Phenolic Metabolites from *Acacia nilotica* Flowers and Evaluation of its Free Radical Scavenging Activity

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Abstract: The study of the chemical constituents of the flowers of *Acacia nilotica* has resulted in the isolation and characterization of nine compounds. These compounds were identified as catechin (**1**), catechin 7-***O***- gallate (**2**), gallic acid (**3**), naringenin 7-***O***-β- glucopyranoside (**4**), quercetin 3-***O***- β- glucoside (2→1) glucopyranoside (**5**), quercetin 3-***O***- β-glucopyranoside (**6**), chalconaringenin 4'-***O***-β-glucopyranoside (**7**), naringenin (**8**) and quercetin (**9**), which were isolated for the first time from *Acacia nilotica* flowers. These compounds were individually identified by spectroscopic analyses and were compared with reported data. The total amount of phenolic compounds of the aqueous methanol extract and fractions was determined by ultraviolet (UV) spectrometry and calculated as gallic acid equivalents. The antioxidant potential of *Acacia nilotica* extract and fractions has been investigated by DPPH radical scavenging assay.

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1. Introduction:

Free radical reactions, especially with participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases (Datta et al., 2000). Reactive oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular diseases (Halliwell, 1997). Many antioxidant compounds, naturally occurring from plant sources, have been identified as a free radical or active oxygen scavengers (Zheng and Wang, 2001). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity (Ito et al., 1983). Natural antioxidants have the capacity to improve food quality and stability and can also act as nutraceuticals to terminate free radical chain react-ions in biological systems, and thus may provide additional health benefits to consumers. The anti-oxidant activities of plants are mainly due to the presence of secondary metabolites of the phenolic compounds, such as simple phenolic acids, flavon-oids, isoflavonoids, hydrolyzable tannins and conden-sed tannins (Chung et al., 1998; Pietta, 2000). There have been numerous studies on the biological activities of phenolics, which are potent antioxidants and free radical scavengers (Kahkonen et al., 1999, Sugihara et al., 1999, Toyokuni et al., 2003).

Acacia is a cosmopolitan genus containing more than 1350 species that found in African and the Middle Eastern countries, with monotypic genus. This genus contains variety of bioactive components such as phenolic acids (Singh et al. 2009a), alkaloids (Clement et al., 1997), terpenes (Mujoo et al., 2001), tannins (Readel et al., 2001) and flavonoids (Fourie et al., 1974) which are responsible for numerous boil-ogical and pharmacological properties like hypo-glycaemic, anti-inflammatory, antibacterial, antipla-telet, aggregatory, antihypertensive, analgesic, anti-cancer, and anti-atherosclerotic due to their strong antioxidant and free radical scavenging activities (Chopra et al., 1999; Singh et al., 2009b).

Plants have played a major role in the introduction of new therapeutic agents. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin (Cusi and Defronzo, 1998). It is our opinion that instead of random search of plants, a selective search based on traditional knowledge would be more focused and productive and certainly more economic. To the best of our knowledge, there is no scientific literature on phenolic constituents and antioxidant activity of *Acacia nilotica* flowers. The present study deals with the isolation and identify-cation of phenolic metabolites from the flowers of *Acacia nilotica* and evaluation of its free radical scavenging activity**.**

2. Materials and Methods:

General

NMR experiments were performed on a Bruker AMX 400 and 500 instruments with standard pulse sequences operating at 400, 500 MHz in ^{1}H NMR and 100, 125 MHz in ^{13}C NMR. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard and DMSO-d_6 as solvent at room temperature. HRESI-MS was taken on a Micromass Autospec (70 eV) spectrometer. UV spectral data was measured on a Shimadzu 240 spectrometer in MeOH. Paper chromatography Whatman 1, using solvent systems A (15% AcOH) and B (n-BuOH: AcOH: H_2O, 4:1:5, upper layer). Compounds were visualized by exposure to UV light (365 nm), before and after spraying with $AlCl_3$ and Naturestoff-polyethylene glycol reagents.

Plant material

Flowers of *A. nilotica* were collected in March 2009 from the Upper Egypt. Identification of the plants was confirmed by Prof. Dr. Ibrahium El-Garf, Department of Botany, Faculty of Science, Cairo, University and comparison with herbarium speci-mens. Voucher Specimens were kept in herbarium, Department of Botany, Faculty of Science, Cairo, University (Boulos, 1999).

Extraction and isolation

The flowers of *A. nilotica* (1 Kg) were defatted with $CHCl_3$ (3 x 1 L) and extracted with CH_3OH: H_2O (7:3; 5 x 3 L) at room temperature. The combined extracts were filtered, evaporated under reduced pressure and lyophilized (80 g). The dry extract was loaded on a polyamide 6S column chromatography (80 x 3 cm). The column was eluted with H_2O, and then H_2O-EtOH mixtures of decree-sing polarity and 10 fractions (1 L, each) were collected. The major phenolic fractions obtained were combined into five fractions after chromatographic analysis. Fraction A (2.5 g) was fractionated by column chromatography on Sephadex LH-20 with aqueous EtOH (0- 70%) for elution to give compou-nds **1** (17 mg) and **3** (25 mg). Fraction B (3 g) was subjected to column chromatography on cellulose and n-BuOH saturated with H_2O as an eluent to give two major subfractions, then each of them was separately fractionated on a Sephadex LH-20 to yield pure samples **6** (22 mg) and **5** (18 mg). Using the same procedure fraction C (2.8 g) and fraction D (2.4 g) gave chromatographically pure samples **4** (15 mg) **2** (25 mg) and **7** (15 mg). Fraction E (1.5 g) was chromatography on Sephadex LH-20 using aqueous acetone (0- 25%) for elution to give pure sample **8** (20 mg) and **9** (25 mg).

Determination of total phenolics concentration

The concentration of total phenolics of the plant extract and fractions was determined according to the method described by (Kumar et al., 2008). Gallic acid was used as standard. Briefly, a mixture of 100 µL of plant extract (100 µg mL^{-1}), 500 µL of Folin-Ciocalteu reagent and 1.5 mL of Na_2CO_3 (20 %) was shaken and diluted up to 10 mL with water. After 2 hours, the absorbance was measured at 765 nm (using a spectrophotometer. All determinations were carried out in triplicate. The total phenolic concentration was expressed as gallic acid equivalents (GAE).

Determination of Total flavonoids concentration

Total flavonoid concentration of plant extract and fractions was determined according to the reported procedure by (Kumaran and Karunakaran, 2007). 100 µL of plant extract (10 mg mL^{-1}) in methanol was mixed with 100 µL of 20 % $AlCl_3$ in methanol and a drop of acetic acid, and then diluted to 5 mL with methanol. The absorbance was measured at 415 nm after 40 min against the blank. The blank consisted of all reagents and solvent without $AlCl_3$. All determinations were carried out in triplicate. The total flavonoid concentration was expressed as rutin equivale-nts (RE).

Antioxidant activity

Free radical scavenging activity by DPPH$^{\bullet}$ (1, 1-diphenyl -2-picryl hydrazyl).

The hydrogen atom-or-electron donation ability of the total extract of *A. nilotica* and fractions was measured from the bleaching of the purple colored methanol solution of DPPH$^{\bullet}$. This spectrophoto-metric assay uses the stable radical, 1, 1-diphenyl-picrylhydrazyl (DPPH$^{\bullet}$), as a reagent (Amarowicz et al., 2004). Different concentrations of the total extract and fractions (0.5-20 µg/ml) were mixed with 1.86 ml distilled water and then added to 1.5 ml of 0.1 mM DPPH$^{\bullet}$ in methanol, and final volume adjusted up to 3.5 ml with distilled water. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm using spectrophotometer (Shimadzu UV-VIS Recording 2401 PC, Japan.). Distilled water was used as blank. The absorbance of distilled water and DPPH$^{\bullet}$ without samples was mea-sured as control. The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH$^{\bullet}$, were calculated according to the formula:

$$I (\%) = [(A_C-A_S)/A_C] \times 100$$

Where: A_C and A_S are the absorbance of the control and sample, respectively. The IC_{50} value represented the concentration of the total extract of *A. nilotica* and fractions that caused 50% inhibition of DPPH$^{\bullet}$.

Hydrogen peroxide (H_2O_2) scavenging activity

The hydrogen peroxide scavenging of fraction C assay was carried out according to the method of Ruch et al. (1989). The principle of this method is that, there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. A solution of 40 mM H_2O_2 was prepared in 0.1 M phosphate buffer (pH 7.4). Different concentration of 1-30 µg/ml of fraction C was added to 0.6 ml of H_2O_2 solution (40 mM) and phosphate buffer (pH 7.4) was added up to a final volume of 4 ml. Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution contained the phosphate buffer without H_2O_2, using spectrophotometer and ascorbic acid (1-30 µg/ml) was used as the reference compound.

The percentage of scavenged $[H_2O_2] = [(A_c-A_t)/A_c] \times 100$

Where: A_c was the absorbance of the control and A_t was the absorbance in the presence of the standard sample or fraction C.

Reducing power

Total reducing capacity of fraction C of *A. nilotica* extract was determined according to the method of (Shi and Dalal, 1991). One ml of fraction C at different concentrations (0.5-20 µg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K_3 Fe $(CN)_6$] (1%). The mixture was incubated at 50 °C for 20 min., and then a portion (2.5 ml) of trichloroacetic acid TCA (10%) was added to mixture, which was centrifuged for 10 min at 1000 x g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml $FeCl_3$ (0.1%). Then the absorbance was measured at 700 nm. Ascorbic acid (0.5-20 µg/ml) was used as the reference compound.

Chemical Characterization of the some isolated compounds

catechin 7-*O*- gallate (2): white amorphous powder UV λ_{max} (MeOH) nm: 216, 268; ^{1}H NMR spectral data (400 MHz, DMSO-*d6*) δ 7.06 (2H, *s*, H-2'',6'' galloyl), 6.76 (1H, *d*, *J* = 1.5 Hz, H-2'), 6.72 (1H, *d*, *J* = 7.8 Hz, H-5'), 6.64 (1H, *dd*, *J* = 7.8,1.5 Hz, H-6'), 6.2 (1H, *d*, *J* = 2.1 Hz, H-6), 6.14 (1H, *d*, *J* = 2.1 Hz, H-8), 4.65 (1H, *d*, *J* = 7.2 Hz, H-2), 3.93 (1H, *m*, H-3), 2.75 (1H, *dd*, *J* = 16.8, 5.1 Hz, H-4α), 2.50 (1H, *dd, J* = 16.8, 7.8 Hz, H-4β), ^{13}C NMR : 156.71 (C-5), 155.74 (C-9), 150.39 (C-7), 146.56 (C-3'), 145.53 (C-4'), 130.84 (C-1'), 118.85 (C-6'), 115.78 (C-5'), 115.02 (C-2'),106.34 (C-10), 101.26 (C-6), 100.99 (C-8), 81.75 (C-2), 66.39 (C-3), 28.18 (C-4); galloyl moiety: 165.04 (C-7''), 146.35 (C-3'',C-5''), 139.76 (C-4''), 120.07 (C-1''), 109.66 (C-2'',6'').

Naringenin-7-O-β-D-glucoside (4): A pale yellow, UV λ_{max} (MeOH) nm: 286, 332sh; ^{1}H NMR spectral data (400 MHz, DMSO-*d6*) δ 7.32 (2H, *d*, *J* = 8.3 Hz, H-2',6'), 6.80 (2H, *d*, *J* = 8.3 Hz, H-3',5'), 6.40 (1H, *d*, *J* = 1.8 Hz, H-8), 6.09 (1H, *d*, *J* = 1.8 Hz, H-6), 5.39 (1H, *dd*, *J* = 12.8, 2.2 Hz, H-2), 3.06 (1H, *dd*, *J* = 17.1, 12.8 Hz, H-3ax), 2.63 (1H, *dd*, *J* = 17.1, 2.2 Hz, H-3eq); 4.72 (1H, *d*, *J* = 7.2 Hz, H-1'') 3..21-3.76 (m, the rest sugar of glucose); ^{13}C NMR :190.43 (C-4), 165.26 (C-7), 164.58 (C-5), 161.03 (C-9), 158.02 (C-4'), 129.30 (C-1'), 128.62 (C-2',6'), 115.51 (C-3',5'), 105.83 (C-10), 99.25 (C-6), 98.11 (C-8), 78.52 (C-2), 44.87 (C-3); 103.80 (C-1''), 77.92 (C-5''), 75.99 (C-3''), 73.83 (C-2''), 70.02 (C-4''), 61.11 (C-6'').

Quercetin 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside (5): yellow amorphous powder, UV λ_{max} nm (MeOH) 256, 268^{sh}, 298^{sh}, 355;. ^{1}H-NMR (DMSO-d6) 7.67 (1H, *dd, J* = 8.4, 2.2 Hz, H-6'), 7.61 (1H, *d, J* = 2.2 Hz, H-2'), 6.92 (1H, *d, J* = 8.4 Hz, H-5'), 6.44 (1H, *d, J* = 1.8 Hz, H-8) , 6.24 (1H, *d, J* = 1.8 Hz, H-6), 5.75 (1H, *d*, *J* = 7.12 Hz, H-1'', glc), 4.82 (1H, *d, J* = 7.7 Hz, H-1''' glc) ^{13}C NMR: *δ* 156.94 (C-2), 133.79 (C-3), 177.88 (C-4), 157.14 (C-5), 99.21 (C-6), 164.61 (C-7), 94.13 (C-8), 156.94 (C-9), 104.47 (C-10), 122.11 (C-1'), 115.74 (C-2'), 145.25 (C-3'), 148.92 (C-4'), 116.72 (C-5'), 121.69 (C-6'), 103.22 (C-1''), 82.31 (C-2''), 76.39 (C-3''), 72.35 (C-4''), 76.35 (C-5''), 61.24 (C-6''), 101.25 (C-1'''), 73.21 (C-2'''), 76.32 (C-3'''), 70.06 (C-4'''), 77.76 (C-5'''), 61.21 (C-6''').

Quercetin-3-*O*-β-D-glucopyranoside (6): yellow amorphous powder; UV λ_{max} nm: (MeOH), 253, 263^{sh}, 294^{sh}, 352; ^{1}H NMR (DMSO-d_6): *δ* 7.53 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.67 (*1H, dd*, *J* = 2.1 Hz and 8.6 Hz, H-6'), 6.82 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.40 (1H, *d*, *J* = 1.8 Hz, H-8), 6.20 (1H, *d*, *J* = 1.8 Hz, H-6), 5.37 (1H, *d*, *J* = 7.6 Hz, H-1'', glc), 3.28-3.65 (m, the rest proton of glucose protons). ^{13}C NMR: *δ* 156.80 (C-2), 133.60 (C-3), 177.50 (C-4), 161.60 (C-5), 98.90 (C-6), 164.60 (C-7), 93.80 (C-8), 156.60 (C-9), 104.00 (C-10), 121.60 (C-1'), 115.80 (C-2'), 145.80 (C-3'), 148.80 (C-4'), 116.20 (C-5'), 122.00 (C-6'), 101.20 (C-1''), 71.60 (C-2''), 74.40 (C-3''), 70.02 (C-4''), 77.70 (C-5''), 61.50 (C-6'').

chalconaringenin 4'-*O*-β-glucopyranoside (7): yellow amorphous powder; UV λ_{max} nm: MeOH, 266, 319^{sh} 369**;** ^{1}H NMR (DMSO-d_6): 7.98 (1H, *d, J* = 15.5 Hz, H-β), 7.64 (2H, *d*, *J* = 8.2 Hz, H-2,6), 7.61 (1H, *d, J* = 15.5 Hz, H-α), 6.82 (2H*, d, J* = 8.2, H-3,5), 6.16 (1H, *d*, *J* = 2.0 Hz, H-5'), 5.96 (1H, *d, J* = 2.0 Hz, H-3'), 5.07 (1H, d, J = 7.4 Hz, H-1''), 3.3-3.72 (m, rest proton of glc). ^{13}C NMR: *δ*: 192.37

(C=O), 143.07 (C-α), 124.57 (C-β), 126.59 (C-1), 131.15 (C-2, 6), 116.31 (C-3, 5), 160.64 (C-4), 105.80 (C-1'), 165.28 (C-2'), 97.32 (C-3'), 166.44 (C-4'), 95.05 (C-5'), 160.23 (C-6'), 100.76 (C-1''), 74.03 (C-2''), 77.12 (C-3''), 69.80 (C-4''), 77.68 (C-5''), 60.83 (C-6'').

3. Results and Discussion:

The flowers extract of *A. nilotica* was fractionated by polyamide 6S column chromatography to give several fractions, which were further chromatogram-phed on Sepadex LH 20 and cellulose to afford two condensed tannins (**1**, **2**), one phenolic acid (**3**), three flavonoids (**5**, **6**, **9**) two flavanones (**4**, **8**) and one chalcone glucoside (**7**) (Fig.1). The compounds were identified catechin (**1**), catechin 7-***O***- gallate (**2**) (Malan and Pienaar, 1987), gallic acid (**3**), naringenin 7-***O***-β- glucopyranoside (**4**), quercetin 3-***O***- β-glucoside (2→1) glucopyranoside (**5**), quercetin 3-***O***-β-glucopyranoside (**6**), chalconaringenin 4'-***O***-β-glucopyranoside (**7**), naringenin (**8**) and quercetin (**9**) by comparison of their 1D and 2D NMR spectral data with the reported data in the literature (Agrawal 1989; Harborne and Baxter,1999).

Plant phenolics possess the ability to scavenge both active oxygen species and electrophiles (Robards et al. 1999). Plant phenolic compounds, including flavonoids, tannins and phenolic acids exhibit a strong antioxidant activity. The total phenolics and flavonoids concentration in the extract and fractions of *A. nilotica* is shown in table 1. Among the extract and fractions, the fraction C is the higher concentra-tion of phenolics and flavonoids content.

Table 1: Total concentration of phenolic and flavonoid compounds from total extract and fractions

Sample	TPC (mg GAE g^{-1} plant extract)	TFC (mg RE g^{-1} plant extract)
Total extract	292.99±5.55	25.2±1.12
Fraction A	259.80±6.40	20.55±0.95
Fraction B	101.17±1.52	19.10±0.29
Fraction C	342.03±1.64	30.33±1.42
Fraction D	164.97±4.02	11.83±0.24
Fraction E	185.31±4.52	15.84±0.65

Values are mean ± SD of three determination, n=3.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity.

Fig 2 represents the radical scavenging activity of the extract from *A. nilotica* and fractions. The antioxidant activity of *A. nilotica* flowers extract and fractions (A-E) were tested by measuring their capacity to scavenge DPPH radical. The fraction C extract showed the highest antioxidant activity with an IC_{50} value of 3.32 µg /mL, while total extract 9.71 µg /mL and the standard antioxidant ascorbic acid showed an IC_{50} value of 2.12µg /mL. The tested samples reduced the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. DPPH radi-cals have been widely used to evaluate the anti-oxidant properties of natural products as well as plant extracts (Wang et al., 2003).

Reactive oxygen species (ROS) are continuously produced by the body's normal oxygen usage such as respiration and some cell mediated immune func-tions. ROS include free radicals such as superoxide anion radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$) and non-free-radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Farombi and Fakoya, 2005; Glc, 2006). It is increasingly being realized that many of common diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of prooxidants. In this respect, ROS play an important role related to the degenerative or pathological processes of various serious diseases, such as aging (Burns et al., 2001), cancer, coronary heart disease, Alzheimer's disease (Smith et al., 1996; Diaz et al., 1997), neurodege-nerative disorders, atherosclerosis, cataracts, and infl-ammation (Aruoma, 1998).

Recently, interest has increased in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage (Scalbert et al., 2005), because these natural antioxidants avoid undesired health problems that may arise from the use of synthetic antioxidants, which may have toxic effects (Aruoma et al., 1992).

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Substances capable of donating electrons/hydrogen atoms are able to conve-rt DPPH radical into their non-radical form 1,1-diphenyl-2- picrylhydrazine. Positive DPPH test suggests that the samples were free radical scaven-gers. In the present study, the scavenging effect of the total extract and fractions on DPPH radical was compared. $DPPH^{\bullet}$ was reduced with the increasing of total extract of *A. nilotica* and fractions concent-ration in a concentration-dependent manner and a higher DPPH radical-scavenging activity is associa-ted with a lower IC_{50} value. The total extract and fractions from the *A. nilotica* flowers showed potent antioxidant activity. Among the extract and fractions, the fraction C exhibits the best antioxidant perfor-mance. Phenolics concentration of the total extract and fractions was responsible for the antioxidant

activity. Extract and fractions from the *A. nilotica* flowers might be valuable antioxidant natural sources for both the medical and food industry.

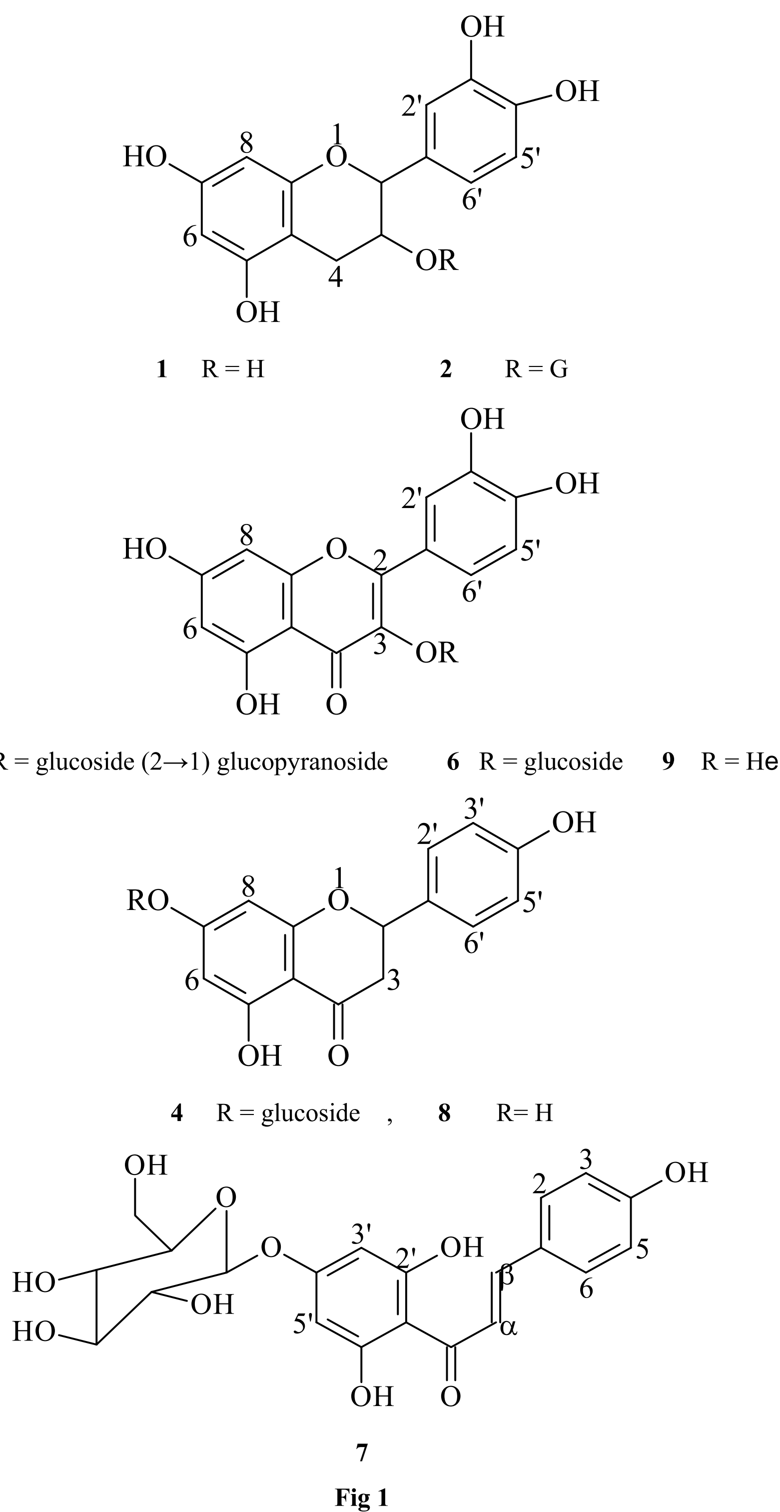

Fig 1

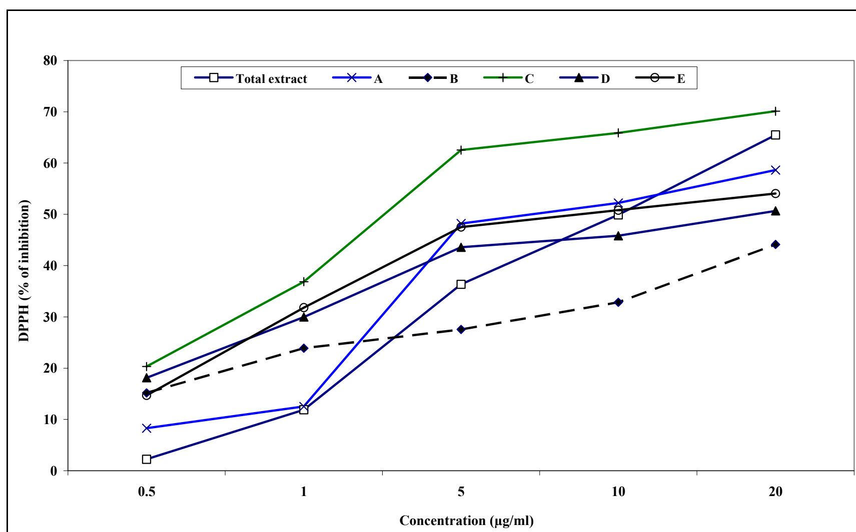

Fig 2: DPPH radical scavenging assay of total extract and fractions (A-E)

Hydrogen peroxide (H_2O_2) scavenging activity

The hydrogen peroxide scavenging ability of fraction C of *A. nilotica* extract and ascorbic acid is shown in Fig (3). Hydrogen peroxide scavenging activity of fraction C at 30 µg/ml was found to be 77.19% and for ascorbic acid at the same concentr-ation was 96.55 %. The IC_{50} value of fraction C was found to be 18.05 µg/ml and for ascorbic acid was 5.75 µg/ml, respectively. The fraction was capable of scavenging H_2O_2 in a concentration dependant manner.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidat-ion of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can prob-ably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Miller et al., 1993). From the results, fraction C was capable of scavenging H_2O_2 in a concentration dependant manner. These results suggest that Fraction C can be a better antioxidant for removing H_2O_2 and thus protecting living or food systems.

Reducing power

As shown in Fig (4), fraction C and ascorbic acid had significant inhibition of reducing power with increasing concentration in the range of 0.5-20 µg/ml. At the concentration of 0.5 µg/ml, the OD values of fraction C and ascorbic acid were 0.19, 0.11 but at concentration 20 µg/ml, the OD values 0.29, 0.34, respectively.

The reducing power reflects the electron donating capacity of bioactive compounds, is associated with antioxidant activity. Antioxidant can be reductants and inactive of oxidants. The reducing capacity of a compound can be measured by the direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. Addition of free Fe^{3+} to the reduced product leads to the formation of intense Perl's Prussian blue complex, $Fe_4[Fe(CN^-)_6]_3$, which has a strong absorbance at 700nm. The reducing ability of a compound greatly depends on the pres-ence of reductones, which have exhibit antioxidative potential by breaking the free radical chain by donating a hydrogen atom (Pin-Der, 1998). In this regarded, increase in Fe^{3+} to Fe^{2+} transformation in presence of test sample implies that sample is elec-tron donor and thus can cause reduction of the oxidized intermediates of lipid peroxidation process. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples (Karimi, et al., 2010). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of an antioxidant compound have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.

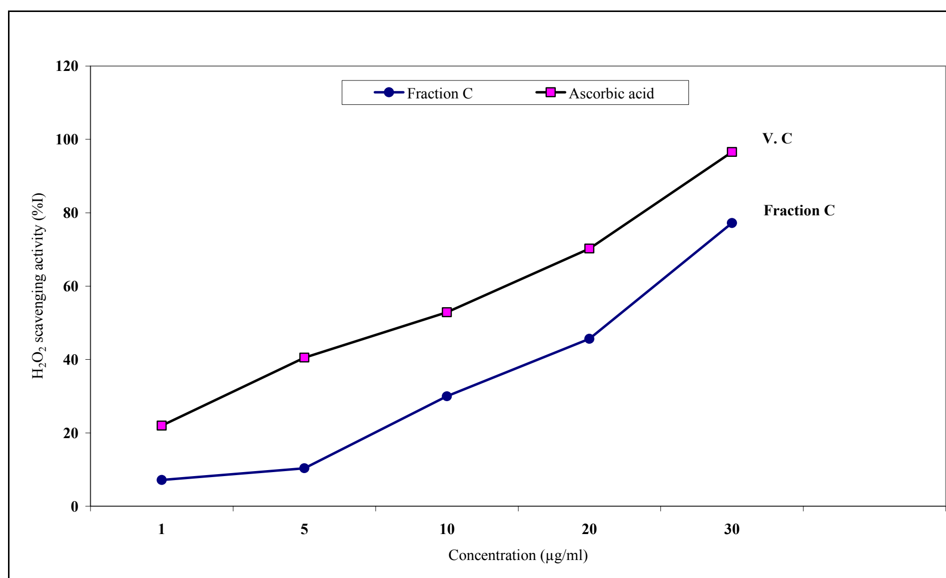

Fig 3: Hydrogen peroxide scavenging activity (H_2O_2) of fraction C and ascorbic acid. Values are mean ± SD.

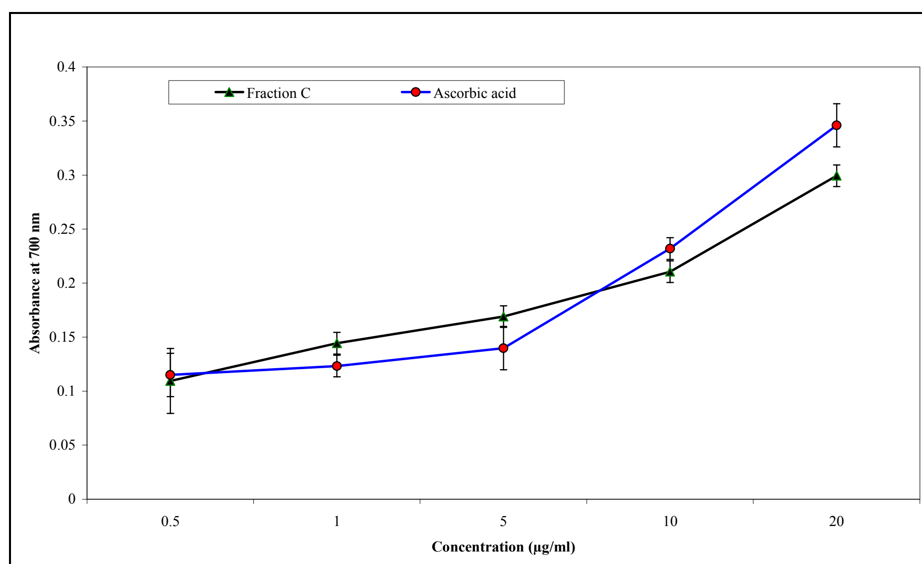

Fig 4: Reducing power of fraction C and ascorbic acid. Values are mean ± SD.

4. Conclusion

In this work, phenolic constituents have been shown to possess various biological properties related to antioxidant mechanism. Thus, in the present study, the antioxidant potential of *A. nilotica* flowers and its fractions may be attributed to the presence of phenolic compounds and the other constituents present there in.

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