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ANTIOXIDANT ACTIVITIES FROM LEAVES OF *ELEPHANTOPUS SCABER* L. EXTRACTED USING DIFFERENT SOLVENTS

Mahood A. Al-Shaheen[1], Mustafa A. R. Al-Shaheen[2] and Omar H. Ismaaiel[2]

[1]Department of Biology, College of Education for Pure Science, University of Anbar, Anbar, Iraq.
[2]Department of Field Crop, College of Agriculture, University of Anbar, Anbar, Iraq.

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ABSTRACT : The present study was carried out to evaluate the antioxidant activities of leaves of *Elephantopus scaber* by using different solvent (ethyl alcohol, acetone and aqueous). The leaves extract from *Elephantopus scaber* using different solvents to extract were evaluated for antioxidant activity by using DPPH method using a different concentrations (1, 10, 20, 30, 40, 60, 80, 100 µg/ml). The results of this method was compared with ascorbic acid as standard. The IC50 value of standard (ascorbic acid) was found to be 20 mg/ml. This study showed the strongest DPPH radical-scavenging activity of (52.54±0.81%) inhibition at concentration of 40 mg/ml and IC_{50} value was 30 mg/ml was found the scavenging activity of (44.92±0.26%) for ethyl alcohol extract. Acetone extract of leaves was found at of concentration 60 mg/ml scavenging activity of 53.26±0.34%. The IC_{50} value of aqueous extract of leaves was (60µg/ml) with percentage inhibition of (54.28±0.28%) followed by (44.57±0.53%) at concentration 40 mg/ml.

Key words : Antioxidant, leaves, *Elephantopus scaber* L, DPPH, solvents.

INTRODUCTION

Elephantopus scaber L. (*Elephant's foot*) is an important of medicinal plants, which is classified worldwide in all tropical regions (Ho *et al*, 2009). The whole of plant (roots, leaves and flowers) is used medicinally, because of the presence from sesquiterpene lactones, deoxyelephantopin, isodeoxyelephantopin, scabertopin, stigma sterol, epifriedelinol, lupeol and stigma sterol throughout the whole plant (Ho *et al*, 2009).

As the traditional system of medicine, it is reported that the leaves are used as cardiotonic, antipyretic and diuretic activities (Miraldi *et al*, 2001). A decoction of the roots and leaves is useb for diarrhea and emollient and given in dysuria, stomach pain and for dysentery (Duke, 2018).

The aqueous extract of leaves is applied externally to treat eczema and ulcers (Cimanga Kanyanga *et al*, 2018). The waters extract of leaf is applied externally to treat ulcers and eczema. The plant is macerated and used on the surface of wounds to acceleration wound healing activity (Rani *et al*, 2016). It is reported that this species is also used for its hepato protective, antitumor, anti-inflammatory activity and wound healing (Asadi-Samani *et al*, 2015).

The growth of this species by seeds is restricted because to poor viability and early death of seedlings in natural environmental conditions (Weber, 2017). In vitroculture is an alternative method of conservation and propagation of this species. Micro propagation of *Elephantopus scaber* L. have been reported through leaves explants (Abraham and Thomas, 2015), but no report have been published regarding plantlet generation from leaves explants, which is the most material for genetic stability of micropropagated plants (Boo *et al*, 2015).

Plants produce a many amount of antioxidants to prevent the oxidative stress caused by reactive oxygen species and are thought to be safer and more healthy than synthetic antioxidants. Biotechnological approaches such as plant tissue culture has been adopted for enhancing bioactive molecules in medicinal plants because to the optimum concentration of growth regulators and nutrients (Franca *et al*, 2016).

This study carried for assess the antioxidant activity of leaves from *Elephantopus scaber* L. extracts using different solvents (ethyl alcohol, acetone and aqueous).

MATERIALS AND METHODS

Collection the plant

The plants collected from Agro Technology Research Station, University Malaysia Perlis Padang Besar, Perlis, Malaysia. The plant has been selected at the flowering stage.

Table 1 : Percentage inhibition of standard (ascorbic acid), leaves extract used solvents (Ethyl alcohol ,acetone and aqueous), at various concentrations (mg/ml) in DPPH scavenging model.

Concentration	Leaves extracts			
	Ethel alcohol	Acetone	Aqueous	Standard
1	28.82±0.38	27.92±0.55	26.87±0.24	33.59±0.19
10	33.36±0.54	30.42±0.42	34.42±0.45	43.89±0.38
20	36.23±0.46	34.04±0.35	38.25±0.48	54.37±0.27
30	44.92±0.26	43.98±0.58	42.55±0.26	61.68±0.38
40	52.54±0.81	45.86±0.28	44.57±0.53	70.04±0.27
60	57.48±0.26	53.26±0.34	54.28±0.28	81.16±0.46
80	60.92±0.52	63.70±0.71	59.34±0.33	88.74±0.19
100	78.73±0.89	70.38±0.28	64.83±0.26	95.62±0.39

Preparation of plant extracts

The selected *Elephantopus scaber* L. was washed thoroughly and separated the leaves from plants then dried under shade. The dried plants material was grounded into fine powder using Waring blender (Cole Parmer, RZ-04245021). The powdered material was extracted by Soxhlet apparatus for 24 hours using different solvents (ethyl alcohol, acetone and aqueous). The solvent was then evaporated using Rotary evaporator at 4^0C for further use (Molla, 2015).

Antioxidant activity assay

Determination of antioxidant activity of leaves extract using different solvent were done by DPPH free radical scavenging activity assay.

DPPH free radical scavenging activity assay

Three samples were prepared at the concentration of 1000ìg/ml. From the stock solution, different concentration, 1, 10, 20, 30, 40, 50, 60, 80 and 100 µg/ml were prepared and used for antioxidant assay. Ascorbic acid was used as standard for study of DPPH scavenging assay. The stock concentration of 1000 µg/ml was prepared in methanol. From the stock concentration all other concentration ascorbic acid, 1, 10, 20, 30, 40, 50, 60, 80 and 100 µg/ml were prepared.

The antioxidant activity will be measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments will be carried out according to the method of Blois, with a slight modification. The reduction of the radical is followed by a decrease in the absorbance at 517 nm. A volume of 4 mL of a water or methanolic stock solution of the extracts will put in test tubes and 4 mL of 2 mM DPPH solution will be added. The tubes will be covered use parafilm and keep again at the dark for 1 hours. Absorbance in 517 nm will be measured with a spectrophotometer UV-visu (Jascos V-531) and compared to an vitamin C calibration curve. The results will be express in mg ascorbic acid (vitamin C)/g dried sample. Each assay will carried out in triplicate. The percentage inhibition from the DPPH radical will be calculate by using the formula:

$$I\% = (A0\text{-}A/A0) \times 100$$

where, I = DPPH inhibition (%), A0 = absorbance of control sample (t = 0 h) and A = absorbance of a tested sample at the end of the reaction (t = 1 h). The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC50) (Gardeli *et al*, 2008; Proestos *et al*, 2013; Gardeli *et al*, 2008; Proestos *et al*, 2013).

RESULTS AND DISCUSSION

Antioxidant activity assay

In the present study, have evaluated the DPPH free radical scavenging activity of *Elephanthopus scaber* from leaves used a different solvent (Ethyl alcohol, acetone and aqueous). The antioxidant activity of the leaves extracts was evaluated by DPPH radical scavenging protocol. DPPH free radical scavenging activity of leaves extract was compared with ascorbic acidas standard (Table 1).

Ethel alcohol extract of leaf part of *Elephanthopus scaber* showed the highest DPPH scavenging activities at concentration of (100µg/ml) where the scavenging activity was (78.73±0.89%) followed by (70.38±0.28%) of acetone extract then (64.83±0.26%) for aqueous extract while at the same concentration, scavenging activity of ascorbic acid was (95.62±0.39%). The IC_{50} value of standard was (20µg/ml) with percentage inhibition of (54.37±0.27%). For Ethel extract, Acetone and Aqueous extract the IC_{50} value was (40µg/ml) with inhibition percentage of (52.54±0.81%), (45.86±0.28%) and (44.57±0.53%), respectively. The IC_{50} value of ethyl alcohol, acetone and aqueous extracts was 80µg/ml with inhibition percentage of (60.92±0.52%), (63.70±0.71%), (59.34±0.33%), respectively. The inhibitory effect of the leaves extracts on DPPH radicals followed dose-dependent manner (Figs. 1, 2 and 3).

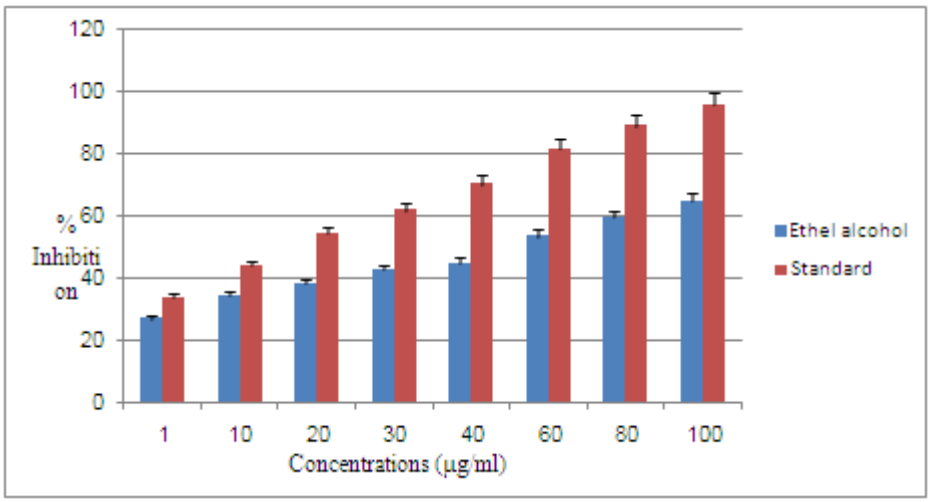

Fig. 1 : DPPH scavenging activity of ethyl alcohol extract of leaf and standard ascorbic acid.

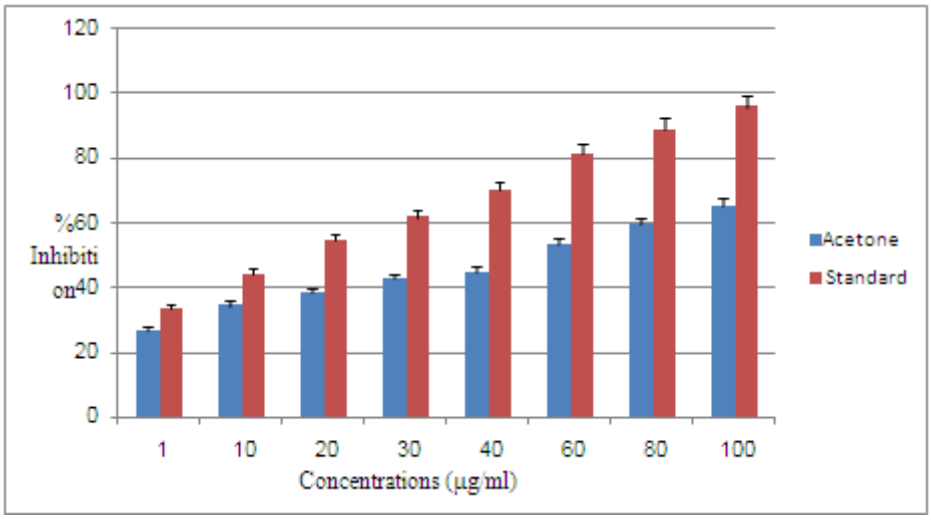

Fig. 2 : DPPH scavenging activity of acetone extract of leaf and standard ascorbic acid.

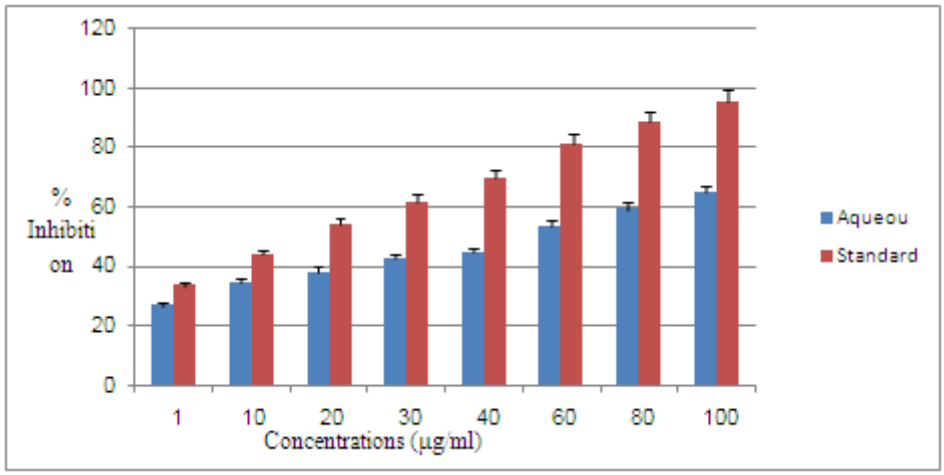

Fig. 3 : DPPH scavenging activity of aqueous extract of leaf and standard ascorbic acid.

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