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# Evaluation of the Antioxidant, Total Phenolics and Total Flavonoids of Suaeda Species Collected in Al Jouf Area

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***Authors' contributions***

*This work was carried out in collaboration between the two authors. Both authors designed the study, performed the extraction, chemical analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Al Jouf area is gifted with a wide range of plant genera belonging to different plant families. In a research program targeting the phytochemical exploration of this area plants, three species representing the genus Suaeda in this area; S. *aegyptiaca*, *S. fruticosa* and *S. mollis* (Family Chenopodiaceae) were subjected for investigation. The present study aimed to explore the secondary metabolite pattern of the three Suaeda species growing in Al Jouf area, through assessment of the total phenolics, total flavonoids and anti-oxidant activity of four different extracts of *S. aegyptiaca*, *Suaeda fruticosa* and *Suaeda mollis*.
**Methodology:** Finely powdered plant material (50 g) of each of the three plant species were exhaustively extracted with ethanol, methanol, ethyl acetate and water. Total phenolics content, total flavonoids content and anti-oxidant activity of all extracts for each species were assessed using Folin Ciocalteu, Aluminium chloride and DPPH assay methods, respectively.
**Results:** Different extracts showed variable percentage yield of each of the three Suaeda species.

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The highest flavonoid content was detected in the methanol extract of *S. aegyptiaca* and *S. mollis*, while the largest content of phenolic compounds are recorded in the ethanol and methanol extracts of *S. mollis*. Estimation of anti-oxidant activity revealed highest activity for the ethanol and methanol extracts of *S. mollis*.

**Conclusion:** The presented results revealed highest anti-oxidant activity of *S. mollis* compared to the activity of *S. aegyptiaca* and *S. fruticosa*, these results could be attributed to highest content of phenolic and flavonoid content of this species.

*Keywords: Suaeda; Al Jouf area; total phenolic content; total flavonoid content; anti-oxidant activity.*

## 1. INTRODUCTION

Al Jouf area is located in the northern part of Saudi Arabia along borders with Jordon. The area has a moderate, rainy climate that is somehow different from the climatic conditions allover Saudi Arabia. This area has a wide variety of plant species belonging to different plant families. Family Chenopodiacea is represented, in this area, by about 15 genera and 36 Species. Genus Suaeda is represented by three species; *Suaeda aegyptica*, *S. vera* and *S. vermiculata* [1]. In addition two more species were collected by our group from Al Jouf area that was identified as *S. fruiticosa* and *S. mollis*. The Name Suaeda is derived from an Arabic word "Suwaid" which means black. This comes from the tendency of these plants to turn black upon drying [2]. Reviewing the on-line data bases for plant names we found a nomenclature overlap between *S. vera*, *S. vermiculata* and *S. fruticosa*. However Schenk and Ferren [3] declared that *S. vera* is a type of Suaeda Section Chenopodina Moq., while *S. fruticosa* is placed under S. Sect. Salsina Moq. Suaeda species are commonly used in folk medicine [4,5]. Some species possess hypoglycemic, anti-inflammatory, hypolipidaemic, cardiotonic, anti-oxidant, antimicrobial and anticancer activity [6-8]. Being a halophyte, Suaeda grow in stressful conditions that encourage the biosynthesis of a wide variety of bioactive metabolites. Halophytes are able to overcome the oxidizing stressful agents due to their powerful antioxidant system which includes enzymatic and non-enzymatic components [9]. The anti-oxidant activity of four Tunisian Suaeda species was evaluated. Results indicated variation in the anti-oxidant potential of the four tested species [5]. Estimation of the total phenolic content, in addition to investigation of the anti-oxidant, anti-inflammatory and anti-cancer activity of the shoot extracts of *S. fruticosa* revealed interesting activities of this edible halophyte [4]. Evaluation of the anti-oxidant potential in addition to quantification of the total flavonoid and total phenolics contents of each of the three species grown in Al Jouf area, could give an indication of their metabolic pattern. Accordingly, the objective of the present study is to evaluate the anti-oxidant activity, total flavonoids and total phenolics content of ethanol, methanol, ethyl acetate and water extracts of the three Suaeda species; *Suaeda aegyptica*, *S. fruticosa* and *S. mollis,* collected from Al Jouf area.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

*S. aegyptiaca, S, fruticosa* and *S. mollis* were collected from Sakaka desert, Al Jouf, KSA in March 2014. *S. aegyptiaca* plant sample was identified by Mr. Hamdan Al-Hassan, Camel and Range Research Center, Sakaka, Al Jouf. While *S, fruticosa* and *S. mollis* were identified by Dr. Helmut Freitag, Institute of Biology, Kassel University, Germany. Voucher specimens are deposited in Pharmacognosy department, Faculty of Pharmacy, Al Jouf University. The total herb of each of the three plants is separately shade dried and powdered using grinding mixer.

### 2.2 Preparation of Extracts

The powdered plants (50 g, each) were, individually, exhaustively extracted by maceration in different polar solvents; ethanol (4x250 ml), methanol (4x250 ml), ethyl acetate (4x250 ml) and water (4x250 ml). The extracts were filtered and the filtrates were evaporated under reduced pressure using rotatory evaporator. The extract yields (% dry weight) of the three species are indicated in Table 1. Stock solution of 1 mg/ml was prepared for each extract.

### 2.3 Preparation of Standard Solutions

2 mg of gallic acid, rutin and ascorbic acid were dissolved, separately, in 2 ml of distilled water.

Serial dilutions were done to prepare (200, 100, 50, 25, 12.5, 10, 6.25 and 5 µg/ml) solutions of each standard compound.

## 2.4 Chemicals

Methanol, ethanol, ethyl acetate, distillated water, gallic acid, rutin, ascorbic acid, 2,2-dyphenyl-1-picrylhydrazyl (DPPH), sodium bicarbonate anhydrous ($NaHCO_3$), aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH) and Folin Ciocalteu's phenol reagent were obtained from Sigma Chemicals Co. (St Louis, MO, USA). All chemicals used were of analytical grade purity.

## 2.5 Determination of Total Phenolic Contents (TPC)

Phenolics are common plant phytochemicals with a wide range of biological activities. They include; simple phenols, coumarins, flavonoids, benzoic and cinnamic acid, tannins, lignins and lignans [10]. Due to the significance of the biological activities of this class of secondary metabolites, chemical tests are used for detecting their occurrence in plant samples and colorimetric and chromatographic methods are adopted for their quantification [11]. In the present study total phenolic content was quantified according to Abu Bakar et al. [12]. The Folin-Ciocalteu reagent (diluted 10-fold) was used to determine the total phenolic contents of the samples. 300 µL stock solution (1 mg/ml) of each extract and of standard Gallic acid solution (200, 100, 50, 25, 10, 5 µg/ml) were mixed with the Folin-Ciocalteu reagent (2.25 mL) and allowed to stand at 22℃ for 5 minutes before adding to sodium bicarbonate solution (2.25 mL, 60 g/L). After 90 minutes at 22℃, the absorbance was measured at 725 nm. The results were expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g).

## 2.6 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using aluminium chloride colorimetric assay method described by [12]. 0.5 ml of the extract and standard rutin solution (200, 100, 50, 25, 12.5, 6.25 µg/ml) were mixed with 2.25 ml of distilled water in test tubes followed by addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3$ solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well with vortex. The absorbance was immediately measured at 510 nm using spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

## 2.7 DPPH Free Radical Scavenging Assay

The scavenging activity of the extracts was estimated by using 1,1-diphenyl-2-pycrylhydrazyl (DPPH) as a free radical model and a method adapted from [13]. An aliquot of 300 µL of samples, control (80% methanol) and standard ascorbic acid solution (250, 200, 100, 50, 25, 12.5 µg/ml) were mixed with 3.0 ml of 500 µM (DPPH) in absolute ethanol. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. The mixture was measured spectrophotometrically at 517 nm. The free radical scavenging activity was calculated as follows:

Scavenging effect (%) = [1-{absorbance of sample/absorbance of control}] x 100

A standard curve was then prepared by plotting the percentage (%) of free radical scavenging activity of ascorbic acid versus its concentration. The final result was expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample.

## 2.8 Statistical Analysis

All experiments were carried out in triplicates and presented as mean ± standard deviation of (SD) using SPSS version 15.0.

## 3. RESULTS AND DISCUSSION

The three species; *S. aegyptiaca, S. fruticosa* and *S. mollis* are subjected to the study as they represent the most prevailing species of genus Suaeda in Al Jouf area.

Herein the % yields of different extracts of the three species are determined (Table 1). Results showed different yields for the four extracts of each of the three species. Methanol was the best among extraction solvents. Yield of methanol extract of *S. mollis* was the largest among all extracts. Total phenolic (TPC) and total flavonoid content (TFC) were determined and compared for ethanol, methanol, ethyl acetate and water extracts of the three suaeda species. Moreover the anti-oxidant activity, using DPPH method, of all extracts are also evaluated.

### 3.1 Determination of Total Phenolic (TPC) and Total Flavonoid Contents (TFC)

Redox properties of phenolic compounds enable them to play an important role in neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [14,15]. The level of phenolic compounds in each of the four extracts of the three Suaeda species is presented in Table 2. Both the ethanol and methanol extracts of *S. mollis* contain the largest amount of phenolic compounds (57.43±7.90) and (49.76±4.70), respectively. On the other hand the methanol extract of *S. aegyptiaca* and *S. fruticosa* exhibited lower content of total phenolics that are estimated as (15.88±0.90) and (10.42±1.30), respectively. These results indicate great difference in the TPC between *S. mollis* and the two other Suaeda species. The colorimetric estimation of the total flavonoids of the three species indicated, highest content in methanol extract of *S. aegyptiaca* (64.22±2.64), which is closely followed by methanol extract of *S. mollis* (60.47±1.63). On the other hand *S. fruticosa* contains the lowest flavonoid content (32.86±1.56). A recent study evaluating the TPC of the shoot extract of Tunisian *S. fruticosa* [4] indicated higher content (31.8 mg GAE/g) compared to the current study results (10.42 mg GAE/g). While TFC was estimated as 26.2 mg CE/g (expressed as catechin equivalent per gram dry weight) [4]. These results are close to the presented results 32.86 mg RE/G that is calculated as rutin equivalent per gram dry weight.

### 3.2 Evaluation of the Anti-oxidant Activity

Several assays are employed for assessment of the antioxidant activity of different samples [16]. These methods depend on scavenging reactive oxygen species [17]. DPPH method is among the most commonly used methods for the determination of anti-oxidant activities of plant extracts [18]. DPPH is a stable free radical. When a solution of DPPH is mixed with a substance with reducing potential, this leads to loss of the violet color [19]. Accordingly; DPPH scavenging activity was measured as the percentage of decreased DPPH free radical adsorption. Evaluation of the anti-oxidant activity of different extracts of the three Suaeda species, revealed high activity for the ethanol and methanol extracts of *S. mollis* (32.66±3.20 and 22.88±0.0005, respectively). Ethyl acetate and water extracts show weak activity. On the other

**Table 1. Percentage yields (g %) of the different extracts of the three Suaeda species**

| | *S. aegyptiaca* | *S. fruticosa* | *S. mollis* |
|---|---|---|---|
| Ethanol | 2.80 | 1.96 | 6.50 |
| Methanol | 9.90 | 5.60 | 10.70 |
| Ethyl acetate | 1.80 | 1.50 | 2.60 |
| Water | 2.40 | 1.70 | 3.60 |

**Table 2. Total phenolics (TPC) and total flavonoids (TFC) content of Suaeda species**

| | *S. aegyptiaca* | | *S. fruticosa* | | *S. mollis* | |
|---|---|---|---|---|---|---|
| | **TFC (mg RE/g)** | **TPC (mg GAE/g)** | **TFC (mg RE/g)** | **TPC (mg GAE/g)** | **TFC (mg RE/g)** | **TPC (mg GASE/g)** |
| Ethanol | 9.0 ± 2.60 | 2.65±0.40 | 14.27±3.80 | 2.45±0.20 | 49.73±7.50 | 57.43±7.90 |
| Methanol | 64.22±2.64 | 15.88±0.90 | 32.86±1.56 | 10.42±1.30 | 60.47±1.63 | 49.76±4.70 |
| Ethyl acetate | 10.14±3.0 | 6.02±0.50 | 10.03±2.80 | 2.39±0.10 | 5.81±0.88 | 5.55±0.40 |
| Water | 12.68±2.30 | 1.62±0.10 | 10.79±0.60 | 0.21±0.10 | 7.43±4.70 | 2.28±0.30 |

*All analyses are the mean of triplicate measurements ± standard deviation; TFC: Expressed as mg Rutin Equivalent/g of dry plant material; TPC: Expressed as mg Gallic acid Equivalent /g of dry plant material*

**Table 3. DPPH scavenging activities of the four extracts of Suaeda species**

| | *S. aegyptiaca* | *S. fruticosa* | *S. mollis* |
|---|---|---|---|
| Ethanol | 1.86±0.0* | 1.33±0.0* | 32.66±3.20 |
| Methanol | 6.73±0.0* | 3.76±0.0* | 22.88±0.0* |
| Ethyl acetate | 3.67±0.50 | 1.02±0.0* | 2.21±0.0* |
| Water | 2.91±0.0* | 1.15±0.0* | 2.44±0.0* |

*All analyses were the mean of triplicate measurements ± standard deviation. Results expressed in percent of free radical inhibition. * SD is too low*

hand all extracts of *S. aegyptiaca* and *S. fruticosa* exhibit either mild or weak anti-oxidant activity. It is clear that the results of assessment of anti-oxidant activity come in great accordance with that for TPC of the tested plant samples. The ethanol and methanol extracts of *S. mollis* show the most significant anti-oxidant activity. This clearly demonstrates the relation between phenolic content and the anti-oxidant potential of the plant. The anti-oxidant activity of four Suaeda species collected from Tunis is previously reported [5]. Different anti-oxidant assay methods are compared for extracts of the four species. Results revealed great variability among the tested species. DPPH method indicates highest anti-oxidant activity of *S. mollis* followed by *S. pruinosa*, *S. fruticosa* and *S. maritma*. The above mentioned report confirms our findings for *S. mollis* species growing in Al Jouf area.

## 4. CONCLUSION

As a conclusion the present study revealed highest phenolics and flavonoids contents of *S. mollis* among the three tested species collected from Al Jouf area. The anti-oxidant activity of the same species is also the greatest using DPPH assay method, compared to the activity of *S. aegyptiaca* and *S. fruticosa*. This could be attributed to high content of phenolics and flavonoids of *S. mollis*. These findings recommend *S. mollis* for biologically guided isolation studies.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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