Indian Journal of Drugs, 2014, 2(2), 65-72 ISSN: 2348-1684

Research Article

EVALUATION OF ANTIOXIDANT, CYTOTOXIC AND CYTOPROTECTIVE ACTIVITIES OF *ALHAGI MAURORUM* EXTRACTS AND PHYTOCHEMICAL PROFILE

Ataa Said[1], Mohamad Abo-El-Fotouh[2], Abdel-Rahman Alshabrawy[2], Jitka Psotova[3], [1]Usama Hawas, Amira Omar[1], Khaled Rashed[1]

[1]National Research Centre, Pharmacognosy Department, Dokki, Giza, Egypt

[2]Faculty of Pharmacy, Cairo University, Cairo, Egypt.

[3]Institute of Medical Chemistry and Biochemistry, Faculty of Medicine, Palacky University, Hnevotínská 3, 775 15 Olomouc, Czech Republic.

[*]**For Correspondence:**
Ataa Said and Khaled Rashed
National Research Centre,
Pharmacognosy Department, Dokki,
Giza, Egypt

Received: 08.04.2014
Accepted: 22.06.2014

Access this article online

Website:
www.drugresearch.in

Quick Response Code:

ABSTRACT

This study was carried out to evaluate antioxidant, cytotoxic and cytoprotective activities of Alhagimaurorum petroleum ether, diethyl ether, chloroform and methanol (70%) extracts and to investigate the main phyto-constituents. Antioxidant activity was carried out by measuring free radical scavenging on model reaction with stable 2, 2-diphenyl-1-picrylhydrazyl radical. Cytotoxic effect was carried out on both primary rat hepatocyte culture, and hepatoma cell line, as well for cytoprotective effect against lipid peroxidation and against oxidantive stress induced by hydrogen peroxide. The results showed that diethyl ether is the best active free radical scavenging agent and it showed IC50 0.083 ± 0.01 (mg/mL). Ether extract showed the highest cytotoxic effect while methanol extract was the least. Concerning cytoprotective effect, all the extracts showed a mild cytoprotective effect. Phytochemical analysis of the extracts proved the presence of triterpenes in petroleum ether, ether and chloroform extracts. Flavonoids, triterpenes and carbohydrates were detected in methanol 70% extract. In conclusion this study may help to discover new natural antioxidant, cytotoxic and cytoprotective substances that could serve as selective agents for infectious diseases.

KEY WORDS: *Alhagimaurorum*, free radical scavenging, cytotoxic, cytoprotective, phyto-constituents.

INTRODUCTION

Free radicals have been demonstrated to be the main initiator for many diseases such as cancer, alzheimer's disease and rheumatoid arthritis (Pulido *et al*., 2000). For this reason, there has been intensive study of the antioxidant properties of plant extracts and isolated phytochemicals, with a view to identifying potentially useful antioxidant treatments. There has been a worldwide trend towards the use of natural phytochemicals present in fruits, vegetables, oil seeds, teas, herbs, berry crops and beans. Natural antioxidants have a wide range of biochemical activities, including inhibition of ROS generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential (Finkel and Holbrook 2002). Flavonoids are always known as a great antioxidant compounds as they are rich in the hydroxyl groups which easily can lose proton to neutralize the odd electron in the free radical harmful species. Different kinds of plants are a big storage for flavonoids as *Alhagimauroum*which belongs to family Leguminosae is native to Mediterranean and central Asia, it's well known as camel thorn and always grows in salty soils and dry environment. It has been used in folk medicine as laxative, purgative, diaphoretic, expectorant and diuretic (Boulos, 1966 and Idem, 1970). The oil of *Alhagi*is used in the treatment of rheumatism and the flowers are used to treat piles, migraine and warts (brown, 1995). Topically water extract of its roots is used to relax the ureter, remove kidney stones and also used as indicator for soil salinity (Habib, 1971). Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain

reaction even with other healthy cells. Their main danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cell damage caused by free radicals appears to be a major reason for aging and degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others which contribute to about 80% of all the diseases (Halliwell, 1994, Halliwell,1995) Free radicals are neutral, short lived, unstable and highly reactive to pair up the odd electron and finally achieve stable configuration. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cells may function poorly or die if this occurs. To prevent free radical damage, the body has a defense system of antioxidants which include extracellular and intracellular antioxidants. Enzymes like Superoxide dismutase [SOD], Catalase [CAT], glutathione peroxidase [GPO], glutathione reductase are intracellular antioxidants. Within biological system, a number of small molecular weight antioxidants are capable of acting as repair or sacrificial antioxidants because they are chain breaking antioxidants which are extracellular antioxidants e.g., uric acid, bilirubin, transferring, albumin, heptaglobin, tocopherol, ascorbic acid, glucose etc. Antioxidants are intimately involved in the prevention of cellular damage, the common pathway for cancer, aging, and a variety of diseases (Nunez et al,1997). Recently there has been growing interest in research into the role of plant-derived antioxidants in food and human health. This study highlights antioxidant, cytotoxic and cytoprotective activities of *AlhagiMaurorum*herb and its main chemical compounds.

MATERIALS AND METHODS

PLANT MATERIAL

Samples of the herb *Alhagimaurorum L.* family Fabeacea were collected from the sides of train lines at Giza, Egypt in Feb. 2008, and were kindly authenticated by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC). The collected herbs of the plant were air-dried, powdered and kept in tightly-closed containers.

EXTRACTION

500 g of the air-dried powder herb of *Alhagimaurorum* was exhaustively extracted with 70% methanol. The methanol extract was evaporated under vaccum to yield 165 gm. The extract was dissolved in distilled water. The aqueous solution was successively extracted with petroleum ether, diethyl ether, chloroform, ethyl acetate, and finally with *n*-butanol, respectively. Each extract was subjected to phytochemical analysis according to method described by (Solomon 2013). All chemicals used in this study were purchased from Sigma Aldrich Chemical Co.Ltd.

BIOLOGICAL ASSAYS

Free radical scavenging activity: The scavenging effect of each extract was evaluated by measuring change in absorbance at 517 nm of 2, 2´-diphenyl-1-picrylhydrazyl radical (DPPH) for 30 min (Deby and Margotteaux, 1970). The reaction mixture contained 0.1 mL of DPPH (20 mg/L, MeOH) and 0.05 mL of stock solution of test extract (0.05-2 mg/ml, MeOH) or MeOH alone as control. The extract's activity was calculated as the result concentration of extract that inhibited the colour reaction by 50% (IC_{50}).

Cytotoxic activity: For cytotoxic studies, the extract stock solution (2-100 mg/ml) was prepared in DMSO. The final concentration of DMSO in medium in course of experiment was lower than 0.5%.The final concentration of extract in growth medium was 0.01, 0.05, 0.1, 0.25, 0.5 mg/ml.

Cell Culture

A-Rat hepatocyte culture: hepatocytes were isolated by two-step collagenase perfusion of liver (Moldeus*et al.*1978) The cells were then suspended in William's medium E, supplemented with glutamine (2 mM), streptomycin (0.1 g/L), penicillin G (10 000 U/L), dexamethasone (1 µg) and insulin (0.1 µg), and washed by centrifugation (50 x g; 1 min), using the trypan blue exclusion test, cell viability was ~90%. Cell suspensions in medium supplemented with 10% bovine serum were seeded on collagen type I pre-coated plates at a density of $1x10^5$ cells/cm^2 and incubated at 37°C in an atmosphere containing 5% CO_2. The medium was changed for a serum-free one after 3 h. Rat hepatocytes were incubated for 24 h with extracts (0.01-0.5 mg/mL). After the experiment, the culture medium was removed and assayed for extracellular lactate dehydrogenase activity (LDH), neutral red uptake assay (NRA) and cell viability was determined by MTT test.

B-FibroblastBalb/c: Fibroblasts Balb/c were cultivated in Dulbecco's modified Eagle's medium, supplemented with glutamine (4 mM), streptomycin (0.1 g/L), penicillin G (100 000 U/L). Cells were seeded in medium supplemented with 10% bovine serum on plates at a density of $1x10^5$ cells/cm^2 and incubated at 37°C in an atmosphere containing 5% CO_2after 24 h the medium was changed for a serum-free one and 24 h toxicity of test extracts (0.01-0.5 mg/mL) was studied. After the experiment, the culture medium was removed and assayed for extra cellular lactate dehydrogenase activity (LDH) and cell viability was determined by MTT test (Hui Yang et al. 2009).

Cytoprotactive activity: Primary rat hepatocytes were prepared by standard way as described in cytotoxicity. Hepatocytes were seeded on collagen type I pre-coated

plates at a density of $1x10^5$ cells/cm^2 and incubated at 37°C in an atmosphere containing 5% CO_2 for 3 h and the medium was changed for a serum-free one, rat hepatocytes were incubated for 24 h with extracts (0.001-1.0 mg/mL) and then they were treated by *t*BH. (0.5 mM; 90 min). After the experiment, the culture medium was removed and assayed for extracellular lactate dehydrogenase activity (LDH) and level of lipid peroxidation products (Buegeand Aust 1978). Cell viability was determined by MTT test.

Methods for estimation of cell toxicity

Lactate dehydrogenase (LDH, EC 1.1.1.27): Its content in the medium was spectrophotometrically measured by the disappearance of NADH during the LDH-catalyzed conversion of pyruvate to lactate as decreased absorbance at 340 nm. The effects of the test compounds were expressed as percent toxicity, respectively protection. (George Fotakis, John A. Timbrell 2006)

Antioxidant activity

MTT assay: The medium free serum supplemented with MTT (5 mg/ml) was applied to cells and cells were incubated for additional 2 h at 37°C. The medium was removed and formed crystals were dissolved DMSO with 1% (v/v) NH_3. The absorbance in well was read on microplate reader (SUNRISE) using a wavelength of 540 nm (Henrik andKassack 2004).

Neutral red assay (NRA): Neutral red (NR; 0.03%) in PBS was added to the cells (40 or 100 µl/well on 96 or 24-well plate). The cells were incubated for additional 2 h at 37 °C, washed with mixture of formaldehyde (0.125%) and $CaCl_2$ (0.25%) and neutral red in cells was dissolved in acetic acid (1%) in methanol (50%). The absorbance in wells was read at 540 nm with a microplate reader (Sunrise Remote, Tecan, Austria), (Psotova*et al.* 2006).

RESULT

The results of antioxidant activity of the different*Alhagimaurorum* extracts using DPPH method are shown in table 1 and fig 1. The highest scavenging activity was resulted from ether extract, while petroleum ether extract was the least effective. The ether extract of *A. maurorum* showed the highest scavenging activity followed by the methanolic extract, then chloroform extract. *A. maurorum*petroleum ether extract showed the last antioxidant activity. The antioxidant effect of methanol extract may be attributed to its high flavonoids content as mentioned in the phytochemical screening of the different extracts, especially that quercetin and kaempferol flavonoids were isolated from *A. maurorum* which are very significant flavonoids as antioxidant.

Table 1. Scavenging activity of*Alhagimaurorum* extracts

Plant Extracts	IC_{50} (mg/mL)
Chloroform	0.17 ± 0.015
70% Methanolic	0.093 ± 0.01
Petroleum ether	0.35 ± 0.04
Ether	0.083 ± 0.01

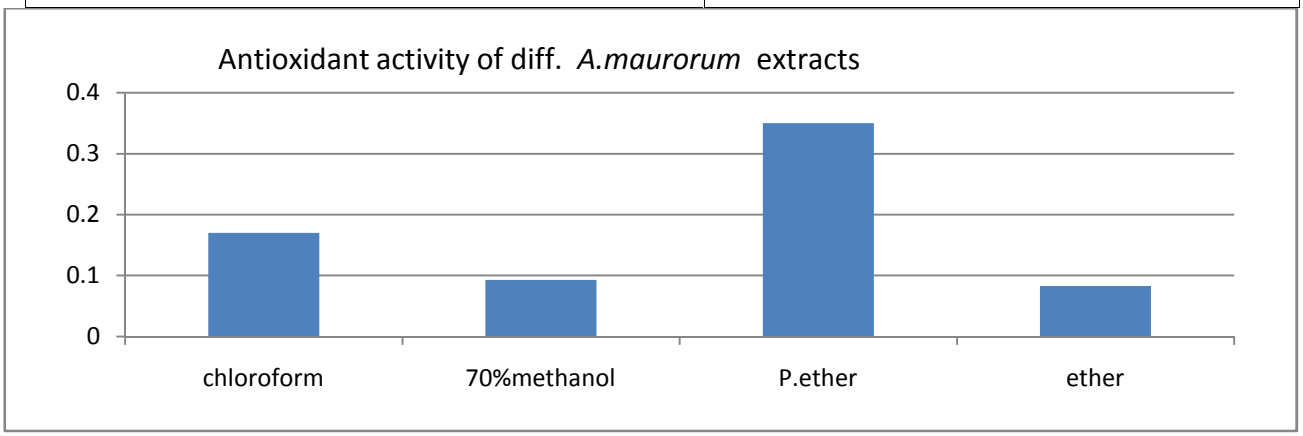


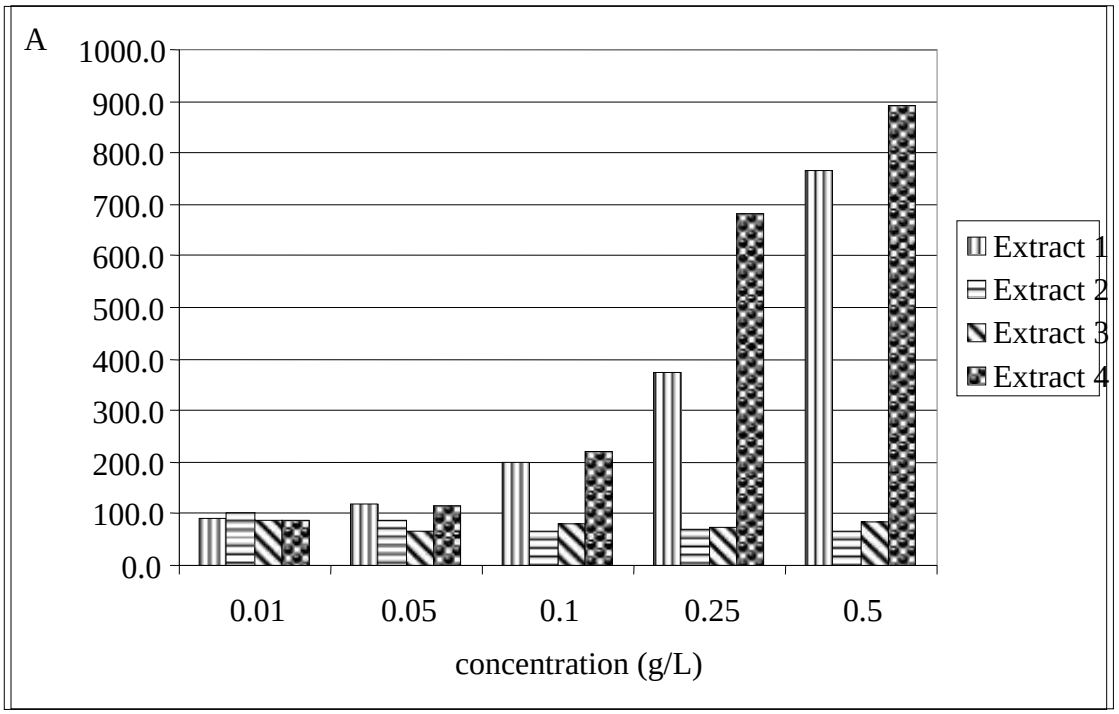
Fig. 1: Antioxidant effect of different *A. maurorum* extracts using DPPH method.

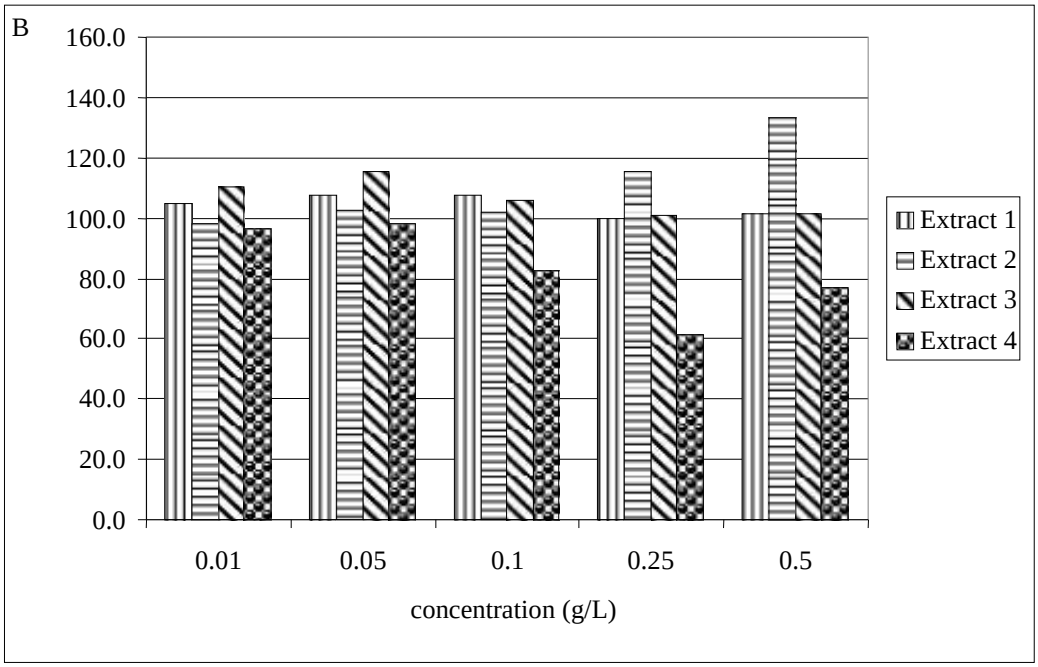
Cytototoxic activity of extracts: The cytotoxic effect of extracts was followed on primary rat hepatocyte cultures. The level of extracellular LDH as parameter of cellular damage (Fig.2A), cell viability (neutral red uptake) (Fig.2B) and MTT as a parameter of mitochondrial function damage (Fig.2C) were also measured. Extracts 1 and 2 were toxic on parameter LDH and MTT. The cytortoxicity is connected with the cellular membrane damage and the disruption of mitochondrial respiration chain. At the highest concentration of extract (0.5 g/L) we observed reversal of parameters which could be given negative or positive influence constituent of extract or worse solubility. All data (MTT and NRA) were corrected on this positive or negative influence of extract compounds (blank).

Toxicity of extracts on fibroblast Balb/c – preliminary result one pilot experiment

The cytotoxic effect of extracts was followed on normal cell line–fibroblast. The level of extracellular LDH as parameter of cellular damage (Fig.3A) and MTT as a parameter of mitochondrial function damage and effect on metabolic processes in cell (Fig.3B) and cell viability (neutral red uptake) (Fig. 3C) were also measured. The extract 2 was the least toxic, but the extract 4 was the most toxic one.

Cytoprotective effect of extracts: The preliminary results are demonstrated there (only from one pilot experiment). The MTT, LDH and NRA were measured. The cytoprotective effect of extracts was followed on primary rat hepatocyte cultures intoxicated by *t*BH. At non-toxic concentration of extracts the very low protection was observed.




B
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.01
0.05
0.1
0.25
0.5
concentration (g/L)
Extract 1
Extract 2
Extract 3
Extract 4

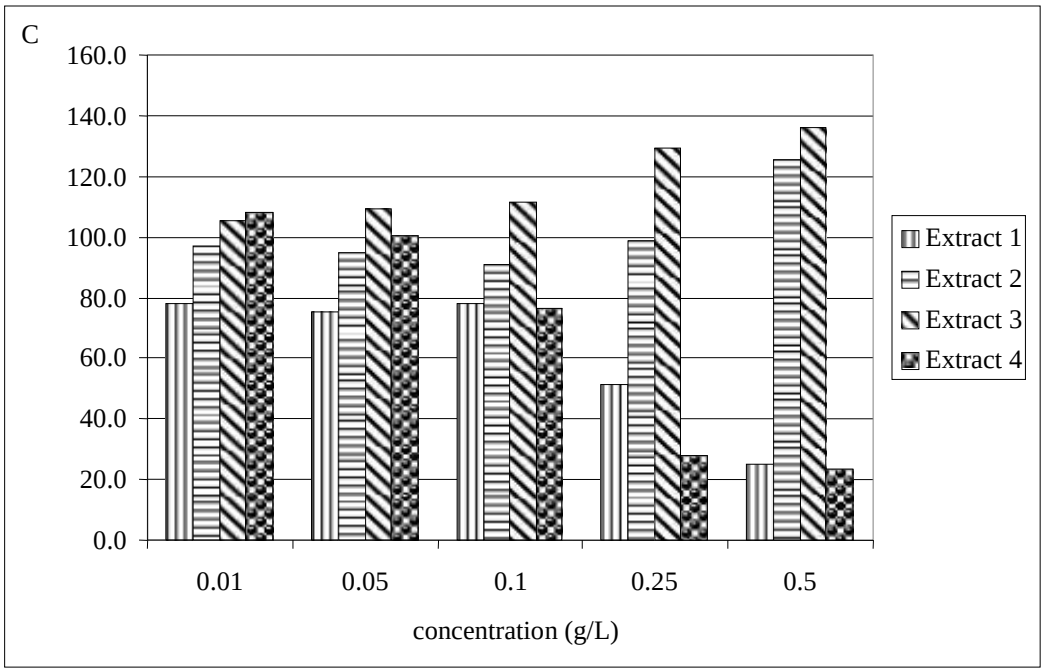
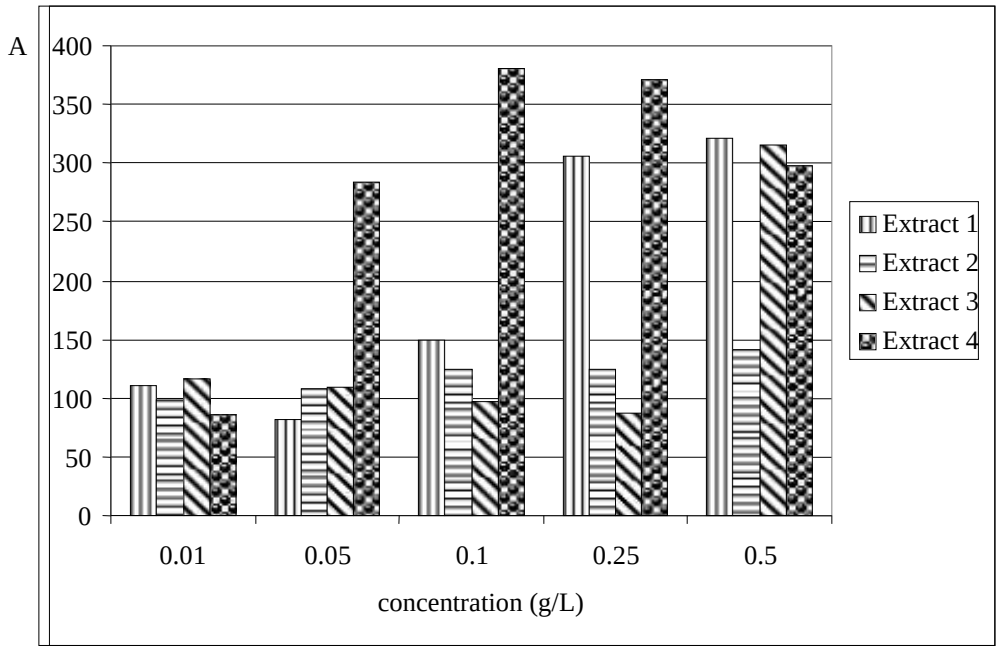
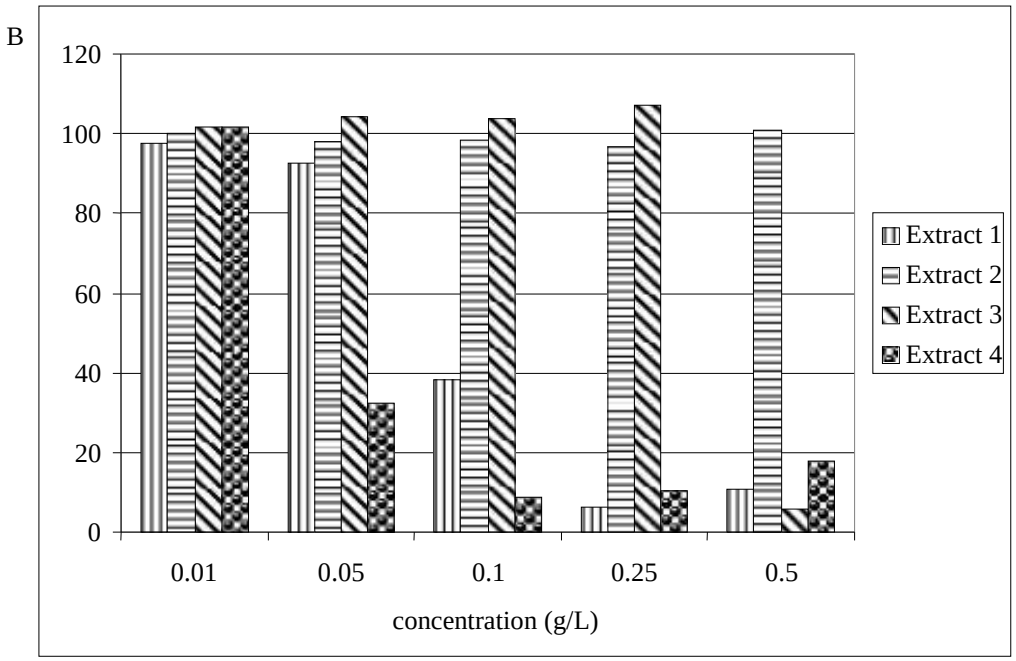

C
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.01
0.05
0.1
0.25
0.5
concentration (g/L)
Extract 1
Extract 2
Extract 3
Extract 4

Fig. 2.Toxic effect of extracts on primary rat hepatocyte culture (A – extracellular level of LDH; B – cell viability; C – mitochondrial function).





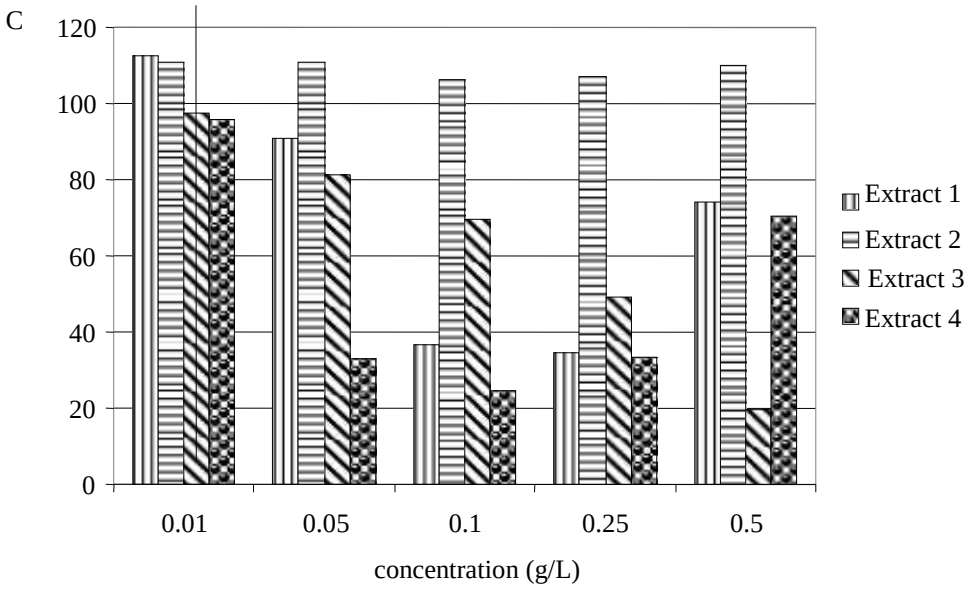


Fig. 3. Shows that extracts 1 and 4 toxicity decreases at 0.5 mg/mL on parameter neutral red uptake. It could be done their worse solubility in these concentrations. These data do not correlate with other used test.

Phytochemical analysis of *AlhagiMaurorum*extracts: Phytochemical analysis of the extracts proved that petroleum ether, ether and chloroform extracts contained triterpenes while methanol extract had flavonoids, triterpenes and carbohydrates. Phytoconstituents play an important role in the activity observed in these extract. The significant antioxidant activity for ether extract can be explained in view of the presence of triterpenes (table 2). Triterpenes are a class of natural products present in most plants and it exhibitunique and important biological and pharmacological activities, including anti-oxidant, cytotoxic andcardiovascular effects (Connolly and Hill, 2008). *β*-sitosterol isolated from *Salvia poculata*proved good antioxidant and anticholinesteraseactivities (Ufuk*et al*., 2009).The identified lupeol (triterpene compound) identified from *Alhagimaromum* (Monica et al., 2014) may be responsible for the observed antioxidant property of the plant. Furthermore, knowing the antioxidant capability of the plant, *F.pseudopalma*can be developed into products which can help prevent the occurrence of oxidative stress related diseases (Librado and Anna 2014).

Table 2. Phytochemical analysis of *A. maromum* extracts

Constituents	Petroleum ether extract	Ether extract	Chloroform extract	Methanol (70%) extract
Triterpenes and /or Sterols	+	+	+	+
Carbohydrates and/or glycosides	-	-		+
Flavonoids	-	-	-	+
Coumarins	-	-	-	-
Alkaloids and/or nitrogenous compounds	-	-	-	-
Tannins	-	-	-	-
Saponins	-	-	-	-
(+) presence of the constituents, (-) absence of the constituents				

CONCLUSION

It can be concluded that *Alhagimaromum* extracts possess a good free radical scavenging, cytotoxic and cytoprotective activities and this may be due to the presence of bioactive compounds as triterpenes and flavonoids. These results also suggest that the ether

extract could serve as potential source as free radical scavenging, cytotoxic and cytoprotective agent.

REFERENCES

1. Boulous, L. (1966): Flora of the Nile Region in Egyptian Nubia. Feddes Report 73,184,215.
2. Brown, D (1995). Encyclopaedia of herbs&their uses. Dorling insersely, London Buege, J. A.; Aust, S. D. (1978). Microsomal lipid peroxidation. Methods Enzymology; 52: 302-31.
3. Connolly J, Hill R (2008). Triterpenoids. Nat. Prod. Res. 25:794-830.
4. Deby, C.; Margotteaux, G. (1970). Relationship between essential fatty acids and tissue antioxidant levels in mice. *CR. Soc. Biol.;* 164: 2675-2681.
5. Habib, I.M.; Al-Ani T. A., Al-Mufti, M.M; Al-Tawil, B. H (1971). Plant Soil 34:405-407.
6. Halliwell, B (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence. Lancet. 721-4.
7. Hallowell, B. (1995). The products formed will initiate the beta-carotene oxidation. Biochem. Soc. Symp., 61, 73.
8. Henrik M.,Matthias U. K (2004). Comparison of the Usefulness of the MTT, ATP, and Calcein Assays to Predict the Potency of Cytotoxic Agents in Various Human Cancer Cell Lines. J Biomol Screen 9 (6): 506-515.
9. Hui Y., Chao L., Danfeng Y., Huashan Z., Zhuge X. (2009). Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition Journal of Applied Toxicology 29(1): 69–78.
10. Finkel T., Holbrook N.J. (2002). Oxidants, oxidative stress and the biology of aging. Nature 408:239-47.
11. George F., John A. T. (2006). In-vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicology Letters 160 (2): 171–177.
12. Jitka P, Alena S, Hana K, Daniela W (2006). Photoprotective properties of *Prunella vulgaris* and rosmarinic acid on human keratinocytes.Journal of Photochemistry and Photobiology B: Biology84 (3): 167–174.
13. Monica R. L., Khaled R., Ataa S., Marco B., Francesco M., Rosa T.(2014). Antiproliferative and antioxidant properties of AlhagimaurorumBoiss (Leguminosae) aerial parts. Industrial Crops and Products 53: 289–295.
14. Nunez-Delicado, E., Sanchez-Ferrer, A., and García-Carmona, F (1997). J. Agric. Food Chem., 45: 28-30.
15. Pulido R, Bravo L, Saura-Calixto F (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/ antioxidant power assay. J. Agric. Food Chem. 48(8): 3396-402.
16. Solomon Charles Ugochukwu, ArukweUche I., OnuohaIfeanyi (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetiatripetala*G. Baker. Asian Journal of Plant Science and Research 3(3):10-13.
17. Ufuk K., Hacibek˙I.R., Mehmet O., Fevzi O., Ayhan U. (2009). Antioxidant and anticholinesterase constituents of *Salvia poculata*. Turk. J. Chem. 33:813-823.

Cite this article as:
Said, A., Abo-El-Fotouh, M., Alshabrawy, A.R., Psotova, J., Hawas, U., Omar, A. and Rashed, K. (2014). Evaluation of antioxidant, cytotoxic and cytoprotective activities of *alhagimaurorum*extracts and phytochemical profile. Indian J. Drugs, 2(2), 65-72.