Research Article

Phytochemical Analysis and Free Radical Scavenging Activity of *Carthamus oxyacantha* Growing in Saudi Arabia: A Comparative Study

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ABSTRACT

The main goal of present studywas to determine the total phenolic and flavonoid contents as well as to evaluate the antioxidant activity and to screen the chemical composition of extracts of different plant organs of *Carthamus oxyacantha* as a comparative study. The study revealed that *Carthamus oxyacantha* characterized chemically by the presence of various secondary metabolites such as flavonoids, cardiac glycosides, triterpenoids/steroids, tannins and carbohydrates at different levels in different extracts of plant organs and the absence of saponins and alkaloids. The antioxidant activity was investigated according to DPPH radical scavenging procedure, using trolox as standard. In addition, the total phenolic and flavonoid contents were estimated using Folin-Ciocalteu reagent. The results showed that the extracts of ethyl acetate, butanol and aqueous of leaves, stems and flowers exhibited a higher antioxidant activity which further confirmed by its higher amounts of phenolic and flavonoid contents among all the tested different organs extracts. These results revealed that the ethyl acetate, butanol and aqueous extracts of different plant organs of *Carthamus oxyacantha* could be developed as a potential natural antioxidant herbal remedy.

Keywords: *Carthamus oxyacantha*, Asteraceae, DPPH,Phytochemical Screening.

INTRODUCTION

Plants are rich and promising source of new herbal remedies for treatment of various diseases. Saudi Arabia is a rich flora of numerous species of medicinal and wild plants. Several of these plants are reputed for their valuable and applicable effects towards many disorders[1-3]. The genus *Carthamus* of the plant family *'Asteraceae'* comprises 25 species which have tremendous applications in both pharmaceutical and commercial aspects [4-5].

A literature survey indicated that, many of *Carthamus* species are used for the treatment of cough, typhoid fever, throat disorders, cardiovascular problems, swelling, menstrual disorders, anti-inflammatory, antioxidant, calcium antagonist and anti-coagulant, anti-microbial and anti-analgesic activities and also used in folk medicine as sedative and anticancer herbal remedy[6-7]. Many studies were conducted on the flowers and seeds of *Carthamus oxyacantha* regarding phytochemical and biological screening without paying the attention that may be the other plant parts could be possibly contain some other constituents or even similar to those of medicinal importance in flowers [8-10].

There is no record on any comparative study on different organs of *Carthamus oxyacantha* regarding their phytochemical contents and antioxidant activities. Therefore the present work was conducted to investigate of the phytochemical contents and to evaluate the antioxidant property of different plant organs fractions.

METHODS

Plant Collection

Carthamus oxyacantha was collected from Al-Hasa, eastern region of Saudi Arabia. Different parts (leaves, stems, flowers and roots) of plant were separated and subjected to air-drying according to the standard protocols. A voucher specimen was kept in Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Al-Hasa, Saudi Arabia.

Extraction and fractionation of different plant organs extracts

The powdered air dried plant material [leaves, stems, flowers and roots of*Carthamusoxyacantha,*500.0, 200.0, 150.0 and 150.0 g respectively] were exhaustively extracted twice at room temperature (each for 5 days) using 4 L 70% MeOH/H_2Oapplying maceration technique at room temperature to protect the potential active constituents from being destructed. The solvent mixture was removed through distillation under vacuum using Rota vapor and dried extracts were directly freeze-dried to give the total extracts of different plant parts(leaves, stems, flowers and roots weighting 31.8, 10.7, 6.3 and 7.1 g respectively) which were kept in -20 ^{o}C for the next steps.

Exactly 31.0, 10.0, 6.0 and 7.0 g of leaves, stems, flowers and roots total extracts respectively were taken and suspended in distilled water (200 ml) using a separating funnel and partitioned with n-hexane (6×500 ml). The resulting n-hexane layers were collected and combined to be concentrated to the least amount using rotary

evaporator and then dried to give 15.0, 4.5, 1.5 and 0.5 g respectively then were stored in a deep freezer in well-closed container. The remaining aqueous part was subjected to partition with chloroform (4×500 ml). The obtained chloroform fractions were also combined and its amount was reduced to the minimal amount through using rotary evaporator and then freeze dried to give 3.5, 1.0, 0.7 and 0.5 g, respectively, and then all were put in fridge in a strong-tight container for later use.

In the same way of fractionation, the ethyl acetate and n-butanol extracts were also obtained using the same above-mentioned method to give 6.0, 2.0, 1.2 and 1.0 g respectively for ethyl acetate fraction and to give 1.1, 1.0, 1.0 and 1.0 g respectively for n-butanol fraction. The remaining mother liquor (aqueous fraction) was also freeze-dried to powder to give 4.2, 1.4, 1.0 and 1.8 g respectively and kept cooled for further use in an air-tight container [11].

Phytochemical Screening of different plant organs extracts

Preliminary phytochemical testes were done on the n-hexane, chloroform, ethyl acetate, butanol and aqueous extracts of different organs using standard methodsto identify the possible nature of its chemical contents[11-14].

Test for flavonoids

Part of dried extracts of each organ was boiled with 10 ml of distilled water for 5 min and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow color, which on addition of acid changed to colorless solution, depicts the presence of flavonoids.

Test for saponins

Part of dried extracts of each organ was separately boiled with 10 ml of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. Demonstration of frothing: 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously to form a stable persistent froth.

Test for steroids and/or triterpenoids

Part of dried extracts of each organ was separately boiled with 10 ml of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. Five milliliters of each extract was mixed in 2 ml of chloroform. Three milliliters of concentrated H_2SO_4 was then added to form a layer. A reddish brown precipitate coloration at the interface formed indicated the presence of steroids and/or triterpenoids.

Test for alkaloids

Part of dried extracts of each specimen was separately boiled with water and 10 ml hydrochloric acid on a water bath and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of Dragendorff's reagent (potassium iodide 0.11 M, bismuth nitrate 0.6 M in acetic acid 3.5 M), the test tubes were observed for orange to brown turbidity.

Test for anthraquinones

Part of dried extracts of each organ was boiled with 2 ml of 10% hydrochloric acid for 5 min. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10% ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any color change; delicate rose pink color showed the presence of an anthraquinone.

Test for tannins

Part of each dried extracts of each organ was separately boiled with 20 ml distilled water for 5 min in a water bath and was filtered while hot. One milliliter of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10% ferric chloride were observed for any formation of precipitates and any color change. A bluish-black or brownish-green precipitate indicated the presence of tannins.

Test for cardiac glycosides

Part of dried extracts of each organ was separately boiled with 10 ml of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. Five milliliters of each extract was treated with 2 ml of glacial acetic acid containing one drop of 10% ferric chloride solution. This was underplayed with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicated the deoxy-sugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

Test for carbohydrates

Part of various extracts were dissolved separately in 4 ml of distilled water and filtered. The filtrate was treated with 2-3 drops of alcoholic alpha-naphthol and 2 ml of concentrated H_2SO_4 was added along the sides of the test tube. Appearance of brownish violet ring at the junction of the two liquids indicates the presence of carbohydrates.

Determination of the total phenolic content:

The total phenolic content was estimated using the Folin-Ciocalteu index protocol[11]. Stock solutions (1 mg/ml) of different extracts will be prepared in methanol. Half ml of Folin-Ciocalteu reagent and Six milliliters of double distilled deionized water were successively added to 0.1 ml of stock solution of each extracts. In addition, 1.5 ml of a 20% Na_2CO_3 solution and water was added to obtain 10 ml. A reaction will take place within 2 hrs. at normal room temperature. Then, the absorbance was recorded at 760 nm. Calibration was done using serial dilution of Gallic acid as a standard (0.5, 0.4, 0.3, 0.2 and 0.1 mg/ml in

distilled water, y = 0.0274x + 0.0011, r2 = 0.9482). The amounts of phenolic components wasdemonstrated as the equivalenceof milligrams of standard Gallic acid per gram of dried plant extract (mg GAE/g).

Determination of the total flavonoidcontent:

The total flavonoid content was calculated in accordance to khalil et al. [11]. Ten mg of extracts will be diluted in 100 ml of deionized water and acetone with ratio of (1:1 v/v). A solution of 0.25 ml of the serially diluted sample was added to 0.75 µl of a $NaNO_2$ (5% w/v) solution, as well as 0.15 ml of a recently prepared aluminum chloride (10% w/v) solution, together with 0.5 ml of 1 M NaOH solution. Then the total volume of reactants was completed to 10 ml with deionized double distilled water. The resultant components were kept for 5 min and the absorption was observed at 510 nm against the same components lacking of the sample. Calibration was done using quercetin as reference substance, from that a standard calibration curve got with solutions of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml (y = 0.0012x + 0.0314, r2 = 0.9358). The results were shown as the equivalence of milligrams of quercetin per gram of dried plant extract (mg QE/g).

DPPH radical scavenging activity

Radical scavenging activity was evaluated using the protocol described by Khalil et al. 2017[15] with some modifications. The absorbance of various dilutions of the test extracts which were previously dissolved in Methanol (1ml) was recorded at 515 nm at zero time as Ab blank. Then, I ml of 200 µM DPPH solution in methanol was add to every test tube, then was kept at room temperature 30 minutes. Followed by, measurement of the absorbance again as Ab sample. Trolox was used as positive control. Each test was performed in triplicates. The percentage of inhibition was measured using this equation:

% of inhibition= (1 –[Ab sample - Ab blank] / {Ab control - Ab blank}) ×100

Where Ab control is the absorbance of mixture (with methanol and all other reactant without test extracts) [16]. IC_{50}wasrecorded as the sample concentration that is essential to cause inhibition of DPPH radical to be formed by 50%.

RESULTS AND DISCUSSION

Phytochemical screening of different plant organs extracts

The preliminary phytochemical screening of extracts of different plant organs showed the presence of different important constituents such as flavonoids, triterpenoids/steroids, carbohydrates, alkaloids, cardiac glycosides and tannins at different levels in different extracts of plant organs and the absence of saponins and anthraquinones as expressed in Table 1.

Table 1: Preliminary phytochemical screening of different fractions of different plant organs.

Plant organ		Flavonoids	Saponins	Triterpenoids/ steroids	Alkaloids	Anthraquinones.	Tannins	Cardiac glycosides	Carbohydrates
Leaves	nH	-	-	+	-	-	-	-	-
	CH	+	-	+	+	-	-	-	-
	EA	+	-	+	+	-	+	+	+
	BT	+	-	+	-	-	+	+	+
	AQ	+	-	-	-	-	+	+	+
Stems	nH	-	-	+	-	-	-	-	-
	CH	+	-	+	+	-	-	-	-
	EA	+	-	+	+	-	+	+	+
	BT	+	-	-	-	-	+	+	+
	AQ	+	-	-	-	-	+	+	+
Flowers	nH	-	-	+	-	-	-	-	-
	CH	+	-	+	+	-	-	-	-
	EA	+	-	+	+	-	+	+	+
	BT	+	-	+	-	-	+	+	+
	AQ	+	-	-	-	-	+	+	+
Roots	nH	-	-	+	-	-	-	-	-
	CH	+	-	+	-	-	-	-	-
	EA	+	-	+	-	-	+	+	+
	BT	-	-	-	-	-	+	+	+
	AQ	-	-	-	-	-	+	+	+

nH, n-Hexane; CH, chloroform ; EA, Ethyl acetate; BT, Butanol; AQ, Aqueous. + (present); - (absent).

Determination of total phenolic constituents

Determination of total phenolic constituents showed that it varies from organ to organ and from fraction to another and ranging from 0.0.028±1.006 to 40.538±1.870 mg GAE/g of dry extract (Fig. 1). Ethyl acetate fraction contains the highest percentage of total phenolic components, followed by butanol fraction then the aqueous fraction. Chloroform fraction contains the least amount while n. hexane extract contains very less of phenolic contents.

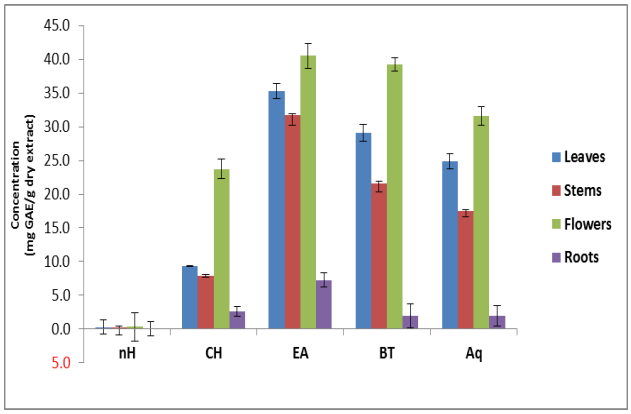

Figure 1: Total phenolic contents of different extracts of *Carthamus oxyacantha*.nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq.; Aqueous fraction, GAE; Gallic acid equivalent. Data are the means ± standard deviation of three replicates.

Determination of total flavonoid constituents

Determination of total flavonoids content illustrated similarity with those of total phenolic where the amount of total flavonoid constituents varies from 0.010±0.013 to 32.746±1.125 mg QE/g of dry extract (Fig. 2). Ethyl acetate fraction is the richest fraction in flavonoid components, next to it the butanol fraction then the aqueous fraction. Similarly, to phenolic contents, the chloroform fraction contains the least amount compared to other fractions, while n-hexane fraction hardly contains any flavonoid constituents.

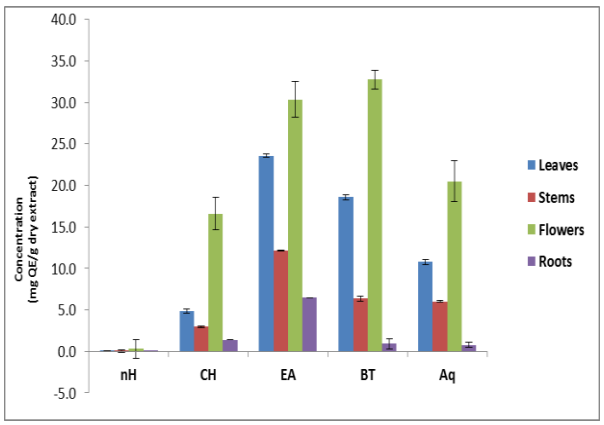

Figure 2: Total flavonoid contents of different extracts of *Carthamus oxyacantha*.nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq.; Aqueous fraction, QE; Quercetin Equivalent. Data are the means ± standard deviation of three replicates.

DPPH radical scavenging activity

All fractions were examined for their radical scavenger activity using the DPPH free radical scavenging assay (Fig. 3). Ethyl acetate, butanol and aqueous fractions of leaves, stems and flowers showed marked scavenging activities ranging from IC_{50}: 49.5to91.1 µg/ml, chloroform fraction of flowers showed noticeable effect with $IC_{50}$of 84.5µg/ml, on the other hand, the rest of chloroform fractions of other plant parts(leaves, stems and roots), hexane fractions of all plant parts and all fractions of root demonstrated much weaker effects with IC_{50} above 100 µg/ml comparable with the standard trolox (IC_{50}:25.1 µM).

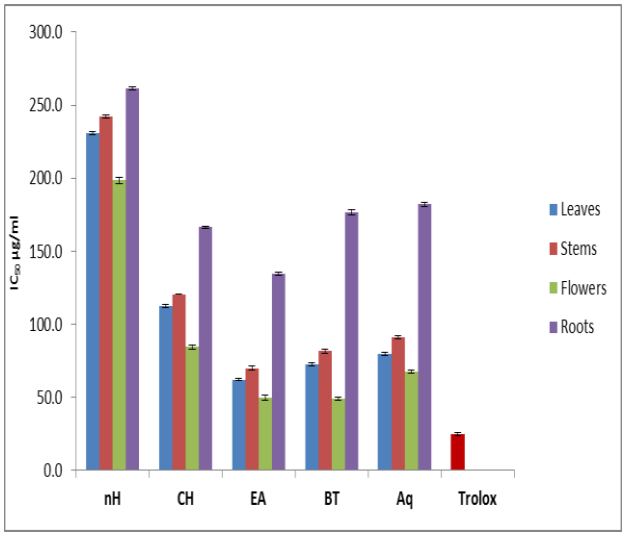

Figure 3: IC_{50} values of DPPH assay of different extracts of *Carthamus oxyacantha*. nH; n-Hexane fraction, CH; Chloroform fraction, EA; Ethyl acetate fraction, BT; Butanol fraction, Aq.; Aqueous fraction,. Data are the means ± standard deviation of three replicates.

CONCLUSION

In conclusion, the current study showed that *Carthamus oxyacantha* characterized chemically by the presence of various secondary metabolites anddifferent organs extracts which exhibited good antioxidant activities specially the ethyl acetate, butanol and aqueous fractions which could be attributed to the higher contents of phenolic and flavonoid components in these fractions.In addition, these findings give a good picture about other waste parts of plant that are not used like leaves and stems. Hence this study demonstrated the importance of leaves and stems compared to that of flowers. So this study has identified a new potential source of compounds with higher antioxidant activity together with the commonly used parts of plant.

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