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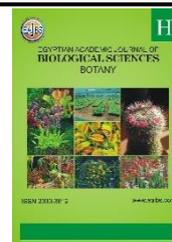
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Phytochemical Investigation, HPLC Analysis and Antimicrobial Activity of Some Plants from Chenopodiaceae Family

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ABSTRACT

The current research was designed to evaluate the phytochemical contents and antimicrobial activity of *Arthrocnemum macrostachyum* (Moric) K. Koch, *Suaeda pruinosa* Lange and *Kochia indica* (Bassia indica) (Wight) A. J. Scott belongs to Chenopodiaceae (Amaranthaceae) family. This study revealed that in the case of *A. macrostachyum* the soil texture was clay loam, in the case of *S. pruinosa* the texture of the soil was sandy clay loam, while in *K. indica* the texture of the soil was loamy sand. *S. pruinosa* results showed the highest contents of total phenolics, flavonoids, lipids, carbohydrates and tannins in comparison with the remaining two plants, while *A. macrostachyum* showed the highest total alkaloids content. HPLC analyses resulted in, *A. macrostachyum* containing resorcinol, kaempferol and quercetin, in case of *S. pruinosa* contain ferulic acid, quercetin, kaempferol and resorcinol while, *K. indica* contain quercetin, kaempferol, resorcinol and phenanthrine. Diethyl ether extract of *S. pruinosa* showed the highest antioxidant activity with scav % 95.25 followed by chloroform extract of *A. macrostachyum* (90.07%). The plants' extracts give moderate activity against *Candida albicans*, but in the case of *Bacillus subtilis*, *S. pruinosa* showed the highest activity. Also, *K. indica* results showed certain activity against *Escherichia coli* and *Proteus Vulgaris*.

INTRODUCTION

Medicinal plants as raw materials play an important role in modern medicine such as some essential antibiotics and drugs which have made a revolution in the control of different diseases (Sridhar *et al.*, 2011). The World Health Organization (WHO) has been active in the development of ethnobotanical medicine standards and strategies (Sridhar *et al.*, 2011). (WHO) has reported that, 70–80% of the world's population relies on herbal plants as a primary source of health care (Muhammad *et al.*, 2011). Medicinal plants resemble a unique natural source of antimicrobials as ethnomedicine, which is used in many countries (El-Shouny *et al.*, 2018). The medicinal value of these plants backs to their active chemical components which found in their secondary phytochemical metabolites, such as phenolics,

alkaloids, flavonoids and terpenoids that have been shown to have significant antimicrobial activities in several studies (Kumar *et al.*, 2015; Nayak *et al.*, 2017 and Annu Ahmed *et al.*, 2018). These natural metabolites provide infinite possibilities for leading new drugs. Oxidative stress is the main reason for different degenerative diseases including, gastric ulcers, cancer, atherosclerosis and other conditions, especially in presence of free oxygen radicals. Many antioxidants found in medicinal plants can scavenge this free oxygen. So, to overcome the drawbacks of synthetic antioxidants, the recent attention was based on searching for natural sources of antioxidants (Gandhia *et al.*, 2018).

Arthrocnemum macrostachyum is native to Mediterranean coastal regions such as France (Murakeo`zy *et al.*, 2007) and Portugal (Rodrigues *et al.*, 2014) and occurs along the Mediterranean coastal region of the Egyptian delta, *A. macrostachyum* has been reported to be a medicinal plant (El-Wahab *et al.*, 2008). This plant contains a large variety of secondary metabolites including, phenolic compounds, alkaloids, flavonoids and tannins. The high phenolic content of *A. macrostachyum* nearly (55%), gave it reductive and antioxidant activities. Also, its higher scavenging activity of free radicals indicates that it can be a potent source of antioxidant compounds (Custodio *et al.*, 2012). It has been documented that, the ethanolic extract of *A. macrostachyum* has an anticholinesterase activity; several phytochemical studies have been done on this plant.

Suaeda pruinosa is a facultative halophyte that tolerates moderate salt soils, dry soils, nitrified and saline. The specific epithet "pruinosa" refers to the presence of pruina, which is a kind of thin waxy coating on stems and leaves, which offers a glaucous appearance. The nutritive value of forage species (crude protein ranges) in *S. pruinosa* Lange is 27.17% (Elsharabasy *et al.*, 2019).

Qualitative analysis of *S. pruinosa*, methanol in water extract showed the presence of alkaloids, flavonoids, terpenoids, steroids, quinones, tannins, saponins and phenols. The obtained compounds were identified to be rutin, quercetin, syringic acid, caffeic acid, catechin, coumaric acid, vanillin, gallic acid and cinnamic acid. The amino acids content of *S. pruinosa* was identified through analyzing the methanolic extract of the aerial parts and the result showed the presence of thirteen different amino acids and the absence of three amino acids namely, valine, isoleucine and phenylalanine. Fatty acid analysis of lipids showed a high percentage of long-chain fatty acids. The presence of β -amyrin, β -sitosterol and stigmasterol was confirmed through thin layer chromatography (TLC) analysis of the lipoidal matter from *S. pruinosa* (Elsharabasy *et al.*, 2019).

Kochia is represented in Egypt by three species, viz. *K. indica*, *K. muricata* and *K. eriophora*. (El-Hadidi and Fayed, 1994-95) and (Boulos, 1999) in their working lists *K. indica* as synonyms to *Bassia indica*. Screening of phytochemicals showed that, *K. indica* contains saponins, flavonoids, oxalates and sterols at a low level that had no adverse effect on their nutritive value for sheep and goat feeding (Friesen *et al.*, 2009).

MATERIALS AND METHODS

Plant Samples:

Arthrocnemum macrostachyum (Moric) K.Koch, *Suaeda pruinosa* Lange and *Kochia indica* (*Bassia indica*) (Wight) A. J. Scott was collected from Northwestern coast, Egypt, during the spring season, 2019 and identified by Dr. Omran Nasser Ghaly, Head of Taxonomy Unit, Desert Research Center, Cairo, Egypt. Voucher specimens were kept under numbers CAIH-1012-R, CAIH-1013-R and CAIH-1014-R, respectively in the herbarium of Desert Research Center, Cairo, Egypt.

Soil Texture:

The soil samples were brought to the laboratory after collection and weighted; dried by air, debris was removed by passing soil through a 2 mm sieve, and then paper bags were used to collect samples for mechanical and chemical analysis. Soil texture was determined by using pipette method according to Allen, (1989) categorized according to their particles size into coarse gravels, fine gravels, coarse sand, fine sand, silt and clay.

Chemical Analysis of Soil:

For chemical analysis, soil water extract (1:5 w/v) was prepared to determine soil pH, electrical conductivity (EC), soluble anions (CO_3^- , HCO_3^- , Cl^- and SO_4^-) and cations (Ca^{++} , Mg^{++} , Na^+ and K^+) (Harris, 1998). The soil pH was determined using pH meter (3510, Jenway, UK). While, EC uses an electrical conductivity meter (Orion 150A+, Thermo Electron Corporation, USA). Sodium (Na^+) and potassium (K^+) were determined using Flame Photometer (PFP 7, Jenway, UK). While, calcium (Ca^{++}) and magnesium (Mg^{++}) were evaluated by the versine titration method (Harris, 1998). Carbonates and bicarbonates were determined by titration against 0.1N HCl using phenolphthalein and methyl orange as an indicator, while sulphates were determined by precipitation as barium sulphates using barium chloride in slightly acidic media using UV/Visible Spectrophotometer, Unicam UV 300, Thermo Spectronic, USA and chlorides were determined by titration against silver nitrate (0.1N) using 1% potassium chromate as an indicator (Jackson, 1967). All these procedures are outlined by Allen, (1989). For DTPA-extractable "available" content of heavy metals and trace elements analyses, soil samples were extracted according to (Soltanpour and Schwab, 1977) using NH_4HCO_3 / DTPA (Diethylene triamine penta acetic acid) solution.

Determination of Minerals Using ICP (Inductively Coupled Plasma):

Minerals content was determined in a known weight of each dried plant sample (0.5 g). The wet digestion procedure was performed as follows: briefly, the sample was mixed with concentrated H_2SO_4 (5 ml) and the mixture was heated for 10 minutes and then with continuous heating 0.5 ml perchloric acid was added until a clear solution was obtained. The digested solution was completed to a 100 ml using distilled water (Piper, 1947). Inductively Coupled Argon Plasma, iCAP 6500 Duo, Thermo Scientific, England. 1000 mg/L multi-element certified standard solution, Merck, Germany was used as a stock solution for instrument standardization.

Phytochemical Analysis:

After authentication, the fresh, healthy plant dry under shade for 2-3 weeks, then pulverize in a blender, sieve and use for further studies. The powdered materials under investigation were subjected to preliminary phytochemical screening (Harborne, 1984; Trease and Evans, 1989).

Metabolites Determination:

Carbohydrates contents were determined following the phenol-sulfuric acid assay (Chaplin and Kennedy, 1994). While lipids contents were determined following the method of Woo *et al.*, (1977).

The total content of phenolics in the tested extracts was determined following the Folin-Ciocalteu method (Singleton and Lamuela-Raventos, 1999), using gallic acid as standard. The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. Total phenolic content was expressed as gallic acid equivalents (GAE) per mg of extract. Total flavonoid content was determined by a colorimetric method of (Zhisen *et al.*, 1999) and calculated using a quercetin calibration curve. The results were expressed as quercetin equivalents (QE) per mg of extract. Alkaloids contents were determined as described by (Snell and Snell, 1953, Christie, 1982).

Antioxidant Activity:

Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox (25 mM in methanol) as a reference substance. The presence of antioxidative substances in the assay leads to the reductive decoloration of the DPPH radical. Depending on the content of antioxidative substances 50 μ L of the sample was adjusted to 1 mL with 50% methanol and then added to 1 mL of DPPH reagent (75 mg in 50 mL of methanol). After 0.5 h in the dark at room temperature, the absorbance was measured against a blank at 515 nm. The blank was a solution where 500 μ L of Trolox and 500 μ L of methanol reacted with 1 mL of DPPH reagent to obtain the complete decoloration of that radical. For the calibration curve, 0.5 - 3 mM of Trolox in 1 mL of methanol was used and results were expressed as Trolox equivalent antioxidant capacity (TEAC) (Liu *et al.*, 2002).

HPLC Analysis Conditions:

- * Column C18 Inertsil ODS 3: 4.6x250mm, 5 μ m.
- * Mobile phase: Buffer (0.1 % phosphoric acid in water) and Methanol.
- * Mode of elution: Gradient.
- * Flow rate: 1 mL/min.
- * Temperature: Ambient.
- * Wavelength: 280 nm.

Antimicrobial Activity:

The agar well diffusion method was used for antimicrobial activity evaluation of tested *A. macrostachyum*, *S. pruinosa* and *K. indica* extracts. The test organisms were separately inoculated in the agar medium. The wells were cut from the agar using a sterile cork-borer (6 mm) and 100 μ L of each plant extract was transferred into them (under aseptic condition). After incubation, the plates were examined and the inhibition zones (mm) were determined (Holder and Boyce, 1994). The fungal and bacterial strains were obtained from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

RESULTS AND DISCUSSION**Mechanical Analysis of Soil Profiles Associated with Plants under Study:**

In the case of *A. macrostachyum* the texture of the soil was clay loam with 8.21 % coarse sand, 28.50 % fine sand, 27.32 % silt and 35.97 % of clay. In case of *S. pruinosa* the texture of the soil was sandy clay loam with 45.63 % coarse sand, 16.42 % fine sand, 16.13 % silt and 21.82 % of clay. While in case of *K. indica* the texture of the soil was loamy sand with 49.16 % coarse sand, 31.37 % fine sand, 11.00 % silt and 8.47 % of clay as indicated in table (1).

Table 1: Mechanical analysis of soil profiles associated with plants under study.

| Plant species | Coarse sand (%) ($\frac{1}{2}$ -1 mm) | Fine Sand (%) (125-250 μ m) | Silt (%) (3.90625-62.5 μ m) | Clay (%) (< 3.90625 μ m) | Texture Class |
|-------------------------|---|------------------------------------|------------------------------------|----------------------------------|-----------------|
| <i>A. macrostachyum</i> | 8.21 | 28.50 | 27.32 | 35.97 | Clay loam |
| <i>S. pruinosa</i> | 45.63 | 16.42 | 16.13 | 21.82 | Sandy clay loam |
| <i>K. indica</i> | 49.16 | 31.37 | 11.00 | 8.47 | Loamy sand |

Chemical Analysis of the Soil Profiles Associated with the Plants under Study:**1. The pH Value and EC:**

The results indicated that, the soil is alkaline in case of *A. macrostachyum*, *S. pruinosa* and *K. indica*, with pH 8.63, 8.06, 7.97, respectively. While the EC was 24.60, 2.06, 0.96, respectively.

2. Soil Cations:

In the case of the *A. macrostachyum* soil sample, the major cation content was Na^+ which has 166.30 meq / L, while Mg^{++} was the second major component that has 55.00 meq / L, moreover, Ca^{++} content was 43.50 meq / L and the lowest content was k^+ (3.49 meq / L).

In the case of *S. pruinosa* the major cation content was Na^+ which has 14.80 meq / L, while, Ca^{++} was the second major component that has 2.80 meq / L, moreover K^+ content was 2.48 meq / L and Mg^{++} was 0.80 meq / L. While, in case of *K. indica* soil sample the major cation content was Ca^{++} which has 7.60 meq / L, while, Mg^{++} was the second major component that has 2.20 meq / L, moreover, Na^+ content was 0.83 meq / L and k^+ content was the lowest one (0.18 meq / L) as indicated in table (2).

3. Soil Anions:

In case of *A. macrostachyum* soil sample, the major anions content was Cl^- which has 184 meq / L, while, SO_4^{--} was the second major component that has 82.85 meq / L, moreover, HCO_3^{--} content was 1.08 meq / L and CO_3^{--} content was 0.36 meq / L. In case of *S. pruinosa* soil sample, the major anions content was Cl^- which has 15.73 meq / L, while, SO_4^{--} was the second major component that has 3.80 meq / L was, moreover, HCO_3^{--} content was 1.17 meq / L and CO_3^{--} content was 0.18 meq / L. In the case of *K. indica* soil sample, the major anions content was SO_4^{--} which has 9.08 meq / L, while, Cl^- was the second major component that has 0.83 meq / L, moreover, HCO_3^{--} content was 0.90 meq / L as indicated in table (2).

4. Available Nitrogen, Potassium, and Phosphorus:

In the case of *A. macrostachyum* soil, it was found that, potassium had the highest concentration with 135.25 ppm, followed by nitrogen with 19.28 ppm and the lowest was phosphorus with 3.06 ppm. In case of *S. pruinosa* soil, it was found that, potassium had the highest concentration with 111.22 ppm, followed by nitrogen with 15.65 ppm and the lowest was phosphorus with 4.38 ppm in case of *K. indica* soil, it was found that, potassium had the highest concentration with 106.24 ppm, followed by nitrogen with 18.24 ppm and the lowest one was phosphorus with 3.94 ppm (Table 2).

Table 2: Chemical analysis of the soil profiles associated with the plants under study.

| Plant species | EC (ds/m) | pH | Cations (meq / l) | | | | Anions (meq / l) | | | | Available Nitrogen (ppm) | Available Phosphorus (ppm) | Available Potassium (ppm) |
|-------------------------|-----------|------|-------------------|------------------|---------------|--------------|------------------|------------------|-----------------|---------------|--------------------------|----------------------------|---------------------------|
| | | | Ca^{++} | Mg^{++} | Na^+ | K^+ | CO_3^- | HCO_3^- | SO_4^- | Cl^- | | | |
| <i>A. macrostachyum</i> | 24.60 | 8.63 | 43.50 | 55.00 | 166.30 | 3.49 | 0.36 | 1.08 | 82.85 | 184.0 | 19.28 | 3.06 | 135.25 |
| <i>S. pruinosa</i> | 2.06 | 8.06 | 2.80 | 0.80 | 14.80 | 2.48 | 0.18 | 1.17 | 3.80 | 15.73 | 15.65 | 4.38 | 111.22 |
| <i>K. indica</i> | 0.96 | 7.97 | 7.60 | 2.20 | 0.83 | 0.18 | 0.00 | 0.90 | 9.08 | 0.83 | 18.24 | 3.94 | 106.24 |

Ash, Organic Matter and Crude Fiber Contents:

Results in table (3) showed that, the total ash content reached its maximum value (27.06 %) for *S. pruinosa*, the optimum value (26.78 %) for *A. macrostachyum*, while, the minimum value (14.82 %) for *K. indica*. On the other side, the organic matter reached its maximum value (85.18 %) for *K. indica*, the optimum value (79.94 %) for *S. pruinosa*, while the minimum value (73.22 %) for *A. macrostachyum*. The amounts of crude fiber reached their maximum value (22.46 %) for *K. indica*, (20.23 %) for *S. pruinosa*, while, the minimum value (18.65 %) for *A. macrostachyum*.

Table 3: Ash, organic matter and crude fiber contents (%) of plants under study.

| Plant species | <i>A. macrostachyum</i> | <i>S. pruinosa</i> | <i>K. indica</i> |
|--------------------|-------------------------|--------------------|------------------|
| Total ash (%) | 26.78 | 27.06 | 14.82 |
| Organic matter (%) | 73.22 | 79.94 | 85.18 |
| Crude fiber (%) | 18.65 | 20.23 | 22.46 |

Total Lipids (mg/g) and Carbohydrates (%) of the Plants under Study:

The total lipid content (Table 4) reached its maximum value (96.98 mg/g) for *S. pruinosa*, followed by 56.42 mg/g for *A. macrostachyum*, while, the minimum value (31.18 mg/g) for *K. indica*. Total carbohydrates content reached its maximum value (17.50 %) for *S. pruinosa*, the optimum value 10.27 % for *A. macrostachyum*, while, the minimum value was detected (4.99 %) for *K. indica*.

Table (4): Total lipids (mg/g) and carbohydrates (%) of the plants under study.

| Plant species | Total lipids (mg/g) | Total carbohydrates (%) |
|-------------------------|---------------------|-------------------------|
| <i>A. macrostachyum</i> | 56.42 | 10.27 |
| <i>S. pruinosa</i> | 96.98 | 17.50 |
| <i>K. indica</i> | 32.18 | 4.99 |

Preliminary Phytochemical Screening of Plants under Study:

From table (5) it can be concluded that, saponins glycosides were detected in all plants. Flavonoids were detected in *A. macrostachyum* and *S. pruinosa* and traces in *K. indica*. Tannins, triterpenes, Phenols and cardiac glycoside were detected in all plants. Proteins and amino acids were detected in *A. macrostachyum* and *S. pruinosa* and traces in *K. indica*. Carbohydrates and/or glycosides were detected in *A. macrostachyum* and *S. pruinosa* and traces in *K. indica*. Finally, alkaloids were detected in *A. macrostachyum* and *S. pruinosa* and traces in *K. indica*, this in agreement with that indicated by Sandberg *et al.*, (1967) and Rizk *et al.*, (1986).

Table 5: Preliminary phytochemical screening of plants under study.

| Constituents | <i>A. macrostachyum</i> | <i>S. pruinosa</i> | <i>K. indica</i> |
|---------------------------------|-------------------------|--------------------|------------------|
| Saponins glycosides | -ve | +ve | +ve |
| Triterpenes | +ve | +ve | +ve |
| Phenols | +ve | +ve | +ve |
| Flavonoids | +ve | +ve | traces |
| Cardiac glycoside | +ve | +ve | +ve |
| Carbohydrates and/or glycosides | +ve | +ve | traces |
| Proteins and amino acids | +ve | +ve | traces |
| Tannins | +ve | +ve | +ve |
| Alkaloids | +ve | +ve | traces |

+ve = Positive, -ve = Negative

Metabolic Products:**Total Phenols, Flavonoids, Tannins and Alkaloids of the Plants under Study (mg/g):**

From table (6) we can conclude that, *S. pruinosa* showed the highest total phenol and contents flavonoid (71.6 mg/g and 8.27 mg/g, respectively), while, total tannins reached their maximum value in *A. macrostachyum*. The total alkaloid content reached its maximum value (0.62 mg/g) for *A. macrostachyum*, followed by 0.46 mg/g for *S. pruinosa*, while the

minimum value (0.16 mg/g) for *K. indica*. This achievement is nearly the same as that obtained by (Rizk *et al.*, 1986).

Table 6: Total phenols, flavonoids, tannins and alkaloids of the plants under study (mg/g).

| Plant species | <i>A. macrostachyum</i> | <i>S. pruinosa</i> | <i>K. indica</i> |
|-------------------------|-------------------------|--------------------|------------------|
| Total phenols (mg/g) | 66.1 | 71.6 | 65.3 |
| Total flavonoids (mg/g) | 7.17 | 8.27 | 3.49 |
| Total tannins (mg/g) | 29.41 | 26.95 | 26.80 |
| Total alkaloids (mg/g) | 0.62 | 0.46 | 0.16 |

HPLC Analysis of (Phenolic and Flavonoid Compounds) Detected in the Studied Plants:

In case of *A. macrostachyum* HPLC of phenolic compounds in the ethanolic extract from the aerial parts revealed that, resorcinol, kaempferol and quercetin was detected. In case of *S. pruinosa* resorcinol, kaempferol, quercetin and ferulic acid, while, in *K. indica* the results indicated that, resorcinol, kaempferol, quercetin and phenantherine (Table 7 and Figs. 1a, 1b and 1c). Our finding in the same way of that achieved by (Elsharabasy *et al.*, 2019).

Table 7: HPLC analysis of (Phenolic and flavonoid compounds) detected in the studied plants.

| Plant species | Peak Name | Retention Time | Area mAU*min | Height Mau | Relative Area% | Relative Height% |
|-------------------------|---------------|----------------|--------------|------------|----------------|------------------|
| <i>A. macrostachyum</i> | Coumarin | - | - | - | - | - |
| | ferulic acid | - | - | - | - | - |
| | Resorcinol | 2.853 | 718.850 | 1370.578 | 89.02 | 79.65 |
| | kaempferol | 3.807 | 49.621 | 214.845 | 6.14 | 12.49 |
| | quercetin | 4.043 | 39.065 | 135.312 | 4.84 | 7.86 |
| | Naphthaline | - | - | - | - | - |
| <i>S. pruinosa</i> | Coumarin | - | - | - | - | - |
| | ferulic acid | 2.88 | 215.799 | 595.126 | 57.12 | 41.49 |
| | Resorcinol | 2.777 | 110.583 | 577.579 | 29.27 | 40.27 |
| | kaempferol | 3.820 | 28.569 | 150.939 | 7.56 | 10.52 |
| | quercetin | 4.297 | 22.818 | 110.686 | 6.04 | 7.72 |
| | Naphthaline | - | - | - | - | - |
| <i>K. indica</i> | Coumarin | - | - | - | - | - |
| | ferulic acid | - | - | - | - | - |
| | Resorcinol | 2.853 | 151.930 | 282.823 | 41.53 | 32.21 |
| | kaempferol | 3.200 | 57.971 | 195.608 | 15.85 | 22.28 |
| | quercetin | 3.927 | 49.016 | 176.926 | 13.40 | 20.15 |
| | Naphthaline | - | - | - | - | - |
| | Phenantherine | 4.603 | 106.898 | 222.696 | 29.22 | 25.36 |

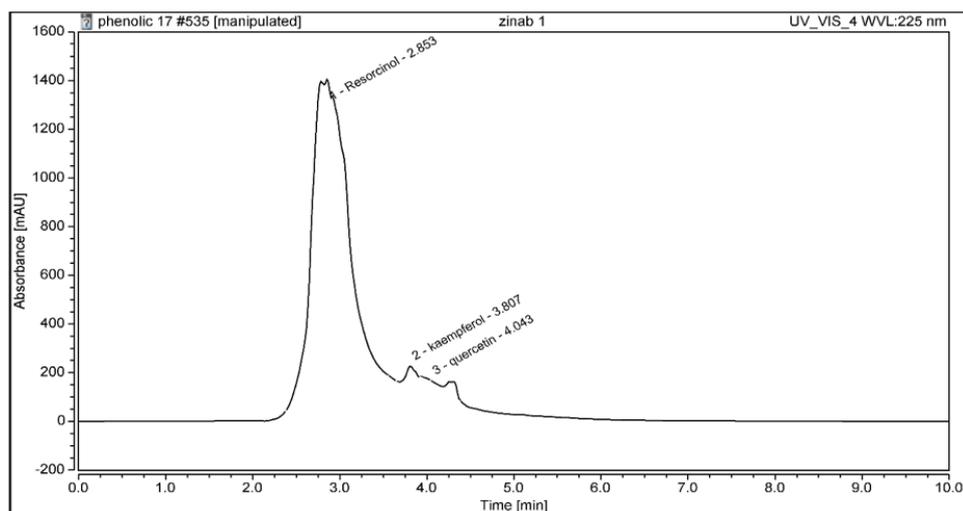


Fig. (1a): HPLC chromatogram of (phenolic and flavonoid compounds) detected in *A. macrostachyum*.

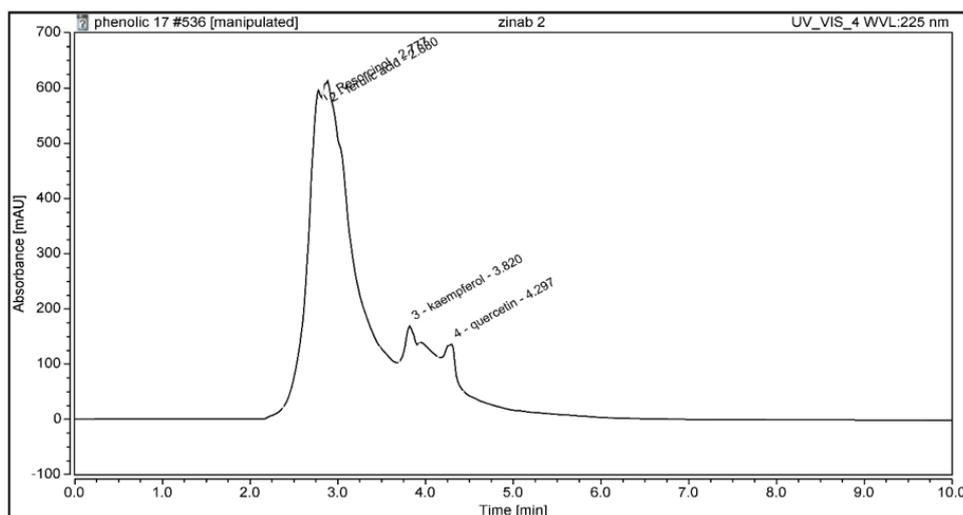


Fig. (1b): HPLC chromatogram of (phenolic and flavonoid compounds) detected in *S. pruinosa*.

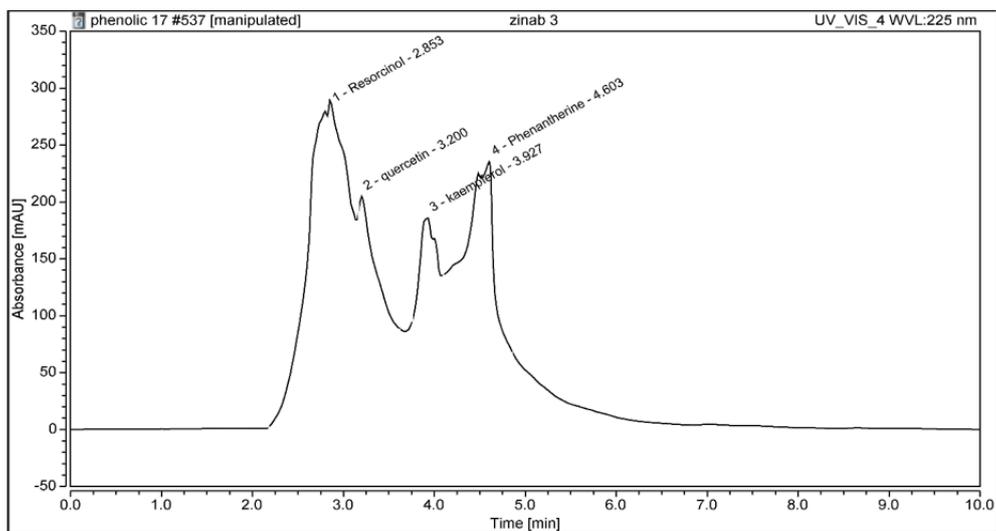


Fig. (1c): HPLC chromatogram of (phenolic and flavonoid compounds) detected in *K. indica*.

Anti-Oxidant Activity of the Studied Plants:

Table (8) represented that, the antioxidant activity *A. macrostachyum* reached its maximum values in case of a chloroform extract (90.07) followed by diethyl ether extract (86.57) followed by ethyl acetate extract (84.43) followed by ethanol 70 % extract (49.58), meanwhile, the minimum value in case of water extract (35.17). In case of *S. pruinosa* the antioxidant activity reached its maximum values in case of diethyl extract (95.25) followed by chloroform extract (88.51) followed by ethyl acetate extract (66.31) followed by water extract (64.96) meanwhile, the minimum value in case of ethanol 70 % extract (59.90). In case of *K. indica* the antioxidant activity reached its maximum values in case of ethyl acetate extract (85.98) followed by chloroform extract (85.79) followed by ethanol 70 % extract (84.82) followed by diethyl ether extract (83.06) meanwhile, the minimum value in case of water extract (47.44). Diethyl extract of *S. pruinosa* showed the highest activity (95.25) in all plants, this may be due to its high phenol and flavonoid contents (table 6), this result is in harmony with that of Roy and Dutta (2021).

Table 8: Anti-oxidant activity of the studied plants (extracts) using DPPH assay.

| Plant | Extract | Scavenging activity % |
|--------------------------------|-------------------------------|------------------------------|
| <i>A. macrostachyum</i> | Diethyl ether (40 – 60 b. p.) | 86.57 |
| | Chloroform | 90.07 |
| | Ethyl acetate | 84.43 |
| | Ethanol (70%) | 49.58 |
| | Water | 35.17 |
| <i>S. pruinosa</i> | Diethyl ether (40 – 60 b. p.) | 95.25 |
| | Chloroform | 88.51 |
| | Ethyl acetate | 66.31 |
| | Ethanol (70%) | 59.90 |
| | Water | 64.96 |
| <i>K. indica</i> | Diethyl ether (40 – 60 b. p.) | 83.06 |
| | Chloroform | 85.79 |
| | Ethyl acetate | 85.98 |
| | Ethanol (70%) | 84.82 |
| | Water | 47.44 |

Antimicrobial Activity of the Studied Plants:

Antimicrobial activity of the studied plants which represented in table (9) indicated that the studied plants give moderate activity against *Candida albicans* 8, 10 and 9 mm (Inhibition zone) for *A. macrostachyum*, *S. pruinosa* and *K. indica*, respectively, but in the case of *Bacillus subtilis*, *S. pruinosa* showed the highest activity 20 mm (Inhibition zone). Also, *K. indica* results showed certain activity against *Escherichia coli* and *Proteus vulgaris* 8 and 9 mm (Inhibition zone), respectively. Generally, *S. pruinosa* represented the highest activity in all plants, in this way our achievement is in agreement with that obtained by Qasim *et al.*, (2011).

Table 9: Antimicrobial activity of the plants under study.

| Plant species / Tested microorganism | <i>A. macrostachyum</i> | <i>S. pruinosa</i> | <i>K. indica</i> | Control |
|---|-------------------------|--------------------|------------------|------------|
| | Inhibition zone (mm) | | | |
| Fungi: | | | | |
| <i>Aspergillus fumigatus</i> (RCMB 002008) | - | - | - | 17 |
| <i>Candida albicans</i> RCMB 005003(1) ATCC10231 | 8 | 10 | 9 | 20 |
| Gram Positive Bacteria: | | | | Gentamycin |
| <i>Staphylococcus aureus</i> ATCC 25923 | - | - | - | 24 |
| <i>Bacillus subtilis</i> RCMB 015 (1) NRRL B-543 | 10 | 20 | 15 | 26 |
| Gram Negative Bacteria: | | | | Gentamycin |
| <i>Escherichia coli</i> ATCC 25922 | - | 9 | 8 | 30 |
| <i>Proteus vulgaris</i> RCMB 004 (1) ATCC 13315 | - | - | 9 | 25 |

The test was done using the diffusion agar technique, well diameter: 6.0 mm (100 µL was tested)

RCMB: Regional Center for Mycology and Biotechnology. The sample was tested at 20 mg/mL concentration.

Conclusion:

The studied plants showed high and moderate activity against tested microorganisms, also it possessed antioxidant activity which may be due to its contents from secondary metabolites.

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Conflict of Interest:

No conflict of interest is associated with this work.

Contribution of Authors:

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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ARABIC SUMMARY

الفحص الفيتوكيميائي ، وتحليل HPLC والنشاط المضاد للميكروبات لبعض النباتات من العائلة الرمرامية.

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3-وحدة المنتجات الطبيعية- قسم النباتات الطبية والعطرية - مركز بحوث الصحراء.

تم تصميم البحث الحالي لتقييم المحتويات الكيميائية النباتية والنشاط المضاد للميكروبات لمجموعة نباتات تنتمي إلى العائلة الرمرامية وهم *Arthrocnemum macrostachyum* و *Suaeda pruinosa* و *Kochia indica* وكشفت هذه الدراسة أنه في حالة (*Arthrocnemum macrostachyum*) كان نسيج التربة طفلي طيني، وفي حالة *Suaeda pruinosa*, كان نسيج التربة طفلي طيني رملي، أما *Kochia indica* فكانت طفلي رملي وأظهرت نتائج أعلى محتويات الفينولات الكلية، والفلافونويدات، والدهون، والكربوهيدرات، بالمقارنة مع النباتين الباقين، في حين أظهر *Arthrocnemum macrostachyum* أعلى محتوى للقلويدات الكلية. وقد أسفرت تحليلات HPLC في حالة *Arthrocnemum macrostachyum* التي تحتوي على مادة "ريزورسينول" و "كايمبرول" و "كوارسيتين" و في حالة *Suaeda pruinosa*, تحتوي على "كوارسيتين" و "كايمبرول" و "ريزورسينول" و "حمض الفريولك" أما نبات *Kochia indica* فيحتوي على "كوارسيتين" و "كايمبرول" و "ريزورسينول" و "فينانثرين" وقد أظهر مستخلص إثير ثنائي الإيثيل في *Suaeda pruinosa* أعلى نشاط مضاد للأكسدة بنسبه % 95.25 يليه مستخلص الكلوروفورم من *Arthrocnemum macrostachyum* بنسبة %90.07. وتعطي مستخلصات النباتات نشاطا معتدلا ضد فطر الكانديدا، ولكن في حالة *Bacillus subtilis*، أظهر نبات *Suaeda pruinosa* أعلى نشاط. وأظهرت النتائج نشاطا إلى حد ما ضد *Proteus vulgaris* و *Escherichia coli* في حالة نبات *Kochia indica*.