Research Article

Phytochemical Analysis and *in-vitro* Antioxdant Activity of Iraqi *Althaea ludwigii* l. Extract

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ABSTRACT

Oxidative stress represents the disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defense which have been implicated in the pathophysiology of diverse disease states. The phytochemical screening and antioxidant property of methanolic extract of *Althaea ludwigii* Linn., were studied. The results of phytochemical analysis were showed the presence of flavonoids, steroids (phytosterols), tannins and terpenoids. The antioxidant activity was done by DPPH (2, 2-diphenyl-1-picryl-hydrazyl) free radical scavenging assay. The *in-vitro* antioxidant assays showed the ethyl acetate fraction of the methanolic extract of *Althaea ludwigii* possess potent antioxidant activity when compared with reference compound ascorbic acid (Vitamin C). The antioxidant activity may relate to the existence of flavonoids which had been approved as potent antioxidant, and identified by Preliminary qualitative phytochemical analysis.

Keywords: *Althaea ludwigii*, phytochemical analysis, antioxidant, DPPH.

INTRODUCTION

Herbal medicines are relevant to herbal remedies, herbal products, herbal medicinal products, phytomedicines, phytotherapeutic agents and phytopharmaceuticals. Rational phytotherapy is the approach of herbal medicines in which an evidence- or science-based fact is used for the curing and avoidance of disease, while the traditional medical herbalism is a completely different approach, which uses herbal medicines depending on a holistic manner and mainly on the basis of their empirical and traditional uses. Although these two approaches – traditional/holistic and rational/evidence-based – in some situations use similar terminology, they are entirely contrasting. For example, traditional herbalism is also identified as 'phytotherapy' and describes preparations of plant material as 'herbal medicines'. Plants have been used medicinally for thousands of years by cultures all over the world.

As reported by the World Health Organization, plant-based medicine is used as a primary form of healthcare by 80% of the world's population [1]. World Health Organization (WHO) has specified herbal drugs as complete, labeled medicinal products that have vigorous ingredients, aerial or secretive parts of the plant or other plant material or combinations. A precise guideline for the evaluation of the safety, efficacy, and quality of herbal medicines has been determined by World Health Organization [2].

The oldest forms of health care known to mankind are Herbal drugs [3]. The overall aim of this research work has been to contribute to the existing body of knowledge about *Althaea ludwigii* (Family: Malvaceae) is an endogenous plant, widely distributed in Iraq.

MATERIALS AND METHODS

Plant material

The whole plant of *Althaea ludwigii* of the Family *(Malvaceae)* was collected from Msayab; a city in Iraq about 47 km south of Baghdad. The plant was authenticated by the National Herbarium at Abu-Graib.

The plant was collected during the month of June (2017), and was cleaned, dried at room temperature in the shade, then pulverized by mechanical mills and weighed.

Extraction [4]

Shade-dried coarsely powdered aerial parts (200g) was packed in a thimble of soxhlet apparatus and extracted with 85% methanol until complete exhaustion.

The alcoholic extract was filtered and then the solvent was evaporated under reduced pressure using rotary evaporator at a temperature not exceeding 40 ˚C to give a dark greenish residue designated as a crude fraction.

The crude fraction was partitioned with Petroleum ether, Chloroform, Ethyl acetate, N-butanol (3x150 ml) for each fraction. The first three fractions were dried over anhydrous sulfate, filtered and evaporated to dryness. Each fraction was weighted and assigned for further analysis.

Preliminary qualitative phytochemical analysis

Chemical tests were carried out using the ethanolic extracts from Plants and or the powdered specimens, using standard procedures to identify the active constituents[5-7].

Test for alkaloids

Alcoholic extract (10 ml) was stirred with 5 ml of 1% HCL on a steam bath. Mayer's (1.35gm mercuric chloride in 60ml water + 5gm potassium iodide in 10ml water)and Wagner's reagents (1.27g of iodine and 2g of potassium iodide in 100ml of water) were added, white and reddish brown color precipitate respectively, were taken as evidence for the presence of alkaloids.

Test for flavonoids

(i)Lead acetate test**:** Lead acetate 10% (1 ml) solution was added to 5ml of alcoholic extract, the formation of a yellowish- white precipitate was taken as a positive test for flavonoids.

(ii)NaOH test: The extract (5 ml) was treated with aqueous NaOH and HCl, and looking for the formation of a yellow orange color.

Tests for steroids

(i) Liebermann-Burchard test: Extract (3ml) was treated with chloroform, acetic anhydride and drops of sulphuric acid was added. The formation of dark pink or red color indicates the presence of steroids.

(ii)H_2SO_4 test: The development of a greenish color was considered as indication for the presence of steroids, when the organic extract (2 ml) was treated with sulphuric and acetic acids.

Test for tannins

Plant material (10mg) in 10ml distilled water was filtered, and then the filtrate (3ml) + 3ml of FeCl3 solution (5%w/v) were mixed. The formation of a dark green or blue black precipitate was considered an indication for the presence of tannins.

Tests for anthraquinones

Borntrager's test: 3ml of alcoholic extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the development of a pink, red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinones.

Test for terpenoids

Alcoholic extract (2ml) was dissolved in chloroform (2ml) and evaporated to dryness. Concentrated sulphuric acid (2ml) was then added and heated for about 2 min. A grayish color was considered an indication for the presence of terpenoids.

Test for cardiac glycoside

Keller-kiliani test: Alcoholic extract (2ml) +1ml glacial acetic acid+ $FeCl_3$+con.H_2SO_4. Formation of green-blue color indicates the presence of cardiac glycoside.

1, 1-diphenyl-2-picrylhydrazyl (dpph) radical scavenging assay

The free radical scavenging activity of the Ethyl acetate fraction of *Althaea ludwigii* extract (X) was measured by DPPH scavenging activity [8] .Briefly, 1ml of 0.1 mM solution of DPPH in methanol was added to 2 ml of fraction dissolved in methanol. This solution was mixed vigorously with different concentrations of x (5-25 µg/ml in methanol) which were prepared through serial dilution. The absorbance reading was measured at wavelength 517 nm by spectrophotometer after 30 min in triplicate. The percentage of reduction of DPPH (Q) was calculated based on the following equation [9]:

$$Q=100(A_0-A_C)\ A_0$$

Where:

A0=Absorbance of control

AC=Absorbance of (X) after 30 min incubation

RESULTS AND DISCUSSION

A- The results of phytochemical analysis are given in table (1) and (2):

Table 1: Phytochemical Screening of *Althaea ludwigii*

Plant part	Alkaloids	Flavonoids	Steroids	Tannins	Saponins	Anthraquinoin	Terpenoids	Cardiac Glycoside
Aerial part	-	+	+	+	-	-	+	-

+, - represent presence and absence of phytoconstituents respectively.

The results of preliminary phytochemical screening of plant extract showed the presence of flavonoids, steroids, tannins and terpenoids in aerial parts of Iraqi species and the absence of, alkaloids, saponins, anthraquinoin and cardiac glycosides in the same plant parts.

Table 2: Qualitative analysis of phytochemical constituents in different fractions of plant.

Fraction used	Flavonoids	Alkaloids	Phenols	Terpenoids
Pet. Ether	-	-	-	+
Chloroform	-	-	-	+
Ethyl acetate	+	-	+	_
n-butanol	+	-	+	_

B-Assay of DPPH Radical Scavenging Activity

Very significant antioxidant activities were found in ethyl acetate fraction of the methanolic extract of *Althaea ludwigii* and positive control (Vitamin C), which increased with increasing concentration in figures (1) and (2):

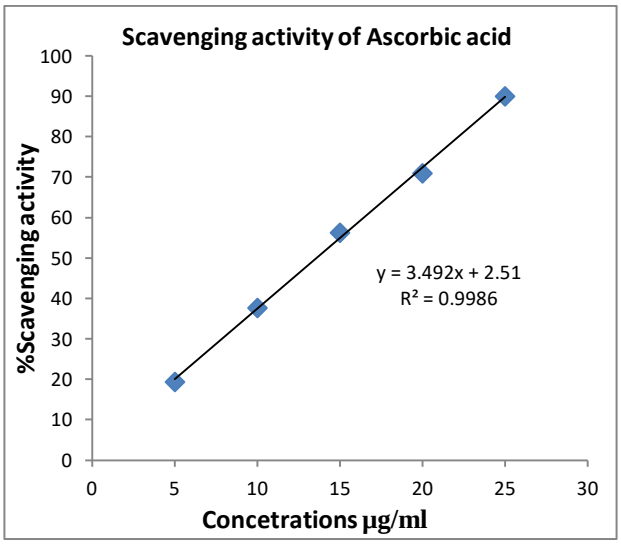

Figure 1: The scavenging activity of ascorbic acid.

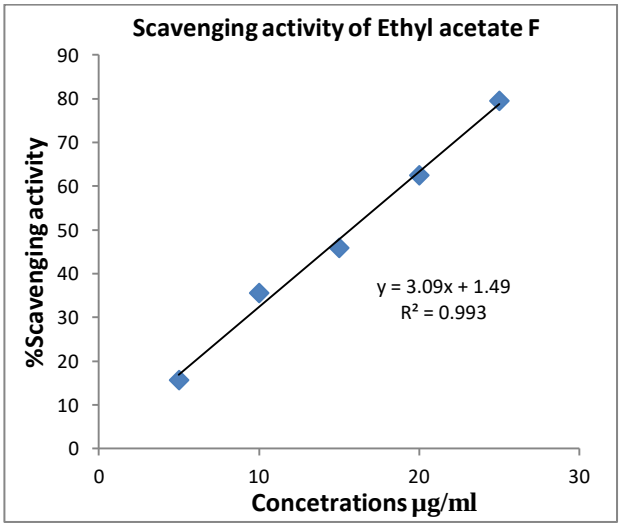

Figure 2: The scavenging activity of Ethyl acetate F.

IC_{50}=49.5 µG/ml of the ethyl acetate fraction (which is the concentration of the sample required to scavenge 50% of the free radicals present in the system), was calculated by the linear regression equation.

It may be possible that the antioxidant activities of this fraction of *Althaea ludwigii* are probably due to the extracted phenols and flavonoids. Flavonoids can prevent injury caused by free radicals by scavenging of ROS, activation of antioxidant enzymes, metal chelating activity, reduction of α-tocopheryl radicals, inhibition of oxidases, and mitigation of oxidative stress caused by NO, increase in uric acid levels and increase in antioxidant properties of low-molecular antioxidants. [10]

CONCLUSION

In the present study, experimental evident revealed that the Ethyl acetate fraction of Iraqi plant *Althaea ludwigii* methanolic extract contains flavonoids. Extractions were performed using the conventional method sohxlet and methanol as solvent. The existence of flavonoids in this plant was confirmed by preliminary tests. The antioxidant capacity was measured by the free radical scavenging method DPPH and was proven to be high.

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