

# Journal of Pharmacognosy and Phytotherapy

Volume 5 Number 3 March 2013

ISSN 2141-2502



*Academic  
Journals*

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Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for *Striga* suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

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## Review

# An overview on *Hypericum* species of Turkey

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Accepted 28 January, 2013

The genus *Hypericum* (Hypericaceae) is represented by nearly 100 taxa grouped under 19 sections in Turkey. Among them, 45 species are endemic. All members of the genus may be referred to as St. John's wort in the world. The genus is known as "sari kantaron, kantaron, binbirdelik otu, mayasil otu" and most of them, especially *H. perforatum*, have been used for the treatment of burns, wounds, haemorrhoids, diarrhoea and ulcers in Turkish traditional medicine. The pharmacological studies showed that this species has several activities: anti-depressant, anti-inflammatory, anti-microbial, anti-viral, anti-nociceptive and wound healing. The chemical composition of the *Hypericum* species is composed of naphthodianthrones (especially hypericin and pseudohypericin), acylphloroglucinol derivatives (especially hyperforin and adhyperforin), flavonoids (especially quercetin, quercitrin, hyperoside and biapigenin), tannins and volatile oils. Investigations on the chemical composition and biological activities, as well as hypericin content of Turkish *Hypericum* species, have been carried out for about 25 years. The aim of this study was to review and summarise important studies about Turkish *Hypericum* species. Endemic species are indicated with (e) in the text.

**Key words:** *Hypericum* species, Turkey, endemic, chemical composition, biological activity, agricultural study, traditional use.

## INTRODUCTION

### Traditional uses of *Hypericum* species in Turkey

*Hypericum* species have been used in traditional medicine in Anatolia for centuries. The existence of four species including; *H. crispum*, *H. perforatum*, *H. perforatum* and *H. coris* as medicinal plants was reported in Dioscorides' Materia Medica. Various traditional uses have been reported for *H. atomarium*, *H. aviculariifolium*, *H. cerastoides*, *H. calycinum*, *H. confertum* subsp. *confertum*, *H. confertum* var. *stenobotrys*, *H. heterophyllum*, *H. hyssopifolium*, *H. hyssopifolium* subsp. *elongatum* var. *elongatum*, *H. lydium*, *H. montbretii*, *H. olympicum*, *H. orientale*, *H. scabrum*, *H. ternatum*, *H. thymifolium*, *H. triquetrifolium* and mainly *H. perforatum* (Yesilada et al., 1993; Yesilada et al., 1995; Honda et al., 1996; Tuzlaci and Tolon, 2000; Tuzlaci and Aymaz, 2001; Keskin and Alpinar, 2002; Ezer and Avci, 2004; Bulut, 2006; Buyukgebiz, 2006; Ecevit and Ozhatay, 2006; Ezer and Mumcu, 2006; Mart, 2006; Cimen, 2007; Akgul, 2008;

Demirci, 2010; Aktan, 2011; Kizilarslan, 2008) in the form of infusion, decoction, ointment and oleat (dried herbs with flowers are kept in olive oil for 1 month and filtered through muslin) in Turkey. Table 1 shows species having traditional uses.

## AGRICULTURAL STUDIES

A study was undertaken to enhance the germination rate of *H. aviculariifolium* subsp. *depilatum* var. *depilatum* (e) seeds. Results revealed that the seeds have exogenous dormancy and light is required for germination (Cirak et al., 2007a). The ontogenetic and morphogenetic variation of hypericin, chlorogenic acid and flavonoids was determined in *H. origanifolium*. Hypericin, quercetin and quercitrin content in whole plant increased during the course of ontogenesis, and the highest level was reached in blooming stage whereas, hyperoside content of whole plant decreased linearly with advancing of development stages, and the highest level was observed at vegetative stage. Among different tissues, reproductive parts accumulated the highest level of hypericin, quercetin and

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**Table 1.** The traditional uses of *Hypericum* species in Turkey.

<b>Hypericum species</b>	<b>Traditional uses</b>	<b>Administration</b>	<b>References</b>
<i>H. atomarium</i>	Stomach diseases, enteritis, sedative	Inf. (int)	Vural (2008)
<i>H. aviculariifolium</i>	Urethra diseases	Inf. (int)	Keskin and Alpınar (2002)
<i>H. cerastoides</i>	To against diarrhea	Inf. (int)	Kızılarıslan (2008)
<i>H. calycinum</i>	To abolish spasm, anti-asthmatic	Dec. (int)	Aktan (2011)
<i>H. confertum</i> subsp. <i>confertum</i>	Anti-asthmatic, wound healing	Inf. (int), Oleat (ext)	Buyukgebiz (2006)
<i>H. confertum</i> var. <i>stenobotrys</i>	To abolish spasm, stomach diseases	Inf. (int)	Buyukgebiz (2006), Bulut (2006) and Mart (2006)
<i>H. heterophyllum</i>	Anti-inflammatory	Oleat (ext)	Unal et al. (2008)
<i>H. hyssopifolium</i> and <i>H. hyssopifolium</i> subsp. <i>elongatum</i> var. <i>elongatum</i>	To abolish spasm, against diarrhea, hemorrhoid, and as a sedative, anti-helminthic, antiseptic against eczema and as an anti-fungal for various fungal disorders, psoriasis	Inf. (int), Oleat (ext)	Unal et al. (2008)
<i>H. lydium</i>	To treat indigestions and stomach diseases, hemorrhoid	Inf. (int)	Yesilada et al. (1995), Sezık et al. (2001) and Yesil (2007)
<i>H. montbretii</i>	Eczema	Dec. (int)	Keskin and Alpınar (2002)
<i>H. olympicum</i>	For stomach ache, inflamed wounds, cuts.	Dec., int. Dec., ext.	Tuzlacı et al. (2001)
<i>H. orientale</i>	Stomach diseases, sedative	Inf. (int)	Ezer (2006) and Tatlı et al. (2009)
<i>H. perforatum</i>	Kidney stones, urinary diseases, diabetes, antihypertensive, cold, stomachache, enteritis, eczema, antifungal, cardiac diseases, arteriosclerosis, antihemorrhagic	Dec. (int)	Yesilada et al. (1993), Yesilada et al. (1995), Tuzlacı et al. (2000), Sezık et al. (2001), Tuzlacı et al. (2001), Ezer (2004), Ecevit and Ozhatay (2006), Kultur (2007), Cimen Oral (2007) and Demirci (2010)
	Asthma, insomnia, uroclepsia(forbabies), gall bladder ailments, facial paralysis, gastritis, chestdiseases, internal hemorrhage, bronchitis, anti-inflammatory, tuberculosis, pharyngitis	Inf. (int)	
	Wounds	Ointment (ext)	
	Wounds, burns, cuts, herpes labialis, lip chap	Ointment (ext)	
	Stomach diseases, diabetes, enteritis, ulcers	Ointment (ext)	
	Stomach disease	Dec. (int)	
<i>H. scabrum</i>	Hemmorroid, constipation, peptic ulcer	Inf. (int), Oleat (int)	Yesilada et al. (1996), Ezer and Mumcu-Arısan (2006) and Unal et al. (2008)
<i>H. ternatum</i>	Antiasthmatic, wound healing	Inf. (int), Oleat (ext)	Bulut (2006) and Mart (2006)
<i>H. thymifolium</i>	Stomach diseases	Inf. (int)	Mart (2006)
<i>H. triquetrifolium</i>	Cardiac diseases, diabetes	Inf. (int)	Akgul (2008)

Dec. = decoction, Inf. = infusion, int. = internal, ext. = external.

quercitrin, however, leaves produced substantially higher amount of chlorogenic acid and hyperoside. Rutin and apigenin-7-O-glucoside were detectable in all tissues only during fruit maturation (Cirak, 2007b).

Chemical and morphological variability was studied in *H. perforatum* samples collected from different locations of Northern Turkey. Hypericin content was found to be correlated positively with leaf dark gland density, however, negatively with leaf area and no correlation was detected between the other morphological traits and bioactive substances examined (Cirak et al., 2007c).

Ontogenetic, morphogenetic and diurnal variation of the total hypericins content was determined in *H. aviculariifolium* subsp. *depilatum* var. *depilatum* (e), *H. perforatum* and *H. pruinatum*. The hypericin content of leaves and whole plant was higher in *H. aviculariifolium* subsp. *depilatum* var. *depilatum* (e) whose leaves had more numerous dark glands than those of the two other species (Cirak et al., 2006a). Ontogenetic, morphogenetic and diurnal variations in total phenolic contents was investigated in *H. aviculariifolium* subsp. *depilatum* var. *depilatum* (e), *H. perforatum* and *H. pruinatum*. Phenolic contents of *H. perforatum* and *H. pruinatum* were the highest during flowering stage, although no diurnal fluctuations were observed in those species (Ayan et al., 2006).

The ability to predict the number of days for seeds of four *Hypericum* species (*H. perforatum*, *H. bupleuroides*, *H. nummularioides* and *H. pruinatum*) to germinate was investigated by using mathematical models based on temperature. Optimum seed germination temperature in the tested species was determined and germination time was calculated using coefficients obtained from regression models (Cirak et al., 2006b). The possibilities of domesticating *H. crenulatum* (e) collected from the Nigde-Demirkazik (Camardi) mountains were investigated. It was observed that the *H. crenulatum* (e) seeds did not germinate, thus did not adapt to the region (Inan and Kirici, 2003).

## CHEMICAL COMPOSITION

The chemical composition of the *Hypericum* species is composed of naphthodianthrone (especially hypericin and pseudohypericin), acylphloroglucinol derivatives (especially hyperforin and adhyperforin), flavonoids (especially quercetin, quercitrin, hyperoside and biapigenin), tannins, n-alkanes, xanthenes and essential oils (Bombardelli and Morazzoni, 1995; Bruneton, 1995).

### Naphthodianthrone and acylphloroglucinol derivatives

Table 2 shows quantitative determination of hypericin, pseudohypericin and hyperforin in Turkish *Hypericum* species.

## Volatile compounds

The volatile compounds of ten taxa have been investigated by using Gas chromatography (GC) and Gas chromatography/Mass spectrometry (GC/MS). Caryophyllene oxide was found as a major component in *H. hyssopifolium* var. *microcalycinum* and *H. lysimachioides* var. *lysimachioides* (Toker et al., 2006). Thirty components representing 92% of the total volatiles were characterized in *H. Bupleuroides*, and sesquiterpenes such as  $\beta$ -sesquiphellandrene (33.2%) and  $\beta$ -caryophyllene (20.2%) were assigned as major compounds (Demirci and Baser, 2006).

The essential oil of *H. linarioides* was found to contain 74 compounds, mainly  $\delta$ -cadinene (6.9%), (Z)- $\beta$ -farnesene (5.2%),  $\gamma$ -muurolene (5.5%), spathulenol (4.8%), hexahydrofarnesyl acetone (4.5%) and selinene (4.0%). The oil was also characterized by high content of sesquiterpenes (64.2% of total oil) (Cakir et al., 2005). *H. adenotrichum* (e), *H. calycinum*, *H. cerastoides*, *H. montbretii*, and *H. perforatum* have been investigated and their major volatile compounds were determined as follows:

Germacone D (38%) in *H. adenotrichum*;  $\alpha$ -pinene (24%) and  $\beta$ -pinene (14%) in *H. calycinum*;  $\alpha$ -pinene (58%), undecane (5%) and  $\beta$ -pinene (3%) in *H. cerastoides*;  $\alpha$ -pinene (26%),  $\beta$ -pinene (19%) and undecane (5%) in *H. montbretii*;  $\alpha$ -pinene (50%) and carvacrol (22%) in *H. perforatum* (Erken et al., 2001). The volatile oil of *H. hircinum* was obtained with an efficiency of 0.73%. The main components of the oil were determined as follows,  $\alpha$ -pinene (88.3%), mircene (3%),  $\beta$ -pinene (2.8%),  $\beta$ -caryophyllene (1.5%) and (E)- $\beta$ -ocimene (1.4%) (Demirci et al., 2008).

## Phenolic compounds

*H. hyssopifolium*, *H. pamphylicum* (e), *H. calycinum* and *H. perforatum* have been investigated for their phenolic compounds. Five flavonoids (I3, I18-biapigenin, quercetin, quercetin-3-O- $\alpha$ -arabinofuranoside, quercetin-3-O- $\beta$ -D-galactopyranoside, quercetin-3-O- $\beta$ -D-galactopyranoside-7-O- $\beta$ -D-glucopyranoside) and a naphthodianthrone (hypericin) were isolated, and their structures were determined by Ultraviolet (UV), Infra red (IR), Nuclear magnetic resonance (NMR) and Mass spectrometry (MS) spectroscopic methods in *H. hyssopifolium* (Cakir et al., 2003).

Quercetin, quercetin 3-glucoside, and quercetin 3-galactoside were isolated from *H. pamphylicum* (e) (Eroglu, 2007). A capillary zone electrophoretic (CZE) method for the determination of rutin in an ethanolic extract of the aerial parts of *H. perforatum* is described and the amount of rutin in the total plant material was found to be 0.21% (Dogrukol-Ak et al., 2001).

Two caffeoylquinic acid derivatives (chlorogenic acid

**Table 2.** Quantitative determination of hypericin, pseudohypericin and hyperforin.

Species	Method	Hypericin (mg/g)	Pseudohypericin (mg/g)	Hyperforin (mg/g)	Reference
<i>H. androsaemum</i>	LC/MS	< 0.01	< 0.01	0.09	
<i>H. aviculariifolium</i>	LC/MS	0.66	0.58	0.02	
<i>H. bithynicum</i>	LC/MS	1.05	2.03	0.15	Smelcerovic et al. (2008)
<i>H. heterophyllum</i>	LC/MS	0.51	0.32	0.08	
<i>H. hirsutum</i>	LC/MS	0.54	0.38	0.20	
<i>H. hyssopifolium</i>	LC/MS	0.52	0.46	0.04	
	HPLC	0.03	0.051	-	Ayan et al. (2004)
<i>H. linarioides</i>	LC/MS	0.34	0.56	< 0.01	Smelcerovic et al. (2008)
<i>H. lydium</i>	UV	1.21	-	-	Cirak (2006)
<i>H. montanum</i>	LC/MS	1.13	1.56	< 0.01	Smelcerovic et al. (2008)
<i>H. montbretii</i>	LC/MS	0.74	2.10	3.45	
	HPLC	2.52	3.58	-	Ayan et al. (2008)
<i>H. nummularioides</i>	LC/MS	0.20	0.18	0.25	
<i>H. orientale</i>	LC/MS	0.02	0.04	0.03	Smelcerovic et al. (2008)
<i>H. origanifolium</i>	LC/MS	< 0.01	0.01	< 0.01	
	HPLC	-	0.93	1.63	Cirak et al. (2008)
<i>H. pamphylicum</i> (e)	HPLC	0.00016	-	trace	Eroglu (2007)
<i>H. perfoliatum</i>	LC/MS	0.29	0.23	0.14	Smelcerovic et al. (2008)
	HPLC	-	2.62	1.84	Ayan et al. (2008)
<i>H. perforatum</i>	LC/MS	3.47	3.54	5.46	Smelcerovic et al. (2008)
	HPLC	2.9	-	-	Oktayoglu (2003)
	HPTLC	2.7	-	-	Kırmızıbekmez et al. (2008)
<i>H. pruinatum</i>	LC/MS	0.36	1.18	0.05	Smelcerovic et al. (2008)
<i>H. scabrum</i>	LC/MS	0.04	0.07	0.02	
	HPLC	0.0046	0.0035	-	Yesilada et al. (1995)
<i>H. triquetrifolium</i>	LC/MS	4.56	3.49	0.05	Smelcerovic et al. (2008)
<i>H. venustum</i>	HPLC	0.03	-	-	Ayan et al. (2004)

and butyl chlorogenate), seven flavonoids (quercetin, quercitrin, hyperoside, isoquercitrin, miquelianin, rutin and I3, I18-biapigenin) and two flavanols [(+)-catechin and (-)-epicatechin] were isolated from *H. calycinum* (Kirmizibekmez et al., 2008a). Four major quercetin glycosides (rutin, miquelianin, hyperoside and quercitrin) were separated and quantitatively determined in methanolic extracts of *H. perforatum* by employing High performance thin layer chromatography (HPTLC)-densitometry (Kirmizibekmez et al., 2008b).

## BIOLOGICAL ACTIVITIES

### Antioxidant activity

Antioxidant activity of ethanol and water extracts of the flowers of *H. venustum* was investigated. They were found to possess strong reducing power, free radicals and hydrogen peroxide scavenging activity, as well as

metal chelating ability (Spiteller et al., 2008). *H. lysimachoides* var. *lysimachoides* was investigated for *in vitro* antioxidant activity. It was observed that antioxidative activities of ethanol extracts of *H. lysimachoides* are comparable with vitamin E, and it was concluded that the use of this extract could be useful in the management of cardiovascular disease in which atherosclerosis is important (Hakimoglu et al., 2007). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities of fruiting and flowering samples of *H. pamphylicum* (e) have been investigated. Both plant samples were active in DPPH radical-scavenging activities assay which was carried out in comparison with ascorbic acid (Eroglu, 2007). Fruiting and flowering samples of *H. montbretii* and *H. perforatum* have been investigated for DPPH radical-scavenging activities and all plant samples were active, compared with ascorbic acid and  $\alpha$ -tocopherol (Oktayoglu, 2003). Antioxidant activity of phenolic compounds isolated from *H. hyssopifolium* subsp. *elongatum* var. *elongatum* was

determined, and all the compounds were found to be active (Cakir et al., 2003). The free radical scavenging activities of the compounds isolated from *H. calycinum* were determined. Compounds showed strong DPPH and nitric oxide (NO) scavenging activities in a concentration dependent manner. (+)-Catechin and (-)-epicatechin were found to be the most active compounds (Kirmizibekmez et al., 2008a).

### Analgesic activity

The mechanism of the analgesic activity caused by *H. perforatum* was investigated. The authors reported that endogenous opioid mechanisms related to OP1-receptors play an important role in *H. perforatum* induced analgesia (Ozturk, 2001).

### Wound-healing activity

Wound-healing effect of St. John's wort extract was investigated on cultured chicken embryonic fibroblasts. It was reported that *H. perforatum* extract exhibits a wound-healing activity whose mechanism of action is similar to that of titrate extract of *Centella asiatica* (Ozturk et al., 2007).

### Hepatoprotective activity

The hepatoprotective activity of *H. perforatum* was investigated *in vivo*. The authors suggested that *H. perforatum* has a protective effect on the liver (Ozturk et al., 1992).

### Anti-inflammatory activity

The probable anti-inflammatory effect of *H. triquetrifolium* was explored in a rat model of carrageenan induced inflammation. It was concluded that *H. triquetrifolium* extract may exert an antiinflammatory effect in rats (Ozturk et al., 2002).

### Anti-nociceptive activity

Total extract of *H. triquetrifolium* exhibited anti-nociceptive activity in the mouse (Apaydin et al., 1999).

### Anti-Helicobacter pylori activity

The anti-*Helicobacter pylori* effect of the extracts and fractions obtained from *H. perforatum* was studied by using agar dilution method. *H. perforatum* extract showed inhibitory activity against the microorganism (Yesilada et

al., 1999).

### Human leukocyte myeloperoxidase activity

*In vitro* effects of *H. perforatum*, *H. empetrifolium* and *H. triquetrifolium* were investigated on human polymorphonuclear leukocyte myeloperoxidase (MPO) activity.

Each extract of *Hypericum* species reduced the peroxidative and chlorinating activity of human leukocyte MPO in concentration-dependent manner. The anti-inflammatory activity of these species may be related with inhibition of MPO activity (Pabuccupglu et al., 2003).

### Antidepressant activity

Anti-depressant effect of certain *Hypericum* species on animal models was summarized. It was observed that anti-depressant activity with the alcoholic extract of *H. Calycinum*, whose effects on the central nervous system of mice are almost equal to the extract prepared from *H. perforatum*. *H. hyssopifolium* ssp. *elongatum* var. *elongatum*, seems to have no anti-depressant activity (Ozturk, 1997). The effects of *H. perforatum* and *H. calycinum* on the central nervous system were investigated using various behavioural models, including swimming time, locomotor activity, tail-flick and hole-board experiments.

According to the results, it was found that the extracts prepared from *H. perforatum* and *H. calycinum* were as effective as anti-depressant drugs, desipramine and trimipramine used as reference. This conclusion suggested that the anti-depressant effect of *H. calycinum* may be as potent as that of *H. perforatum* and may be used for therapeutic purposes in depression (Ozturk et al., 1996).

### Substance dependence

In a review study on *H. perforatum* and substance dependence, Uzbay (2008) discussed the effects of *H. perforatum* on substance dependence and its possible benefit. The results suggest that an extract of *H. perforatum* (HPE) has some beneficial effects on ethanol withdrawal syndrome and that HPE blocks caffeine-induced locomotor hyperactivity in mice. Furthermore, it was reported that HPE may be useful for the treatment of alcoholism in clinical trials (Uzbay et al., 2007; Coskun et al., 2006; Uzbay, 2008).

## DISCUSSION

Among 100 *Hypericum* taxa growing widely in Turkey, some of them were investigated and many articles were published. Table 3 shows published articles about Turkish



**Table 3.** Investigation on the *Hypericum* spp. of Turkey.

Species	Investigation											References			
	Traditional Uses	Agricultural	Quantitative Determination	Chemical Compositions	Antioxidant Activity	Antimicrobial Activities	Antinociceptive Activity	Wound-Healing Activity	Hepatoprotective Activity	Antiinflammatory Activity	Substance Dependence		Anti-H.pylori Activity	MPO Activity	Antidepressant Activity
<i>H. adenotrichum</i> (e)				+											Erken et al. (2001)
<i>H. androsaemum</i>	+		+												Kultur (2007) and Smelcerovic et al. (2008)
<i>H. atomarium</i>	+														Kultur (2007)
<i>H. aviculariifolium</i>	+		+												Smelcerovic et al. (2008)
<i>H. aviculariifolium subsp. depilatum var. depilatum</i> (e)		+	+												Cirak et al. (2006a), Cirak et al. (2007c) and Ayan et al. (2004)
<i>H. bithynicum</i>			+												Smelcerovic et al. (2008)
<i>H. bupleuroides</i>		+		+											Cirak et al. (2006b) and Demirci et al. (2006)
<i>H. calycinum</i>	+		+	+	+								+	+	Erken et al. (2001), Kırmızıbekmez et al. (2008), Ozturk et al. (1996) and Ozturk (1997)
<i>H. capitatum</i>						+									Sokmen et al. (1999)
<i>H. cerastoides</i>	+			+											Erken et al. (2001)
<i>H. crenulatum</i> (e)		+													Inan et al. (2003)
<i>H. empetrifolium</i>	+												+		Pabuccuoğlu et al. (2003) and Kultur (2007)
<i>H. heterophyllum</i>	+		+												Cirak et al. (2008)
<i>H. hircinum</i>				+											Demirci et al. (2008)
<i>H. hirsutum</i>			+												Smelcerovic et al. (2008)
<i>H. hyssopifolium</i>	+		+	+											Cakir et al. (2003) and Cakir et al. (2005)
<i>H. hyssopifolium subsp. elongatum var. elongatum</i>	+				+								+		Ozturk (1997) and Cakir et al. (2003)
<i>H. hyssopifolium var. microcalycinum</i>				+		+									Toker et al. (2006)
<i>H. imbricatum</i>						+									Dulger (2005a)
<i>H. kazdagensis</i> (e)						+									Dulger and Gonuz (2005b)
<i>H. kotschyuanun</i> (e)			+	+	+	+									Unsal et al. (2008, 2009)
<i>H. linarioides</i>			+	+		+									Cakir et al. (2005), Smelcerovic et al. (2008) and Ayan et al. (2008)
<i>H. lydium</i>			+												Cirak (2006c)
<i>H. lysimachioides var. lysimachioides</i>				+	+	+									Toker et al. (2006) and Hakimoglu et al. (2007)
<i>H. montanum</i>			+												Smelcerovic et al. (2008)

Table 3. Contd.

<i>H. montbretii</i>			+	+	+	+										Erken et al. (2001) and Oktayoglu (2003)
<i>H. nummularioides</i>			+	+												Cirak et al. (2006b) and Smelcerovic et al. (2008)
<i>H. olympicum</i>		+														Kultur (2007)
<i>H. orientale</i>		+		+												Ayan et al. (2008)
<i>H. origanifolium</i>			+	+												Cirak et al. (2007c), Smelcerovic et al. (2008) and Ayan et al. (2008)
<i>H. pamphylicum</i> (e)				+		+	+									Eroglu (2007)
<i>H. perforiatum</i>				+	+											Smelcerovic et al. (2008) and Cirak et al. (2008)
<i>H. perforatum</i>		+	+	+	+	+	+	+	+		+	+	+	+		Cirak et al. (2006a, b, 2007c), Ayan et al. (2006), Ozturk et al. (2007), Kirmizibekmez et al. (2008) and Uzbay (2008)
<i>H. pruinatum</i>			+	+												Cirak et al. (2006a) and Ayan et al. (2004)
<i>H. rupestre</i>															+	Dulger (2005a)
<i>H. salsugineum</i> (e)				+	+	+	+									Unsal et al. (2008, 2009)
<i>H. scabroides</i> (e)				+	+	+	+									
<i>H. scabrum</i>		+		+												Smelcerovic et al. (2008), Kultur (2007) and Ayan et al. (2008)
<i>H. thymopsis</i> (e)				+	+	+	+									Unsal et al. (2008, 2009)
<i>H. triquetrifolium</i>		+		+				+			+				+	Ozturk et al. (2002), Apaydin (1999), Pabuccuoglu et al. (2003) and Kultur (2007)
<i>H. uniglandulosum</i> (e)				+	+	+	+									Unsal et al. (2008, 2009)
<i>H. vacciniifolium</i>															+	Dulger (2005a)
<i>H. venustum</i>				+		+										Spiteller et al. (2008) and Ayan et al. (2004)
<i>H. xylostrifolium</i>		+														Kultur (2007)

Turkish *Hypericum* species. Sixteen *Hypericum* have traditional uses. Hypericin, pseudohypericin and hyperforin percentages were determined in 20 species. The highest content of hypericin was found in *H. triquetrifolium* (4.56 mg/g) and *H. perforatum* (3.47 mg/g). Hyperforin was the highest in *H. perforatum* (5.46 mg/g) (Smelcerovic, et al., 2008). Volatile compounds were isolated and identified in 10 species, and phenolic compounds in only 4 species. Twenty-one species were investigated for their activities in wound healing, hepatoprotective, anti-inflammatory, anti-ulcerogenic, analgesic, anti-oxidant, anti-nociceptive, anti-depressant, anti-microbial activities.

*H. triquetrifolium* showed anti-inflammatory and anti-nociceptive activities (Ozturk et al., 2002; Apaydin et al., 1999). The anti-depressant effect of *H. calycinum* was as potent as that of *H. Perforatum*, therefore it may be used for therapeutic purposes in depression (Smelcerovic et al., 2008). *H. hyssopifolium* subsp. *elongatum* var. *elongatum*, *H. lysimachoides* var. *lysimachoides*, *H. montbretii*, *H. pamphylicum*, *H. venustum* and *H. perforatum* were all found to have antioxidant properties (Cakir et al., 2003; Eroglu, 2007). All *Hypericum* extracts investigated were found to have anti-bacterial activity against *Staphylococcus aureus*. The essential oils of *H. linarioides* and *H. capitatum* showed anti-fungal

and slight anti-retroviral activity against human immunodeficiency virus I (HIV-I), respectively (Cakir et al., 2005; Sokmen et al., 1999).

Although several *Hypericum* species have been used in folk medicine, only *H. perforatum* exists as its pharmaceuticals in the market. Studies on Turkish *Hypericum* species continued with an increasing trend and we hope that these species will become valuable in the future.

## REFERENCES

- Akgul A (2008). Ethnobotany at Midyat (Mardin). Master Thesis, Ege University, Institute of Science. Izmir.  
Aktan T (2011). Ethnobotanical Studies of Yenisehir (Bursa) Villages. Master Thesis, Celal Bayar University, Institute of

- Science, Department of Botany, Manisa.
- Apaydin S, Zeybek U, Ince I, Elgin G, Karamenderes C, Ozturk B, Tuglular I (1999). *H. triquetrifolium* Turra. extract exhibits antinociceptive activity in the mouse. *J. Ethnopharmacol.* 67:307-312.
- Ayan AK, Cirak C (2008). Hypericin and Pseudohypericin Contents in Some *Hypericum* Species Growing in Turkey. *Pharm Biol.* 46(4):288-291.
- Ayan AK, Cirak C, Kevseroglu K, Zen T (2004). Hypericin in some *Hypericum* species from Turkey. *Asian J. Plant Sci.* 3(2):202-204.
- Ayan AK, Yanar O, Cirak C, Bilgener M (2006). Variations in total phenolic during ontogenetic, morphogenetic and diurnal cycles in *Hypericum* species from Turkey. *J. Plant Biol.* 49(6):432-439.
- Bombardelli E, Morazzoni P (1995). *Hypericum perforatum*. *Fitoterapia* 66:43-68.
- Bruneton J (1995). *Pharmacognosy, Phytochemistry, Medicinal Plants*. Lavoisier Publishing, Paris 367-370.
- Bulut Y (2006). Useful Plants of Manavgat District (Antalya). Master Thesis, Suleyman Demirel University, Institute of Science, Department of Biology, Isparta.
- Buyukgebiz T (2006). Non-Wood Forest Products Of Sutculer District (Isparta). Master Thesis, Suleyman Demirel University, Department of Forest Engineering, Isparta.
- Cakir A, Kordali S, Kilic H, Kilic H, Kaya E (2005). Antifungal properties of essential oil and crude extracts of *H. linarioides*\_Bosse. *Biochem. Syst. Ecol.* 33(3):245-256.
- Cakir A, Mavi A, Yildirim A, Duru ME, Harmandar M, Kazaz C (2003). Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *H. hyssopifolium* L. by activity-guided fractionation. *J. Ethnopharmacol.* 87:73-83.
- Cimen OD (2007). Ethnobotanical Studies About Traditional Medicines in Konya. Master Thesis, Gazi University, Institute of Health Science, Program of Phytotherapy. Ankara Cirak C (2006c). Hypericin in *H. lydium* growing in Turkey. *Biochem. Syst. Ecol.* 34:897-899.
- Cirak C, Kevseroglu K, Ayan AK (2007a). Breaking of seed dormancy in a Turkish endemic *Hypericum* species: *H. aviculariifolium* subsp. *depilatum* var. *depilatum* by light and some pre-soaking treatments. *J. Arid Environ.* 68(1):159-164.
- Cirak C, Odabas MS, Ayan AK, Kevseroglu K (2006b). Modeling the temperature effect on days to germinate some *Hypericum* species from Turkey. *Seed Technol.* 28(1):58-63.
- Cirak C, Radusiene J, Camas N (2008). Pseudohypericin and Hyperforin in two Turkish *Hypericum* Species: Variations Among Plant Parts and Phenological Stages. *Biochem. Syst. Ecol.* 36:377-382.
- Cirak C, Radusiene J, Ivanauskas L, Janulis V (2007b). Variation of bioactive secondary metabolites in *H. origanifolium* during its phenological cycle. *Acta Physiol. Plant* 29(3):197-203.
- Cirak C, Radusiene J, Karabuk B, Janulis V (2007c). Variation of bioactive substances and morphological traits in *H. perforatum* populations from Northern Turkey. *Biochem. Syst. Ecol.* 35(7):403-409.
- Cirak C, Saglam B, Ayan AK, Kevseroglu K (2006a). Morphogenetic and diurnal variation of hypericin in some *Hypericum* species from Turkey during the course of ontogenesis. *Biochem. Syst. Ecol.* 34(1):1-13.
- Coskun I, Uzbay TI, Ozturk N, Ozturk Y (2006). Attenuation of ethanol withdrawal syndrome by extract of *Hypericum perforatum* in Wistar rats. *Fundam. Clin. Pharmacol.* 20:481-488.
- Demirci B, Kıyan T, Başer KHC (2008). Chemical composition of volatile oil of *Hypericum hircinum* L. *J. Scient. Phytother. Fitomed Turkey* 2(6):52.
- Demirci F, Baser KHC (2006). Volatiles of *H. bupleuroides* Griseb. *J. Essential Oil Res.* 18(6):650-651.
- Demirci S (2010). Ethnobotanical Study In Andirin (Kahramanmaraş) District. Master Thesis, Istanbul University, Institute of Health Science, Department of Pharmaceutical Botany. Istanbul.
- Dogrukol-Ak D, Kirimer N, Tuncel M, Aboul-Enein HY (2001). Determination of Rutin in *H. perforatum* extract by Capillary Electrophoresis. *Anal. Lett.* 34(2):185-191.
- Dulger B (2005a). Antimicrobial studies on three *Hypericum* species from Turkey. *S. Afr. J. Bot.* 71(1):100-103.
- Dulger B, Gonuz A (2005b). Antibacterial activity of the endemic *H. kazdaghensis*. *Fitoterapia* 76(2):237-239.
- Ecevit GG, Ozhatay N (2006). An Ethnobotanical Study in Catalca (European Part of Istanbul) II. *Turk J. Pharm. Sci.* 3(2):73-89.
- Erken S, Malyer H, Demirci F, Demirci B, Baser KHC (2001). Chemical Investigations on some *Hypericum* Species Growing in Turkey-I. *Chem. Nat. Compounds* 37(5):434-438.
- Eroglu E (2007). Investigaton of the *H. pamphylicum* in the Sense of Hypericin Content and Biological Activity. Master Thesis, Istanbul University, Department of Pharmacognosy.
- Ezer N, Avcı K (2004). Traditional Medicines of Cerkes (Cankiri) Region. *J. Hacettepe University, Faculty of Pharmacy* 24(2):67-80.
- Ezer N, Mumcu AO (2006). Folk Medicines in Merzifon (Amasya, Turkey). *Turk. J. Bot.* 30:223-230.
- Hakimoglu F, Kizil G, Kanay Z, Kizil M, Isi H (2007). The effect of ethanol extracts of *H. lysimachioides* on lipid profile in hypercholesterolemic rabbits and its *in vitro* antioxidant activity. *Atherosclerosis* 192:113-122.
- Honda G, Yesilada E, Tabata M, Sezik E, Fujita T, Takeda Y, Takaishi Y, Tanaka T (1996). Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydın provinces. *J. Ethnopharmacol.* 53:75-87.
- Inan M, Kirici S (2003). The possibilities of domestication of some endemic *Achillea* and *Hypericum* species. *Agric. Mediterr.* 133(2):124-129.
- Keskin M, Alpınar K (2002). Ethnobotanical Studies About Kışlak (Yayladağı-Hatay). *J. OT Syst. Bot.* 9(2):91-100.
- Kırmızıbekmez H, Celep E, Bardakçı H, Yeşilada E (2008). Quantitative determination of hypericin on *Hypericum perforatum* by using HPTLC. *J. Sci. Phytother. Fitomed Turk.* 2(6):43.
- Kızılarslan C (2008). An Ethnobotanical Survey in The South Part of İzmit Gulf. Master Thesis, Istanbul University, Institute of Health Science, Department of Pharmaceutical Botany. Istanbul.
- Kırmızıbekmez H, Atay I, Yesilada E (2008b). Determination of Four Major Flavonoids in the Methanolic Extract of *Hypericum perforatum* by HPTLC-Densitometry. *Planta Medica* 74(9):1103.
- Kırmızıbekmez H, Bassarello C, Pizza C, Celep E, Atay I, Mercanoglu G, Yesilada E (2008a). Antioxidant Phenolics From *Hypericum calycinum*. *Planta Medica* 74(9):951.
- Kultur S (2007). Medicinal plants used in Kırklareli Province (Turkey). *J. Ethnopharmacol.* 111:341-364.
- Mart S (2006). An Ethnobotanical Investigation of The Natural Plants Using By Inhabitants in Bahce and Hasanbeyli Districts of Osmaniye Province. University of Cukurova, Institute of Natural and Applied Sciences, Department of Biology, Adana.
- Oktayoglu E (2003). Investigaton of the *H. montbretii* in the Sense of Hypericin Content and Biological Activity. Master Thesis, Istanbul University, Department of Pharmacognosy, Istanbul.
- Ozturk B, Apaydin S, Goldeli E, Ince I, Zeybek U (2002). *H. triquetrifolium* Turra. extracts exhibits antiinflammatory activity in the rat. *J. Ethnopharmacol.* 80:207-209.
- Ozturk N, Korkmaz S, Ozturk Y (2007). Wound-healing activity of St. John's Wort (*H. perforatum* L.) on chicken embryonic fibroblasts. *J. Ethnopharmacol.* 111:33-39.
- Ozturk Y (1997). Testing the Antidepressant Effects of *Hypericum* Species on Animal Models. *Pharmacopsychiatry* 30:125-128.
- Ozturk Y (2001). Possible mechanism of the analgesic effect of St. John's Wort. *Fundam. Clin. Pharmacol.* 15(1):113-153.
- Ozturk Y, Aydin S, Baser KHC, Kirimer N, Kurtar-Ozturk N (1992). Hepatoprotective Activity of *H. perforatum* L. Alcoholic Extract in Rodents. *Phytother. Res.* 6:44-46.
- Ozturk Y, Aydin S, Beis B, Baser KHC, Berberoglu H (1996). Effects of *H. perforatum* and *H. calycinum* extracts on Central Nervous System in mice. *Phytomedicine* 3(2):139-146.
- Pabuccuoğlu A, Konyalıoğlu S, Bas M, Elgin-Meral G (2003). The *in vitro* effects of *Hypericum* species on human leukocyte myeloperoxidase activity. *J. Ethnopharmacol.* 87:89-92.
- Sezik E, Yesilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T (2001). Traditional medicine in Turkey X. Folk medicine in Central Anatolia. *J. Ethnopharmacol.* 75:95-115.
- Smelcerovic A, Zuehlke S, Spittler M, Raabe N, Özen T (2008). Phenolic constituents of 17 *Hypericum* species from Turkey. *Biochem. Syst. Ecol.* 36(4):316-319.

- Sokmen A, Jones BM, Erturk M (1999). Antimicrobial Activity of Extracts from the Cell Cultures of some Turkish Medicinal Plants. *Phytother. Res.* 13:355-357.
- Spiteller M, Ozen T, Smelcerovic A, Zuehlke S, Mimica-Dukic N (2008). Phenolic constituents and the *in vitro* antioxidant activity of the flowers of *H. venustum*. *Fitoterapia* 79(3):191-193.
- Tatlı I, Sahpaz S, Kupeli AE, Martin-Nizard F, Gressier B, Ezer N, Bailleul F (2009). Antioxidant, anti-inflammatory and antinociceptive activities of Turkish medicinal plants. *Pharm Biol.* 47(9):916-921.
- Toker Z, Kizil G, Ozen HC, Kizil M, Ertekin S (2006). Compositions and antimicrobial activities of the essential oils of two *Hypericum* species from Turkey. *Fitoterapia* 77(1):57-60.
- Tuzlaci E, Aymaz PE (2001). Turkish folk medicinal plants, Part IV: Gönen (Balıkesir). *Fitoterapia* 72:323-343.
- Tuzlaci E, Tolon E (2000). Turkish folk medicinal plants, Part III: Şile (Istanbul). *Fitoterapia* 71:673-685.
- Unal E, Mavi A, Kara A, Cakir A, Sengul M, Yildirim A (2008). Antimicrobial and Antioxidant Activities of Some Plants Used as Remedies in Turkish Traditional Medicine. *Pharm Biol.* 46(3):207-224.
- Unsal C, Kultur S, Eroglu E (2009). Investigation On Some Endemic *Hypericum* Species in Turkey. University of Istanbul, Project No: 442/27122005, Istanbul.
- Unsal C, Ozbek B, Eroglu E, Kultur S (2008). Antimicrobial activity of some *Hypericum* species from Turkey. *J. Sci. Phytother. Fitomed Turkey* 2(6):74.
- Uzbay TI (2008). *Hypericum perforatum* and Substance Dependence: A Review. *Phytother. Res.* 22:578-582.
- Uzbay TI, Coskun I, Kayir H, Ozturk N (2007). Extract of *Hypericum perforatum* blocks Caffeine-induced Locomotor Activity in Mice: A Possible Role of Nitric Oxide. *Phytother. Res.* 21:415-419.
- Vural G (2008). Ethnobotanical Features Some Of The Wild Plants On The Honaz Mountain And Its Environment Ethnobotanic. Master Thesis, Afyon Kocatepe University, Graduate School of Natural and Applied Sciences, Afyon.
- Yesil Y (2007). An Ethnobotanical Study in Kürecik District (Malatya/Akçadag). Master Thesis, Istanbul University, Institute of Health Science, Department of Pharmaceutical Botany, Istanbul.
- Yesilada E, Gurbuz I, Shibata H (1999). Screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity. *J. Ethnopharmacol.* 66:289-293.
- Yesilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T, Takeda Y, Takaishi Y (1995). Traditional medicine in Turkey V. Folk medicine in the inner Taurus Mountains. *J. Ethnopharmacol.* 46:133-152.
- Yesilada E, Honda G, Sezik E, Tabata M, Goto K, Ikeshiro Y (1993). Traditional medicine in Turkey IV. Folk medicine in the Mediterranean subdivision. *J. Ethnopharmacol.* 39:31-38.

Full Length Research Paper

# Phenolic content, radical scavenging activity and cytotoxicity of *Tamarix nilotica* (Ehrenb.) bunge growing in Egypt

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Accepted 28 January, 2013

The radical scavenging activity using 1,1-diphenyl, 2-picrylhydrazyl (DPPH) and cytotoxicity using sulphorhodamine B (SRB) assay of the aqueous methanolic extract of *Tamarix nilotica* (Ehrenb.) Bunge (Tamaricaceae) flowers and its subextracts (CHCl<sub>3</sub>, EtOAc and Pet.ether) were evaluated. Total phenolic and flavonoid contents were estimated using colorimetric assays. Ethyl acetate (EtOAc) showed the highest free radical scavenging activity with inhibitory concentration (IC<sub>50</sub>) 7.25 ± 0.86 µg/ml in addition to potential cytotoxic effect on liver cell carcinoma (Huh-7) (IC<sub>50</sub> 49.1 ± 0.96 µg/ml) whereas effect on lung cell carcinoma (A-549) was much lower (IC<sub>50</sub>: 137.9 ± 1.85 µg/ml). EtOAc had the highest flavonoid content (1.75 ± 1.5 mg/g QE) compared to other subextracts. These results indicated that ethyl acetate fraction contains bioactive compounds worthy of more sophisticated studies as free radical scavenger and cytotoxic agent.

**Key words:** *Tamarix nilotica*, cytotoxic activity, sulphorhodamine B (SRB) assay, scavenging activity, 1,1-diphenyl, 2-picrylhydrazyl (DPPH) assay, flavonoid, phenolic.

## INTRODUCTION

Genus *Tamarix* is the largest genus in family Tamaricaceae (Tamarisk). *Tamarix* is represented in Egypt with two indigenous species which are *Tamarix aphylla* (L.) H.Karst and *T. nilotica* (Ehrenb.) Bunge (Boulos, 1999). *T. nilotica* (Ehrenb.) Bunge has its root deep in the Egyptian history where it was mentioned in ancient papyri in pharaonic times to expel fever, relieve headache, to draw out inflammation and as an aphrodisiac, in addition, it was used in Egyptian traditional medicine as an antiseptic agent (Abouzid and Sleem, 2011). In Egypt, different parts of *Tamarix* are used; the leaves and young branches are cooked for oedema of spleen and mixed with ginger for uterus infections, while the bark, when boiled in water with vinegar is used as

lotion against lice (Boulos, 1983).

Flavonoid and phenolic constituents have been reported from the leaves, roots and flowers of *T. nilotica* (Ehrenb.). Roots revealed the presence of gallic acid derivatives, a lignan (syringaresinol) and isoferulic acid, niloticol, and 3-hydroxy-4-methoxycinnamaldehyde (Barakat et al., 1987). Leaves revealed the presence of nilotinins M1, M4, D2, D3, D7, D8, D9, hirtellins B, C, F, tamarixinin A, 1,2,6-tri-O-galloyl-D-glucose, methyl ferulate 3-O-sulphate, coniferyl alcohol 4-O-sulphate, kaempferol 4'-methyl ether, tamarixetin and quercetin 3-O-beta-D-glucopyranuronide (Orabi et al., 2009, 2010; Abouzid et al., 2009). Moreover, flowers revealed isolation of the methyl and ethyl esters of gallic acid, p-methoxygallic acid, kaempferol, quercetin 3-O-glucuronides, the 3-O-sulphated kaempferol 7,4'-dimethyl ether and the free flavonols, besides the digalloylglucose and nilotycin (Nawwar et al., 1982, 1984a, b; Nawwar and Souleman, 1984). However, in spite of intensive studies

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on its constituents, few reports were achieved concerning its biological activities.

Antioxidant and hepatoprotective activities were evaluated for total flower extract (Abouzid et al., 2008; Abouzid and Sleem, 2011), moreover, nilotinin D8 and hirtellin A isolated from leaf extract were found active against the human oral tumor cell lines, human squamous cell carcinoma (HSC-2, HSC-3, and HSC-4) and human promyelocytic leukemia (HL-60) cells (Orabi et al., 2010).

In our investigation to discover new drug candidates from natural sources, *T. nilotica* appeared promising. Phenolic compounds are known to have antioxidant activity (Tepe et al., 2006) in addition, cytotoxic drugs play a major role in cancer chemotherapy (Zunino and Capranico, 1997). Through previous studies, *T. nilotica* is a very rich phenolic source but correlation between bioactive components and biological activity was not well studied. In addition, flowers were not intensively studied as leaves. Therefore, the aim of this study was to enlighten two activities of *T. nilotica* (Ehrenb.) Bunge flowers in total aqueous methanolic and its successive subextracts in relation to phenolic and flavonoid contents. Radical scavenging activity was assessed using DPPH method, cytotoxic activity was tested using SRB assay against two human tumor cell lines, liver (huh-7) and lung (A-549) cancer cell lines while total phenolic and flavonoid contents were measured using colorimetric methods.

## MATERIALS AND METHODS

### Plant

The flowers of *T. nilotica* (Ehrenb.) Bunge were collected from Ismaila road, Egypt, in October, 2011. Authentication of the plant was performed by Dr. Mona M. Marzouk (PhD), Department of Phytochemistry and Plant Chemosystematics, National Research Center (NRC) of Cairo. Voucher specimen (No RS01) was deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy MSA University, Egypt.

### Chemicals

All chemicals used, including solvents, were of analytical grade. DPPH, Folin Ciocalteu's phenol reagent, quercetin, and gallic acid were purchased from (Sigma-Aldrich Chemie, Steinheim, Germany).

### Preparation of plant extract and successive fractions

The powdered air-dried flowers of *T. nilotica* (Ehrenb.) Bunge (1 kg) were exhaustively extracted with 70% methanol. The combined aqueous methanolic extract was concentrated by evaporation under reduced pressure then, the residue was weighed and suspended in water, then exhaustively defatted with petroleum ether (60 to 80°C) (Petroleum ether) (300 × 15). Combined Petroleum ether subextracts were evaporated under reduced pressure. Methanol was removed from the remaining extract and diluted with distilled H<sub>2</sub>O to

400 ml and successively extracted with chloroform (CHCl<sub>3</sub>) (20 × 500 ml) and Ethyl acetate (EtOAc) (20 × 500 ml). Each solvent extract was evaporated to dryness under reduced pressure to give CHCl<sub>3</sub> (yield: 0.47%) and EtOAc (yield: 5.27%), respectively. The remaining aqueous extract was further extracted with n-butanol (BuOH) (20 × 500 ml) and evaporated to dryness to yield BuOH (yield: 9.5%). The final aqueous phase was also evaporated to dryness (yield: 38.09%)

## Biological activity

### 1,1-diphenyl, 2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of different plant subextracts was measured spectrophotometrically using the stable free radical DPPH (Shimada et al., 1992).

Total extract and all successive subextracts (CHCl<sub>3</sub>, EtOAc, BuOH and aqueous) were dissolved in methanol and screened at 100 µg/ml where the most potent active extracts (gave more than 90%) were assayed at 25 to 75 µg/ml. 0.1 mM solution of DPPH in methanol was prepared. Then, 1 ml of this solution was added to 3 ml of extract solution at different concentration (25 to 75 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm carried out in triplicate. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was calculated by the following formula: DPPH scavenging effect (%) = 100 - [(A<sub>0</sub> - A<sub>1</sub>) / A<sub>0</sub>] × 100], where: A<sub>0</sub> was the absorbance of the control reaction and A<sub>1</sub> was the absorbance in the presence of the sample (Oktay et al., 2003). The concentration of sample required to scavenge 50% of DPPH was calculated from a graph plotted for the % inhibition against the concentration in µg/ml.

### Cytotoxic activity

The cytotoxicity of the total extract and its successive fractions was tested against two human cancer cell lines of economical importance in Egypt, liver (Huh-7) and lung (A-549) cells by Sulforhodamine (SRB) assay as described by Skehan et al. (1990). Exponentially growing cells were collected using 0.25% Trypsin-Ethylenediaminetetraacetic acid (EDTA) and plated in 96-well plates at 1000 to 2000 cells/well. Cells were exposed to test extracts [concentrations 0.1, 1, 10, 100, 1000 µg/ml, dissolved in Dimethyl sulfoxide (DMSO)] for 72 h and subsequently fixed with trichloroacetic acid (TCA) (10%) for 1 h at 4°C. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm. The dose response curve of different fractions were analyzed using E<sub>max</sub> model.

$$\% \text{ Cell viability} = (100 - R) \times \left( 1 - \frac{[D]^m}{K_d^m + [D]^m} \right) + R$$

[R] is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, "K<sub>d</sub>" is the dose of the drug that produces a 50% reduction in cell viability and "m" is a Hill-type coefficient. IC<sub>50</sub> was defined as the drug concentration required to reduce absorbance to 50% of that of the control (that is, K<sub>d</sub> = IC<sub>50</sub>, when R = 0 and E<sub>max</sub> = 100 - R) (Al-Abd et al., 2008). The concentration required to reduce cell viability by 50% (IC<sub>50</sub>) was

determined using the sigmoid  $E_{max}$  model.

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD)

### Phytochemical analysis

#### Phytochemical screening

The Petroleum ether,  $\text{CHCl}_3$ , EtOAc, BuOH subextracts and aqueous remaining, obtained from successive fractionation of flowers of *T. nilotica* (Ehrenb.) Bunge, were exposed to preliminary phytochemical analyses to explore the major classes of active constituents responsible for activity using standard procedures of analysis (Harborne, 1993; Sofowora, 1993; Trease and Evans, 2002).

#### Quantitative estimation of total phenolic and flavonoid contents

The total phenolic content of each fraction was determined by the Folin-Ciocalteu Reagent (FCR) using gallic acid as standard (Sellappan et al., 2002) and measured at maximal absorption 765 nm. Measurements were carried out in triplicate and calculations based on a calibration curve obtained with gallic acid. The total phenolics were expressed as milligram of gallic acid equivalents (GAE) per milligram dry extract. The total flavonoid content was determined by aluminium chloride colorimetric assay (Kosalec et al., 2004). This method is based on the formation of a complex flavonoid aluminium having the absorptivity maxima at 415 nm. Calculations are based on quercetin calibration curve. The total flavonoid content was expressed as milligram of quercetin equivalent per milligram dry extract.

## RESULTS

### Biological activities

#### 1, 1-diphenyl, 2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH free radical scavenging activity of different *T. nilotica* subextracts has been screened at 100  $\mu\text{g/ml}$ . EtOAc (100%), BuOH (93%) and total (90%) exhibited potential antioxidant activity while  $\text{CHCl}_3$  exhibited the lowest effect (26%), followed by aqueous remaining (75%). Comparing the  $\text{IC}_{50}$  of promising subextracts (> 90%) with ascorbic acid as positive control ( $\text{IC}_{50}$  4.8  $\pm$  0.54  $\mu\text{g/ml}$ ), EtOAc showed the best effect (7.25  $\pm$  0.86  $\mu\text{g/ml}$ ), with lower  $\text{IC}_{50}$  followed by BuOH (8.25  $\pm$  0.65  $\mu\text{g/ml}$ ) and total extract (45  $\pm$  0.73  $\mu\text{g/ml}$ ) (Figure 1). These results imply the presence of antioxidant principles in the extracts.

### Cytotoxic activity

SRB assay was used to assess the cytotoxicity pattern

(dose-response profile) of  $\text{CHCl}_3$ , EtOAc, BuOH, and aqueous subextracts compared to total extract of *T. nilotica* (Ehrenb.) against two human tumor cell lines Huh-7 and A-549 cell lines. Most of the tested samples exerted cytotoxic activity against hepatocellular carcinoma and lung carcinoma cell lines with different concentrations. The EtOAc exhibited the most potent cytotoxic activity against Huh-7 ( $\text{IC}_{50}$  49.1  $\pm$  0.96  $\mu\text{g/ml}$ ), followed by  $\text{CHCl}_3$  ( $\text{IC}_{50}$  84.5  $\pm$  1.64  $\mu\text{g/ml}$ ). The resistant fraction of Huh-7 was 0%, which denoted the potency of all fractions on liver cell carcinoma (Table 1). Effects of different subextracts on A-549 was lower, still EtOAc was the most potent ( $\text{IC}_{50}$  137.9  $\pm$  1.85  $\mu\text{g/ml}$ ), followed by  $\text{CHCl}_3$  ( $\text{IC}_{50}$  271.1  $\pm$  3.23  $\mu\text{g/ml}$ ), while  $\text{IC}_{50}$  of BuOH and aqueous cannot be detected at the used concentrations. Additionally, substantial R-fraction of A-549 ranged from 6.7 to 15.8%, which indicate the partial resistance of lung cell carcinoma (Table 1).

### Phytochemical analysis

#### Phytochemical screening

Phytochemical screening revealed the presence of flavonoids in all fractions except Pet.ether and remaining aqueous. Sterols were only found in Pet.ether and  $\text{CHCl}_3$  subextracts. Tannins were present only in considerable amount in EtOAc, BuOH and aqueous subextracts. Additionally, alkaloids, saponins and anthraquinones were totally absent in all extracts.

#### Phenolic and flavonoid contents

Results of total phenolic and flavonoid contents in their chemical equivalents (gallic acid and quercetin, respectively) are shown in Table 2. Contents of total phenols were measured by (FCR) in terms of gallic acid equivalent (standard curve equation:

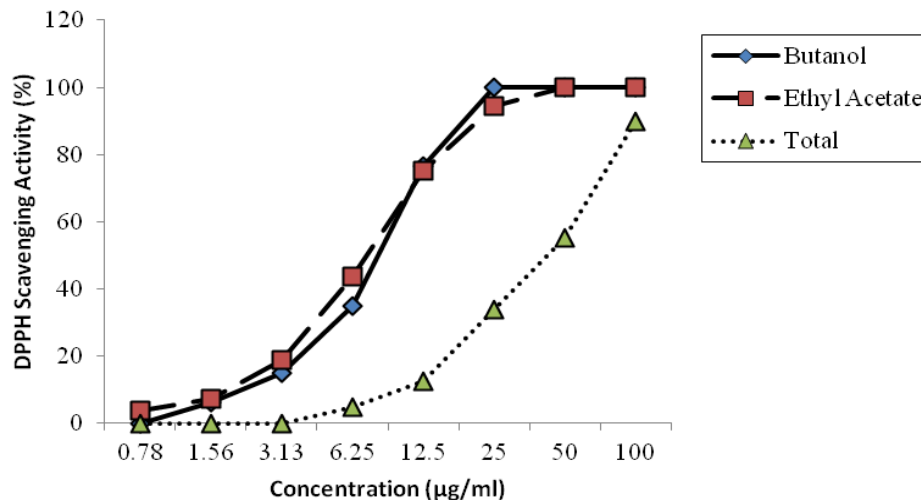
$$y = 0.0011x + 0.0009, r^2 = 0.9867).$$

The total phenolic content varied from 7.37  $\pm$  0.9 to 119.63  $\pm$  0.9 mg/g GAE. Total extract showed higher concentration (119.63  $\pm$  0.9 mg/g GAE), followed by n-BuOH (22.12  $\pm$  mg/g GAE),  $\text{CHCl}_3$ , EtOAc and finally aqueous.

Flavonoid content (FC) was measured by aluminum chloride colorimetric assay in terms of quercetin equivalent (standard curve equation:

$$y = 0.005x - 0.0198, r^2 = 0.9774)$$

FC ranged between 0.44  $\pm$  1.5 and 2.55  $\pm$  0.19 mg/g QE. Total extract was higher in concentration (2.55  $\pm$  0.19 mg/g QE) followed by EtOAc (1.75  $\pm$  1.5 mg/g QE),  $\text{CHCl}_3$  (0.79  $\pm$  2.4 mg/g QE), n-BuOH fraction (0.58  $\pm$  2.3



**Figure 1.** DPPH scavenging activity of butanol, ethyl acetate and total aqueous methanolic fractions of *Tamarix nilotica* and data are expressed as mean  $\pm$  SD (n = 3).

**Table 1.** Cytotoxicity of *T. nilotica* extracts against different solid tumor cell lines.

Fraction	Cytotoxic effect			
	Huh-7 (liver)		A549 (lung)	
	IC <sub>50</sub> (µg/ml)	R-fraction %	IC <sub>50</sub> (µg/ml)	R-fraction %
Ethyl acetate	49.1 $\pm$ 0.96	0	137.9 $\pm$ 1.85	15.8
Chloroform	84.5 $\pm$ 1.64	0	271.1 $\pm$ 3.23	6.7
Butanol	132.9 $\pm$ 2.23	0	-*	-
Aqueous	285.35 $\pm$ 3.4	0	-	-
Total	162 $\pm$ 2.05	0	727 $\pm$ 3.11	0

\*At the maximum used concentration, no cytotoxic effect was observed.

**Table 2.** Phenolic and flavonoid contents in *Tamarix nilotica* flowers.

Fraction	Total phenolic (mg/g GAE)	Total flavonoid (mg/g QE)
Chloroform	21.67 $\pm$ 2.1	0.79 $\pm$ 2.4
Ethyl acetate	20.6 $\pm$ 1	1.75 $\pm$ 1.5
Butanol	22.12 $\pm$ 2.4	0.58 $\pm$ 2.3
Aqueous	17.2 $\pm$ 1.4	-
Total	119.63 $\pm$ 0.09	2.55 $\pm$ 0.19

Each value in the table was obtained by calculating the average of three experiments  $\pm$  standard deviation. GAE = gallic acid equivalent, QE= quercetin equivalent.

mg/g QE) while aqueous subextract appeared devoid of flavonoid content.

## DISCUSSION

Herbal medicines are the primary form of healthcare known to mankind. Natural products are important

sources of antioxidant and anti-cancer lead molecules and this is mainly due to the high degree of diversity and novelty. The increased interest in the measurement and use of plant antioxidants for scientific research, as well as industrial purposes, is mainly due to their strong biological activity, exceeding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis, in addition to safe and effective use with

fewer side effects (Suhaj, 2006; Tadhani et al., 2007).

Phenolics are known for their strong reactive oxygen species (ROS) scavenging capacities (Atmani et al., 2009) and inhibition of free radical producing enzymes (Berboucha et al., 2010). In addition, cytotoxic effect of dietary polyphenols has been proved through many studies of tumor cells, therefore these compounds could contribute in the prevention and treatment of cancer (Kampa et al., 2000). DPPH offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic anti-oxidants (Cao et al., 1997). The degree of its discoloration is attributed to hydrogen donating ability of tested extracts.

In addition, for evaluation of cytotoxic activity, SRB assay offer a simple, rapid, sensitive and inexpensive method for measuring the cellular protein content. It relies on the uptake of the negatively charged pink amino-xanthine dye, SRB, by basic amino acids in the cells. The greater the number of cells, the greater the amount of dye is taken up and after fixing when the cells are lysed, the released dye will give a more intense colour and greater absorbance (Houghton et al., 2007). The types of cancers examined in this study were selected based on epidemiological evidence and high health related problem as representing two main cancer problems present in Egypt. Comparing the efficacy of successive subextracts with the total fraction for their antioxidant and cytotoxic activities, EtOAc showed the best cytotoxic effect against liver cell carcinoma ( $49.1 \pm 0.96 \mu\text{g/ml}$ ) beside the lowest  $\text{IC}_{50}$  while estimating DPPH scavenging activity ( $7.25 \pm 0.86 \mu\text{g/ml}$ ).

Two colorimetric assays were used to compare the phenolic and flavonoid content extracted by different type of solvents based on Folin-Ciocalteu and  $\text{AlCl}_3$  reagents. Although the total extract showed the highest phenolic and flavonoid contents ( $119.63 \pm 0.09 \text{ mg/g GAE}$ ;  $2.55 \pm 0.19 \text{ mg/g QE}$ ) but its effect on hepatocellular cancer cell line (Huh-7) was the least, and its scavenging activity was lower than EtOAc and BuOH. This suggests the presence of other phenolics which did not contribute in this activity. However, the bioactive subextract EtOAc showed the highest flavonoid content ( $1.75 \pm 1.5 \text{ mg/g QE}$ ) representing twice  $\text{CHCl}_3$  content and thrice the BuOH.

Findings in this study are in good agreement with previous reports estimating phenolic content of flowers of *T. gallica*, it was  $135.35 \pm 7.7 \text{ mg GAE/g}$  with flavonoid  $12.33 \pm 2.10 \text{ mg Catechin equivalent/g}$  (Ksouri et al., 2009), while upon fractionation of *T. ramossissima* aqueous acetone extract and estimating the radical scavenging activity, EtOAc showed  $\text{IC}_{50}$   $8.6 \mu\text{g/ml}$  (Sultanova et al., 2001). In addition, Abouzid et al. (2008) denotes the efficient antioxidant power of the *T. nilotica* flower ( $89.34 \pm 0.82\%$  at  $500 \mu\text{g/ml}$ ). This study is the first report concerning cytotoxic activity, phenolic and flavonoid contents of total aqueous methanolic and successive subextracts of *T. nilotica* flowers.

## Conclusion

The investigated species *T. nilotica* (Ehrenb.) Bunge appeared to be a potential good candidate for further phytochemical and chromatographic studies to isolate and identify the bioactive compounds related to the antioxidant and cytotoxic activities.

## REFERENCES

- Abouzid S, Sleem A (2011). Hepatoprotective and antioxidant activities of *Tamarix nilotica* flowers. *Pharm Biol.* 49(4): 392-395.
- Abouzid SF, Elshahaat A, Ali S, Choudhary MI (2008). Antioxidant activity of wild plants collected in Beni-Sueif governorate, Upper Egypt. *Drug Discov. Ther.* 2 (5): 286-288.
- Abouzid SF, Ali SA, Choudhary MI (2009). A new ferulic acid ester and other constituents from *Tamarix nilotica* leaves. *Chem Pharm Bull (Tokyo)*. 57(7):740-742.
- Al-Abd AM, Lee JH, Kim SY, Kun N, Kuh HJ (2008). Novel application of multicellular layers culture for in situ evaluation of cytotoxicity and penetration of paclitaxel. *Cancer Sci.* 99:423-31.
- Atmani D, Chaher N, Atmani D, Berboucha M, Debache N, Boudaoud H (2009). Flavonoids in human health: from structure to biological activity. *Curr. Nutr. Food Sci.* 5:225-237.
- Barakat HH, Nawwar MAM, Buddrus J, Linscheid M (1987). Niloticol, a phenolic glyceride and two phenolic aldehydes from the roots of *Tamarix nilotica*. *Phytochemistry* 26 (6):1837-1838.
- Berboucha M, Ayouni K, Atmani D, Benboubetra M (2010). Kinetic study on the inhibition of xanthine oxidase by extracts from two selected Algerian plants traditionally used for the treatment of inflammatory diseases. *J. Med. Food* 13(4): 896-904.
- Boulos L (1999). *Flora of Egypt*. Al Hadara Publishing, Cairo, Egypt. Vol. 2, p 124.
- Boulos L (1983). *Medicinal Plant of North Africa*, Michigan. 286 p.
- Cao G, Sofic E, Prior RL (1997). Antioxidant and pro-oxidant behavior of flavonoids: structure activity relationship. *Free Radic. Biol. Med.* 22(5):749-760.
- Harborne JB (1993). *Phytochemistry*. Academic Press, London. pp. 89-131.
- Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ, Lee CC (2007). The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. *Methods* 42:377-387.
- Kampa M, Hatzoglou A, Notas G, Damianaki A, Bakogeorgou E, Gemetzi C, Kouroumalis E, Martin PM, Castanas E (2000). Wine antioxidant polyphenols inhibit proliferation of human prostate cancer cell lines. *Nutr. Cancer* 37:223-333.
- Kosalec H, Bakmaz M, Pepeljnjak S, Vladimir-kne S (2004). Quantitative analysis of the flavonoids in raw propolis from Northern Croatia. *Acta Pharm.* 54:65-72.
- Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magné C, Abdely C (2009). Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolics constituents. *Food Chem. Toxicol.* 47:2083-2091.
- Nawwar MAM, Buddrus J, Bauer H (1982). Dimeric phenolic constituents from the roots of *Tamarix nilotica*. *Phytochemistry* 21:1755 - 1758.
- Nawwar MAM, Souleman AMA (1984). 3,4,8,9,10-Pentahydroxy-dibenzo[b, d]pyran-6-one from *Tamarix nilotica*. *Phytochemistry* 23(12):2966-2967.
- Nawwar MAM, Souleman AMA, Buddrus J, Linscheid M (1984a). Flavonoids from the flowers of *Tamarix nilotica*. *Phytochemistry* 23:2347-2349.
- Nawwar MAM, Souleman AMA, Buddrus J, Bauet H, Linscheid M (1984b). Polyphenolic constituents of the flowers of *Tamarix nilotica*: The structure of nilocitin, a new digalloylglucose. *Tetrahedron Lett.* 25:49-52.
- Oktay M, Gulcin I, Kufrevioglu OI (2003). Determination of *in vitro*

- antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT. Food Sci. Technol. 36(2):263-271.
- Orabi MAA, Taniguchi S, Hatano T (2009). Monomeric and dimeric hydrolysable tannins of *Tamarix nilotica*. Phytochemistry 70:1286–1293.
- Orabi MAA, Taniguchi S, Yoshimura M, Yoshida T, Kishino K, Sakagami H, Hatano T (2010). Hydrolyzable tannins of Tamaricaceous plants. III. Hellinoyl- and macrocyclic-type ellagitannins from *Tamarix nilotica*. J. Nat. Prod. 73:870–879.
- Sellappan S, Akoh CC, Krewer G (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J. Agric Food Chem. 50(8):2432-2438.
- Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR, Natl J (1990). New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. Cancer Inst. 82:1107–1112.
- Shimada K, Fujikawa K, Yahara K, Nakamura T (1992). Antioxidative properties of xanthan on the autooxidation of soybean in cyclodextrin emulsion. J. Agric. Food Chem. 40:945-948.
- Sofowora H (1993). Screening plants for Bioactive Agents In: Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd, Sunshine House, Ibadan. Nigeria 2<sup>nd</sup> Ed. pp.134-156.
- Suhaj M (2006). Spice antioxidants isolation and their antiradical activity: a review. J. Food Compos. Anal. 19(6-7): 531-537.
- Sultanova N, Makhmoor T, Abilov ZA, Parween Z, Omurkamzinova VB, Atta-ur-Rahman, Choudhary MI (2001). Antioxidant and antimicrobial activities of *Tamarix ramosissima*. J. Ethnopharmacol.78:201–205.
- Tadhani MB, Patel VH, Subhash R (2007). *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. J. Food Compos Anal. 20:323-329.
- Tepe B, Sokmen M, Akpulat HA, Sokmen A (2006). Screening of the antioxidant potentials of six *Salvia* species from Turkey. Food Chem. 95:200-204.
- Trease GE, Evans WC (2002). Pharmacology. 15<sup>th</sup> Ed. Saunders Publishers, London.
- Zunino F, Capranico G (1997). In: Teicher B. A. (Ed.), Cancer Therapeutics: Experimental and Clinical Agents. Cancer Drug Discovery and Development, Humana Press, Totowa, NJ. pp. 195-214.



## *UPCOMING CONFERENCES*

**International Conference on Biological, Health and Environmental Sciences,  
London, UK, 19 Jan 2014**



**International Conference on Psychology, Psychiatry, Neurological, Behavioral  
and Cognitive Sciences, Barcelona, Spain, 27 Feb 2014**



## Conferences and Advert

### **September 2013**

61st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Muenster, Germany, 1 Sep 2013

### **January 2014**

International Conference on Medical, Biological and Pharmaceutical Sciences, London, UK, 19 Jan 2014

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