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

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**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*<sub>f</sub> values. It was found that flavonols, flavones, 1soflavone, 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.

**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 as flavonoids, saponins and diterpenes. 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 $R_t$-values, MS/MS fragmentation trends and molecular formulae.\(^3\) There is a growing tendency in comparing the phytochemical constituents isolated from plants and their pharmacological actions.\(^4\) 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.\(^5,6\) 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\(^a\), 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\(^b\), analytical grade; Darmstadt, Germany). 2,2-Diphenyl-2-picrylhydrazyl (DPPH), potassium ferri-cyanide, Trolox, ferrozine, 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonicacid (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 and100% 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\(_3\)OH 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 C\(_2\)H\(_5\)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 µl stock (50/ 1000 µl) was diluted in 1000 liter reconstitution solvent. Finally, a concentration of 1 µg/ µl was injected. Inject 25 l µl of total extract and 25 µl 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. DPPH, ABTS percentage, FRAP and reducing power values of the studied extract and compounds were calculated. Metal chelating activity assay was determined according to the previous protocol.

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 ATCC8739andC. albicans ATCC 10213according to the Clinical and Laboratory Standards Institute(CLSI) (2019). 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 200µl of 1: 100 v/v of overnight cultures of the S. aureus and P. aeruginosain TSB inoculum size 5 × 10⁵ 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. aeruginosain overnight cultures were diluted 1: 100 in TSB to produce 1x10⁵ 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 200l 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 Ck40Japan) 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). 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.

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.
Synadenium grantii Hook f.
Phenolic Acids

The HPLC/QTOF-MS² analysis (in positive ion mode) revealed the presence of six isomers of monocaffeoylquinic acid like-structures (31-36), including chlorogenic acid (3-O-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-caffeoyl]⁺ or [quinic+H]⁺, and 163 [M+H-quinoyl]⁺ or [caffeic+H]⁺, together with a third positive ion at m/z 137amu. 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. 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 flavonoids 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/z595.16598 produced fragment ions at m/z 541( loss of 44amu, C-CH₂-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 146amu) 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 (Isororientin).

Peak 66 produced a molecular anion at m/z 609.18197 and diosmetin aglycon, MS² fragment at m/z 301.0719 (M-308, neohesperidoside) 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 rutinoys unit). Peaks (43-45) had \([\text{M}+\text{H}]^+\) 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 \([\text{M}+\text{H}-(\text{glucose}+\text{CO})]\), 257 \([\text{M}+\text{H}-(\text{glucose}+\text{CO}+\text{H}_2\text{O})]\), 229 \([\text{M}+\text{H}-(\text{glucose}+2\text{CO}+\text{H}_2\text{O})]\), 165\([\text{M}+\text{H}-(\text{glucose}+3\text{CO}+3\text{H}_2\text{O})]\) and 153 corresponding to \([\text{M}+\text{H}-(\text{glucose}−\text{H}_2\text{O}−\text{C}_8\text{H}_6\text{O}_3)]^+\) 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). Compound 48 was identified as quercetin 3-O-glucuronide with a pseudomolecular ion at m/z 479.08283 \([\text{M}+\text{H}]^+\) and fragment ion 303.0497 after the loss of a glucuronide unit (176 amu). 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. 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, 287, 270, 255, 245, 229, 218, 181, 165, 153 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. 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 maritimein. 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, 255.138452, 229.0486, 181.014252, 165.0164, 153.019952, 147.0237 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. Aglycons Peaks (60-62) showing a \([\text{M}+\text{H}]^+\) 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. 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 \((\text{C}_7\text{H}_4\text{O}_3\cdot\text{H})\) radical, M-136) from the base molecular ion. Isoflavone Single peak (40) of ipriflavone with protonated molecular ion \([\text{M}+\text{H}]^+\) at 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). Anthocyanins Derivatives One peak with retention time 0.73min and formula \(\text{C}_2\text{H}_2\text{O}_{12}\) 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 MS² fragments 449, 287, resulted in the loss of 324amu for two glucose units was characterized as cyanidin-3, 5-O-diglucoside. Two isomers (75, 76) at m/z = 611.2858, 595.28978 and MS² 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

**Amino Acids**

**Aliphatic Amino Acid**

Four isomers of 2-Aminobutyric acid and three derivatives of glycine (C₄H₉NO₂) 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+H₂O), 57.06333, 87.0447 (loss of NH₃) 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-H₂O), and product ions at m/z 74.05824, [M-(CO+ H₂O)] 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 (Rò.65 min) with the molecular formulaC₄H₇N₁O₄ and having the precursor ion at m/z 134.04499 in the ESI+ mode, was been tentatively proposed as L-aspartic acid Peak(15) which showed product ions at m/z 115.03751, 116.03751, 117.052052 (loss of H₂O).32

The compounds (25) and (26) possessed a molecular ion at m/z146.11736 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 C₅H₈O₄]+ are characteristic of transzeatin-riboside, compound (27). A component (16) with molecular ion [M+H]+ at m/z 152.05477 was proposed to be guanine with fragment ions at m/z 135.029852 (loss of NH₃), 111.0206, 110 (loss of 42amu, -HNCNH), 68.024552 [loss of 84amu, NH₃(-HCNCCO)]. Adenosine and 2’-deoxyguanosine monohydrate both had identical formula C₁₀H₁₃N₅O₄ with different (Rt, 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-C₅H₁₀O₃]+. 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-H₂O), 274.10598, (M-2H₂O), 232.10598, (M-H₂O+C₂H₄NO.) revealed that peak (21) was tentatively proposed as N-acetyleneuraminic acid. There was a dominant neutral loss of 17amu, which could be due to the loss of NH3 with fragment ion at m/z 149.09353and 120.07898, (M-CO+H₂O) in positive ionization mode. Compound (28) with a precursor ion [M+H]+ at m/z 160.08616 and with formula C₉H₁₁O₁ 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 C₂₀H₂₂O₈. These fragments were fitted to the resveratrol 3-O-glucoside, peak...
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-CH₂. 153.093652, (M-149, C₉H₉O₂•), 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-valonieic acid bilactone. Four isomers of gallic acid derivatives (74, 79, 81, 82) with molecular formula C₂₁H₁₄O₁₃, C₂₀H₂₀O₁₄, C₂₇H₂₂O₁₇ and C₂₇H₂₄O₁₈ exhibited molecular ions at m/z 475.24853, 485.25135, 619.27619 and 637.25234 with fragment ions at 323, 425, 303, 153, 171, 485, 467 were assigned as Trigallic acid, Trigalloyllevoglucosan, di and tri galloyl hexoside. 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 galloylgucose) as the main fragment.

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

| Peak | Formulae | Rt (min) | LC-MS [M+H]+ | QTOF–MS-MS | Tentative Assignment | Intensity |
|------|----------|----------|---------------|-------------|----------------------|-----------|
| 1    | C₁₅H₁₂O₅ | 0.53     | 277.10608     | 208, 193, 207, 163, 121, 125, 235, 231, 229 | Capensine | 5841 |
| 2    | C₁₉H₁₆N₂O₅ | 0.57 | 104.07079 | 58, 57 | N-Methyl-DL-Alanine | 3498 |
| 3    | C₁₅H₁₆O₇ | 0.57 | 104.07079 | 58, 57 | N,N-Dimethylglycine | 3498 |
| 4    | C₁₅H₁₄N₂O₇ | 0.57 | 104.07079 | 58, 57 | N,N-Dimethylglycine hydrochloride | 3498 |
| 5    | C₁₅H₁₄O₇ | 0.57 | 104.07079 | 58, 87 | L-α-Amino-n-butryic acid | 3498 |
| 6    | C₁₅H₁₄O₇ | 0.57 | 104.07079 | 58, 57 | L-2-Aminobutyric acid | 2755 |
| 7    | C₁₅H₁₄O₇ | 0.57 | 104.07079 | 58, 57 | 2-Aminoisobutyric acid | 3498 |
| 8    | C₁₅H₁₄N₂O₇ | 0.57 | 104.07079 | 58, 57 | D-L-2-Aminobutyric acid | 3498 |
| 9    | C₁₅H₁₄O₇ | 0.61 | 120.06603 | 102, 60 | D-β-Homoserine | 2382 |
| 10   | C₁₅H₁₄N₂O₇ | 0.61 | 120.06603 | 102, 60, 84 | L-β-Homoserin | 2382 |
| 11   | C₁₅H₁₄N₂O₇ | 0.61 | 120.06603 | 102, 74, 84, 56 | L-allo-threonine | 2382 |
| 12   | C₁₅H₁₄N₂O₇ | 0.62 | 120.06603 | 102, 85, 76, 74 | D-β-Homoserine [(S)-3-Amino-4-hydroxybutyric acid (-)] | 2382 |
| 13   | C₁₅H₁₄N₂O₇ | 0.63 | 120.06566 | 102, 74, 92, 73, 56 | L-Homoserine | 2755 |
| 14   | C₁₅H₁₄N₂O₇ | 0.61 | 120.06566 | 102, 85 | L-β-Homoserine [(R)-3-Amino-4-hydroxybutyric acid (+)] | 2755 |
| 15   | C₁₅H₁₄N₂O₇ | 0.65 | 134.04499 | 115, 117, 116 | L-Aspartic acid | 1917 |
| 16   | C₁₅H₁₄O₇ | 0.65 | 152.05747 | 135, 110, 111, 68 | Guanine | 2534 |
| 17   | C₁₅H₁₄O₇ | 0.66 | 275.09285 | 169, 153, 121, 125, 235, 231, 229 | Phloretin | 1255 |
| 18   | C₁₅H₁₄N₂O₇ | 0.68 | 268.10440 | 136, 135, 119, 118, 116, 107 | Adenosin | 3699 |
| No. | Molecular Formula | Molecular Weight | Exact Mass | MS/MS Fragment | Description |
|-----|------------------|------------------|------------|----------------|-------------|
| 19  | C_{20}H_{25}O_{3} | 391.13934        | 231, 287, 269ni, 265ni, 147, 146 | Resveratrol 3-O-glucoside | 1561 |
| 20  | C_{14}H_{16}NO_{3} | 120.06603        | 74, 102, 84 | L-Threonine | 2368 |
| 21  | C_{13}H_{15}NO_{9} | 310.11343        | 292,274, 232, 169, 103 | N-Acetylneuraminic acid | 1955 |
| 22  | C_{6}H_{8}NO_{2}  | 104.07061        | 87, 69     | γ-Amino-n-butyric acid | 3565 |
| 23  | C_{10}H_{13}N_{4}O_{4} | 268.10445 | 150 | 2′-Deoxyguanosine monohydrate | 3699 |
| 24  | C_{23}H_{25}O_{12} | 494.14094        | 374, 331, 181, 121 | Malvidin 3-glucoside | 883 |
| 25  | C_{13}H_{15}NO_{2} | 146.11736        | 86, 69, 55 | L-β-Homoleucine-HCl | 1613 |
| 26  | C_{13}H_{15}NO_{2} | 146.11736        | 86, 68, 55, 83 | L-β-Homoisoleucine hydrochloride | 1613 |
| 27  | C_{15}H_{21}N_{5}O_{3} | 352.16039 | 220, 136, 333, 251, 202 | Trans-Zeatin-riboside | 1271 |
| 28  | C_{6}H_{11}NO_{2}  | 166.08616        | 149, 120, 118, 105 | L-(−)-Phenylalanine | 130007 |
| 29  | C_{15}H_{16}O_{3}  | 239.07192        | 103, 115 | Daphniflucin | 1421 |
| 30  | C_{10}H_{12}O_{7}  | 353.06511        | 165, 310, 254, 208, 147 | Chlorogenic acid | 52946 |
| 31  | C_{16}H_{16}O_{6}  | 355.10299        | 193, 163, 137 | l-Caffeoylquinic acids | 52946 |
| 32  | C_{16}H_{16}O_{6}  | 355.10299        | 193 | 1-O-Caffeoylquinic acids | 52946 |
| 33  | C_{16}H_{16}O_{6}  | 355.10299        | 193 | 1-O-Caffeoylquinic acids | 52946 |
| 34  | C_{16}H_{16}O_{6}  | 355.10299        | 193 | 3-Caffeoylquinic acids | 52946 |
| 35  | C_{16}H_{16}O_{6}  | 355.10299        | 193 | 4-Caffeoylquinic acids | 52946 |
| 36  | C_{16}H_{16}O_{6}  | 355.10299        | 193, 162, 192, 116 | 5-Caffeoylquinic acids | 52946 |
| 37  | C_{27}H_{30}O_{15} | 595.16598        | 541, 475, 355, 325 | Apigenindi-C-hexoside | 839 |
| 38  | C_{27}H_{30}O_{15} | 595.16598        | 457, 439, 490, 307, 295 | Di-C,C hexosyl-Apigenin | 835 |
| 39  | C_{27}H_{30}O_{15} | 595.16598        | 449, 336, 309, 282, 271 | Saponarin | 839 |
| 40  | C_{38}H_{30}O_{5}  | 281.11585        | 165, 137, 105, 102 | Ipriflavone | 1649 |
| 41  | C_{27}H_{36}O_{16} | 611.16034        | 303, 465, 247, 153 | Quercetin-3-Oβ-gluco-pyranosyl-7-Oα rarhamno-pyranoside | 9795 |
| 42  | C_{27}H_{36}O_{16} | 611.16034        | 302, 465, 322, 285, 181, 153, 272 | Rutin | 9795 |
| 43  | C_{21}H_{26}O_{12} | 465.10307        | 303, 302, 257, 201, 165, 153 | Speriaesoside | 20911 |

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| No. | Molecular Formula | Molecular Weight (Da) | Mass (Da) | Retention Time (min) | Identified Compounds                                                                 | Literature References |
|-----|------------------|-----------------------|-----------|----------------------|-------------------------------------------------------------------------------------|----------------------|
| 44  | C_{21}H_{30}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_{30}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_{10}S_{2} | 3.63         | 479.08085 | 465, 301, 293, 261 | 4-Methoxy-3-indolylmethyl glucosinolate                                               | 599                 |
| 47  | C_{20}H_{26}O_{4}  | 3.64                  | 325.14386 | 221, 165, 135, 283, 269, 240, 222, 189 | Glaabranine                                                                        | 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_{10}H_{12}O_{6}  | 3.76                  | 347.14937 | 239, 227, 213, 143 | Gibberelin A3                                                                       | 624                 |
| 50  | C_{21}H_{30}O_{11} | 3.84                  | 449.10801 | 353, 287 | Luteolin –C-hexosyl                                                               | 136475               |
| 51  | C_{21}H_{30}O_{11} | 3.84                  | 449.10801 | 353, 412, 368, 330, 328 | Homo-orientin (Isoorientin)                                         | 136475               |
| 52  | C_{21}H_{30}O_{10} | 3.92                  | 433.11541 | 287, 229 | Kaempferol-3-O-rhamnose                                                        | 708                 |
| 53  | C_{19}H_{25}O_{10} | 3.92                  | 433.11541 | 287 | Rhamnosyl-kaempferol                                                                | 708                 |
| 54  | C_{21}H_{30}O_{10} | 3.92                  | 433.11541 | 287 | Kaempferol 7-O-rhamnoside                                                        | 137258               |
| 55  | C_{21}H_{30}O_{11} | 4.02                  | 449.10825 | 287, 286, 258, 153, 165 | kaempferol-3-O-glucoside                                                            | 135868               |
| 56  | C_{21}H_{30}O_{11} | 4.02                  | 449.10825 | 287, 286, 258, 185, 165, 229, 257 | kaempferol-3-glucoside                                                               | 135868               |
| 57  | C_{21}H_{30}O_{11} | 4.02                  | 449.10825 | 287, 284, 201, 177 | Luteolin-4′-O-glucoside                                                              | 135868               |
| 58  | C_{21}H_{30}O_{11} | 4.02                  | 449.10825 | 287, 284, 258, 201 | Luteolin-7-O-glucoside                                                               | 135868               |
| 59  | C_{21}H_{30}O_{11} | 4.02                  | 449.10825 | 287, 284, 258, 152, 135, 123, 107, 105 | Maritimein                                                                          | 135868               |
| 60  | C_{15}H_{16}O_{6}  | 4.26                  | 287.05534 | 231, 165, 258, 197 | Fisetin                                                                             | 36814                |
| 61  | C_{15}H_{16}O_{6}  | 4.26                  | 287.05539 | 231, 229, 153, 165 | Kaempferol                                                                           | 36727                |
| 62  | C_{15}H_{16}O_{6}  | 4.26                  | 287.05539 | 153 | Luteolin                                                                             | 36727                |
| 63  | C_{21}H_{30}O_{11} | 4.31                  | 449.10805 | 303, 287, 270 | Quercetin-3-rhamnoside                                                              | 136374               |
| No. | Formula     | MW   | M/z   | Components Description                                                                 | References |
|-----|-------------|------|-------|---------------------------------------------------------------------------------------|------------|
| 64  | C$_{21}$H$_{30}$O$_{11}$ | 346.2 | 449.10805 | 165,229, 147, 153, 121 Quercetin-3-O-rhamnose                                           | 136374     |
| 65  | C$_{34}$H$_{32}$N$_{4}$O | 548.3 | 383.18691 | 216, 351, 281, 145, 132 1-(2,3-dibenzimidazolyl-2-propyl)-2-methoxybenzene               | 1142       |
| 66  | C$_{25}$H$_{32}$O$_{15}$ | 609.2 | 609.18197 | Neodiosmin                                                                              | 720        |
| 67  | C$_{21}$H$_{20}$O$_{11}$ | 303.1 | 449.1077 | 165, 229, 147, 153, 121 Quercetin-3-O-rhamnopyranoside                                  | 137258     |
| 68  | C$_{21}$H$_{16}$O$_{13}$ | 5.13  | 477.10238 | 303 Ellagic acid acetyl pentoside                                                       | 37564      |
| 69  | C$_{24}$H$_{18}$O$_{15}$ | 10.91 | 547.20978 | 471, 395, 171 Dihydroxybenzoic acid-digallate                                           | 6356       |
| 70  | C$_{27}$H$_{31}$O$_{16}$ | 11.6  | 611.3249 | 449, 287 cyanidin-3, 5-O-diglucoside                                                   | 20351 2013 |
| 71  | C$_{27}$H$_{20}$O$_{15}$ | 13.9  | 575.32723 | 472 Galloxy-valonoe acid bilactone                                                      | 4539       |
| 72  | C$_{22}$H$_{22}$O$_{21}$ | 14.17 | 623.32565 | 471, 395, 171, 141 Galloyl-valonoe acid bilactone                                       | 263226     |
| 73  | C$_{20}$H$_{20}$O$_{14}$ | 14.17 | 485.25135 | 425, 333, 171 Digalloyl-hexoside                                                       | 20719      |
| 74  | C$_{27}$H$_{22}$O$_{16}$ | 15.5  | 611.28587 | 287 cyanidin-3-O-sophoroside                                                           | 1972       |
| 75  | C$_{27}$H$_{22}$O$_{15}$ | 15.64 | 595.28978 | 449, 287 cyanidin-3-O-rutinoside                                                       | 12534      |
| 76  | C$_{27}$H$_{15}$O$_{16}$ | 15.65 | 613.17775 | 345, 315, 303 Suffruticoside B                                                         | 1385       |
| 77  | C$_{27}$H$_{12}$O$_{15}$ | 15.65 | 613.17775 | 345, 315, 303 Suffruticoside D                                                          | 1385       |
| 78  | C$_{27}$H$_{12}$O$_{13}$ | 15.66 | 475.24853 | 323, 171 Trigallic acid                                                                | 34122      |
| 79  | C$_{27}$H$_{12}$O$_{15}$ | 15.67 | 615.3256 | 301 7-O-Methyl-cyanidin-3-O-(2'-galloyl)-galactosid                                      | 14055      |
| 80  | C$_{27}$H$_{20}$O$_{17}$ | 15.72 | 619.27619 | 303, 153 Trigalloylevoglucosan                                                         | 11231      |
| 81  | C$_{27}$H$_{22}$O$_{18}$ | 15.76 | 637.25234 | 485, 467, 171 Tri-galloyl-hexosid                                                       | 8080       |
| 82  | C$_{30}$H$_{24}$O$_{12}$ | 15.88 | 579.33614 | 427, 409, 291 proanthocyanid dimer                                                      | 59025      |
| 83  | 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 15.65 min of peaks (77, 78) had the molecular formula \( \text{C}_{27}\text{H}_{32}\text{O}_{16} \), exhibiting an ion \([\text{M}+\text{H}]^+\) at \( m/z \) 613.17775 which yielded major fragment ions at \( m/z \) 345.183592 resulting from loss of 152 (gallate moiety), 132 (pentose unit), \((\text{M}-\text{C}_2\text{H}_3\text{O})\) and OH radical from glucose unit respectively. Fragment ions at \( m/z \) 315.183592 corresponding to (loss of 298, \( \text{C}_{13}\text{H}_{15}\text{O}_8 \)) and 303.183592 (loss of \( \text{C}_2\text{H}_3\text{O}_7+\text{CH}_3 \)) 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 82.40±0.24% at the concentration of 500 μg/ml. This activity is very close to that of the synthetic antioxidant BHA at the same concentration (92.85±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 μg/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 ±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 μg/ ml, where it is ranged from 38.35±0.22 to 90.27 ± 0.16%. Furthermore, the results of FRAP are expressed in μmol Trolox / g dry matter (Fig.-3E). The investigated extract recorded very promising FRAP. The values obtained by FRAP assay were 2787 ± 27.10 μmol Trolox/100 g D.W.at the concentration of 500 μg/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.
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.\(^\text{38}\) Data found by Gülçin 2010\(^\text{39}\), 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.\(^\text{39}\)

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.\(^\text{40}\) 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.\(^\text{41}\)

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

| IC mg/ml | MIC | S. a<sup>1</sup> | B. s<sup>2</sup> | E. c<sup>3</sup> | P. a<sup>4</sup> | C. a<sup>5</sup> |
|----------|-----|-----------------|-----------------|-----------------|----------------|-----------------|
| 6.0      |     | 5.0            | 2.0             | 4.0             | 5.0           | 4.0             |

*mg/ml; of (AM) extract milligram / milliliter, IC; initial concentration1; S. aureus2; Bacillus subtilis3; E. coli4; P. aeruginosa, and5; C. albicans

Fig.-5: Antibiofilm of (AM) Extract against p. aurginosa 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 (CtoJ) 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.
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