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NUTRITIVE POTENTIALITIES AND BIOLOGICAL FEATURES OF *Emex spinosa* NATURALLY GROWING IN EGYPT

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ABSTRACT

The present study assesses the morphological and anatomical features of *Emex spinosa* (L.) compd., in addition to Investigation of the nutritive value, the bioactive constituents, antioxidant and antimicrobial potential of this polygonaceous herb. The moisture content (6.4%), fiber content (20.5%), lipid content (1.1%), total carbohydrates content (41.5%) and crude protein content (19.05%) and the nutritional value (253.9 k cal./ 100g) of *Emex spinosa* were detected. Also, the methanolic extract of *Emex* aerial parts contained relatively higher concentrations of Polyphenols, flavonoids, Alkaloids, Tannins and Saponins than that of the water extract. The methanol extract showed high antioxidant activity in terms of 2,2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid ($ABTS^+$) cation radical and reducing power assays in comparison with ascorbic acid. Both water and methanolic extracts of *E. spinosa* display antimicrobial activity against the bacterial strains, *Bacillus subtilis* and *Erwinia Spp.* and the pathogenic fungus *Candida albicans*.

Keyword: *Emex spinosa*, Anatomy, metabolites, Antioxidant, Antimicrobial.

INTRODUCTION

In contrast to the view proposed weeds as foe, weeds are gift of Allah to human. They play an important role in providing a forage source for animals, as food for man, providing fuel for local inhabitants, used as herbal medicine (Shaltout *et al*., 2009 and Al Kraeeshi, 2015). *Emex spinosa* is commonly Known as little jack and locally named Dirs el-agooz. *E. spinosa* is a Mediterranean taxon belonging to the family *Polygonaceae* occurring along railway tracks, waste sandy places, beside the edges of drains, and fields of winter grain crops (Boulos, 1999 and Jan *et al*., 2014). The phytochemical screening revealed the presence of anthraquinones, alkaloids and coumarins in *E. spinosa* (Rizk, 1986 and Abdel-Fattah *et al*., 1990). Luteolin and rutin were isolated from this plant, showed strong scavenging activity (Donia *et al*., 2014). The pharmacological importance related to the detected omega-3 fatty acids, and omega-6 fatty acids in the leaves (Freije *et al*., 2013). It is one of the important medicinal plant used to relief dyspepsia; stimulate appetite and a remedy for stomach disorders and to relief colic. It is believed to be purgative and diuretic (Watt & Breyer-Brandwjik, 1962). Thirteen compounds were isolated from the aerial parts of *Emex spinosa* in Egypt (Kader *et al*., 2006). The foliage leaves and the thick carrot-like roots are edible (Mandaville, 1990 and Boulos & El-Hadidi, 1994). The boild leaves of *E. spinosa* were used by African tribes to relieve stomach disorder (Abbas and Al-Saleh, 2002).

The high phenolics content of *E. spinosa* may contribute towards the anti-inflammatory and antioxidant properties (Shanker *et al*., 2008). Animals

treated with ethanolic extracts of *E. spinosa*, showed major improvement of the relative weight of reproductive organs, sperm motility, sperm count and total abnormality of sperm (Gamal *et al*., 2012). Under different conditions *E. spinosa* have the ability to germinate but percentage germination that will differ under different ecological conditions (Shoab *et al*., 2012). Aloe-emodin glucoside and four fractions from *Emex spinosa* were evaluated for their cytotoxic and antimicrobial activities (Raheim *et al*., 2014).

The current work was designed to know more details regarding the morphological and anatomical characteristics as well as evaluating the bioactive metabolites, antioxidant and antimicrobial activities of *E. spinosa*.

MATERIALS AND METHODS

Morphological characteristics of *E. spinosa* were described from fresh mature plants in flowering stage. The descriptive terminology based on LAWG (1999) and the texts of flora of Egypt Boulos (1999).

For anatomical investigation, cross-sections of the plant parts were prepared and described by Peacock and Hardbury (1973), then examined and photographed.

Phytochemical investigation:

Mature plants of *E. spinosa* were collected; shade dried at room temperature, crushed into fine powder and kept in dark well tight bottle for chemical analyses.

Quantitative estimations of moisture, ash, fiber, lipid, crude protein and total carbohydrate contents were carried out according to the methods described by Cakilcioglu and Khatun (2011), AOAC (2002), Arlington (1995), Allen *et al*. (1986).

The nutritive value of the plant was calculated as reported by Indrayan *et al*. (2005). Nutritive value = 4(% protein) + 9 (% lipid) + 4(% carbohydrate)

Determination of the secondary metabolites was carried out using standard procedure as described by Arlington (1995), Sadasivan and Manickam (2008), Lin and Tang (2007) and Obadoni Ochuko (2001).

Antioxidant activity

The reducing power was determined according to (Oyaizu, 1986).

$ABTS^{+}$ (2,2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was done as described by Re *et al*. (1999).

Antimicrobial activity

The antimicrobial activity of aqueous and methanolic extracts was examined by the filter paper disc assay (Murray *et al*., 1995) using inoculums of 10^6 bacterial or fungal cells/ml against *Klebsiella pneumonia*, *Escherichia coli*, *Bacillus subtilis*, *Erwinia spp*. and *Candida albicans*.

RESULTS

Morphology

E. spinosa (plate 1) is an autumn-winter active annual herb only reproduced by seeds, weak competitor but has several strong colonizing characteristics including: drought tolerance, rapid growth, abundant seed

production, high dispersal ability. *E. spinosa* is heterocarpy, produces both subterranean and aerial achenes. The plant develops a thick, carrot-like taproot, 12-20 cm depth (plat 2).

Stem decumbent to erect, rounded, solid, ribbed, sometimes reddish, dichotomously branched at periodic nodes. Leaves are ovate-cordate, with ochreate stipules, have pinnate reticulate primary vein category with a single main vein and weak brochidodromous secondaries joint together in a series of arches. The lower leaves may be simple lobed sinnatified with entire-sinuate morgin and acuminate apex.

Inflorescence possesses sessile female and pedicellated flowers inserted in dense axillary clusters. *Emex* is an amphicarpic plant that produces chasmogamous flowers early after seedling emergence at ground level in the axils of the rosette leaves. Fruit is simple dry indehiscent short spingy achene, contains a single trigonous brown seed weighing 28-33 mg (Plate c & d).

ANATOMY

Stem: Microscopic examination of the cross-sections in stem of *E. spinosa* (plate a) revealed that the stem has the general characters of herbaceous dicotyledonous mesophytes, composed of: epidermis of a single layer of isodiametric cells have thin protective cuticle; highly developed cortex differentiated outer zone adjacent to the epidermis of angular collenchyma interrupted by patch of assimilatory chloronchyma cells, followed by pharenchymatous cells having intercellular spaces, the inner layer of distinguishable cells constitutes the endodermis. The vascular tissues represented by collateral bundles forming continuous ring. The cambium is located between the outer phloem and xylem. It is not clearly observed. Pith is wide and forms the central part of the stem with a large hollow space.

Leaf: Transverse section of *E. spinosa* leaf (Plate 2b) passing through the midrib showed abaxial and adaxial epidermis covered by thin cuticle. The epidermal cells of the midrib were larged on both surfaces and projection from the blade. Mesophyll tissue comprised one-layered palisade cells and 4-layered spongy tissue. The vascular tissues represented by three bundles within the midrib zone. Close to the abaxial epidermis, angular collenchyma cells were observed.

Root: (Plate c) shows cross section of *Emex spinosa* root with normal secondary thickening. Its main features include concentric vascular tissues that forming a solid core and the presence of well-defined periderm. The epidermis is formed of a single layer of cells, mostly crushed and disintegrated due to increasing pressure from developing secondary tissues. Beneath the epidermis there is 3-4 layered cortex followed by multilayered periderms. A complete cambial ring is found and give narrow cylinder of secondary phloem outward and second xylem inner ward. Pith is narrow and has few parenchyma cells.

Phytochemical Analysis

Primary metabolites and nutritive value

The proximate metabolic constituents of *E. spinosa* are presented in Table 1. The moisture content, ash, fiber, total fat, crude protein and total carbohydrates were 6.4%, 11.45, 20.50%, 1.1%, 19.05% and 41.50, respectively. In turn the nutritive value of *E. spinosa* aerial parts was 253.9 Kcal/100g.

Secondary metabolites

All estimated secondary constituents were higher in methanol extract of *E. spinosa* than that of aqueous extract, except saponins which take reverse trend (Table 2).

The total phenolics content was higher in methanol extract (6.61%) than in water extract (8.76%). The flavonoids content was (1.05%) in methanol extract and (1.48%) in water extract. The alkaloid content was (0.55%) in methanol extract and (0.84%) in water extract. The tannins content was higher in methanol extract (0.33%) than water extract (0.54%). In contrast, the saponins content was higher in water extract (0.56%) than methanol extract (0.55%) of *E. spinosa*.

Table (1): The proximate primary metabolites in *Emex spinosa*

Primary metabolites %	
Moisture	6.4
Ash	11.45
Fiber	20.50
Protein	19.05
Lipid	1.1
Carbohydrates	41.50
Energy (Kcal/100g)	253.9

Table (2): The estimated secondary metabolites in *Emex spinosa*

Compounds (g/100 dry wt.)	Extract	
	Water	Methanol
Total phenolic	6.61	8.76
Flavonoids	1.05	1.48
Alkaloids	0.55	0.84
Tannins	0.33	0.54
Saponins	0.56	0.55

a) Reducing power assay

The reducing power assay was applied through measuring the absorbance of the reaction mixture. Higher absorbance of the reaction mixture indicated greater reducing power. This assay depends on analysis of the capability of the extracts to chelate metal ion iron (II) to different extents as a measure for antioxidant activity. Ascorbic acid was employed as standard compound in this experiment.

In comparison with the absorbance values for Ascorbic acid, water and methanol extracts of *E. spinosa* were high in their antioxidant activity in considerable values (Table 3). In addition methanol extracts (2.320) showed activity higher than water extract (1.605).

b) $ABTS^{+}$ cation radical assay

The free radicals of $ABTS^{+}$ was used for evaluation of the lipophilic and hydrophilic antioxidants present in the plant extracts through measuring the percent of inhibition of absorbance results from decolorisation. The obtained results in (Table 3) showed that the methanolic extracts (82.77%) were higher in their activity than the water extract (59.2 %), but not exceed the ascorbic acid.

Table (3): Antioxidant activity of water and ethanol extract of *E. Spinosa*.

Method	Reducing power assay		ABTS	
	Optical density		% Inhibition	
Extract	Water	Methanol	Water	Methanol
Emex spinosa	1.605	2.320	59.20	82.77
Ascorbic acid	1.153		91.44%	

Table (4): Antimicrobial activity of water and ethanol extract of *E. Spinosa*.

Microorganism	Emex spinosa	
	Water extract	Methanol extract
Bacillus subtilis	7	13
Escherichia coli	-	-
Klebsiella pneumonia	-	-
Erwinia spp.	-	6.5
Candida albicans	7	10

Values indicate zone of inhibition in mm and include filter paper disk diameter (6 mm); "-": no inhibition.

The obtained results elucidate that the methanol extracts, generally, possess a broader antimicrobial spectrum (Table 4). The *E. spinosa* extracts showed activity against *Bacillus subtilis, Erwinia spp.* and *Candida albicans.* Meanwhile it didn't show any activity against *Escherichia coli* and *Klebsiella pneumonia*.

DISCUSSION

E. spinosa is characterized by foliage leaves, ovate-cordate, with covering ochrear stipules and have pinnate reticulate primary vein category with weak brochidodromous secondaries. Flowers are green, unisexual and the male flowers emerging between the female flowers in axillary clusters.

Fruits are dimorphic with subterranean and aerial achenes. Seeds are trigonous with pointed end and brown colour.

Anatomically, *Emex* stem has highly developed cortex, bicollateral vascular bundles forming continuous ring and central pith with a large hollow space. The leaf has anisocytic stomata, dorsiventral lamina and three vascular bundles within the midrbib zone. The root showed normal secondary thickening. The findings of the present study correlated to those obtained from the previous studies (Fahn, 1982; Varma *et al*., 1984; Sanchez & Kron, 2008; and Shaltout *et al*., 2009).

Providing information about the nutritional values about indigenous plant would be important to the achievement of efforts for promoting the use of these plants as an alternative and bio diverse resources of food. *E. spinosa* rich with considerable amount of protein, fat, fiber, carbohydrates with energy of 253.9 Kcal per 100 g. Proteins and carbohydrates are the building blocks of the body and so are required in high amounts in regular diet. Similarly, lipids are the integral part in the cellular membranes and are required for various body functions (Norton, 2003). These finding may explain their uses as food.

In this study qualitative phytochemical screening of water and methanol extracts of *E. spinosa* revealed the presence of pharmacologically active ingredients like phenolics, flavonoids, alkaloids, tannins and saponins in methanol extracts than that in water extracts. Aldamegh *et al*. (2013) detected qualitatively the presence of Flavonoids, alkaloids and anthraquinones in *E. spinosa* from Saudi Arabia. Such these compounds present in the studied plant are pharmacologically helpful for human. For example, flavonoids as a class of phenolics have various properties such as antimicrobial, anti-inflammation and anti-carcinogenic activity. Alkaloids and terpenoids protect against several chronic diseases (Kaya *et al*., 2010; Aslam *et al*., 2012).

Synthetic antioxidants were found to be injurious to health whereas most of the natural antioxidants from plant sources proved to be safer for health and possess better biological activities (Kumari and Kakkar, 2008). As reported by Saikia and Upadhyaya (2011) Natural antioxidants from plants can use as nutraceuticals.

Methanol extract of *E. Spinosa* showed inhibition and cidal effect against *Bacillus subtilis*, *Candida albicans* and *Erwinia spp.*, while water extract showed inhibition activity only against *Bacillus subtilis* and *Candida albicans*. On the other side *E. spinosa* didn't show any activity against *Escherichia coli* and *Klebsiella pneumonia*. In this respect, Aldamegh *et al*. (2013) agree with our results where *E. spinosa* showed activity against *Candida albicans*, while he mentioned that *E. spinosa* didn't show any activity against *Proteus vulgaris*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*. Donia *et al*. (2014) revealed that plant extracts of *E. spinosa* possess antibacterial potential against *Staphylococcus aureus* and *Streptococcus pyogenes*.

In conclusion, *E. spinosa* that growing naturally at different habitats in Egypt possesses a good nutritional value as food or fodder. In addition it has good phytochemical content, which shows that the plant has good medicinal value and extracts can be used for medicinal formulations.

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الامكانات الغذائية والسمات البيولوجية لنبات ضرس العجوز النامى طبيعيا بمصر

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تهدف هذه الدراسة لتقيم السمات المورفولوجية و التشريحية لنبات ضرس العجوز بالاضافة الى دراسة القيمة الغذائية، المواد الفعالة و مضادات الأكسدة و القدرة ضد الميكروبية لهذا النبات، حيث كان محتوى نبات من الرطوبة (٦,٤%) ، و الالياف (٢٠,٥%) ، الدهون (١,١%) ، الكربوهيدرات (٤١,٥%) ، محتوى البروتين الخام(١٩,٠٥%) والقيمة الغذائية(٢٥٣,٩ كيلو سعرة حراري). و أيضا أظهرت النتائج ان المستخلص الكحولي للاجزاء الهوائية لنبات ضرس العجوز كان يحتوي على مركبات فينولية ، فيلافونيدات، والقلويدات، التانينات و الصابونين أكبر من محتواها للمستخلص المائى. أيضا كان المستخلص الكحولى أكثر كفائة لمضادات الاكسدة من المستخلص المائى. حيث أظهر المستخلص الكحولى و المائى لنبات ضرس العجوز نشاط ضد *Bacillus subtilis* and *Erwinia Spp.* and the pathogenic fungus *Candida albicans*.