

## Anti-inflammatory and Antioxidant Activity of *Solenostemma argel* extract

Eman A. Ibrahim, Alaa A. Gaafar\*, Zeinab A. Salama, Farouk K. El Baz

Plant Biochemistry Department, National Research Centre, (NRC), 33 EL Bohouth st. (former EL Tahrir st.), Dokki,  
Giza, Egypt, P.O.12622

Available Online: 03<sup>rd</sup> June, 2015

### ABSTRACT

The potency of *Solenostemma argel* (*asclepiadaceae*) as anti-inflammatory and antioxidant effects was investigated. Three solvent extract were used for extracting (polyphenolic, flavonoids and tannins) from the leaves of *Solenostemma argel* (*S. argel*). Three complementary antioxidant systems: (DPPH· free radical scavenging, Fe<sup>2+</sup>-chelating and iron reducing power) as well as the total phenolic, total flavonoids and total tannins contents of the extracts was also investigated. *In-vitro* anti-inflammatory effects of *S. argel* were assessed by using albumin denaturation assay. Our findings indicated that *S. argel* acetone extract exhibited greater scavenging activity than those extracted by other solvents. The same trend was achieved by using Fe<sup>2+</sup>-chelating and reducing power. The results revealed that the aqueous, acetone and ethanol extracts of *S. argel* showed anti-inflammatory activity (74.19%, 69.44% and 66.58%) respectively by inhibiting the heat induced albumin denaturation. The outcomes from HPLC study showed that acetone extract from *S. argel* had the highest amounts of e-vanillic, benzoic, pyrogallol, chlorogenic and ellagic acids. Whereas, rosmarinic, quercetrin and naringin acids were the major flavonoids compounds in *S. argel* acetone extract. The obtained results showed that, the solvent play a vital role in the extraction of the plant constituents and *S. argel* is a very promising source of bioactive compounds. Therefore, it could be concluded that flavonoids and related polyphenols present in the *S. argel* extract may be responsible for the anti-inflammatory and antioxidant activities which may be attributed to the radical scavenging and anti-inflammatory effects of some of its active constituents.

**Keywords:** *Solenostemma argel*, Anti-inflammatory, Antioxidant

### INTRODUCTION

The increase in prevalence of multiple drug resistance has shown the development of new synthetic antioxidative and anti-inflammatory drugs; moreover, the new drug is necessary to search for new antioxidant and anti-inflammatory sources from alternative sources. Phytochemicals from medicinal plants showing antioxidant and anti-inflammatory activities<sup>1</sup>. Screening of various bioactive compounds from plants has led to the discovery of new medicinal drug which have efficient protection and treatment roles against different diseases.

*Argel* (*Solenostemma argel*) is a desert plant of traditional medical used in folk medicine in different places in the world especially in African country. *Argel* belongs to the *Asclepiadaceae* family<sup>2</sup>. *Argel* is considered to be medicinally important in Sudan, Libya and Chad<sup>3</sup>. *Argel* leaves are used in herbal medicine for the treatment of some liver and kidney diseases and some allergies. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica<sup>4</sup>. It is reported that the aqueous extracts of *Argel* have antifungal and antibacterial properties. It is widely used in Sudanese traditional folkloric medicine as antispasmodic<sup>5</sup>, anti-inflammatory and anti-rheumatic agent<sup>6</sup>. Smoke inhalation and infusion

of the whole of this plant is used in treatment of hypercholesterolemia, diabetes mellitus, cold, cough, jaundice and measles. The plant also possesses insecticidal effect and hence was used to combat insect pests. Moreover, it was reported to have antimicrobial properties, as well as antibacterial and antioxidant activity<sup>7</sup>.

Chemical investigations, and phytochemical of *S. argel* leaves revealed the presence of numerous biochemical ingredients<sup>8</sup>, flavonoids, kaempferol quercetin, rutin, flavanones, and alkaloids<sup>7</sup>. The leaves of *S. argel* were also characterized by having a high percentage of carbohydrates (64.8), slightly low percentage of protein, low percentage of crude fiber (6.5), crude oil (1.6), about 7.7% as ash and 4.4% as moisture. Together with all these, phytic acid of 3.2 g/100g and tannin content of about 0.4% were reported<sup>9</sup>. The objective of this study was undertaken to evaluate *in-vitro* the antioxidant and the anti-inflammatory potential activities of *Solenostemma argel* (*asclepiadaceae*).

### MATERIALS AND METHODS

#### Chemicals

ABTS<sup>+</sup> (2, 2'-azinobis (3-ethyl benzothiazoline – 6 - sulfonic acid)), Folin–Ciocalteu reagents, Gallic acid,

Table 1. Total phenolic, flavonoids and tannins contents of *Solenostemma argel* extracts

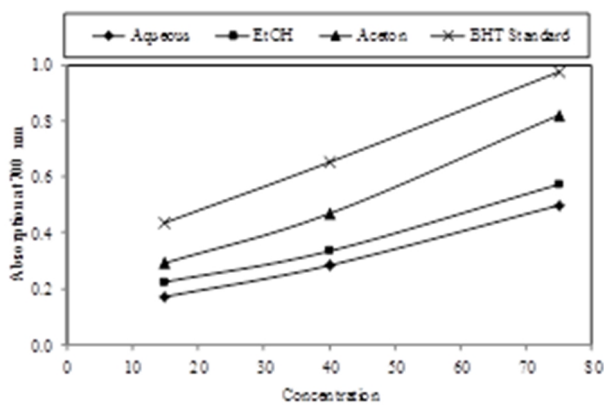
Extracts	Phenolics mg/g	Flavonoids mg/g	Tannins mg/g
Aqueous	46.72 ± 0.47 <sup>a</sup>	18.42 ± 0.37 <sup>a</sup>	34.49 ± 0.31 <sup>a</sup>
Ethanol 80%	62.58 ± 0.70 <sup>b</sup>	23.90 ± 0.23 <sup>b</sup>	42.37 ± 0.32 <sup>b</sup>
Acetone 80%	81.45 ± 0.39 <sup>c</sup>	37.38 ± 0.31 <sup>c</sup>	54.04 ± 0.31 <sup>c</sup>
LSD at 0.05	0.40	0.82	0.33

All values are the means of three replicates and are significantly different at  $p \geq 0.05 \pm$  standard deviation

Table 2. IC<sub>50</sub> of DPPH radical of *Solenostemma argel* leaves extracts

Extracts	IC <sub>50</sub> µg/ml
Aqueous	57.41 ± 2.26 <sup>d</sup>
Ethanol 80%	53.48 ± 1.84 <sup>c</sup>
Acetone 80%	48.87 ± 0.54 <sup>b</sup>
BHT standard	8.18 ± 0.04 <sup>a</sup>
LSD at 0.05	2.74

All values are the means of three replicates and are significantly different at  $p \geq 0.05 \pm$  standard deviation.

Figure 1. EC<sub>50</sub> of *Solenostemma argel* extracts

quercetin, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, Ferrozine:

(3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine, BHT: Butyl Hydroxy toluene and, potassium ferricyanide, were from (Sigma Chemical Co., St. Louis, MO, USA).

#### Preparation of plant extract

Argel, was purchase from local market in Egypt. The dried leaves were ground, powdered, stored at 4 °C and protected from light prior to further use. The *S. argel* (leaves) was extracted. Briefly, 10 g of the dried powder from skin and peel were soaked with 100 ml of water, 80% ethanol, and 80% acetone and shaking at room temperature for 48 h. The extracts were filtered and the extraction was repeated twice. The resulting of different extracts was used for the determination of total phenolic, flavonoid, and tannins and their antioxidant and anti-inflammatory activity.

#### Phytochemicals

##### Total phenolic content

The total phenolic content (TPC) of argel extracts were spectrophotometrically determined by Folin Ciocalteu reagent assay using gallic acid for the preparation of calibration curve (20 – 120 mg/l)<sup>10</sup>. A suitable aliquot (1

ml) of each extract or standard solution was added to 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min. 10 ml of 7 % Na<sub>2</sub>CO<sub>3</sub> solution were added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min. at room temperature, the absorbance was determined at 750 nm with spectrophotometer (Unicum UV 300) against prepared reagent as blank. A total phenolic content in samples was expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicates.

##### Total flavonoid content

Total flavonoid content (TFC) of argel extracts were spectrophotometrically determined by the aluminum chloride method using quercetin as a standard<sup>11</sup>. One ml of extract or standard solution (quercetin, 20–120 mg/l) was added to 10 ml volumetric flask, containing 4 ml of distilled water. To the flask 0.3 ml 5 % NaNO<sub>2</sub> was added and after 5 min 0.3 ml 10 % AlCl<sub>3</sub> was added. At 6<sup>th</sup> min, 2 ml 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The solutions were mixed well and the absorbance was measured against prepared reagent blank at 510 nm by using spectrophotometer (Unicum UV 300). Total flavonoids in sample were expressed as mg quercetin equivalents (QE)/ g dry weight. Samples were analyzed in triplicates.

##### Total tannins content

Total tannins content (TTC) of different extracts was measured using the Folin-Ciocalteu reagent assay<sup>12</sup>. One ml of *S. argel* extracts or standard solution of (tannic 20-120 mg/l) was added to 7.5 ml distilled water (dH<sub>2</sub>O) then add 0.5 ml of Folin reagent and 1 ml of 35% sodium carbonate solution. The volume was made up for 10 ml with distilled water and absorbance was measured against prepared reagent blank at 775 nm by using spectrophotometer (Unicom UV e300). Total tannins in sample were expressed as mg tannic acid equivalent (TAE)/g dry weight. All samples were analysed in triplicates.

##### Antioxidant activity

##### DPPH Free radical scavenging assay

The DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging activity of argel extracts was assayed by using 0.1 mM of DPPH<sup>•</sup> in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of argel extracts at different concentrations (25, 50, 75, 100 µg/ml). Methanol was used as blank. The mixture was shaken vigorously and allowed to stand at room temperature. Butyl Hydroxytoluene (BHT, Sigma) was used as positive control; and negative control contained the entire reaction

Table 3. Fe<sup>2+</sup>- chelating activity of *Solenostemma argel* leaves extracts

Extracts	Inhibition %		
	15 µg/g	40 µg/g	75 µg/g
Aqueous	4.25 ± 0.31 <sup>a</sup>	14.52 ± 0.48 <sup>a</sup>	31.60 ± 0.31 <sup>a</sup>
Ethanol 80%	9.12 ± 0.63 <sup>b</sup>	19.03 ± 0.47 <sup>b</sup>	44.39 ± 0.60 <sup>b</sup>
Acetone 80%	13.10 ± 0.64 <sup>c</sup>	24.63 ± 0.24 <sup>c</sup>	55.40 ± 0.24 <sup>c</sup>
EDTA standard	27.31 ± 0.55 <sup>d</sup>	52.15 ± 0.40 <sup>d</sup>	80.61 ± 0.33 <sup>d</sup>
LSD at 0.05	1.25	0.60	0.87

All values are the means of three replicates and are significantly different at  $p \geq 0.05 \pm$  standard deviation.

Table 4. Reducing power activity of *S. argel* leaves extracts

Extracts	EC <sub>50</sub> µg/ml
Aqueous	76.49 ± 0.82 <sup>d</sup>
Ethanol 80%	63.93 ± 0.13 <sup>c</sup>
Acetone 80%	40.13 ± 0.20 <sup>b</sup>
BHT standard	22.48 ± 0.21 <sup>a</sup>
LSD at 0.5	0.70

All values are the means of three replicates and are significantly different at  $p \geq 0.05 \pm$  standard deviation

reagent except the extracts. Then the absorbance was measured at 515 nm against blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity<sup>13</sup>.

The capacity to scavenge the DPPH<sup>•</sup> radical was calculated using the following equation:

$$\text{DPPH}^{\bullet} \text{ scavenging effect (Inhibition \%)} = [(A_c - A_s / A_c) \times 100]$$

Where A<sub>c</sub> was the absorbance of the control reaction and as the absorbance in the presence of the plant extracts.

#### Metal chelating activity

Metal chelating effects on ferrous ions was carried out by using one ml of argel extracts, or EDTA solution as a positive control at different concentrations (25, 50, 75, 100 µg/ml) were spiked with 0.1 ml of 2 mM FeCl<sub>2</sub>·4H<sub>2</sub>O and 0.2 ml of 5 mM ferrozine solution and 3.7 ml methanol were mixed in a test tube and reacted for 10 min, at room temperature then the absorbance was measured at 562 nm. Mixture without extract was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity<sup>14</sup>.

The percentage of ferrous ion chelating ability was calculated using the following equation:

$$\text{Iron chelating activity (Inhibition \%)} = [(A_c - A_s / A_c) \times 100]$$

Where A<sub>c</sub> was the absorbance of the control reaction and as the absorbance in the presence of the plant extracts.

#### Reducing power

Different concentrations (25, 50, 75, 100 µg/ml) of argel extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. After, 2.5 ml of trichloroacetic acid (10 %) were added to the mixture, centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and 0.25 ml FeCl<sub>3</sub> solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. Increased

absorbance values indicate a higher reducing power. The extract concentration providing 0.5 of absorbance (EC<sub>50</sub>) was calculated from the graph of absorbance at 700 nm against the extract concentration. BHT was used as standard<sup>15</sup>.

#### In-vitro anti-inflammatory activity inhibition of albumin denaturation

Inhibition of albumin denaturation activity was assayed with slight modifications<sup>16,17</sup>. The reaction mixture was consisting of *S. argel* extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

#### Identification of phenolic acid and flavonoid compounds

The phenolic and flavonoid compounds of *S. argel* were extracted according to the method described by<sup>18,19</sup>

Ten grams sample were extracted using 10 ml of aqueous, 80% ethanol and 80% acetone by homogenization for 2 min then centrifuged at 25,000 g for 10 min. The supernatant was decanted into a round-bottom flask. The pellet was resuspended in aqueous, 80% ethanol and 80% acetone (2x5ml) and centrifuged, and the supernatants were combined, evaporation by rotary at 40 °C until dryness of different extracts and resolve in (methanol-HPLC). Extracts were filtrated through 0.20µm millipore membrane filter and set up to a known volume (10 ml). Three milliliters were collected in a vial for subsequent HPLC separation. HPLC instrument (Agilent Technologies 1100 series, equipped with a quaternary pump (G131A model) Separation was achieved on ODS reversed phase column (C18, 25×0.46 cm i.d. 5 µm). Injection volume was 75 µl carried out with autosampling injector. The column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1.0 ml/min. Elutes were monitored using UV detector set at 280 nm for phenolic acid and 330 nm for flavonoid. Chromatographic peaks were identified by comparing the retention times with the respective retention times of known standard reference material. Retention time and peak area were used to calculation of phenolic acid and flavonoid compounds concentration by the data analysis of Hewlett Packard software. Phenolic acid and flavonoid compounds were expressed as µg/g sample on dry weight basis.

Table 5. Evaluation of anti-inflammatory of *S. argel* extracts with bovine albumin serum

Extracts	Inhibition %			
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
Aqueous	69.27 ± 0.36 <sup>c</sup>	70.90 ± 0.13 <sup>c</sup>	72.87 ± 0.88 <sup>c</sup>	74.19 ± 0.41 <sup>c</sup>
Ethanol 80%	63.83 ± 0.18 <sup>a</sup>	64.89 ± 0.17 <sup>a</sup>	65.99 ± 0.36 <sup>a</sup>	66.58 ± 0.16 <sup>a</sup>
Acetone 80%	66.71 ± 0.56 <sup>b</sup>	68.38 ± 0.60 <sup>b</sup>	69.24 ± 0.46 <sup>b</sup>	69.44 ± 0.39 <sup>b</sup>
Diclofenac Sodium	74.05 ± 0.37 <sup>d</sup>	81.74 ± 0.64 <sup>d</sup>	85.50 ± 0.21 <sup>d</sup>	88.48 ± 0.30 <sup>d</sup>
LSD at 0.05	0.88	0.84	1.12	0.72

All values are the means of three replicates and are significantly different at  $p \geq 0.05 \pm$  standard deviation.

Table 6. Phenolic compounds of *Solenostemma argel* with different solvent (mg/100g DW)

Phenolic compounds (mg/100g DW)	Aqueous	Ethanol	Acetone
Gallic	9.78	53.70	127.30
Pyrogallol	228.91	466.63	964.47
3-Hydroxy tyrosol	86.56	158.99	330.60
4-amino benzoic	4.56	20.62	92.17
Protocatechuic	59.98	67.71	277.00
Chlorogenic	74.77	251.53	785.34
Catechol	233.62	129.22	374.46
Epicatechin	46.54	74.98	308.28
Catechin	287.31	-	297.57
Caffeine	107.70	42.25	114.40
<i>P</i> -hydroxy benzoic	21.34	194.07	85.50
Caffeic	19.90	62.38	128.49
Vanillic	97.32	93.88	151.99
Ferulic	175.16	261.13	290.29
Iso-ferulic	47.97	36.40	127.82
<i>e</i> -vanillic	1173.81	2628.94	6736.32
Reversetrol	13.04	11.52	114.22
Ellagic	272.04	345.18	619.87
$\alpha$ - coumaric	30.62	63.84	114.18
Benzoic	500.69	215.37	1104.30
3,4,5-methoxy cinnamic	33.01	145.80	101.78
Coumarin	28.68	38.64	81.25
Saiyilic	145.32	-	340.40
<i>p</i> -coumaric	13.34	18.94	-
Cinnamic	5.75	47.77	18.67

Table 7. Flavonoids compounds of *S. argel* with different solvent (mg/100g DW)

Flavonoids compounds (mg/100g DW)	Aqueous	Ethanol	Acetone
Narengin	325.19	146.21	835.16
Rutin	78.16	94.71	289.83
Hesperdin	212.12	386.33	500.34
Rosmarinic	181.53	34.61	1216.52
Quercetrin	61.70	69.97	896.86
Quercetin	42.19	121.33	156.47
Narengenin	18.99	21.68	113.77
Kampferol	8.77	37.25	66.43
Hesperitin	62.50	99.88	236.06
Apegenin	7.24	78.30	32.76
7-hydroxyflavone	0.98	60.66	29.38

#### Statistical analysis

Data were statistically analyzed using Costat statistical package <sup>20</sup>.

## RESULTS AND DISCUSSION

Total phenolic (TPC), total flavonoids (TFC) and total tannins (TTC) contents of *Solenostemma argel* extracts

Data of polyphenolic contents of *S. argel* samples are presented in Table 1. Results revealed that the total phenols in 80% acetone extracts were (81.45 ± 0.39 mg GA/g DW), lower than in ethanol (62.58 ± 0.7039 mg GA/g DW) and water (46.72 ± 0.47 mg GA/g DW). The highest concentration of total phenolic was found in 80% acetone extract. The extractive capacity of phenolic components

from *S. argel* is considerably depend on the type of solvents. The best extraction efficiency was achieved by 80% acetone and 80 % ethanol whereas water resulted in poor phenolic contents. The yields of TFC and TTC obtained by using various (solvents) are shown in Table 1. The highest content of TFC was obtained with acetone 80% ( $37.38 \pm 0.31$  mg Q/g DW) and the lowest one with aqueous ( $18.42 \pm 0.37$ mgQ/g DW).The same trend was observed with TTC. The highest value was gained with 80% acetone ( $54.04 \pm 0.31$ mg TAE/g DW) and the lowest value ( $34.49 \pm 0.31$  TAE/g DW) was observed with aqueous extract. This study showed that TP extracts obtained using different solvents, had different total phenolic ,total flavonoids and total tannins contents . The differences observed could be related to the polarity of particular solvent used in the extraction.

#### Antioxidant activity

##### DPPH scavenging radical activity of *Solenostemma argel* extract

DPPH<sup>•</sup> free radical test is based on the exchange of a proton between the antioxidant and the stable DPPH<sup>•</sup> free radical and shows absorption at 515 nm. In principle, the reaction brings about the reduction of DPPH<sup>•</sup> radicals to the corresponding hydrazine, which can be observed by a rapid color change from purple to yellow and can be monitored spectrophotometrically <sup>21</sup>. The decrease in absorbance of DPPH<sup>•</sup> radical is caused by antioxidant through the reaction between antioxidant molecule and radical results in the scavenging of the radical by hydrogen donation The DPPH<sup>•</sup> radical contains an odd electron, which is responsible for the absorbance at 515 nm and also for a visible deep purple color. When DPPH<sup>•</sup> accepts an electron donated by an antioxidant compound, the DPPH<sup>•</sup> is decolorized, which can be quantitatively measured from the changes in absorbance. From the results, it may be postulated that the plant extracts have hydrogen donors thus scavenging the free radical DPPH<sup>•</sup> <sup>22</sup>. The results for aqueous, 80% ethanol and 80% acetone extracts are shown in (Table 2).

The scavenging ability of all the crude extracts on DPPH<sup>•</sup> radicals exhibited concentration dependence. Statistical analysis revealed that the difference between the 80% acetone extract and the other samples was significant ( $p < 0.05$ ). Similar results were also found with respect to IC<sub>50</sub> values as indicated in (Table).

Acetone had the least value of IC<sub>50</sub> ( $48.87 \mu\text{g/mL}$ ), followed by ethanol ( $53.48 \mu\text{g/mL}$ ) and aqueous ( $57.41 \mu\text{g/mL}$ ). A lower IC<sub>50</sub> value means better efficiency of antioxidant activity of the sample. It could be attributed that the phenolic compounds in different extracts had different mechanism against diverse free radicals scavenging activity. These results also demonstrated that 80% acetone might be a more suitable solvent for extracting antioxidants from *S. argel* leaves extract.

##### Fe<sup>2+</sup>-chelating activity of *Solenostemma argel* leaves extracts

Iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Iron is essential for oxygen transport, respiration, and enzyme activity; it is a

reactive metal that catalyzes oxidative damage in living tissues and cells <sup>23</sup>.

Ferrozine can quantitatively form complexes with Fe<sup>2+</sup>. Results indicate that *S. argel* acetone extract exhibited a significantly higher Fe<sup>2+</sup>-chelating activity ( $55.40 \% \pm 0.24$ ) compared to 80% ethanol ( $44.39\% \pm 0.60$ ) and aqueous extract ( $31.60 \pm 0.31$ ) (Table 3).

##### Reducing power activity

Another reaction pathway in electron donation is the reduction of an oxidized antioxidant molecule to regenerate the active reduced antioxidant. Reducing power is a very important aspect for the estimation of the antioxidant activity <sup>24</sup>. As shown in (Table 4 and Figure 1), the reducing power, expressed as EC<sub>50</sub> of *S. argel*.

The presence of antioxidants caused the reduction of the Fe<sup>3+</sup> / ferricyanide complex to the ferrous form and the yellow color of the test solution changed to green and blue depending on the reducing power of each compound. Table 4 and Figure 1 shows the reducing power of different solvent extracts at different concentrations from the *S. argel* leaves extract.

Different solvent extracts showed significant ( $p \geq 0.05$ ) differences in their reducing power, indicating that *S. argel* reducing power can also be influenced by the type of extraction solvent. The reducing power of the different extracts was in the following order: 80% acetone >80% ethanol and aqueous. This data indicates that *S. argel* extracts are capable of donating electrons, which can react with free radicals to convert them to stable products and strongly inhibit radical chain the potency of *S. argel* power reducing may be attributed to the presence of natural antioxidants such as phenolic compounds in the plants <sup>25</sup>.

##### Anti-inflammatory activity of *S. argel* leaves extracts

*In vitro* anti- inflammatory effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds. Denaturation of proteins is a well-documented cause of inflammation. The ability of *S. argel* extract in inhibiting heat induced albumin denaturation was studied (Table 5). Maximum inhibition (74.19 %) was observed from *S. argel* aqueous extract followed by 80% acetone extract (69.44%) and ethanol extract (66.58%) respectively at 200  $\mu\text{g/mL}$ . Diclofenac Sodium, a standard anti-inflammation drug showed the maximum inhibition (88.48%). This may be attributed to the ability of *S. argel* extracts to inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal constituents include bactericidal enzymes and proteinases, which upon extracellular release cause further tissue inflammation and damage <sup>17, 26</sup>. Hence, the presence of bioactive compounds in the plant extract of *S. argel* may contribute to its, antioxidant and anti-inflammatory activity. The present investigation has shown that strong antioxidant and anti-inflammatory properties were confirmed in the acetone extract. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenolics and Saponins <sup>27</sup>.

### Identification of Phenolic and flavonoids compounds of *Solenostemma argel* extracts

It is obvious that the total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of the quality or quantity of the phenolic constituents in the extracts<sup>28, 29</sup>. Various factors affect HPLC analysis of phenolics, including sample purification, mobile phase, column types and detectors<sup>30</sup>. The HPLC analysis of the phenolic compounds in different extracts (Aqueous, 80% and 80 % acetone) were employed using the previous condition and were compiled in Table 6. Data presented in Table 6 reveals that the acetone extracts from *Solenostemma argel* had the highest amounts of e-vanillic, benzoic, pyrogallol, chlorogenic and ellagic acids (6736.32, 964.47, 785.34 and 619.87 mg/100 g DW respectively). The 80 % ethanol extract showed the highest amount of e-vanillic, pyrogallol acid (2628.94, 466.33 mg/100 g DW respectively). e-vanillic and benzoic acid were gained with aqueous extract (1173.81 and 500.69 mg/100 g DW). It was observed that *p-cumaric* not detected with 80% acetone extract, whereas, catechin and salycelic acid not detected in 80% ethanol extract. It has been established that the phenolics and flavonoids content of *S. argel* extracts are strongly depend on the type of the solvent on the different concentrations used as well as the solvent polarity<sup>31, 32, 33</sup>.

Among the studied flavonoids, the rosmarinic, quercetin and naringenin acids were the most potent antioxidants among the simple phenolic acids. In general, rosmarinic, quercetin and naringenin acids were the major phenolic compounds in *S. argel* acetone extract (1216.52, 896.16 and 835.16 mg/100 g DW) followed by hisperidin, rutin, hesperidin and quercetin (500.34, 289.83, 236.06, 156.47 mg/100g DW). In contrast, ethanol and aqueous showed relatively lower content in phenolic compounds in *S. argel* compared to acetone extract (Table 7). It demonstrates that naringenin (21.68 mg/100 g DW), rosmarinic acid (34.61 mg/100g DW), showed lower content of flavonoids. The least flavonoids constituent was apigenin (7.24 mg/100 g DW) and kaempferol (8.77 mg / g100 DW) which was detected in *S. argel* leaves aqueous extract.

### CONCLUSION

From the results obtained it could be concluded that the solvent play a vital role in the extraction of the plant constituents and *S. argel* is a very promising source of novel anti-inflammatory and antioxidant drugs. Further investigations are required to find active component in *S. argel* extract and to confirm the mechanism of action.

### REFERENCES

1. Fabricant DS, Fansworth NR. The value of plants used in traditional medicine for drug discovery. *Environment Health Perspective*. 2001; 109: 69-75.
2. Elkamali HH, Khalid SA. The most common herbal remedies in Dongola province, Northern Sudan. *Fitoterapia*. 1996; 69:118-121.
3. Ahmed MA. The efficacy of four systemic insecticides using two methods of application against the green date palm pit scale insect (*Asterolicanium phoenicis* Rao)

(Homoptera: Asterolicaniidae) in northern Sudan. *Acta Horticulturae*. 2007; 736:369-389.

4. Tharib SM, El-Migdirab, Veitch GBA. A preliminary investigation of the potential antimicrobial activity of *Solenostemma argel*. *Pharm Biol.*, 1986; 24: 101-104.
5. Innocenti, G., Dall'Acqua, S., Minesso, P., Budriesi, R., Micucci, M. and Chiarini, A. Evaluation of muscarinic M3-receptor antagonism of *Solenostemma argel* leaves. *Planta Med*. 2010; 76: 634.
6. Shayoub, M., Haj, E., Makawy, A., Rasha, R. Mona, A. Adverse reaction of *Solenostemma argel* leaves, extraction and alkaloids tablets administered to patients. *Global J Trad. Med. Sys.*, (2013); 2: 14-18.
7. Shafek RE, Michael HN. Antibacterial and antioxidant activity of two new kaempferol glycosides isolated from *Solenostemma argel* stem extract. *Asian journal of plant sciences*. (2012); 11: 143-147.
8. Plaza A, Perrone A, Balestieri M, Felice F, Balestrieri C. New unusual pregnane glycosides with anti proliferative activity from *Solenostemma argel*. *Steroids*. (2005); 70: 594-603.
9. Murwan K, Sabah E, Murwa A. Chemical composition, minerals, protein fractionation, and anti-nutrition factors in leaf of Hargel plant (*Solenostemma argel*). *Euro. J. Sci. Re.*, 2010; 43: 430-434.
10. Singleton VL, Rossi JA. Colorimetric of total phenolics with phosphomolibdic-phosphor tungstic acid reagents. *American Journal of Enology and Viticulture*. 1965; 16, 144-158.
11. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 1999; 64: 555-559.
12. Polshettiwar SA, Ganjiwale RO, Wadher SJ, Yeole PG. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. *Indian Journal of Pharmaceutical Sciences*. (2007); 69: 574-576.
13. Chu YH, Chang CL, Hsu HF. Flavonoids content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agricultural*. 2000; 80, 561-566.
14. Su C, Chen W, Weng Y, Tseng C. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry*. 2003; 83, 85-92.
15. Kuda T, Tsunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *J. Food Compos. Anal*. 2005; 18: 625-633.
16. Mizushima Y and Kobayashi M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol*. 20: 169-173
17. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *I. J. Pharm. Pharm. Sci.*, 2010; 2: 146-155.
18. Hakkinen SH, Karenlampi SO, Heinonen IM, Mykkanen HM, Torronen AR. HPLC method for

- screening of flavonoids and phenolic acids in berries. *J. Sci. Food Agric.*, 1998; 77: 543-551.
19. Mattila P, Astola J, Kumpulainen J. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *J. Agric. Food Chem.*, 2000; 48: 5834-5841.
20. Anonymous A. Cohort Soft Ware Crop. Costat user manual version 3.03, 1989; Berkeley CA, USA.
21. Matthus B. Antioxidant activity of extracts obtained from residues of different oilseeds. *J. Agric. Food Chem.* 2002; 50: 3444-3452.
22. Valko M, Leibfritz D, Moncol J, Cronin MT. Free radicals and antioxidants in normal physiological functions and human disease *Int. J. Biochem. Cell. Biol.* 2007; 39: 44-84.
23. Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R. Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. *Lebens Wissenun Technol.* 2007; 40: 239-248.
24. Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A, et al. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. *C R Biol* 2008; 331:865-873.
25. Lee YL, Yang JH, Mau JL. Antioxidant properties of water extracts from *Monascus* fermented soybeans. *Food Chem.* 2008; 106: 1128-1137.
26. Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant induced paw edema in rats and inflammatory mediators release. *Phytother Res.* (1997); 11:152-154.
27. Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) .*Journal of Pharmacognosy and Phytotherapy*, 2011; 3: 3-51.
28. Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anurad E, Shiwaku K, and Yamane Y. Screening for antioxidant activity in edible plant products: Comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin-Ciocalteu assay. *Journal of the Agricultural and Food Chemistry.* 2004; 52: 2391-2396.
29. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of the Agricultural and Food Chemistry.* (2004); 52: 4026-4037.
30. Stalikas CD. Review: Extraction, Separation, and detection methods for phenolic acids and flavonoids. *J Sep Sci.*, 2007; 30: 3268-32958.
31. Lapornik B, Prosek M, Wondra AG. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *J Food Eng.*, 2005; 71: 214-222.
32. Rodtjer A, Skibsted LH, Andersen ML. Antioxidative and prooxidative effect of extracts made from cherry liqueur pomace. *Food Chem.*, 2006; 99: 6-14.
33. Turkmen N, Sari F, Velioglu YS. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chem.*, 2006; 99: 835-841.
34. Su C, Chen W, Weng Y, Tseng C. Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry.* 2003; 83, 85-92.
35. Tharib SM, El-Miggirab, Veitch GBA. A preliminary investigation of the potential antimicrobial activity of *Solenostemma argel*. *Pharm Biol.*, 1986; 24: 101-104.
36. Turkmen N, Sari F, Velioglu YS. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chem.*, 2006; 99: 835-841.
37. Valko M, Leibfritz D, Moncol J, Cronin MT. Free radicals and antioxidants in normal physiological functions and human disease *Int. J. Biochem. Cell. Biol.* 2007; 39: 44-84.
38. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of the Agricultural and Food Chemistry.* (2004); 52: 4026-4037.
39. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 1999; 64: 555-559.