

Phytochemical and Antioxidant Evaluation of the Flavonoids and Tannins from *Synadenium grantii* Hook f, (Ephorbiaceae)

Asmaa S. Abd Elkarim^{1,*}, Sahar Abdelaziz², Hany Gouda Attia^{3,4}, Hanan A.A. Taie⁵, Rafik Monir⁶

Asmaa S. Abd Elkarim^{1,*}, Sahar Abdelaziz², Hany Gouda Attia^{3,4}, Hanan A.A. Taie⁵, Rafik Monir⁶

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

²Pharmacognosy department, Faculty of Pharmacy, Zagazig University, 44519 Zagazig, EGYPT.

³Department of pharmacognosy, faculty of pharmacy, Najran University, Najran 55461, SAUDI ARABIA.

⁴Department of pharmacognosy, faculty of pharmacy, Kafr EL- Shiekh university, EGYPT.

⁵Plant Biochemistry Department, Division of Agriculture and Biological Researches, National Research Centre, 33 El-Bohouth St. (Former El-Tahrir St.), Dokki 12622, Giza, EGYPT.

⁶Pharmacognosy department, Faculty of Pharmaceutical sciences and drug manufacturing, Misr University for Science and Technology, 6th October City, EGYPT.

Correspondence

Asmaa S. Abd Elkarim

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

E-mail: asmaa_nrc@yahoo.com

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ABSTRACT

Background: The methanol extract (MeE) of the aerial parts from *Synadenium grantii* Hook f, (Ephorbiaceae) has more Polyphenols and used to treat several diseases. **Objective:** The aim of this study is isolation, purification and identification of Polyphenols that showed good antioxidant activity.

Materials and Methods: In the present study, purification of the MeOH-H₂O extract (70:30) from the air-dried aerial parts of *S. grantii* resulted by different chromatographic tools. The antioxidant activities were evaluated for the examined compounds by several methods using DPPH, Reducing power, ABTS and FRAP assays. **Results:** A total of 14 compounds were isolated from this plant species for the first time. The results obtained showed that all the examined compounds possess remarkable antioxidant activity through all assays especially compounds 2 and 4 where they showed the highest antioxidant activity among the other compounds and very close to the results of the standard compound BHA. **Conclusion:** The compounds examined have exhibited antioxidant activity with promising value as natural source antioxidant drugs.

Key words: *Synadenium grantii*, *Ephorbiaceae*, Flavonoids, Tannins, Antioxidant.

INTRODUCTION

Plants are an significant source of natural antioxidants that counter oxidative stress induced by an increase in free radicals / ROS such as superoxide O₂⁻ anion, hydroxyl radical HO⁻ and peroxide as well¹. The phytoconstituents research of these bioactive compounds in a plant is therefore of paramount importance, resulting to further biological and pharmacological studies. This study can be presented to develop herbal medicines that require the isolation of bioactive compounds from medicinal plants². In recent research advances around the world the properties of medicinal plants have been studied because of no side effects, safety in contrast to the synthetics that are unsafe to human and environment. As a result people are more favourable to use natural compounds obtained from plants. It is great interest for scientists to focus on the search of biologically active compounds from plants as a natural source of useful drugs against infectious diseases³. Biological studies of herbal products (active ingredient) may be due to a variety of chemical components including flavonoids, phenolics, glycosides, anthocyanins, tannins, etc.⁴. Natural products are considered a drug source for several years, and have been chosen to interact with biological studies as a target⁵. The bioactive constituents in plant Phenolic compounds (PCs) for example, flavonoids are greater antioxidant activity⁶. Several studies have shown that many plants are rich source of antioxidants. One of these plants is *S. grantii* (Ephorbiaceae) that a plant species which belongs to a small genus of about 15 species. Plants from this genus for example

(*S. umbellatum*, *S. glaucescens*, *S.compactum*, *S.claycinum*) have different biological activities as anti-ulcer activity⁷, antiasthmatic effect⁸, anti-tumoral and anti-angiogenic effects⁹, antinociceptive anti-inflammatory, anti-plasmodial activity¹⁰. The aim of this work is to study the antioxidant activity of the isolated pure compounds from MeOH-H₂O extract (70:30) of *S. grantii* for the first time.

MATERIALS AND METHODS

General methods

NMR spectra were recorded at 400 MHz for NMR and 100 MHz for ¹³C NMR on Bruker AMX-400, Avance 400, and Avance 300 spectrometers (Bruker, Rheinstetten, Germany) with standard pulse sequences operating at 400MHz in ¹H NMR and 100, MHz in ¹³CNMR.chemical shifts was expressed in δ values (ppm) using DMSO as the internal standard. QTOF-HR-TOF4500scan (HPLC system was coupled to quadruple time-of-flight mass spectrometer), Column chromatography (CC) was carried out on Polyamide 6S (Riedel-DeHaen, Hannover, Germany) and sephadex LH-20 (Fluka, Pharmazia, Uppsala, Sweden).

Plant material

A sample of *S. grantii* (aerial parts) was collected from National Research Centre (NRC) garden, Egypt in 2017 (flowering stage). The plant species was identified by Dr. M. EL-Gibaly, Lectuer of Taxonomy and Consultant for Central Administration of Plantation and Environment. Avoucher sample (No: A79.) was deposited at chemistry of tanning

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materials and leather technology department, (NRC), Dokki, Cairo, Egypt.



Extraction and isolation

The aerial parts of *S. grantii* (1.75 kg) were crushed and extracted by macération with 70% aqueous CH₃OH, the methanol water extract was evaporated under reduced pressure and lyophilized (250gm). A sample (150gm) of the dry extract was fractionated by chromatography on polyamide 6S column (60x4cm). The column was eluted with H₂O and H₂O-MeOH step gradient by decreasing polarities at a flow rate 1ml/minute was then carried out. The bands migrated along the column were traced under UV light during elution to note their characteristics and to control the fractionation process as well. The obtained fractions (1litre, each) were subjected to paper chromatography using BAW and 15% AcOH/H₂O as a developing solvents, and the similar fractions were collected together to give four major fractions (I-IV). The sub-fractions were then subjected to different chromatographic techniques including, cellulose and repeated sephadex LH-20 column using eluents of different polarities. This led to the isolation and purification of six flavonoid compounds (1-6). Some sub fractions precipitated with diethyl ether, then subjected to sephadex LH-20 column with absolute ethanol to give compounds (7-14).

Separation of fraction I (8g) on sephadex column LH-20 CC with saturated n-Bu-H₂O gave three sub-fractions, then the first and third sub-fractions were purified on sephadex LH-20CC using EtOH-H₂O (1:1) as solvent system and gave a pure samples of 1 (28 mg) and 2 (35 mg). The second sub-fraction was subjected to sephadex LH-20CC with EtOH-H₂O (30:70) as an eluent to give two major compounds which were further purified on sephadex CC using MeOH (HPLC) as solvent to give pure samples of 3 (30 mg) and 4 (35 mg).

Fraction II (6.5 gm) was chromatographed on sephadex LH-20CC and eluted with EtOH for further times to afford pure compounds of 5 (25 mg) and 6 (18 mg)

Fraction III (13 gm) has been separated on cellulose column chromatography with n-BIW as eluent to give six sub-fractions. Four of them subjected to sephadex LH-20CC with absolute EtOH to give four pure samples of 7(20 mg), 8(22 mg) and 9(18 mg). The last two subfractions were purified by precipitation with diethyl ether the filtrate and precipitate were applied on sephadex CC with HPLC methanol to afford pure compounds of 10(20 mg), 11(16 mg) and 12(18 mg).

Fraction IV (3.5 gm) was fractionated by the same way of fraction I to give two pure samples 13(1.5 gm) and 14(1.1gm) which identified by compared with authentic samples on paper chromatography (PC).

Structure elucidation established by using NMR data ¹H NMR (400MHz, DMSO-d₆) and ¹³C NMR (100MHz, DMSO-d₆).

Quercetin3-O-β-D-glucopyranoside (1)

Yellow amorphous powder, UV λ_{max} (MeOH)nm: 351, 253; ¹H NMR (400MHz, DMSO-d₆) δ(ppm): 7.67 (1H, dd, J = 8.6, 2 Hz, H-6'); 7.36 (1H, d, J = 2 Hz, H-2'), 6.87 (1H, d, J = 8.6 Hz, H-5'), 6.41(1H, d, J = 1.8Hz, H-8), 6.21(1H, d, J = 1.8Hz, H-6), 5.34 (1H, d, J = 8Hz, H-1''), 5.28 (1H, m, H-2''), 3.66 (1H, dd, J = 12.5 Hz, H-6'a), 3.42 (1H, br d, J = 11.5 Hz, H-6'b), 3.23 (1H, t, J = 8.4 Hz, H-3''), 2.98 (1H, m, H-4''), 2.95 (1H, m, H-5''). ¹³C NMR (100MHz, DMSO-d₆) δ(ppm): 176.90 (C-4), 165.80 (C-7), 162.30 (C-5), 156.60 (C-2), 156.50 (C-9), 147.80 (C-4'), 144.60 (C-3'), 134.30 (C-3), 122.00 (C-1'), 121.50 (C-6'), 116.00 (C-2''), 115.70 (C-5'), 104.00 (C-10), 98.00 (C-6), 93.68 (C-8), 102.00 (C-1''), 76.70 (C-5''), 73.65 (C-3''), 71.60 (C-2''), 71.10 (C-4''), 61.85 (C-6'').

(Quercitrin) quercetin3-O-α-L-rhamnoside (2)

Yellow amorphous powder, UV λ_{max} (MeOH)nm: 344, 253; ¹H NMR (400MHz, DMSO-d₆) δ (ppm): 7.77 (1H, dd, J = 8.5, 2 Hz, H-6'), 7.29 (1H, d, J = 2 Hz, H-2'), 6.93(1H, d, J = 8.6 Hz, H-5'), 6.39 (1H, d, J = 2 Hz, H-8), 6.21(1H, d, J = 2 Hz, H-6), 5.29 (1H, br s, H-1''), 5.26 (1H, m, H-2''), 3.98 (1H, br d, H-3''), 3.529 (1H, t-like, J=8.8Hz, H-4''), 3.23 (1H, dq (sextet), J = 9.2, 6.4 Hz, H-5''), 1.07 (3H, s, CH₃ of rhamnose), ¹³C NMR (100MHz, DMSO-d₆) δ(ppm): 177.50 (C-4), 165.34 (C-7), 162.34 (C-5), 156.22 (C-2), 156.99 (C-9), 147.60 (C-4'), 144.11 (C-3'), 134.27 (C-3), 121.18 (C-1'), 120.91 (C-6'), 115.81 (C-5'), 115.61 (C-2''), 98.90 (C-6), 94.39 (C-8), 103.23 (C-10), 101.87 (C-1''), 71.25 (C-5''), 71.00 (C-3''), 70.88 (C-2''), 70.60 (C-4''), 17.88 (C-CH₃).

Quercetin3-O-β-D-galactopyranoside (3)

Yellow amorphous powder, UV λ_{max} (MeOH)nm: 352, 256; ¹H NMR (400MHz, DMSO-d₆) δ (ppm): 7.68 (1H, dd, J = 8.5, 2 Hz, H-6'), 7.57 (1H, d, J = 2 Hz, H-2'), 6.86 (1H, d, J = 8.6Hz, H-5'), 6.41(1H, d, J = 2 Hz, H-8), 6.20 (1H, d, J = 2 Hz, H-6), 5.45(1H, d, J = 7.6Hz, H-1''), 5.39 (1H, dd, J=10.8 Hz, H-2''), 3.65 (1H, m, H-6'a), 3.59 (1H, dd, J = 6 Hz, H-3''), 3.44 (1H, br s, H-4''), 3.32 (1H, m, H-5''), 3.24 (1H, dd, J=13Hz, H-6'b), ¹³C (100MHz, DMSO-d₆) δ(ppm): 176.20 (C-4), 164.20 (C-7), 163.90 (C-5), 155.90 (C-2), 155.5 (C-9), 146.90 (C-4'), 144.61 (C-3'), 134.55 (C-3), 122.73 (C-1'), 122.30 (C-6'), 115.96 (C-2'), 115.60 (C-5'), 103.91 (C-10), 102.03 (C-1''), 98.70 (C-6), 94.65 (C-8), 76.90 (C-5''), 75.83 (C-3''), 74.65 (C-2''), 70.55 (C-4''), 61.79 (C-6'').

Kaempferol3-O-β-D-galactopyranoside (4)

Yellow amorphous powder, UV λ_{max} (MeOH)nm: 346, 266 ¹H NMR (400MHz, DMSO-d₆) δ(ppm): 8.05 (2H, d, J=9.6Hz, H₂'/6'), 6.90 (2H, d, J = 9.6 Hz, H₃'/5'), 6.44 (1H, d, J = 2 Hz, H-6), 6.22 (1H, d, J = 2 Hz, H-8), 5.37 (1H, d, J = 7.5 Hz, H-1''), 5.37(1H, m, H-2''), 3.35(1H, dd, J=11.6, 2.5 Hz, H-6'a), 3.58 (1H, dd, J=8.8, 3.0 Hz, H-3''), 3.47 (1H, br s, H-4''), 3.21 (1H, dd, J = 8.8 Hz, H-5''), 3.15 (1H, dd, J=13, 6 Hz, H-6'b), ¹³C (100MHz, DMSO-d₆) δ(ppm): 176.81 (C-4), 165.02 (C-7), 163.73 (C-5), 155.30 (C-2), 155.5 (C-9), 147.90 (C-4'), 144.61 (C-3'), 133.68 (C-3), 122.56 (C-1'), 122.31 (C-6'), 115.96 (C-2'), 115.20 (C-5'), 103.91 (C-10), 101.82 (C-1''), 98.70 (C-6), 94.55 (C-8), 77.10 (C-5''), 76.60 (C-3''), 74.75 (C-2''), 70.94 (C-4''), 62.00 (C-6'').

Kaempferol3-O-α-L-rhamnoside (5)

Yellow amorphous powder, UV λ_{max} (MeOH)nm: 346, 266 ¹H NMR (400MHz, DMSO-d₆) δ(ppm): 7.76 (2H, d, J = 8.8 Hz, H₂'/6'), 6.93 (2H, d, J = 8.8 Hz, H₃'/5'), 6.41(1H, d, J=2 Hz, H-6), 6.21(1H, d, J = 2 Hz, H-8), 5.30 (1H, s, J=1.5, H-1''), 3.99 (1H, br s, H-2''), 3.54 (1H, dd, J=9.5, 3.4 Hz, H-3''), 3.48 (1H, m, H-4''), 3.17 (1H, dq (sextet), J=9.5, 6 Hz, H-5''), 1.18 (3H, s, CH₃ of rhamnose), ¹³C (100MHz, DMSO-d₆) δ(ppm): 178.15 (C-4), 164.71 (C-7), 161.70 (C-5), 160.45 (C-2), 157.73 (C-9), 156.95 (C-4'), 134.63 (C-3), 131.05(C-3'/5'), 120.95 (C-1'), 115.78 (C-2'/6'), 104.56 (C-10), 102.19 (C-1''), 99.21 (C-6), 94.24 (C-

8), 71.56 (C-5"), 71.06 (C-3"), 70.78 (C-2"), 70.51 (C-4"), 17.86 (CH₃ of rhamnose).

Kaempferol aglycone (6)

Yellow amorphous powder, UV λ_{\max} (MeOH)nm: 366, 266 ¹H NMR (400MHZ, DMSO-d₆) δ (ppm): 8.07 (2H, d, *J* = 8.8 Hz, H2'/6'), 6.94 (2H, d, *J* = 8.8 Hz, H3'/5'), 6.44 (1H, d, *J* = 2 Hz, H-6), 6.18 (1H, d, *J* = 2 Hz, H-8)

Ellagic acid (7)

White amorphous powder, UV λ_{\max} (MeOH)nm: 364, 255 characteristic for ellagic acid (Tanaka et al., 1986). ¹H NMR(400MHZ, DMSO-d₆) δ (ppm): 7.49(2H, s, H-5/5').

4, 4'-di-O methyl ellagic acid (8)

Yellow powder, UV λ_{\max} (MeOH)nm: 375, 247 ¹H NMR (400MHZ, DMSO-d₆) δ (ppm): 7.69 (2H, s, H-5/5'), 3.98 (6H, s, 2OCH₃), LC-Q/TOF - MS² m/z: 330.97297 [M+H]⁺

3, 3'-di-O-methyl ellagic acid-4'-O- α -L-rhamnoside (9)

Brownish yellow powder ¹H NMR (400MHZ, DMSO-d₆) δ (ppm): 7.69 (1H, s, H-5'), 7.43(1H, s, H-5), 5.52 (1H, s, H-1"), 1.81(3H, s, CH₃of rhamnose)

3, 3', 4'- tri-O-methyl ellagic acid (10)

Yellow powder, UV λ_{\max} (MeOH) nm: 370, 249, ¹H NMR (400MHZ, DMSO-d₆) δ (ppm): 7.59, 7.48(2H, s, H-5', 5), 4.01, 4.02,3.98(9H,s, 3OCH₃).

3, 3'-di-O-methyl ellagic acid (11)

Yellow powder, UV λ_{\max} (MeOH) nm: 375, 247¹H NMR(400MHZ, DMSO-d₆) δ (ppm): 7.53(2H, s, H-5/5'), 4.00, 3.98, (6H, s, 2OCH₃).

3, 3', 4, 4'-tetra -O- methyl ellagic acid (12)

Yellow powder¹H NMR (400MHZ, DMSO-d₆) δ (ppm): 7.74 (1H, s, H-5/5'), 4.08, 4.06, 4.12, 3.99 (12H, s, 4OCH₃)

3, 4, 5-trihydroxy benzoic acid (gallic acid). (13) and methyl gallate (14)

UV λ_{\max} (MeOH) nm: 272 characteristic for phenolic acids, were proved by co chromatography with authentic samples

Quantification of total phenolic content (TPC)

TPC was carried out using the Folin-Ciocalteu colorimetric method described by (Saboo et al 2010). Five mgs of MeOH extract dissolved in 5 ml of 50% MeOH to prepare a solution of final concentration, 1 mg/ml. An aliquot (2 ml) of the extract and standard solution of gallic acid (8-100 μ g/ml) was added to 25 ml volumetric flask containing 1.5 ml of Folin-Ciocalteureagent and 4ml of 20% Na₂CO₃ solution, then the solution was diluted to 25 ml with distilled water. The absorbance was measured, after 30 min at 765 nm using spectrophotometer, against a blank prepared at the same time using 2 ml of distilled H₂O instead of the standard solution. All concentrations were carried out in triplicate. (TPC) were expressed as gallic acid equivalent (GAE).

Quantification of total flavonoid content (TFC)

TFC of plant extract was determined according to Saboo S et al. 2010²⁰. 10 mgs of the extract were dissolved in 10 ml of 70% MeOH to prepare final concentration 1 mg/ml. An aliquot (0.5 ml) of the extract and standard solution of quercetin (10-100 μ g/ml) was added to 5 ml volumetric flask containing 1.5 ml of MeOH, 0.1 ml of 10% AlCl₃, 0.1

ml of 1M potassium acetate aqueous solution and 2.8 ml of distilled H₂O. The solution was mixed well and the absorbance was measured spectrophotometrically at 415 nm against blank. Each measurement was performed in triplicate. (TFC) was expressed as quercetin equivalent (QE).

Investigation of antioxidant activity

Antioxidant activity (DPPH Assay)

The free radical scavenging activity using the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent was determined according to Brand-Williams²¹. The isolated compounds (1-9) were dissolved in 85% methanol: water. To 0.5 ml of each, 1.0 ml of freshly prepared methanolic DPPH solution (20 μ g/ ml-1) was added and stirred. The discoloration processes was recorded after 5 min of reaction at 517 nm and compared with a blank control.

Antioxidant activity = [(control absorbance - sample absorbance) / control

absorbance] \times 100%

In this test, data can only be compared when obtained under identical settings.

Reducing power Assay (RPA)

The reducing power of the examined compounds was determined according to the method of Oyaizu 1986²². 0.5ml of each isolated compound were added to Phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5ml). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml,10%) were added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared FeCl₃ solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of their reaction mixture indicated increased reducing power.

ABTS radical scavenging assay

ABTS assay was carried out using the method of Re et al.²³. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS⁺ Solution with 60 ml methanol to obtain an absorbance of 0.706 \pm 0.001 units at 734 nm using the spectrophotometer. ABTS⁺ Solution was freshly prepared for each assay. The isolated compounds were allowed to react with 3 ml of the ABTS⁺ Solution and the absorbance were taken at 734 nm after 7 min using the spectrophotometer. The ABTS⁺ scavenging capacity of different concentrations percentage inhibition calculated as ABTS radical scavenging activity using the following equation.

(%) = Abs.control - Abs.sample] / (Abs.control)] \times 100

Where Abs. control is the absorbance of ABTS radical + methanol; Abs. sample is the absorbance of ABTS radical + sample extract. Each of the above assays was carried out in triplicate.

This test does not distinguish between the kinetics of radical trapping and stoichiometry.

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was done according to Benzie and Strain²⁴ with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃•6H₂O solution. The fresh working solution

was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution and then warmed at 37 °C before using. Compounds (1-9) were allowed to react with 2850 μl of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. Results are expressed in $\mu\text{mol Trolox} / \text{g dry matter}$. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve. This method essentially provides the stoichiometry of antioxidants.

RESULTS AND DISCUSSION

Flavonoids isolated from 70% ($\text{CH}_3\text{OH}/\text{H}_2\text{O}$) extract

The purification of the aerial parts extract from *S. grantii* afforded 14 known compounds were separated for the first time by different chromatographic technique, their structures established based on NMR spectroscopic data and by comparing them to those reported in the literature (Figure 1). Compounds (1-6) were glycosides of flavonoids

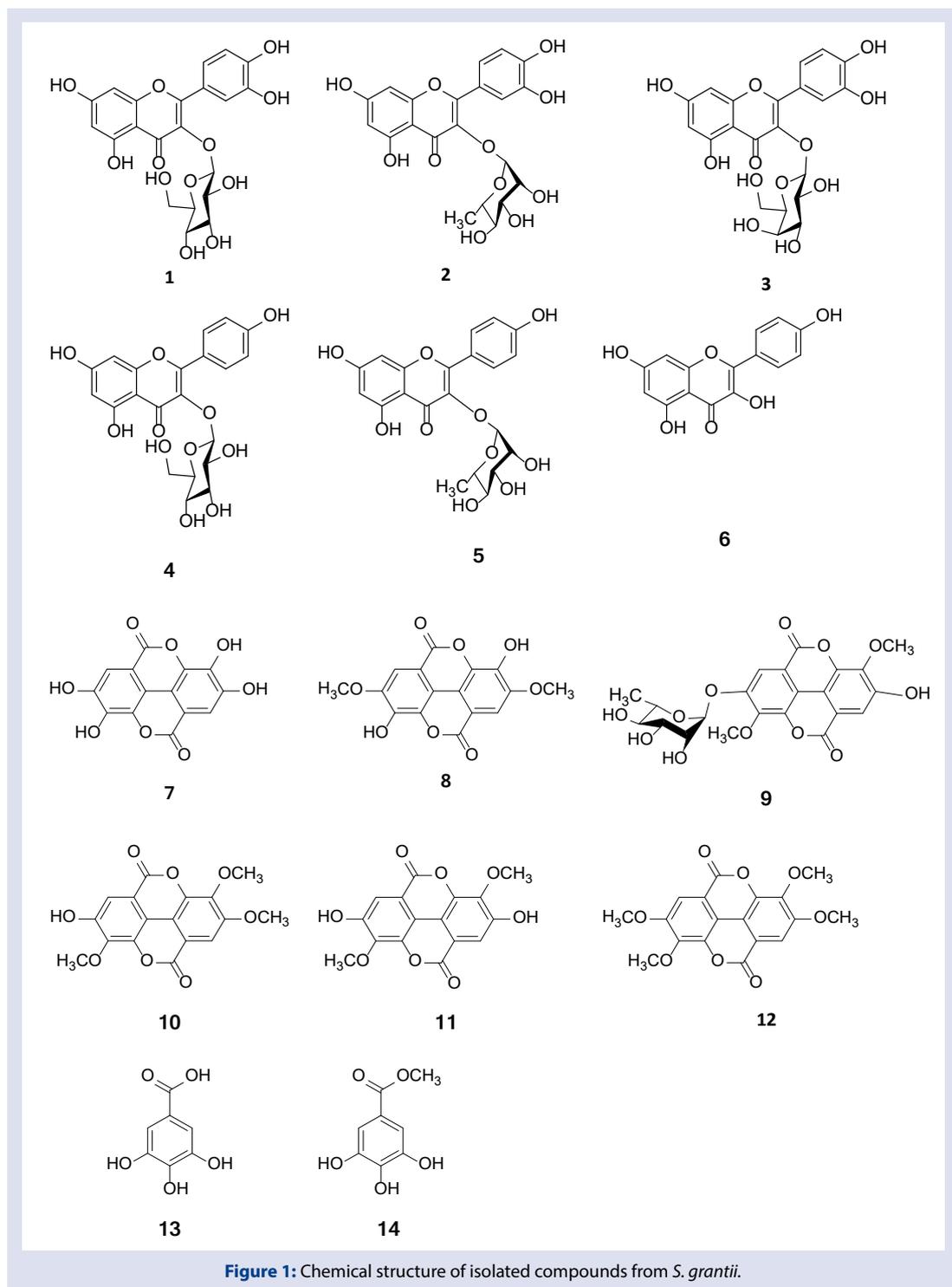


Figure 1: Chemical structure of isolated compounds from *S. grantii*.

and aglycon, obtained as yellow solid powder, appear as dark purple spot under UV light on PC, changed to bright yellow colour and green fluorescence when exposed to ammonia vapour, after spraying with aluminium chloride.

¹H NMR spectrum of compound **1** showed in its aromatic region an ABX-spin coupling system, each of one proton, at δ ppm 7.67, 7.36 and 6.87 assignable to H-6', 2' and 5', respectively. In addition, an AM-spin coupling system described in the form of two *meta* coupled protons at 6.41, 6.21 for H-8 and 6, respectively. Thus, the aglycone moiety was confirmed as a quercetin 3-*O*-substituted. In the aliphatic region one anomeric proton with large *J* value was assigned at 5.34 (d, *J* = 8 Hz) for β-configuration and the appearance of H-3" at 3.23 (1H, t, *J* = 8.4 Hz, H-3") established compound **1** as quercetin3-*O*-β-D-glucopyranoside.

Compounds **2**, **3** were very similar to **1**. The difference between them was in the aliphatic region (sugar moiety). In compound **2** two singlet signals appeared at δ ppm 5.29 and 1.07 assignable for the anomeric and methyl rhamnose, revealed rhamnose sugar with α-linkage. Compound **2** was confirmed to be quercetin3-*O*-α-L-rhamnoside. In compound **3** the appearance of H-3", *J* = 6 Hz and H-4" as br s differentiated the glycoside moiety as galactoside rather than glucoside (*J* = 9.5-10 Hz), so compound **3** was identified as quercetin3-*O*-β-D-galactopyranoside.

¹H NMR spectrum of compound **4** showed in its aromatic region an A₂X₂-spin coupling system as two *o*-doublets, (*J* = 9.6 Hz), each of two protons, at δ 8.05 and 6.90 assignable to H-2'/6' and H-3'/5', respectively. In addition, an AM-spin coupling system described in the form of two *m*-coupled protons (*J* = 2 Hz), at 6.44 and 6.22 for H-8 and H-6, respectively. Thus, the glycone moiety was confirmed as a kaempferol 3-*O*-substituted. In the aliphatic region the anomeric proton was assigned at 5.37 (d, *J* = 7.5 Hz) exactly the same pattern in previous mono glycoside. Accordingly, compound **4** was finally identified as Kaempferol3-*O*-β-D-glucoside.

Compound **5** has the same chemical structure of **4**, but its aliphatic region showed signal at δ ppm 5.30 s, *J* = 1.5 was attributed to α-anomeric proton and a singlet at 1.18 for the methyl rhamnose protons, indicating presence of rhamnosyl moiety, and confirmed compound **5** to be Kaempferol3-*O*-α-L-rhamnoside. The disappearance of the anomeric in the aliphatic region exhibited that compound **6** is aglycone not glycoside and identified as Kaempferol¹¹.

Tannins isolated from methanol water extract

Six ellagic acid derivatives were separated. Compound **7** obtained as white amorphous powder that displayed shiny buff fluorescence spot on PC under UV light, changed to dull yellow fluorescence with ammonia vapours. Spraying with FeCl₃ gave blue colour indicating its phenolic nature. The UV spectral data exhibited two absorption bands (λ_{max} 364, 255) characteristic for ellagic acid. ¹H-NMR spectrum showed a singlet signal at δ ppm 7.49 integrated for two equivalent protons in aromatic region assigned for H-5 and H-5', so the compound **7** elucidated as ellagic acid¹².

Compound **8**, isolated as yellow powder exhibited dark violet fluorescence under short and long UV light turned greenish-yellow fluorescence with NH₃ vapours. Spraying with FeCl₃ gave blue colour, R_f (x100) 56 (15% HOAc) 30 (BAW) indicating its phenolic nature. The UV spectral data exhibited two absorption bands at (λ_{max} 247, 375) characteristic for ellagic acid derivatives¹³. The molecular formula C₁₆H₁₀O₈ from LC-Q/TOF - MS² with pseudo molecular ion at m/z: 330.97297[M+H]⁺, ¹H-NMR spectrum showed a down field singlet signal at δ ppm 7.69 (down field) integrated for two aromatic protons assigned for H-5/5' and a singlet of six protons at δ ppm 3.98 assigned for two equivalent methoxy groups established compound **8** as 4, 4'-di-*O*-methyl ellagic acid¹⁴.

Compound **9**, was identified as 3, 3'-di-*O*-methylellagic acid 4'-*O*-α-L-rhamnoside. Its ¹H-NMR revealed presence of down field singlet signal at 7.69, and upfield singlet at 7.43 assignable for H-5', H-5 respectively, suggested the rhamnose and *O*-methyl in position 3' and 4'. The aliphatic region exhibited the singlet anomeric proton at 5.52 and a singlet methyl for rhamnosyl moiety at 1.8¹³. Finally, its molecular formulae was C₂₂H₂₀O₁₂ from (LC-Q/TOF - MS²) at m/z: 477.10238[M+H]⁺ for the pseudo molecular ion.

Compound **10**, yellow powder, molecular formula C₁₇H₁₂O₈, LC-Q/TOF - MS² m/z: 344.22763 [M+H]⁺, similar to **8** it exhibited the same physical and chemical properties of **8**, R_f (x100) 60 (15% HOAc), 29 (BAW). ¹H-NMR spectrum showed two singlet signals at 7.59, 7.48 assigned for two aromatic protons at H-5', H-5 and three non-equivalent signals at 4.01, 4.02, 3.98 for three methoxy group on the basis of the above data and by comparison with reported data, compound **10** could be identified as 3, 3', 4'-tri-*O*-methyl ellagic acid¹³.

Compounds **11**, **12** showed the intrinsic chromatographic behaviour of ellagic acid derivatives and have the same physical and chemical properties of **8**. In compound **11** the two *O*-methyl groups are substituted at position 3, 3' due to the appearance of a singlet signal at 7.53 (UP field) assignable for two equivalent aromatic protons (H-5/5') and a singlet of six protons at 4.00, 3.98 assignable for two methoxy groups at position 3 and 3, compound **11** is deduced to be 3, 3', di-*O*-methyl ellagic acid. In compound **12** ¹H-NMR spectrum showed four singlet signals at 4.08, 4.06, 4.12, 3.99 revealed four *O*-methyl in aliphatic region and one down field signal in aromatic assigned at 7.74 for H-5/5', compound **12** is deduced to be 3, 3', 4, 4'-tetra-*O*-methyl ellagic acid¹⁴.

The gallic acid **13** and methyl gallate **14** were identified from chromatographic properties, by comparing UV spectra with reported data and by co-chromatography on TLC with authentic sample^{15,16}.

The existence of this kind of compounds in the aerial parts of *S. grantii* was confirmed by the TPC assessment, by the Folin-Ciocalteu method and TFC. *S. grantii*, 258.04 ± 15.67 mg/g content and 380.48 ± 31.07 mg/g content such results confirm the significance of assessing antioxidant activity, as it depends on the structural features of organic compounds, mainly due to the presence of phenolics and flavonoids so the antioxidant properties of this species can be responsible. Analyzes of NMR revealed the presence of phenolics and flavonoids compounds in the extract of the aerial parts. Hence its ability to prevent the development of free radicals assessed.

The phenolic and flavonoid contents were 258.04 ± 15.67 mg GAE/G extract and 380.48 ± 31.07 mg QE /G extract, respectively.

BIOLOGICAL EVALUATION

In vitro Antioxidant Activity

The antioxidant was determined by using four assays DPPH, RPC, ABTS, and FRAP.

DPPH Radical Scavenging Activity

Free radicals of (DPPH) are widely used for screening of medicinal plants to investigate their antioxidant potential. The DPPH radical scavenging activity was measured and compared with that of BHA. Results in (Figure 2) showed that DPPH radical scavenging activity of compounds (**1-9**) ranged from 24.83 ± 0.32% for compound **5** (500 µg/ml) to 88.47 ± 0.37% for compound **2** (100 µg/ml). Compound **4** also exhibited a very high DPPH radical antioxidant activity.

Reducing power capacity (RPC)

In reducing power assay, the presence of the antioxidant agents can improve the reduction of Fe (III)/ferric cyanide complex on the

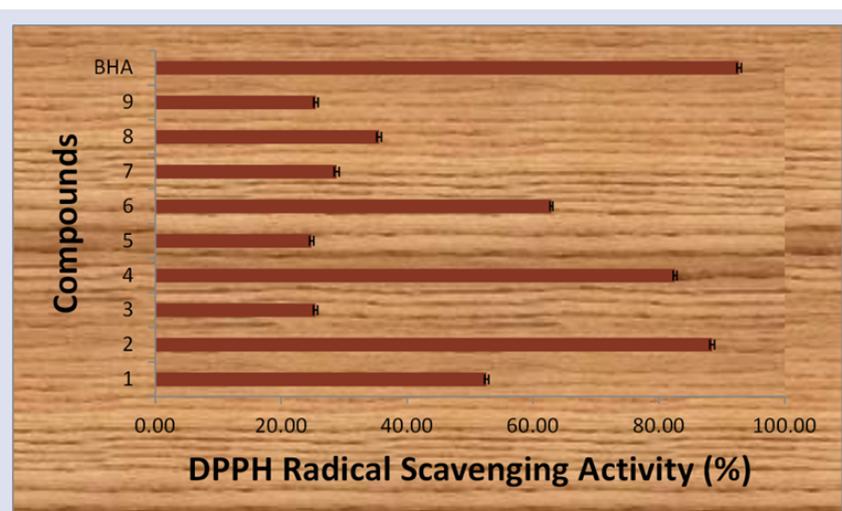


Figure 2: Radical Scavenging ability of the isolated compounds (1-9) from *S. grantii* on DPPH radical, (Data are means \pm standard deviation of triplicate experiments).

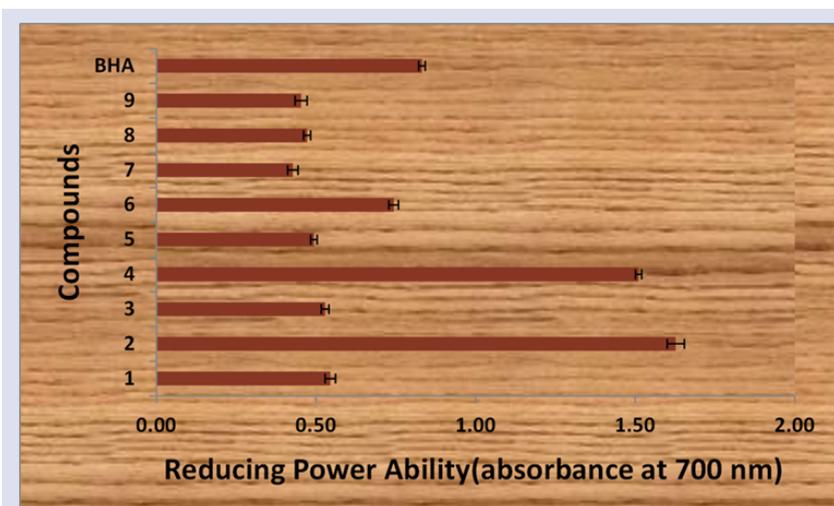


Figure 3: (RPC) of the isolated compounds (1-9) *S. grantii* (Data are means \pm standard deviation of triplicate experiments).

ferrous form [Fe (II)] by donating an electron. The occurrence of reluctant in the sample would cause the reduction of (Fe³⁺) to (Fe²⁺) ion through the donation of an electron and the creation of the Perl Prussian blue complex. Results illustrated in (Figure 3) indicated that all the examined compounds have good reducing power ability. Compounds 2, and 4 have the superiority their absorbance at 700 nm found to be 1.63 ± 0.03 and 1.51 ± 0.01 respectively at the concentration 500 $\mu\text{g/ml}$. Compound 7 recorded the lowest reducing power capacity at the same concentration (0.43 ± 0.02).

ABTS scavenging activity

The isolated flavonoids and tannins as well as the synthetic antioxidant BHA revealed considerably different in their ABTS radical scavenging activities. All the isolated compounds caused an inhibition in ABTS⁺ activity (Figure 4). The highest ABTS scavenging activity obtained corresponded to compound 2 ($71.65 \pm 0.42\%$ at the concentration 100 $\mu\text{g/ml}$), followed by compound 6. ($70.77 \pm 0.34\%$) but at a higher concentration 250 $\mu\text{g/ml}$. Also compound 4 exhibited a good ABTS radical scavenging activity ($60.89 \pm 0.18\%$) at a low concentration 100 $\mu\text{g/ml}$. Compounds 8, 9 and 1 recorded 63.15 ± 0.14 , 59.82 ± 0.26 and 55.42 ± 0.20 respectively at the same concentration (500

$\mu\text{g/ml}$), while compound 5 showed the lowest activity (31.85 ± 0.34) compared to the standard BHA (92.38 ± 0.26). The highest activity may be attributed to the presence of the high molecular weight flavonoids which are responsible for quenching of ABTS cation as reported by¹⁷.

Ferric reducing power ability (FRAP)

The iron reducing power test based on the reduction of Fe³⁺ iron to Fe²⁺ iron was used in this study to highlight the antioxidant potential of the isolated compounds (1-9). Similar trend was noticed in the obtained results of ferric reducing power ability as compound 2 exhibited the highest ferric reducing power ability, it found to be $3574 \pm 19.14 \mu\text{mol Trolox/100g DW}$ at the concentration 100 $\mu\text{g/ml}$ followed by compound 6 where the compound is achieved $3080 \pm 17.43 \mu\text{mol Trolox/100g DW}$ 500 $\mu\text{g/ml}$ also compounds 8, 5 and 3 recorded good ferric reducing power ability they recorded 1352 ± 10.02 , 1149 ± 13.50 and $1129 \pm 11.93 \mu\text{mol Trolox/100g DW}$ respectively at the same concentration (Figure 5).

Analyzes of NMR showed that extract of aerial parts from *S. Grantii* has a high level of flavonoids and tannins, so its ability to inhibit free radical formation was assessed. All of the compounds tested possessed strong antioxidant activity across all assays used. Compounds 2,

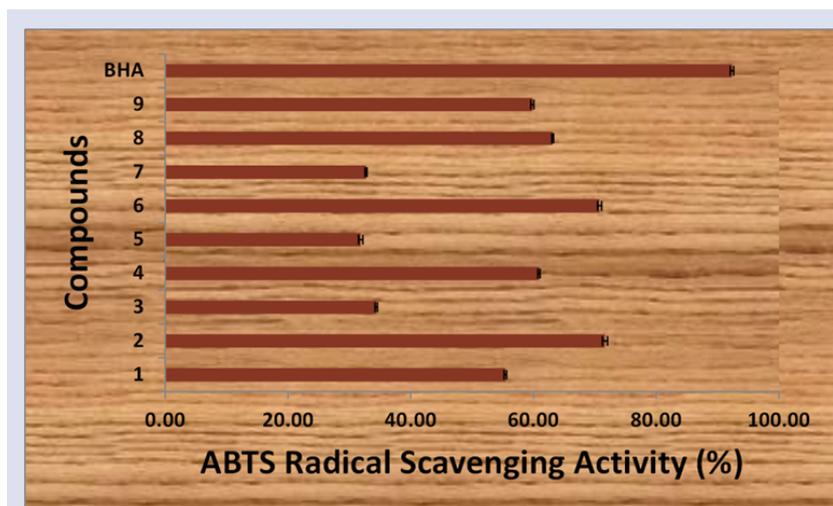


Figure 4: Scavenging ability of the isolated compounds (1-9) from *S. grantii*, ABTS radicals, (Data are means \pm standard deviation of triplicate experiments).

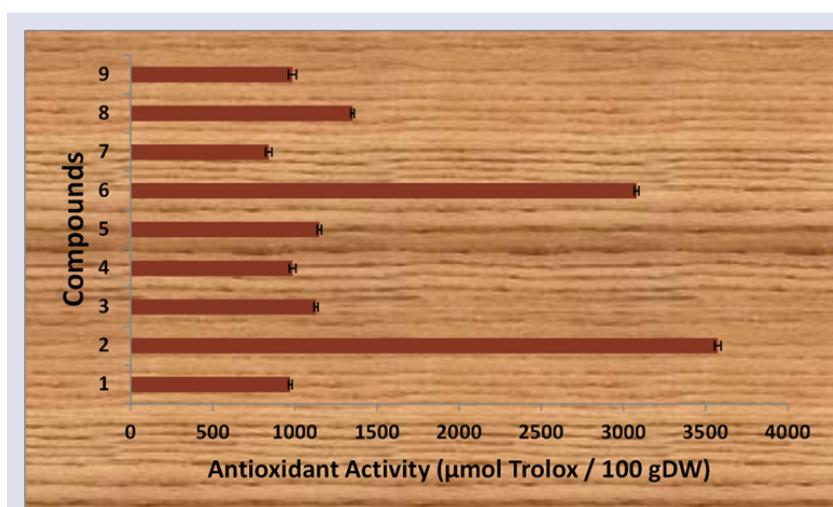


Figure 5: (FRAP) of the isolated compounds (1-9) from *S. grantii* (Data are means \pm standard deviation of triplicate experiments).

4 and often 6 consistently displayed higher antioxidant activity at lower concentrations than the majority of the compounds used. This high capacity can be attributed for their chemical configuration and contains multiple effective groups which were the main reason for the radical scavenging operation. Antioxidant property is widely used as a parameter for medicinal bioactive components [4]. Phenolic compounds and flavonoids may be responsible for the alcoholic extract's antioxidant properties^{18,19}.

CONCLUSIONS

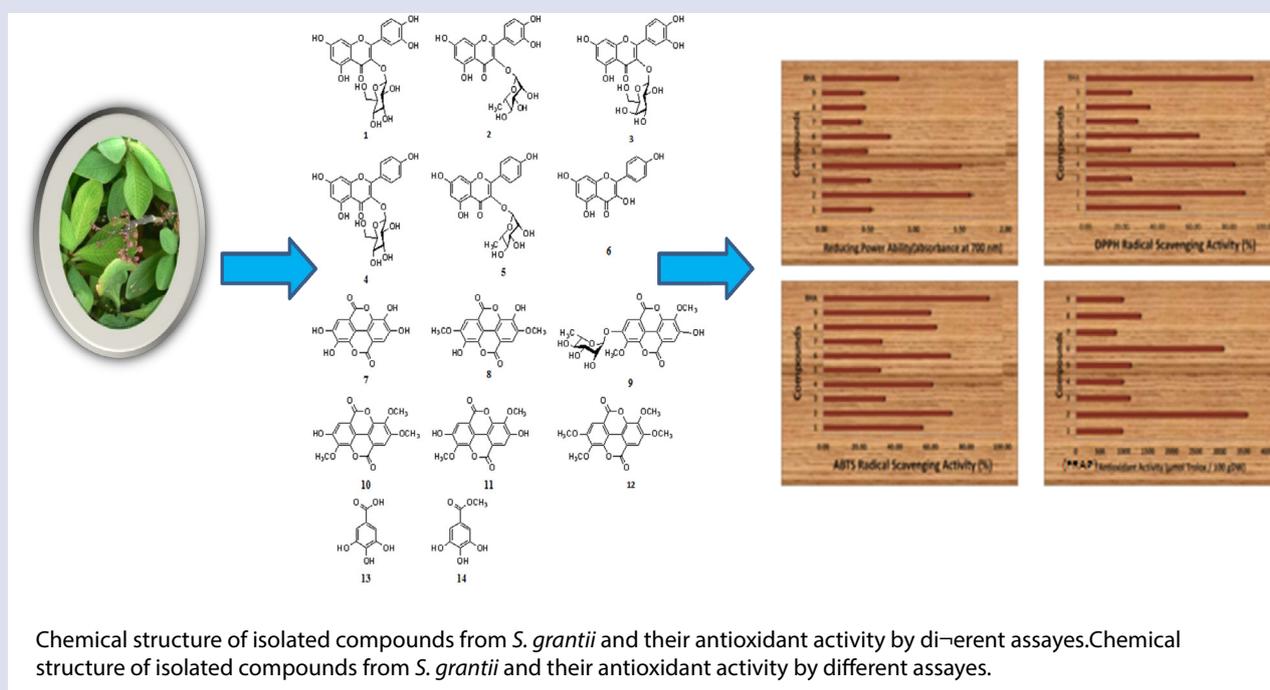
The compounds tested had strong antioxidant function as a natural source of antioxidant medicinal products.

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GRAPHICAL ABSTRACT



ABOUT AUTHORS

- **Asmaa S. Abd Elkarim^{1,*}**: Researcher, ¹Chemistry of Tanning Materials and Leather Technology Department, National Research Centre.
- **Sahar Abdelaziz²**: Associate Professor, Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, 44519 Zagazig, Egypt.
- **Hany Gouda Attia^{3,4}**: Department of pharmacognosy, faculty of pharmacy, Najran University, Najran 55461, Saudi Arabia. Department of pharmacognosy, faculty of pharmacy, Kafr EL- Shiekh university, Egypt.
- **Prof. Hanan Anwar AlyTaie⁵**: Research professor of Plant Biochemistry - Plant Biochemistry Department, National Research Centre. Awarded M.Sc. In Biochemistry from Cairo University in 1999 and awarded Ph.D. in Biochemistry from the same university in 2005. Reviewer in many international journals. She supervised several M. Sc. And Ph. D. Thesis.
- **Rafik Monir⁶**: Associate Professor, Pharmacognosy Department, Faculty of Pharmacy, Misr University for Science and Technology.

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