₁ H ₂₀ O ₁₁	4.71	449.1077	302, 287,285, 245, 201, 165, 153, 147	Quercitrin	137258
68	C ₂₁ H ₂₀ O ₁₁	4.71	449.1077	303, 287, 286, 270, 245, 229, 181, 165, 153,	Quercetin-3-O-α-L-rhamnopyranoside	137258
69	C ₂₁ H ₁₆ O ₁₃	5.13	477.10238	303	Ellagic acid acetyl pentoside	37564 Dinche va I
70	C ₂₄ H ₁₈ O ₁₅	10.91	547.20978	471, 395, 171	Dihydroxybenzoic acetate-digallate	6356
71	C ₂₇ H ₃₁ O ₁₆	11.6	611.3249	449, 287	cyanidin-3, 5-O-diglucoside	20351 2013
72	C ₃₂ H ₄₆ O ₉	13.9	575.32723	472	Ganoderic acid K	4539
73	C ₂₂ H ₂₂ O ₂₁	14.17	623.32565	471, 395, 171, 141	Galloyl-valoneic acid bilactone	263226
74	C ₂₀ H ₂₀ O ₁₄	14.17	485.25135	425, 333, 171	Digalloyl-hexoside	20719
75	C ₂₇ H ₃₁ O ₁₆	15.5	611.28587	287	cyanidin-3-O-sophoroside	1972
76	C ₂₇ H ₂₉ O ₁₅	15.64	595.28978	449, 287	cyanidin-3-O-rutinoside	12534
77	C ₂₇ H ₃₂ O ₁₆	15.65	613.17775	345, 315, 303	Suffruticoside B	1385
78	C ₂₇ H ₃₂ O ₁₆	15.65	613.17775	345, 315, 303	Suffruticoside D	1385
79	C ₂₁ H ₁₄ O ₁₃	15.66	475.24853	323, 171	Trigallic acid	34122
80	C ₂₉ H ₂₆ O ₁₅	15.67	615.3256	301	7-O-Methyl-cyanidin-3-O-(2"-galloyl)-galactosid	14055
81	C ₂₇ H ₂₂ O ₁₇	15.72	619.27619	303,153	Trigalloyllevoglucosan	11231
82	C ₂₇ H ₂₄ O ₁₈	15.76	637.25234	485,467, 171	Tri-galloyl-hexosid	8080
83	C ₃₀ H ₂₄ O ₁₂	15.88	579.33614	427, 409, 291	proanthocyanidin dimer	59025
84	C ₂₇ H ₂₂ O ₁₈	19.05	635.28597	483, 303	galloyl-HHDP-glucose (corilagin isomer)	1311 2013

Other Organic Compounds

Two isomers with high retention time 15.65min of peaks (77, 78) had the molecular formula ($C_{27}H_{32}O_{16}$), exhibited an ion $[M+H]^+$ at m/z 613.17775 which yielded major fragment ions at m/z 345.183592 resulted from loss of 152 (gallate moiety), 132 (pentose unit), $(M-C_2H_5O^-)$ and OH^- radical from glucose unit respectively. Fragment ions at m/z 315.183592 corresponding to (loss of 298, $C_{13}H_{15}O_8$) and 303.183592 (loss of $C_{13}H_{15}O_8 + CH_3$) indicating the presence of glucose, pentose and galloyl units in their structures. These compounds were assigned to suffruticoside B and D.³⁷

Pharmacological Evaluation

Antioxidant Capacity

Several studies associate antioxidant activities with terpenes compounds that are present in plant extracts. Results in Fig.-3A showed that DPPH radical scavenging activity of AME increased gradually by increasing the concentration of the extract. It reached to be $82.40 \pm 0.24\%$ at the concentration of $500 \mu\text{g/ml}$. This activity is very close to that of the synthetic antioxidant BHA at the same concentration ($92.85 \pm 0.35\%$). RPC also increased gradually by increasing the concentration of the extract. Figure-3B indicates that all tested concentrations exhibited a potent reducing power capacity even more than BHA at the same concentration ($500 \mu\text{g/ml}$) they recorded 1.41 ± 0.02 and 0.83 ± 0.02 (absorbance at 700 nm), respectively. Results in figure 3C indicated that the AME possesses the good iron metal chelating ability, it found to be $75.69 \pm 0.30\%$ at the concentration 3 mg/ml . On the other hand, ABTS radical scavenging activity results are given in Fig.-3D, which is demonstrated the same trend, i.e. increased gradually by increasing the concentration of the extract at concentrations beginning from 50 to $500 \mu\text{g/ml}$, where it is ranged from 38.35 ± 0.22 to $90.27 \pm 0.16\%$. Furthermore, the results of FRAP are expressed in $\mu\text{mol Trolox / g dry matter}$ (Fig.-3E). The investigated extract recorded very promising FRAP. The values obtained by FRAP assay were $2787 \pm 27.10 \mu\text{mol Trolox/100 g D.W.}$ at the concentration of $500 \mu\text{g/ml}$. Similar results were found by Elkarim et al., 2020¹⁰, they stated that the NMR analyses of the aerial parts of *S. Grantii* possess an abundance content of flavonoids and tannins which are mainly attributed to the ability of the isolated compounds to inhibit the free radical formation, therefore the high capacity of the compounds as antioxidant agents which clearly explains and confirms the high radical scavenging activity of the methanol extract.

Fig.-3: Antioxidant Activity of (AM) Extract using Different Antioxidant Assays: (A) Scavenging Ability on DPPH radical, (B) Reducing Power, (C) Metal Chelating Activity, (D) Scavenging Ability on ABTS Radicals, (E) Antioxidant Capacity FRAP Assay. Data are means $\pm$ Standard Deviation of Triplicate Experiments

Also, Our results are very close to the newly published results which suggested that *S. grantii* has different types of phenolic compounds and therefore possesses the ability to scavenge free radicals.³⁸ Data found by Gülçin 2010³⁹, explains that phenolic compounds, such as resveratrol, affect a wide range of intracellular mediators as well as inhibiting lipid peroxidation more strongly than the antioxidants BHA, BHT, α -tocopherol, and trolox.³⁹

On the other hand LC-MS/MS analysis clearly showed the presence of many phenolic compounds in (MSP) prepared from *S. grantii* aerial parts which possessed the ability to inhibit the formation of free radicals. These findings show and explain the importance of evaluating antioxidant activity, since it depends on the structural characteristics of organic compounds, in most cases due to the presence of terpenes and, mainly, phenolic groups.⁴⁰ Flavonoids and tannins contain many functional hydroxyl groups which are considered the main factor of antioxidant effects by scavenging free radicals and also maybe through chelating metal ions.⁴¹

MIC Determination and Antibiofilm Activity

The minimum inhibitory concentration of (AM) extract was against bacteria and yeast was illustrated in (Table-2). *Bacillus subtilis* was the most sensitive bacterial strain to it where the MIC value is 2 mg/ml. of (AME) exhibited excellent biofilm preventing agent against *S.aureus* at concentrations below the lethal dose without affecting bacterial growth where, concentrations 0.5, 0.25 and 0.125, mg/ml of (AME) reduce *S. aureus* biofilm formation with proportions 97.1, 92.8 and 78.1% respectively in dose-dependent manner (Fig.-4). while on the other hand, gram negative *P. aeruginosa* biofilm formation didn't affect by of AME (Fig.-5).

Further to evaluate the effect of (AM) extract on the surface topology of *S. aureus* biofilm matrix, inverted light microscopic analysis was carried out and the results showed the presence of thick biofilm matrix with micro colony formation in control, while of (AME) treated groups exhibited no colonization and *S. aureus* cells appear as scattered separated cells in dose-dependent manner (Fig.-6).

Fig.-4: Antibiofilm of (AM) Extract against *S.aureus* exhibit inhibition of Biofilm formation up to 98% at concentrations 2.0 -0.125 mg/ml

Table-2: Minimum Inhibitory Concentration (MIC) of of (AM) Extract (mg/ml*)

IC mg/ml	of extract (AM)	<i>S. a</i> ¹	<i>B.s</i> ²	<i>E. c</i> ³	<i>P. a</i> ⁴	<i>C. al</i> ⁵
6.0	MIC	5.0	2.0	4.0	5.0	4.0

*mg/ml; of (AM) extract milligram / milliliter, IC; initial concentration 1; *S. aureus* 2; *Bacillus subtilis* 3; *E. coli* 4; *P. aeruginosa*, and 5; *C. albicans*

Fig.-5: Antibiofilm of (AM) Extract against *p. aeruginosa* exhibit no activity against Biofilm formation in concentrations.

Fig.-6: Microscopic Images 100x of *S. aureus* biofilm A) represent positive control of *S. aureus*, cells aggregated together with biofilm matrix (B) represent negative control untreated TSB media, Panels (C to J) represent of (AM) extract treated samples at concentrations (4 to 0.3 mg/ml) respectively which exhibited prevention of biofilm production in a dose-dependent manner.

CONCLUSION

These data suggest that *S. grantii* aerial parts extract is a very good source as a natural antioxidant, antimicrobial and antibiofilm against bacterial pathogens agent, may be potential in the nutraceutical and pharmaceutical industries after the safety examinations.

ACKNOWLEDGEMENT

The authors would like to thank Dr. M. EL-Gibaly, Lecturer of Taxonomy and Consultant for Central Administration of Plantation and Environment, Egypt; for plants identification and supply.

REFERENCES

1. A.P. Rogerio, C.R. Cardoso, C. Fontanari, M.A. Souza, S.R. Afonso-Cardoso, EVG. Silva, N.S. Koyama, F.L. Basei, E.G. Soares and J.B. Calixto, *Glicobiology*, **17**, 795(2007), DOI:10.1093/glycob/cwm053
2. L.I. Zhuan-Hong, G.U.O. Han, X.U. Wen-Bin, G.E. Juan, L.I. Xin, A.L. Mireguli and H.E., *Journal of Chromatographic Science*, **54**(5), 805(2016), DOI:10.1093/chromsci/bmw016
3. M. George, L. Joseph and Ramaswamy, *African Journal of Traditional, Complementary and Alternative Medicines*, **6**(4), 554(2009), DOI:10.4314/ajtcam.v6i4.57206
4. B. Saranya, T. Sulfikarali, S. Chindhu, A. M. Muneeb, N. K. Leela and T. J. Zachariah, *Journal of Spices and Aromatic Crops*, **26**(1), 9(2017), DOI:10.25081/josac.2017.v26.i1.803
5. F. J. Firdaus, N. H. Sami, Soekamtoand J. Latip, *Rasayan Journal of Chemistry*, **14**(2), 676(2021), DOI:10.31788/RJC.2021.1425976
6. A. Karunanidhi, E. Ghaznavi-Rad, and R. A. Hamat, *BioMed Research International*, **2018**,1(2018), DOI:10.1155/2018/9845075
7. S. E. Desouky, M. S. El-Gamal, A. F. Mohammed, *World Applied Sciences Journal*, **32**, 731(2014), DOI:10.13140/RG.2.1.2311.9768
8. A. Hamed, A. S. Abdel-Razek, M. Araby, M. Abu-Elghait, D.G El-Hosari, M. Frese, H. S. M. Soliman, H. G. Stammler, N. Sewald and M. Shaaban, *Natural Product Research*, 1-9, (2020), DOI:10.1080/14786419.2020.1741583
9. E. Ahmed, W.A. Elkhateeb, H.A.A. Taie, M. Rateb and W. Fayad, *Journal of Applied Pharmaceutical Science*, **7**(1), 98(2017), DOI:10.7324/JAPS.2017.70113
10. A.S.A. Elkarim, S. Abdelaziz, H.G. Attia, H.A.A. Taie and R. Monir, *Pharmacognosy Journal*, **12**(6), 1421(2020), DOI:10.5530/pj.2020.12.196
11. M.B. Hossaina, N.P. Bruntonb, C. Barry-Ryana, A.B. Martin-Dianaa, and M. Wilkinsonc, *Rasayan Journal of Chemistry*, **1**(4), 751(2008).
12. H.A.A. Taie, H.I. Abd-Alla, S.A. Ali and H.F. Aly, *International Journal of Pharmacy and Pharmaceutical Sciences*, **7**(6), 311(2015).
13. Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, Twenty-fifth Informational Supplement, CLSI Document, M100-S29, Wayne, Pa, USA. 2019.
14. K. R. C.Goly, Y. Soro, A. Dadie, A. B. B. Kassi and D. J. E. Marcellin, *Rasayan Journal of Chemistry*, **8**(4), 396(2015).
15. Y.Yinjun, S. Xinguang, L. Jinjun, L. Kang, S. Chen, B. Ma and B. Guo, *Molecules*, **21**(10), 1306(2016), DOI:10.3390/molecules21101307
16. N. Fabre, I. Rustan, E. Hoffmann and J. Quetin-Leclercq, *Journal of the American Society for Mass Spectrometry*, **12**(6), 707(2001), DOI:10.1016/S1044-0305(01)00226-4
17. I.M. Abu-Reidah, D. Arráez-Román, A. Segura-Carretero, and A. Fernández-Gutiérrez, *Food Research International Journal*, **51**, 354(2013), DOI:10.1016/J.FOODRES.2012.12.033
18. B. Anghel, E. R. Javier, A. Carlos, B. Sepúlveda and, M. J. Simirgiotis, *Molecules*, **19**(11), 17400(2014), DOI:10.3390/molecules191117400
19. I.M. Abu-Reidah, S. A. Mohammed, M. J. Rana and A. Segura-carretero, *Food Chemistry*, **166**, 179(2015), DOI:10.1016/j.foodchem.2014.06.011
20. D. Ivayla, B. Ilian, k.Violeta, P. Dobson, G. McDougall and D. Stewart, *International Journal of Agriculture Science and Research*, **3**(3), 127(2013).
21. L. Regazzoni, E. Arlandini, D. Garzon, N. A. Santagati, G. Beretta and M. R. Facino, *Journal of Pharmaceutical and Biomedical Analysis*, **72**, 202(2013), DOI:10.1016/j.jpba.2012.08.017
22. M. M. Shabana, A. M. El Sayed and M. F. Yousif, *Pharmacognosy Magazin*, **7**, 298(2011),

DOI:10.1016/J.FOODCHEM.2014.06.011

23. P. Kachlicki, A. Piasecka, M. Stobiecki and T. Marczak, *Molecules*, **19**(11), 17400(2014), DOI:10.3390/molecules21111494
24. H. Rini , M. Lamek, I.A. Ketut and S. Partomuan , *Rasayan Journal of Chemistry*, **12**(3), 1822(2019), DOI:10.31788/RJC.2019.1235353
25. C. Yeqing, Y. U. Hongli, W. U, Hao, P. Yaozong Pan , W. Kuilong, J.I.N. Yangping Jin and Z. Chengchao. *Molecules*, **20**(10), 18352(2015), DOI:10.3390/molecules201018352
26. S. Sabina , L. Dae-Young, P. Ji-Hae, C. Jin-Gyeong S. Woo-Duck , K. Hee Cheol , J. Yong-Jin Y. Seung-Woo , B. Myun-Ho and B, *Journal of the Korean Society for Applied Biological Chemistry*, **55**, 689(2012), DOI:10.1007/s13765-012-2133-2
27. R. Perestrelo, Y. Lu, S. A. O. Santos, A. J. D. Silvestre, C. P. Neto, J. S. Câmara and S. M. Rocha, *Food Chemistry*, **135**, 94(2012), DOI:10.1016/j.foodchem.2012.04.102
28. H. McNab, E.S.B. Ferreira, A.N. Hulmea and A. Quye, *International Journal of mass Spectrometry*, **284**, 57(2009), DOI:10.1016/j.ijms.2008.05.039
29. W. Schänzer, H. Geyer, A. Gotzmann, U Mareck (eds.) Recent Advances in Doping Analysis (14), Sport BuchStrauss GmbH., Köln, pp. 91-100 (2006).
30. C. W. Kirby, T. Wu, R. Tsao and L. M. C Jason, *Phytochemistry*, **94**, 284(2013), DOI:10.1016/j.phytochem.2013.06.017
31. S. De Pascual-T, J. C. Rivas-Gonzalo, *The Royal Society of Chemistry*, pp.48-62(2003).
32. P. Monique, V.S Christine, P. Konstantinos, E. Claire, S. Jean-Paul , M. Aymeric and B. Denis, *Rapid Communication in Mass Spectrometry*, **17**(12), 1297(2003), DOI:10.1002/rcm. 1054
33. H.Q. Zhao, X.Wang, H.M. Li, B. Yang, H.J. Yang, and L. Huang, *Molecules*. **18**(8), 9755(2013), DOI:10.3390/molecules18089755
34. W. Zhijun, W. Xiaowei, M. Chen, C. Mo, H. U Hongyan, C. Jie, C.Tuanyao and W. Hong, *Molecules*. **24**(6), 1058(2019), DOI:10.3390/molecules2406105
35. M. Sanz, E. Cadahía, , E. Esteruelas, A.M. Muñoz , S.B. Fernandez de, T. Hernandez, and I. Estrella, *Journal of Agriculture and Food Chemistry*, **58**(17),9631(2010), DOI:10.1021/JF102718T
36. B. Fröhlich, R. Niemetz, G. G. Gross, Gallotannin, *Planta Medica*, **216**(1), 168(2002), DOI:10.1021/jf102718t
37. H. Matsuda, T. Ohta, A. Kawaguchi and M. Yoshikawa, *Chemical and Pharmaceutical Bulletin*, **49**(1), 69(2001), DOI:10.1248/cpb.49.69
38. N.P. Dasari, B.G Rao, E.S Rao, T.M Rao and V.S. Praneeth, *Free Radicals Antioxidants*, **2**(2), 68(2012), DOI:10.5530/ax.2012.2.12
39. I. Gülçin, *Innovative Food Science and Emerging Technologies*, **11**, 210(2010), DOI:10.1016/j.ifset.2009.07.002
40. A.C. Munhoz, B. R.Minozoo, L. S. Cruz, T. L. Oliveira, W. M. Machado, A.V Pereira, D. Fernandes, F. A. Manente, J. C. Velloso, A. Nepel and A. Barison. Oliveria, *Planta Medica*, **80**(06), 458(2014), DOI:10.1055/s-0034-1368300
41. S.Kumar, A. Mishra and A. K. Pandey, *BMC Complementary and Alternative Medicine*, **13**(1),120 (2013), DOI:10.1186/1472-6882-13-120

[RJC-6165/2020]