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Evaluation of the Antioxidant Activity and the Acute Oral Toxicity of Three Plant Extracts on Albino Mice

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ABSTRACT

In recent years much attention has been devoted to antioxidants and their association with health benefits. Plants are important sources of natural antioxidants have potential to neutralize the reactive oxygen species (ROS) and led to delay the oxidation process. Therefore, they may be used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. The present study aimed to determine the total phenolic and flavonoid contents of the methanolic extracts of three plants growing in Egypt *Cassia fistula, Cynara scolymus* and *Glycine max*. Also, evaluation and determination of the antioxidant activity and preliminary acute oral toxicity of the methanol extracts of the three plants were carried out respectively. Folin–Ciocalteu and aluminum trichloride reagents were used for determination of phenolic and flavonoid contents respectively. The obtained results appeared that each plant extract has a considerable content of phenolic and flavonoids. Also, three different methods such as: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, total antioxidant capacity and reducing power were used for determination of the antioxidant activity of each plant extract. The results exhibited that each methanolic extract of the three plants has high antioxidant activity and *G.max* methanol extract displayed the highest activity. Also, a positive correlation between the antioxidant activity of the methanol extracts of the three tested plants and their phenolic contents was observed. On the other hand, the results of the preliminary acute oral toxicity of the three plant extracts on the mice proved that these extracts can be considered as safe or nontoxic up to 10.000 mg/kg body weight of mice (the lethal dose; LD_{50} values >10.000 mg/kg for mice).

Key words: Antioxidant, *Cassia fistula, Cynara scolymus*, *Glycine max*, total phenolics and flavonoid content, acute oral toxicity and mice.

Introduction

Free radicals are well known to be the major cause of various chronic and degenerative diseases as they are associated with pathogenic mechanisms of many diseases including nonalcoholic fatty liver disease, atherosclerosis, neurodegenerative diseases, cancer, diabetes, inflammatory diseases, as well as aging processes (Nooman *et al*., 2008; Veeru *et al*., 2009). The free radicals and reactive oxygen species (ROS) are produced through frequent physiological and biochemical processes in the human body as by products (Braga *et al*., 2012). ROS includes a number of chemically reactive molecules derived from oxygen, such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical (OH^-) etc. Over production of such free radicals might lead to oxidative damage of biomolecules in the body. These free radicals are capable of reacting with membrane lipids, nucleic acids, proteins, enzymes and other small molecules, resulting in cellular damage (Gulcin *et al*., 2002; Jayanthip and Lalitha 2011).

The harmful effect of the free radicals can however, be blocked by antioxidant substances. Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Current research has confirmed that antioxidants are the most effective tools to eliminate free radicals and can protect the cells from ROS and retard the progress of many diseases (Cai *et al*., 2003; Devasagayam *et al*., 2004; Saeed *et al*., 2012) Also, antioxidants play an important role in nutritional by lengthening the shelf life of food and reducing nutritional losses and formation of harmful substances. However, the safety of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), is now in doubted (Moein *et al*.,2008**)**. These findings directed the use of dietary and/or medicinal supplements particularly during the disease attack (Huda-Faujan *et al*., 2009; Saeed *et al*.,2012).

Many researchers have been searching for powerful of non-toxic antioxidants from natural sources, especially edible or medicinal plants. Natural antioxidants have been studied in order to find out compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage (Yang *et al*., 2007; Maoulainine *et al*., 2012). Results have shown that the raw extracts or isolated pure compounds from them were more effective antioxidants *in vitro* than BHT or vitamin E So, medicinal plants can be a potential source of natural antioxidants (Moein *et al*., 2008**)**.

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Plants produce wide array of secondary metabolites such as phenolic compounds, alkaloids, vitamins, terpenoids and other secondary metabolites that have been proved to have antioxidant activities (Kaur and Kapoor, 2002; Wojdyło *et al*.,2007). Recently, the polyphenols have been found to be beneficial as strong antioxidants. In this context, evaluation of the polyphenols and their antioxidant activity in herbs has become important tool to understand the healing property of medicinal plants. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Chang *et al*., 2007; Yang *et al*., 2007; Kumar *et al*.,2008).

The three Egyptian investigated plants; *Cassia fistula, Cynara scolymus* and seeds of *Glycine max* were reported to show plenty of medicinal applications (Bahorun *et al*., 2005; Alghazeer *et al*.,2012; Gupta and Suman 2012). Nevertheless, their acute oral toxicities were poorly studied (Fukuda *et al*., 2011; Jothy *et al*., 2011). Therefore, it was thoughtful to carry out this study in order to determine of the total contents of phenolic and flavonoid of the methanolic extracts of three plants ; *C. fistula, C. scolymus* and *G. max*. Also, investigation the antioxidant properties of these extracts and determination the acute oral toxicity of these extracts (LD_{50} value) was carried out. As the acute oral toxicity test is the simplest and often the first toxicity test to be conducted on a sample (WHO, 2004), so the acute oral toxicity study will also be carried out as a preliminary step before proceeding to further *in vivo* studies on these extracts.

Materials and Methods

I.Materials:

I.1.Collection and identification of plant materials:

The plants were collected during 2009 where *Cassia fistula* was collected from El-Orman Botanical Garden while *Cynara scolymus* from Horticulture Research Center and *Glycine max* from Field Crops Research Institute. The plants were kindly identified by Prof Dr. Wafaa Amer, Professor of Plant Taxonomy, Faculty of Science, Cairo University. Voucher specimens of the plants were kept in Medicinal Chemistry Department, Theodor Bilharz Research Institute.

I.2.Chemicals for antioxidant studies:

DPPH (1,1-diphenyl-2-picrylhyrazyl) was purchased from Sigma-Aldrich Co. (St. Louis MO, USA). Also, gallic acid, sodium phosphate, ammonuim molybdate, Folin-Ciocalteu reagent, ascorbic acid, rutin, sodium carbonate, aluminium trichloride and other chemicals of high quality and were purchased from common sources.

I.3.Animals:

A healthy albino male mice aged 8 weeks were obtained from Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute. All the animals received standard animal care and they were housed in standard polypropylene cages with stainless steel covers. The animals were acclimatized for 1 week under laboratory conditions (20-23°C, relative humidity 55-60%, 12 h light/dark cycle) before each study. After, acclimatization, the animals were divided randomly into groups with six mice per group. The animals had free access to tap water and a standard pellet diet, except for a short fasting period of four hours before and after the oral administration of single doses of each extract.

II. Methods:

II.1.Preparation of plant extracts:

500 gm of each plant under investigation was shade dried at room temperature, grounded into fine powder using a mixer grinder and then extracted for several times using 70% methanol. The methanolic extract of each plant was combined and filtered through Whatmann filter paper then concentrated under reduced pressure using a rotatory evaporator at 40^{o}C till complete removal of the methanol. The dried methanolic extract of each plant was weighed and kept a well from moisture in a well plastic vials in desiccator for determination of their effects in present study.

II.2.Determination of total phenolic content:

Phenolic content of the three tested methanolic extracts was estimated using Folin-Ciocalteu reagent by a colorimetric assay which based on procedures described by Kumar *et al*., 2008 with some modifications, gallic acid was used as standard. In this method, 100 µl of each extract in concentration of 100 µl/ml was mixed with 500 µl of the Folin–Ciocalteu reagent and 1.5 ml of sodium carbonate (20 %). The mixture was shaken and adjusted to 10 ml using distilled water and allowed to stand for 2 h. The absorbance was measured at 765 nm against a blank with distilled water and gallic acid as standard. Estimation of the phenolic content was carried out in triplicate. The total phenolic content was expressed as mg gallic acid equivalent per gram dry weight extract (mg GAE/g extract).

II.3.Determination of total flavonoid content:

The total flavonoid content of each extract was determined according to the procedures described by Kumaran and karunakaran 2006 using rutin as a standard. Plant extract (100 µl) in methanol (100 µg/ml) was mixed with 100 µl of aluminium trichloride in methanol (20 mg/ml) and then diluted with methanol to 500 µl. After 40 min, the absorption of each test was evaluated at 415 nm against the blank. All determinations were carried out in triplicate. The total flavonoid in each plant extract was estimated as mg rutin equivalents per gram extract (mg RE /g extract).

II.4.Determination of DPPH radical scavenging activity:

The ability of each extract to scavenge DPPH radicals was measured according to the procedure described by Mansour and Khalil 2000; Liu *et al*., 2009. Three ml of each plant extract at a concentration of 100 µg /ml were mixed well with 1 ml of (0.1 mM of 1,1-diphenyl-2-picrylhydrazyl; DPPH) in methanol. The mixture was then shaken and left for 30 min in the dark at room temperature. Absorbance was measured at 517 nm against the blank using UV/VIS spectrophotometer. Ascorbic acid was used as a reference standard. Controls contain only solvent and DPPH without any extract. All experiments were carried out in triplicate.

Free radical scavenging activity (antioxidant activity) of each extract is expressed as the percentage of DPPH decrease. It was expressed as SC_{50} which is defined as the concentration of each extract required for scavenging of 50% of DPPH radicals compared with that of ascorbic acid. The lower SC_{50} value is an indication of higher scavenging activity or higher antioxidant activity of plant extracts.

II.5. Determination of total antioxidant capacity:

The total antioxidant capacity of the each extract was determined by phosphomolybdate method using ascorbic acid as a standard (Preretto *et al*., 1999; Saeed *et al*., 2012). An aliquot of 0.5 ml of sample solution was mixed with 5 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were sealed then incubated at 95°C in a water bath for 90 min. Thereafter, the tubes were left to cool at room temperature then the absorbance of the mixture was measured at 695 nm against a blank. The blank consisted of 5 ml of the reagent solution and the appropriate volume of the solvent and was also incubated under the same conditions. The experiment was repeated for 3 times. As ascorbic acid was used as standard, so the antioxidant activity of the extracts was expressed as the number of equivalents of ascorbic acid (AAE).

II.6. Reducing Power Assay:

Reducing power of extracts was evaluated according to the method of Oyaizu, 1986. Each sample 1 ml in methanol was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50 °C for 20 min. Then adding 2.5 ml of trichloro acetic acid (TCA), the mixture was centrifuged at 3000 rpm for 10 min. The supernatant solution (2.5 ml) had taken out and immediately mixed with 2.5 ml of methanol and 0.5 ml of 0.1% ferric chloride. After incubation for 10 min, the absorbance against blank was measured at 700 nm. Increase in absorbance of the reaction mixture indicates increased reducing power. All the tests were performed in triplicates. Ascorbic acid was used as standard.

III. Acute toxicity studies:

The acute oral toxicity study was performed according to the OECD guideline for testing of chemicals (OECD 1981) and WHO guideline (WHO, 2000). In order to determine any possible toxic effect or changes in normal behavior of a single oral administration of the three plant extracts, the present studies were carried out on

78 male albino mice (31.5-34.9 g /mouse at the beginning of experiment) fed normal diet and supplied with tap water. Regulations of the ethical committee of Theodor Bilharz Research Institute were strictly followed. These mice were divided into 13 groups (each of 6 mice). All the animals were fasted overnight before the experiment. On the other hand, four doses from each tested plant extract ; ***C.fistula, C.scolymus*** **and** ***G.max*** (4.000, 6.000, 8.000, 10.000 mg/kg body weight) were prepared in distilled water. In case of each plant extract, the mice were administered a single oral dose from them as shown in table (3). These doses took into account the solubility of the extracts in distilled water, while control group received only the same volume of distilled water.

After administration of these doses of each plant extract, the mortality and general behavior of the animals were observed continuously for the first 3 h for any toxic symptoms followed by observation at regular intervals for 24 h up to 14 days. The parameters observed were some behavioral signs of toxicity such as CNS depression (hypoactivity, relaxation), CNS stimulation (hyperactivity, irritability, tremors, convulsions), respiratory pattern, state of stool. At the end of the study, the animals were also observed for general organ toxicity and mortality then sacrificed under anesthesia (Ramesh *et al*., 2007; Yang *et al*., 2010; Singh and Kumar 2011; Thounaojam *et al*., 2011).

Initial and final body weights and food intake were observed. At the end of experiment some vital organs such as liver, kidneys, heart, lung and spleen were isolated and weighted. Relative organ weight was calculated and compared with the control group.

IV. Absolute and relative organs weight:

Before and after the experiment, the body weight of mice was recorded. Food and water intake were observed. Some vital organs such as liver, kidneys, heart, lung, spleen were isolated and weighted. Relative organ weight (ROW) was calculated and compared with the control group as follows :
ROW= (Absolute organ weight X100 %) / Body weight of mice on sacrifice day (g) (Sahgal *et al*., 2010).

V. Statistical analysis:

Results were expressed as mean ± standard deviation. Statistical significance was determined by one-way analysis of variance (ANOVA) The data obtained from acute toxicity studies were analyzed using Student's t-test. *P* values less than 0.05 were considered significant.

Results and Discussion

The oxidative stress, defined as ''the imbalance between oxidants and antioxidants. It led to cellular damage, inflammation , fibrosis, aging and many other serious diseases (Takayama *et al*., 2009). Several plant extracts showed antioxidant properties and can be of great significance in therapeutic treatments for different diseases resulted to oxidative stress (Mansour and Khalil, 2000; Sharma *et al*., 2010; Chung *et al*., 2012). In the present study ,the phenolic and flavonoid contents and the antioxidant of the methanolic extracts of three plants growing in Egypt *C. fistula* ,*C. scolymus* and *G. max* were determined. Also, preliminary acute oral toxicity of each plant extract was calculated in order to determine the lethal dose (LD_{50})of these extracts.

Total phenolic content:

It has been reported that the antioxidant of the plant extracts depend on their phenolic contents. The phenolic components play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides due to of their redox properties (Osawa 1994). Also the antioxidant extracts have several pharmacological activities including anti-inflammatory, antiproliferative and antiviral effects (Mohamed *et al*, 2008; Jimenez- Eshada *et al.*, 2013). Therefore, in the present study, the phenolic and flavonoid contents of the methanol extracts of three plants were determined. The results in table (1) showed that the total phenolic contents of the methanolic extracts of *C. fistula*, *C. scolymus* and *G. max* were 138.63±0.79, 135.58±1.69 and 220.37±1.65 respectively. This mean that the three extracts contain a considerable of phenolic compounds and *G. max* had high phenolic content. Therefore, the three plant extracts may be expected to have antioxidant properties.

Total flavonoid content:

Flavonoids have reducing properties due to the multiple hydroxyl groups attached to their aromatic ring. Therefore, it is recognized that flavonoids are able to scavenge different reactive oxygen radicals such as the hydroxyl and superoxide radicals (Moein *et al*, 2008; Noorhajati *et al* 2012; Jimenez-Eshada *et al.*, 2013). The total flavonoid content of the methanolic extracts of the three plants *C. fistula* ,*C. scolymus* and *G. max* was

evaluated by colorimetric assay and expressed in mg rutin. Results in table (1) revealed that *Glycine max* extract showed highest content of flavonoids (69.36±1.29 mg rutin equivalent/gm plant) followed by *C. fistula* (45.53±0.81 mg rutin equivalent/gm plant) and *C. scolymus* (40.53±1.79 mg rutin equivalent/gm plant).

The obtained results revealed that the methanol extracts of each plant under investigation contain high phenolic and flavonoid content. This means that the extract of each plant will recommended as antioxidant agents as well as it becomes medically important.

DPPH radical scavenging activity:

Although, many methods are available for determination of free radical scavenging activity but 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity has received the maximum attention owing to its ease of use and its convenience. This method has been used widely in the model system to investigate the scavenging activities of several natural compounds especially phenolic compounds (Mansour and Khalil,2000; Malenecic *et al.*,2008). In the DPPH radical scavenging assay, when the DPPH is exposed to antioxidant compounds, its purple color changed into yellow after reduction. The more yellowish color of DPPH was observed, This change can be quantified by decrease of absorbance at wave length 517 nm.

In the present study, DPPH radical scavenging activity of the methanol extracts of the three plants were determined using ascorbic acid as reference. Results in table (2) exhibited that the methanol extract of *G.max* has the highest radical-scavenging activity. The order of activity of the tested extracts was *G.max* (SC_{50} = 74.37 ± 4.86), *C.fistula* (SC_{50} = 132.86 ± 1.94) and *C.scolymus* (SC_{50} = 140.25 ± 1.45). These results revealed that the high DPPH scavenging activity was associated with the high phenolic content of extract of *G.max* .The activity of the other plants were in order *C.fistula* followed by *C.scolymus*. It was appeared that there is a positive correlation between the antioxidant activity of the methanol extracts of the three tested plants and their phenolic contents (R2 =0.99) as shown in fig (1). These results are in full agreement with other previous studies on many plant extracts (Preretto *et al*., 1999; Frankel and Meyer 2000; Awika *et al*., 2003; Prakash *et al*., 2007; Mohamed *et al*., 2008; Irshad *et al*., 2012; Noorhajati *et al.*, 2012).

Total antioxidant activity:

Some methods were developed recently for measuring the total antioxidant capacity of food and natural products. These assays differ in their chemistry and measurements (Pellegrini *et al*., 2003). In the present work ,the phosphomolybdenum method was used .This method is based on the reduction of Mo(IV) to Mo(V) by the tested extract and the subsequent formation of green phosphate /Mo(V) compound with a maximum absorption at 695 nm. A high absorbance value of the sample indicates its strong antioxidant capacity. From results in table (2), it is appeared that all the tested plants showed a good total antioxidant capacity. The methanolic extract of *G.max* was the most active (225.72±4.18 mg equivalent to ascorbic acid/ g extract). Also, as shown in fig (2), there is a relationship between the total antioxidant capacity of the methanolic extracts of the tested extracts and their phenolic contents (R2 =0.94). These results are in good accordance with previous studies which reported a linear relationship between the total antioxidant capacity of some plant extracts and their phenolic contents (Preretto *et al*., 1999; Awika *et al*., 2003; Mohamed *et al*., 2008). Also these results are in full agreement with the previous studies on the antioxidant potency of the extracts of bark and seeds of *C.fistula* extracts and other plant extracts *of C.scolymus* and *G.max* extracts (Jimenz -Escrig et al 2003; Bhatnagar *et al*., 2010; Fukuda *et al* 2011; Zhang *et al.*, 2011; Noorhajati *et al.*,2012; Jiméneze –Eshada *et al*., 2013).

Reducing power:

It has been reported that the reducing powers of any plant extract may serve as a significant indicator of its potential antioxidant activity (Oyaizu,1986; Mohamed *et al*., 2008). In this assay the yellow color of the test solution changes to green and blue. This is depend upon the reducing power of each compound which can break the free radical chains by donating a hydrogen atom. The presence of antioxidant radicals causes the conversion of the Fe^{3+} in ferric chloride to the ferrous (Fe^{2+}) form. Therefore by measuring the formation of pearls Prussian blue at 700nm, the Fe^{2+} concentration can be monitored. A higher absorbance at 700nm indicates a higher reducing power (Rehman *et al*., 2003). The results of the reducing power assay given in table (2) showed that all the methanol extracts have reducing power. The activity of the plant extracts is probably due to the presence of phenolic compounds which might act as electron donors. These results are in full agreement with the previous studies which reported that the reducing power of plant extracts are correlated with their phenolic content (Alabsalvar *et al*., 2006; Mohamed *et al*., 2008).

Acute oral toxicity study:

In many cases, the natural products contain bioactive principles with potential to cause some adverse effects (Bent and Ko 2004). Therefore, the investigation of acute oral toxicity of any plant extract is the first step in the toxicological analysis of herbal drug (Sim *et al.*, 2010). A part from giving a clue on the range of doses that could be used in any subsequent testing ,it could reveal the possible clinical signs elicited by the substance under investigation. It is also a useful parameter to investigate therapeutic index (i.e. LD_{50}) of drugs (Bent and Ko 2004).

In the present study several parameters was assessed: the animals death, the body weight, the behavior adverse effects, the relative organ weights and the water & food consumed.

Results indicated that no death was observed after the administration of doses from 4.000 to 10.000 mg/kg.b.wt. of the methanol extract of the three tested plants (Table 3). This is an indication that the extracts have negligible level of toxicity when administered orally as their LD_{50} > 10.000 mg/kg (10 g/kg). As according to Vaghasiya *et al*., 2010 substances with LD_{50} higher than 5 g /kg by oral route are regarded as being safe or practically non-toxic. These findings are in full agreement with Jothy *et al*., 2011; Fukuda *et al*., 2011 as they reported the safety of 5.000 mg/kg body weight of *C. fistula* extract and 2500 mg/kg body weight of *G. max* in mice.

The body weight of the animals treated with the extracts once, did not show any significant change when compared with the control group, although it had a tendency to decrease body weight in groups treated with *G. max* but this decrease was insignificant. The mean value of body weight of control and treated groups ranged from 31.5-34.9 g/mice and 32.2- 36.4 g/mice at the beginning and the end of the experiment respectively (Table 3). Generally, the reduction in body weight is a simple and sensitive index of toxicity after exposure to toxic substances. Body weight changes are of adverse effects of drugs and chemicals and it would be significant if the body weight loss occurred is more than 10% from the initial body weight (Teo *et al*., 2002).

The behavioral signs of toxicity such as CNS depression (hypoactivity, relaxation), CNS stimulation (hyperactivity, irritability, tremors, convulsions) and others were observed (Table 3). No signs of toxicity were observed either in the control or treated groups.

Organ weight also is an important index of physiological and pathological status in man and animals. The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are the primary organs affected by metabolic reaction caused by toxicant (Dybing *et al*., 2002) .

The relative organ weights were almost the same in control and treated groups, sometimes the weight was slightly more in treated groups but it was statistically insignificant from control group (results not included). Therefore, it can be concluded that administration of methanolic extracts did not show any effect on organ weight of all target organs. Moreover, gross examination of internal organs of all mice revealed no detectable abnormal changes in color and texture when compared with the control group.

It has been reported that determination of food consumption is important in the study of safety of a product with therapeutic purpose as proper intake of nutrients is essential to the physiological status of the animal (Vaghasiya *et al*., 2010). Also treatment with the extracts did not decrease the water and food consumption (data not shown).

In conclusion the three extracts appeared safe at the doses used in this study. According to FDA (1988) the oral LD_{50} value in mice which is higher than 10.000 mg/kg falls into class 5 (safe) of the classified system for chemical substances.

Conclusion:

It can finally be concluded from this study that the methanol extracts of the three plants *C.fistula*, *C.scolymus* and *G.max* possess strong antioxidant potency and there is a strong correlation between the antioxidant potency of the plant extracts and their phenolic contents. Also, the results of the preliminary acute oral toxicity of the three plant extracts on the mice proved that these extracts can be considered as safe or nontoxic up to 10.000 mg/kg body weight of mice (the lethal dose; LD_{50} values >10.000 mg/kg for mice).

Table 1: Total contents of phenolic and flavonoid of methanol extracts of *C.fistula*, *C.scolymus* and *G.max*.

Plant	Total phenols (mg gallic acid equivalent/g plant extract)	Total flavonoids (mg rutin equivalent/g plant extract)
Cassia fistula	138.63 ± 0.79	45.53 ± 0.81
Cynara scolymus	135.58± 1.69	40.53±1.79
Glycine max	220.37±1.65	69.36±1.29

Values are expressed as mean of triplicate determinations ± standard deviation

Table 2: Yield, free radical scavenging activity, total antioxidant capacity and reducing power of methanol extracts of *C.fistula*, *C.scolymus* and *G.max.*

Plant	Yield (%)	DPPH free radical scavenging activity SC_{50}(µg/ml)[a]	Total antioxidant capacity (mg equivalent to ascorbic acid/g)[b]	Reducing power
C.fistula	20.2	132.86 ± 1.94	198.5 ± 7.00	0.305±0.002
C.scolymus	23.7	140.25±1.45	188.46±2.98	0.211 ±0.006
G.max	24.7	74.37±4.86	225.72±4.18	0.371 ±0.004
Ascorbic acid	-----	7.90±0.20	----------	1.69±0.011

Values of SC_{50} and total antioxidant capacity are expressed as mean of triplicate determinations ± standard deviation
[a]SC_{50}, Concentration in µg/ml required scavenging the DPPH radical (100 µg/ml) by 50 %.
[b]Antioxidant capacity monitored by the phosphomolybdenum method.

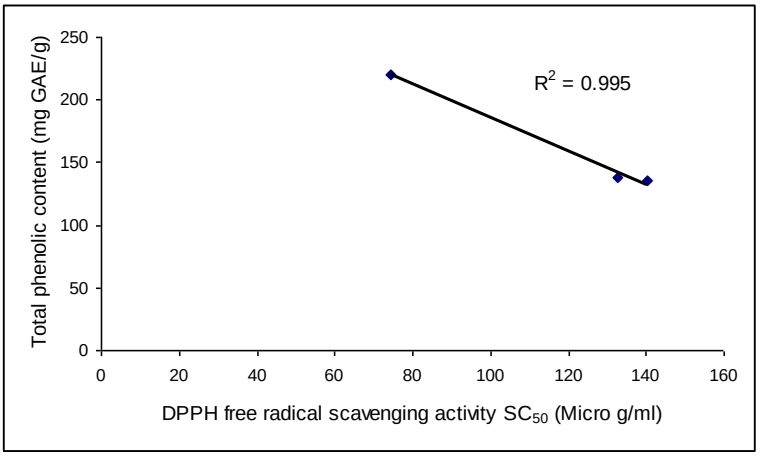

Fig. 1: Correlation between DPPH scavenging activity and total phenolic contant.

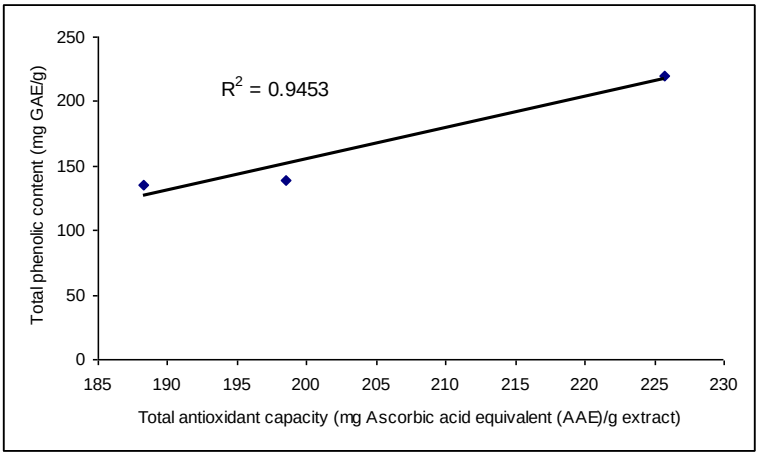

Fig. 2: Correlation between total antioxidant capacity and total phenolic content.

Table 3: Effect of acute toxicity test (single oral doses) of *C.fistula*, *C.scolymus* and *G.max* on body weight and potential toxic effect in mice.

Dose of extract(mg/kg.b.wt)	Initial body weight	Final body weight	No Dead/ treated mice	Mortality ratio (%)	Symptoms
C. fistula					
4.000	33.4±1.7	35.1±1.8	0/6	0	None
6.000	32.2±2.7	33.7±2.5	0/6	0	None
8.000	33.8±2.4	36.4±1.9	0/6	0	None
10.000	31.9±2.1	32.7±2.2	0/6	0	None
C.Scolymus					
4.000	34.9±1.5	36.3±3.1	0/6	0	None
6.000	33.8±3.1	35.7±2.9	0/6	0	None
8.000	32.4±1.9	34.8±1.5	0/6	0	None
10.000	31.5±2.2	32.2±2.1	0/6	0	None
G.max					
4.000	33.4±2.7	32.2±2.8	0/6	0	None
6.000	34.1±2.6	32.9±2.4	0/6	0	None
8.000	33.5±1.8	32.5±2.6	0/6	0	None
10.000	34.9±1.7	34.2±3.5	0/6	0	None
Control	32.8±1.9	35.7±2.8	0/6	0	None

Control group (received only distilled water without any extract).
No significant difference was observed in any group.
Symptoms (the behavioral signs of toxicity).

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