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Phytochemical and antimicrobial activity of two Egyptian xerophytes: *Artemisia jaudica* L. and *Artemisia monosperma* Delile

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ABSTRACT

This study aimed to investigate the various bioactive constituents of the leaves of two desert plants belonging to family Asteraceae *Artemisia jaudica* and *Artemisia monosperma* consumed in the Egyptian folk medicine.Nutritional value,antioxidant activity, antimicrobial activity, phytochemicals; total phenolics, flavanoids, saponins, alkaloids andterpenoids which have different therapeutic uses were studied. Proximate composition of the plants was determined following AOAC method. Phytochemical screening, antioxidant activity and antimicrobial spectrum of methanol and water extracts were performed using several assays according to the standard procedures.

Results revealed that the nutritional leaves of *A. monosperma* and *A. jaudica* are good source of energy, dietary fiber, proteins, carbohydrates and fats**.**The phytochemicals results revealed that the prepared extractshavegood antioxidant activity. The extracts showed good antimicrobial activity against gram positive bacteria, gram negative bacteria and one fungal strain.

Conclusively, the dried leaves of *A. monosperma* and *A. jaudica*are good source of health promoting constituents which can be used for therapeutic and nutritional purposes, therefore there is need for further studies on the active compounds of these xerophytic plants so as to maximize their medicinal and nutritional values.

Key words: *A. monosperma*, *A. jaudaica,* nutritional value, phytochemicals, antioxidant activity, antimicrobial activity, Egyptian xerophytes.

INTRODUCTION

Since ancient times, plants have been used in folk medicine as pharmaceuticals and thought to provide nutritional powers for people (Venkataswamy *et al*., 2010). The World Health Organization (WHO) announced that about 80% of populations in the developing countries in Africa still depend upon traditional medications using restorative plants due to their social insurance needs and therapeutic potential (Joy *et al*, 2001; WHO, 2002).

The restorative plants in Egypt considered to be promising source of new medications for the treatment of many human infections. Many studies have illustrated that restorative plants are sources of different bioactive compounds that have antimicrobial properties and can protect the human body from oxidative stress caused by free radicals that can cause coronary heart diseases, neurodegenerative disorder, iron deficiency, joint inflammation, Parkinson's disease, asthma, ischemia, cancer and many other diseases. It is consequently vital to describe distinctive kinds of therapeutic plants for their cancer prevention agent and antimicrobial potential (Mothana and Lindequist, 2005; Bajpai *et al*., 2005;Wojdylo *et al*., 2007; Zheng *et al*,

2008; Brooks *et al.*, 2009;Ghasemzadeh *et al*, 2010).

The species *Artemisia* is an economically important variety of the Asteraceae family that comprise more than 500 species. It is a cosmopolitan and growing mostly in the calm zones of the northern half of the hemisphere (McArthur and Plummer, 1978; Ling, 1982, 1994; Mabberley, 1990;Oberprieler, 2001;Valles and McArthur, 2001;Valles and Garnatge, 2005;). A review of different systematic and evolutionary aspects of the genus, with special emphasis on cytogenetic and molecular data was given by Vallès and McArthur (2001).

In Egypt, *Artemisia* is represented by four wild species (*Artemisia monosperma* Delile, A. judaica L., *A. scoparia* Waldst and *A. verlotiorum* Lamotte) and one more species (*A. vulgaris* L.) is cultivated (Boulos, 2002).

Artemisia monosperma (Delile) is a green aromatic perennial shrublet that grows widely in the deserts of Middle East, Africa and China (Abad *et al.*, 2012; Bora and Sharma, 2011; Migahid and Hammouda, 1974). It is widespread in the desert plains and wadis, both inland and in the Mediterranean coastal region, often not too far from the coast in northern Sinai (Badr *et al.,* 2012). The plant is used in traditional medicine for treatment of gastrointestinal diseases (Dabe and Kefale, 2017) diabetics, rheumatism, fever, hypertension and helminths (Hijazi and Salhab, 2010).

A. monosperma has been reported for the high antioxidant and antimicrobial activities (Zaki .*et al*, 1984), in addition to its insecticidal and antimalarial potential (Abou-Taleb *et al.*, 2016; Abdel-Shafy *et al.*, 2009; Maia and Moore, 2011; Saleh, 1984). It contains many phytochemicals such as flavonoids, alkaloids and coumarins. A few constituents of *A. monosperma* was investigated to be dynamic against: 12-lipooxygenase, colorectal and bosom tumor cell lines, *Mycobacterium* and *Staphylococcus aureus* (Stavri *et al*., 2005; Hammoda *et al*., 2008; Abu-Niaaj and Katampe, 2018).

Artemisia Judaica L. is a perennial fragrant shrublets with pubescent leaves (Dob and Chelghoum, 2006) which grows extensively in wadi beds, terraces and stony plains (Nofal *et al*., 2009; Badr *et al.*, 2012) in the Sinai Peninsula in Egypt, its Arabic name is "shih balady" and this plant is a restorative herb in Egypt. It has distinct scent due to its high volatile oils with anti-inflammatory, antitumor, antimicrobial and antioxidant activity (Ahmed *et al.*, 2017). It is widely used in traditional medicine in improving vision, cardiovascular health, capillary strength and stimulate immune system functions, as well as decreased risk of atherosclerosis, cancer, arthritis and gastrointestinal diseases (Janaćković *et al.*, 2015).

Phytochemical investigation of *A. judaica* demonstrated that the plant is rich in flavonoids (Bakr, 2015), sesquiterpene lactones and the high oil content that act as cancer prevention agent (Al-Wahaibi *et al*, 2018), anthelmintic, calming, pain relieving, antipyretic, antimalarial, antiviral, antitumor, antispasmodic, and antimicrobial (Mahmoud and Gairola, 2013; Abu-Darwish *et al*., 2016).

Thus, the present work had been designed to evaluate the nutritional value, phytochemical constituents, antioxidant activityand antimicrobial potential of two Egyptian medicinal xerophytes (*A. monosperma* and *A. judaica*).

MATERIALS AND METHODS

Plant material:

The leaves of *Artemisia jaudaica* were collected from Saint Catherine protectorate, while those of *Artemisia monosperma* were collected from the

northern deltaic cost at region of Marsa Matrouh. The plant materials were collected in cool and dry containers. The plants were identified and voucher specimens were deposited in the Herbarium of Botany Department at Damietta University. The collected plant materials were air dried in shade for 15 days and then finely grinded in blender and stored in clean and dry containers for further analysis**.**

Biochemical estimations:

Determination of nutritive value

Crude fat, crude fiber, crude protein, ash, moisture and total carbohydrates were determined according to the methodology of AOAC (AOAC, 2016). Fats were extracted with petroleum ether (boiling point 40–60°C) using a Soxhlet fat extraction unit. Crude protein was determined according to Kjeldahl method for determination of total nitrogen content and using a conversion factor of 6.25. the total soluble sugars were determined with the slightly modified phenol–sulphuric acid method according to Masuko and co-workers (2005), while reducing sugars were determined by the modified method of Miller (1959). Non-reducing sugars and total carbohydrates were calculated according to the following formulae:

Total carbohydrates = 100 – (crude fiber **+**percentage of ash + percentage moisture + percentage fat + percentage protein) (Burlingame, 2000).

% Non-reducing sugars = Total sugars – Reducing sugars

Nutritive value was finally determined using the following formula:

Nutritive value = 4 x (percentage of protein) + 9 x (percentage of fat) + 4 x (percentage of total carbohydrate).

The energy produced expressed as kcal/100 g dry weight of the plant material taking into consideration that 1g protein = 4.1kcal; 1g carbohydrates = 4.1kcal and 1g lipids = 9.2kcal (AOAC, 2016).

Extraction of the active ingredients:

Aqueous extract of Artemisia was prepared using 5 g of each of the air-driedleaves that were extracted upon shaking at 240 rpm for 20 minutes at 70°C using 100 ml distilled water then filter, while 5 g of the air dried plant materials were extracted with 80% methanol using Soxhlet extraction unit, then obtained extracts were evaporated under vacuum to dryness using rotary evaporator followed by lyophilizer for complete dryness, then the crude extractsweighed and the yielding percent was defined.

Total phenolics, flavonoids, alkaloids, tanins, saponins content, antioxidant activity and antimicrobial assays were performed on each of the obtained extracts.

Total phenolics

It was measured using the modified FolinCiocalteu colorimetric assay developed by Wolfe *et al*, 2003.The quantity of the phenolics present in the extracts was calculated using the standard curve of gallic acid (Y=0.006X, r2 =0.98) and expressed as gram gallic acid equivalent/100 gram dried plant material.

Total flavonoids

It was measured using aluminum chloride colorimetric assay developed by Zhishen*et al*, 1999.Total flavonoids contentwas calculated using standard curve of catechine (Y=0.003X, r2=0.99) and expressed as gram catechin equivalent/100 gram dried plant material.

Total Tannins

It was estimated using Vanillin hydrochloride assay (Sadasivam and Manickam, 2008).The quantity of the tannins present in the extracts was calculated using the standard curve of gallic acid (Y=0.0009X, r2 =0.955) and expressed as gram gallic acid equivalent/100 g dried plant material.

Total Alkaloids

It was measured according to the method described by Harborne (1999) and calculated as a percentage of the dry plant material.

Total Saponins

These were determined according to the method described by Obadoni and Ochuko (2001) and were calculated as a percentage of the dry plant material.

Evaluation of antioxidant activity of the plant extracts

Determination of free radical scavenging activity using DPPH assay

The effect of the extracts on DPPH radical was estimated using the method described by Kitts *et al.* (2000) with slight modifications (Liyana- Pathirana&Shahidi, 2005). The radical scavenging activity of the extracts was expressed as the concentration of the extract at which 50% of the radical scavenged ($IC_{50\%}$) using ascorbic acid as reference for comparison.

Antioxidants

Of plant extracts determined by (ABTS+) cation radical assayABTS (2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was done according to the method described by Re *et al.* (1999) and ascorbic acid was used as reference compound for comparison.

Antimicrobial activity

Microbial susceptibility testing

The antimicrobial activity of the extracts of *A. monosperma* and *A. jaudaica* was estimated using filter paper disc assay (Murray *et al*., 1998). Gentamycin was used as standard antibiotic for comparison.

Four gram positive bacteria (*Bacillus subtilius, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogenes),* four Gram negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumonia*, *Proteus vulgaris*, *Erwinia carotovora)* and one fungal strain *(Candida albicans)* were used. Stock cultures of the tested organisms were obtained from the microbiological Lab at Faculty of Science at unit of Genetic Engineering and Biotechnology Mansoura University.

RESULTS AND DISCUSSION

Biochemical analysis

Nutritive value

Primary metabolites are essential for growth, development and reproduction of plants in addition to its nutritional value for human and animal fodder. Many primary metabolites also are precursors for many pharmacologically active metabolites (Pagare *et al.*, 2015; Ncube and Staden, 2015).

The Proximate composition of leaves of *A. monosperma* and *A. jaudica*is presented in Table (1). The obtained results indicated that moisture content, crude fat and crude fiber content were higher in leaves of *A. jaudica*, while crude protein levels, reducing sugars percent, total sugars percent, crude ash and total nitrogen percent were higher in *A. monosperma* leaves. The analysis showed that there was not much difference in the content of non-reducing sugars and total carbohydrates in the studied plants. The obtained results showed that the leaves of both plants contain appreciable levels of nutritive content considering that the nutritive value of *A. monosperma* leaves

(272 kcal/100 g dry weight) was slightly higher than that of *A. jaudica* (264 kcal/100 g dry weight).

Table (1): Proximate analysis of the leaves of*A. monosperma*and *A. jaudica*

	A. monosperma	***A. jaudica***
Moisture %	9.76	11.62
Crude Ash %	6.77	5.02
Crude Protein %	14.82	11.58
Crude fat %	1.46	2.19
Crude Fiber %	28.15	33.44
Reducing sugars %	22.52	19.85
Total sugars %	49.50	45.04
Non reducing sugars %	26.98	25.15
Total carbohydrate %	48.8	47.77
Total Nitrogen %	3.603	1.853
Nutritive value kcal/100 g dry weight	272	264

The abiotic environmental stress factors are considered as precursors for plants to produce different kinds of secondary metabolites for their defense (Tsao, 2010; Stanojevic *et al.*, 2009; Ramamoorthy and Bono, 2007; Pourmorad *et al*., 2006). The dry habitat of the studied plants is considered as precursor for them to synthesize many secondary metabolites such as phenolics, flavonoids, alkaloids, saponins and many other compounds that have protective and therapeutic effects.

The phytochemical screening of the water and 80% methanol extracts of the leaves of the studied plants are presented in Table (2). The results showed that the total phenolics, total flavonoids and total tannins contents were higher in water extracts than 80% methanol extracts while alkaloids content was higher in methanol extract than that of water. The extracts of *A. monosperma* were rich in flavonoids, phenolics, alkaloids and saponins in considerable amounts than those present in the extracts from *A. Judaica*. The total saponins content were higher in the 80% methanol extract than water extract for both plants, whereas, *A. monosperma* extracts represent higher values for saponins than *A. jaudaica.*

Table (2): Comparison betweenthe phytochemicals in the two species of *Artemisia*

	A. monosperma		***A. judaica***	
Extracts / **Phytochemical constituents**	**Aquous extract**	**Methanol extract**	**Aquous extract**	**Methanol extract**
Phenolics (g gallic acid equivalent/100 g dried plant)	2.661	2.338	1.509	2.012
Flavonoids (g quercetine equivalent/100 g dried plant)	0.438	0.357	0.205	0.247
Tannins (g gallic acid equivalent / 100 g dried plant material)	0.049	0.028	0.102	0.020

Total flavonoids/total phenolics	0.165	0.153	0.102	0.164
Alkaloids %	2.88	3.80	1.28	2.74
Saponins%	12.84	16.14	12.06	11.35

Evaluation of the antioxidant activity

Theantioxidant scavenging activity of the prepared extracts was determined using DPPH and $ABTS^+$ assays. Numerous reports of antioxidant extracts from medicinal plants have appeared recently. Phenolics and flavonoids are probably the best known of the active secondary ingredient due to their high antioxidant activity and therapeutic effects. The highest antioxidant activity of phenolics and flavonoids iscontributed to their chemical structure as they contain hydroxyls that are responsible for the radical scavenging effects of most plants (Agbo *et al.*, 2015; Li *et al.*, 2018; Hernández Zarate *et al.*, 2018).

The free radical scavenging activity was estimated using DPPH assay.

The concentration of an antioxidant needed to diminish the initial concentration DPPH radical by 50% ($IC_{50\%}$) is a parameter widely used for estimating the antioxidant activity. $IC_{50\%}$, is inversely proportional to the antioxidant activity(Sanchez Moreno *et al*, 1998).The data presented in Table (3) indicated that the methanolic extract of *A. jaudica* was higher in its free radical scavenging activity (0.05 mg/ml) than the aqueous extract and was the highest through all the tested extracts followed by water and methanol extracts of *A. monosperma* (0.071 and 0.083 mg/ml, respectively), while the lowest antioxidant scavenging activity expressed by the water extract of *A. jaudica* (0.111 mg/ml). In comparison with the $IC_{50\%}$ of ascorbic acid, all the tested extracts have considerable antioxidant scavenging activities but with values lower than that of ascorbic acid (0.02 mg/ml).

Table (3): The IC_{50} values of DPPH scavenging effect of *A. monosperma* and *A. jaudica* extracts (mg/ml).

Method	(DPPH)	
	$IC_{50\%}$	
Extract	Aquous extract	Methanol extract
A. monosperma	0.071	0.083
A. jaudica	0.111	0.053
Ascorbic acid	0.022	

$ABTS^+$ assay 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 decolorization. The data represented in Table (4) indicated that the methanolic extract of *A. jaudica*was higher in its activity (79.40%) than the aqueous extract (59.36%) and was the highest through all the tested extracts followed by water and methanol extracts of *A. monosperma* (74.59% and 71.50%, respectively), while the lowest activity expressed by the water extract of *A. jaudica*. All tested extracts have considerable percent of inhibition but with values lower than that of ascorbic acid (91.44%).

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Table (4): % inhibition of ***A. monosperma*** **and** ***A. jaudica*** extracts **measured by the ABTS Method.**

Method	ABTS	
	% Inhibition	
Extract	Aquous extract	Methanol extract
A. monosperma	74.59	71.50
A. jaudica	59.36	79.40
Ascorbic acid	91.44	

The results obtained suggestedthe correlation between the increase in total phenolics content, total flavonoids content, as well as total flavonoids/total phenolics ratio (TF/TP) and the higher antioxidant activity and reducing power of the tested extracts of the two investigated *Artemisia* species.

Evaluation of the antimicrobial potential of *A. monosperma* and ***A. jaudica*****:**

This was done using microbial susceptibility testing (disc diffusion assay). The antimicrobial potential of plant extracts was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards gentamycin. The present results revealed that the methanolic extracts of *A. monosperma* and *A. jaudica* showed potent antibacterial activity against both gram positive and gram negative bacteria than their aqueous extracts. The antimicrobial spectrum of each of the tested extracts is presented in Figure (1). The methanolic extract of *A. jaudica* showed inhibitory activity against 55.56%, while that of *A. monosperma* showed inhibitory activity against 67% of the tested pathogenic microorganisms. The water extract of *A. jaudica* showed no activity against any of the tested microorganisms, while that of *A. monosperma* showed inhibitory activity of only 11.11% against the tested pathogenic microorganisms.

Natural antimicrobial agents have been more popular due to their efficacy against antibiotic resistant microorganisms and campaign for consumption of natural products (Farjana *et a.l*, 2014). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of medicinal plants (Behera and Misra, 2005; Patel,2014; Mahesh and Satish, 2008; Joe *et al.*, 2009; Bereksi *et al.*, 2018; Babotă *et al.*, 2018). Secondary metabolites like Phenolics, flavonoids, terpenoids, alkaloids, saponins, coumarins and sterols were isolated from *Artemisia* species and have anti-malarial, antiviral, anti-hepatitis, antibacterial, antifungal and anti-tumor (Tan *et al.*, 1998; Lee, 2014; Rabe *et al.*, 2015). Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, little reports are available on the exploitation of antifungal or

antibacterial property of plants for developing commercial formulations.

Table (5): Antimicrobial screening test of the prepared plants extracts against several pathogenic microbial strains.

	A. Jaudica	*A. monosperma*		*A. Jaudica*	*A. monosperma*
Solvents	**80% Methanol**	**Water**	**Solvents**	**80% Methanol**	**Water**
Candida albicans	9	-	***Candida albicans***	9	-
ErwiniaCarotovora	9	-	***ErwiniaCarotovora***	9	-
Pseudomonas aeruginosa	-	–	***Pseudomonas aeruginosa***	-	–
Staphylococcus aureus	11	–	***Staphylococcus aureus***	11	–
Klebsiella pneumonia	-	–	***Klebsiella pneumonia***	-	–
Proteus vulgaris	-	_	***Proteus vulgaris***	-	_
Streptococcus pyogenes	–	–	***Streptococcus pyogenes***	–	–
Staphylococcus epidermis	9	–	***Staphylococcus epidermis***	9	–
Bacillus subtilius	9	-	***Bacillus subtilius***	9	-

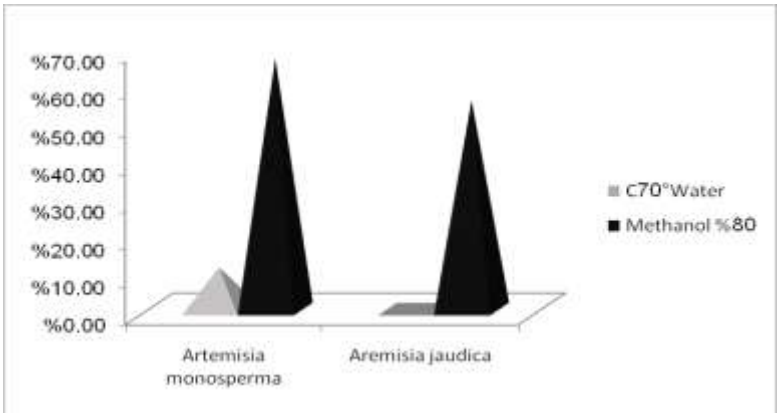

Fig. (1):The antimicrobial spectrum of the studied plants extracts expressed as inhibitory percent against the tested pathogenic microbial strains

Conclusion:

It was found that the extracts of *A. monosperma* and *A. jaudaica* contain some important bioactive components with pronounced antioxidant and antimicrobial activities in addition to some important nutritional components. However, further studies are recommended to be carried out on the plants leaves in order to isolate, identify and characterize the active compounds so as to maximize the nutritional and medicinal value of these Egyptian xerophytic plants.

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دراسة حول النشاط االفيتوكيميائي والمضاد للبكتريا لنباتي شيح وحيد البذرة والبعثران في مصر

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المستخلص

تهدف الدراسة الي التعرف علي المحتوي الكيميائي لنباتين من العائلة المركبة وهما شيح وحيد البذرة والبعثران واللذان يتم استخدامهما في الطب الشعبي في مصر. وقد تم دراسة كلا من القيمة الغذائية والنشاطا لمضاد للاكسدة وكذلك النشاط المضاد للميكروبات كما تم دراسة المحتوي الكيميائي للنباتين حيث ان لهما اهمية واستخدامات علاجية. وقد تم عمل مسح شامل للمحتوي الكيميائي للمستخلص الكحولي والمائي لكلا من النباتين باستخدام طرق مختلفة. ومن خلال دراسة المحتوي الغذائي لاوراق شيحا البذرة الحبة والبعثران تم التوصل الي ان كليهما مصدرجيد للطاقة وان لهما محتوي جيد من الالياف والبروتين والكربوهيدرات والدهون. كماوضحت دراسةالمحتوي الكيميائي لكلاالنباتين ان لهما نشاط مضاد للاكسدة وكذلك نشاط مضاد للميكروبات. نستخلص من هذه الدراسة ان الاوراق الجافة لنبات شيح وحيد البذرة ونبات البعثران تعتبرمصدرغذاائي جيد وايضا يمكن استخدامها للاغراض العلاجية كذلك وعليه فاننا نحتاج الي مزيد من البحث ودراسة المحتويات النشطة لكلا النباتتين لزيادة الاستفادة منهما في المجالات الدوائية وكذلك الغذائية.