Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014-15; 6(4); 698-703

ISSN: 0975-4873

Research Article

Microscopical and Phytochemical Investigation of Egyptian *Artemisia judaica l. Var. Sinaitica tackholm* and its Free Radical Scavenging Activity

*Bakr, R.O

Pharmacognosy Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, Egypt

Available Online: 17th November, 2014

ABSTRACT

Artemisia judaica L. (Asteraceae) is a perennial fragrant small shrub widely growing in desert and coast of Egypt. Microscopical features of the leaves were characterized by the presence of non glandular T-shaped trichomes beside the tube like secretory duct responsible for oil production and storage. Phenolic and flavonoid contents were estimated using colorimetric assays where ethyl acetate had the highest phenolic and flavonoid contents. Orientin was the major identified flavonoid. Radical scavenging activity was estimated using DPPH assay where butanol extract showed the highest potency compared with ascorbic acid and butylated hydroxyanisol. *A. judaica* represents a future antioxidant candidate from natural source.

Keywords: *Artemisia judaica,* T-shaped trichomes, phenolics, DPPH assay, radical scavenger.

INTRODUCTION

Artemisia L. is a genus of small herbs and shrubs, belonging to family Compositae (Asteraceae), found in northern temperate regions, commonly known as wormwood. Asteraceae is one of the most numerous plant groupings, which comprises about 1,000 genera and over 20,000 species. Within this family, Artemisia is included in the tribe Anthemideae and comprises over 500 species, which are mainly found in Asia, Europe and North America [1].

Artemisia judaica L. Var. Sinaitica Tackholm, Arabic name ‘‘Shih Balady’’ is a perennial fragrant small shrub with pubescent leaves, which grows widely in Egypt (desert and coast) and in the Middle East (Sinai Peninsula, Israel, Jordan and Saudi Arabia) [2].

It is widely used in folk medicine and is recommended as a healer plant in traditional medicine by Bedouins. Mixture of the dry leaves of *A. judaica, A. monosperma* and *A. hera alba* is very common anthelmintic drug in the most of North African and Middle East countries under Arabic name of Shih. In addition, they have been used worldwide as tonic, stomachic and stimulant beverage and as antiseptic oils or tinctures for the relief of rheumatic pains [3].

Phytochemical analysis of *A. judaica* showed the plant as rich in flavonoids [4, 5] and sesquiterpene lactones [6]. The characteristic scent of *A. judaica* is due to the high volatile oil content contributing to the antioxidant [7], insecticidal [8], anthelmintic, anti-inflammatory, analgesic, antipyretic effects, anti-malarial, antiviral, anti-tumor, antispasmodic and anti-microbial activity [9, 10].

Therefore, the aim of this study was to investigate the microscopical characters of the Egyptian *A. judaica* leaves as an important tool for identification of the plant. In addition, *A. judaica* extracts were evaluated for their phenolic and flavonoid contents quantitatively using colorimetric assays and qualitatively using HPLC analysis. Two main activities closely related to the flavonoid contents were assessed, radical scavenger activity (DPPH assay) as well as cytotoxic activity (SRB assay) against liver and lung cancer cell lines.

MATERIAL AND METHODS

Plant material: Aerial parts of *Artemisia judaica L. var. sinaitica Tackholm* were collected from Saint-Catherine Sinai, Egypt, in September 2011. The plant was authenticated by *Ass.Prof.Dr*. Mona M. Marzouk, Department of Phytochemistry and Plant systematics, National Research Center, Egypt. A voucher specimen of the plant was deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, MSA University (RS008). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Chemika (Buchs, Switzerland). All solvents and chemicals were of analytical grade and obtained from local suppliers.

Microscopical investigation**:** Microscopical investigation of *A. judaica* leaves was performed on cross sections of leaves which were preserved in 70% alcohol and on air-dried finely powdered samples. The sections were prepared by the gliding technique using rotary gliding microtome, stained using phloroglucinol-HCl. The slides were examined and photographed using Leica DMLB Image microscope equipped with leica Q.550 lw image

**Author for correspondence*

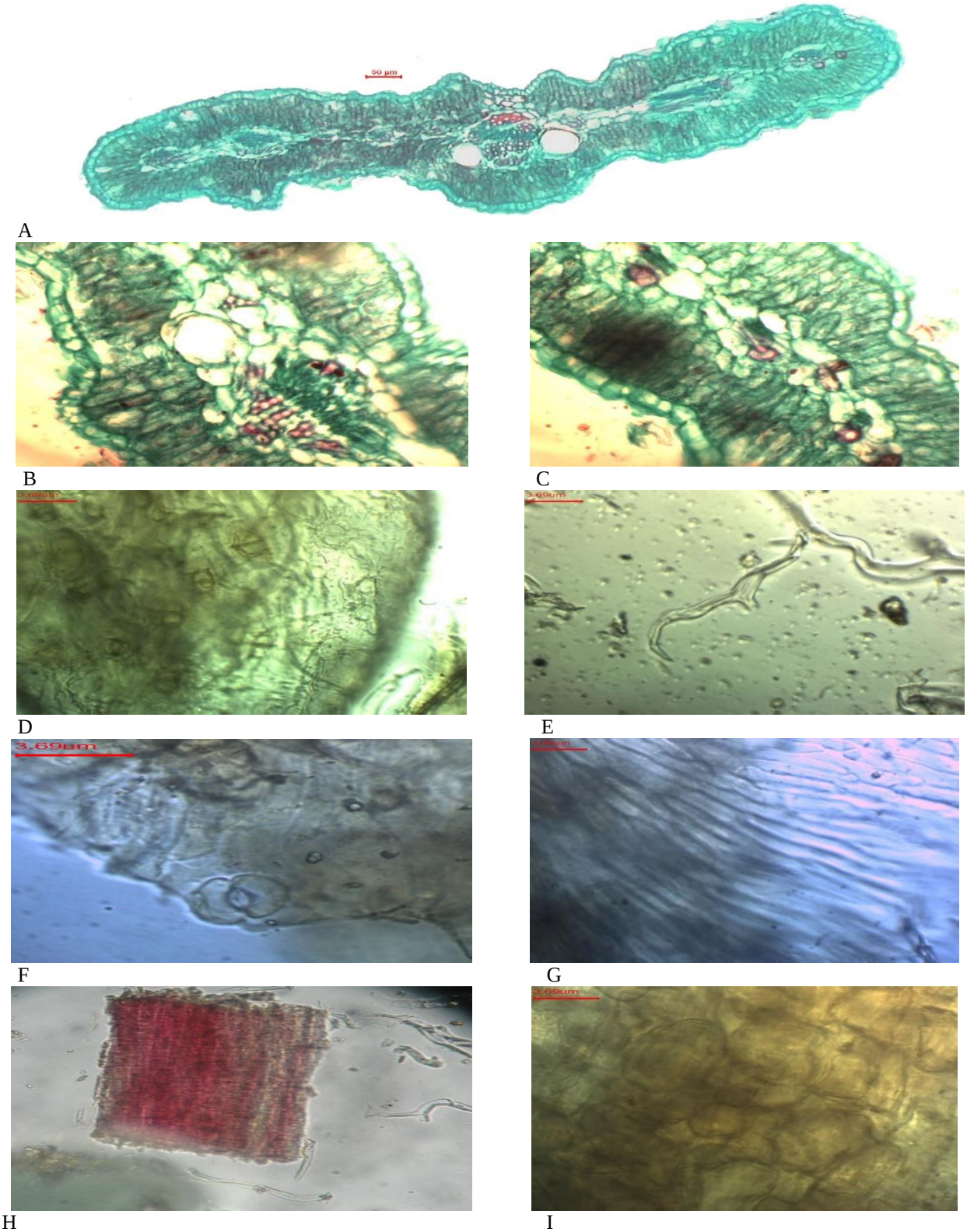

Fig. 1: Micromorphology of the leaf of A.judaica. A: Detailed T.S sector (X=50µm), B: tube like secretoty duct (X=2µm) C,D: Compositae hair, E: Non glandular trichome, F: Anomocytic stomata, G: Epidermal cells, H: Xylem vessels (X=2µm), I: Parenchyma cells

and peripheral software version 2.1

Phytochemical studies

Extraction: The fresh aerial parts of *A. judaica* were dried in the shade in an air draft at room temp. (250 g) were comminuted to powder and refluxed with MeOH / H_2O (3:1) (4 L × 6, 70°C, and 4 h). The residue left after evaporation of the solvent (70 g) was dissolved in 400 mL MeOH/H_2O (9:1) and then successively extracted with petroleum ether (Pet.ether) (4 × 300 mL); dichloromethane (CH_2Cl_2) (7 × 300 mL), EtOAc (5 × 300 mL) and n-butanol (BuOH) (5 × 300 mL). Pet. ether, CH_2Cl_2, EtOAc and BuOH extracts were evaporated under reduced pressure to

give yields equal to 0.7 g (yield: 1%), 18.2 g (yield: 26%), 4.62 g (yield: 6.6 %) and 6.44 g (yield: 9.2%), respectively. The remaining aqueous extract (RA) was also evaporated to dryness 22.61 g (yield: 32.3%). All extracts were tested for their radical scavenging activity and effect against hepatocellular and breast carcinoma.

Estimation of total phenolic and flavonoid contents**:** The total phenolic content (TPC) of each extract was determined by the Folin–Ciocalteau (FC) colorimetric method [11]. This method depends on the reduction of Folin–Ciocalteau reagent (FCR) by phenol to a mixture of blue oxides which have maximal absorption in the region of 765 nm. Measurements were carried out in triplicate and calculations were based on the calibration curve of gallic acid standard solutions (Standard curve equation: y = 0.0011x + 0.0009, R2 = 0.9867). The total phenolics were expressed as milligram of gallic acid equivalents (GAE) per gram plant extract.

The total flavonoid content (TFC) was determined by aluminium chloride colorimetric assay [12]. This method is based on the formation of a complex flavonoid aluminium having the absorptivity maxima at 415 nm. Measurements were carried out in triplicate and calculations were based on the calibration curve of quercetin standard solution (Standard curve equation: y = 0.005x - 0.0198, R2 = 0.9774). The total flavonoid content was expressed as milligram of quercetin equivalent per gram extract.

HPLC Analysis: The phenolics and flavonoids of *A. judaica* extracts were identified by HPLC method using Agilent HPLC 1200 series equipped with diode array detector (Agilent Technologies, Waldbronn, Germany). Chromatographic separations were performed using a waters column C18. The binary mobile phase consisted of (A) acetonitrile and (B) 0.1% acidified water with formic acid. The elution profile was: 0-1 min 100% B (isocratic), 1-30 min 100-70% B (linear gradient), 30-35 min 70-20% B (linear gradient). The flow rate was 0.3 ml/min and the injection volume was 5 µl. Chromatograms were recorded at 278 nm. Identification and quantitation of the major flavonoid and phenolic acid constituents in the leaves of *A. judaica* was determined adopting the external standard method (%) by comparing chromatographic peaks with the retention time (Rt) of individual standards adopting the procedure of Mattila et al [13]. Standards of flavonoid: quercetin, vitexin, isovitexin, orientin, iso-orientin, luteolin 7-glucoside and phenolic acids: caffeic and isoferulic acids were obtained from Fluka, Sigma, Germany. Results were the average of triplicate experiments.

Biological investigation

Radical scavenging activity: The free radical scavenging activity of extracts against the stable free radical DPPH• (Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically using the method of Shimada et al. [14]. All plant extracts were screened at 100 µg/ mL while the most potent active extracts (gave more 90%) were assayed at 25-75µg/ ml. 0.1mM solution of DPPH• in methanol was prepared. Then, 1 ml of this solution was added to 3 ml of extract solution at different conc. (25-75µg/ 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 in Asys microplate reader. Radical scavenging activity was calculated by the following formula: DPPH scavenging effect (%) = $[(A_0 - A_1)/A_0) \times 100]$, where: A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample [15]. 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 different extracts was tested against Huh-7 and A-549 cells using SRB assay [16, 17]. Color intensity was measured at 540 nm. The dose response curve of compounds was analyzed using Emax model.

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

Where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e., Kd = IC_{50} when R=0 and Emax =100-R). The concentration required to reduce cell viability by 50% (IC_{50}) was determined using the sigmoid Emax model.

RESULTS AND DISCUSSION

Pharmacognostical study

Microscopic features: Microscopic method is one of the cheapest and best method to start with establishing the correct identification of the source material [18]. In addition, microscopic examination of the various plant parts is important in providing information regarding the cellular organization, as well as providing information regarding various phytoconstituents present in the plant and their location [19].

A. Judaica leaves consists of thick midrib less prominent on the upper surface compared with the lower surface and thin lamina. The lamina is about 125 µm thick and the midrib is 208 µm (Fig.1A). Leaf is isobilateral (palisade parenchymatous cells are present on both adaxial and abaxial surfaces), homogenous, the adaxial and abaxial palisade zones are equal in height consisting of three layers. The adaxial part is more lobed compared to the abaxial part which is wide, thick and slightly lobed. The epidermal layers are prominent, squarish with thick cuticle. Epidermal cells are arranged in a single layer, around 19.5 µm. The continuity of both epidermises is interrupted by stomata and trichomes. Trichomes are glandular and non glandular. The marginal parts of lamina are slightly bulged, blunt and semicircular very similar to *A. parviflora* [20-21].

The vascular system consists of collateral vascular bundle with several short parallel lines of xylem elements and a thick adaxial arc of phloem, one large, centrally placed main vascular bundle in addition to small, less prominent accessory lateral bundles. Two tube-like secretory duct, very large and prominent near the xylem tissue (Fig.1B)

Table 1: Phenolic, flavonoid, radical scavenging activity (IC_{50}, IC_{90}) and effect of different extracts of *A.judaica* against liver and lung cell carcinoma.

	CH_2Cl_2	BuOH	EtOAc	RA	BHA	Ascorbic acid
Flavonoid (µg/mg QE)	4.12 ± 0.54	12.6 ± 1	18.3 ± 1.23	10.15 ± 0.96		
Phenolic (µg/mg GAE)	152.38 ± 2.4	241.6 ± 12.6	370.58 ± 3.5	84 ± 7.85		
Radical scavenging activity (IC_{50})	-	23.7 ± 1.67	61.9 ± 1.14	-	53.9 ± 3.1	42.8 ± 1.5
Radical scavenging activity (IC_{90})		46.9 ± 1.21	107.9 ± 2.31	-		
IC_{50} (liver carcinoma), mg/ml	0.58 ± 2.53	1.38 ± 0.74	1.38 ± 0.95	-		
IC_{50} (lung carcinoma), mg/ml	1.07 ± 0.85	1.04 ± 0.65	1.04 ± 0.56	-		
R%	0	0	0	0		

Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

are observed in agreement to *A. campestris* where the entire tube consists of densely cytoplasmic cells that secrete their product into the duct lumen. Shizogenic development of secretory ducts has been also reported. These organelles may play an important role in the oleoresin production, and the transport of the secreted material towards the plasmalemma [22].

The powder is greyish green showing a large number of secretory trichomes, biseriate, two celled stalk and a biseriate head with two or four cells (Fig.1C, D); nonglandular T-shaped trichomes with a short uniseriate stalk capped by a very long, undulating terminal cell tapering at the ends (Fig.1E) in agreement with many species as *A. nova, A. vulgaris* and *A.absinthium* [23-26]. In addition, anomocytic stomata (Fig.1F), fragments of epidermises with sinuous walls (Fig. 1G), annular and spiral xylem vessels (Fig. 1H) and parenchyma cells (Fig. 1I) are observed.

Phytochemical Study

Phenolic and flavonoid contents**:** Quantitation of the phenolic and flavonoid contents has been evaluated in terms of gallic acid and quercetin equivalent using colorimetric assays. Table 1, reports the TPC and TFC. TPC varied from 84 ± 7.85 to 370.58 ± 3.5 mg/g GAE. EtOAc showed the highest concentration (370.58 ± 3.5 mg/g) followed by BuOH (241.6 ± 12.6 mg/g) while RA extract represented the lowest concentration.

TFC varied from 4.12 ± 0.54 mg/g to 18.3 ± 1.23 mg/g. The highest flavonoid content was observed with EtOAc (18.3 ± 1.23 mg/g), followed by BuOH while the lowest concentration was observed with CH_2Cl_2 extract.

Artemisia is well known for its high flavonoid and phenolic contents which have contribution on its previously reported biological activities [27, 28].

HPLC analysis: HPLC analysis of the extracts of high phenolic and flavonoid contents (BuOH and EtOAC) revealed the identification of orientin, vitexin, quercetin and isovitexin while the main identified phenolic acids were caffeic acid and isoferulic acid. Orientin is the major identified flavonoid in both extracts followed by vitexin and quercetin in BuOH extract and quercetin and caffeic acid in EtOAc extract. Vitexin was previously identified in *A.vulgaris* [29] while quercetin, caffeic acid and ferulic were found in *A.absinthium* [27].

Free radical Scavenging Activity: Free radical scavenging activity of different *A. judaica* extracts has been checked at 100 µg/mL. BuOH exhibited 100% scavenging activity followed by EtOAc 81.9% while CH_2Cl_2 exhibited 48%. No activity was observed with RA extract.

Comparing the IC_{50} of the bioactive extracts with ascorbic acid and butylated hydroxyanisol (BHA) as positive control (Table 1), BuOH showed the best effect (23.7 ± 0.86 µg/ml) compared with ascorbic acid (42.8 ± 1.5) followed by EtOAc (61.9 ± 0.65µg/ml). Several studies reported the high antioxidant potential of genus Artemisia [27, 28, 30] in relation to its high phenolic and flavonoid contents. Two possible mechanism for the antioxidant potential are the hydrogen transfer mechanism for the phenolic acid and the electron-transfer mechanism for the flavonoid [31].

Cytotoxic Activity: Cytotoxic activity of each extract, CH_2Cl_2, EtOAc, BuOH and RA, was investigated using SRB assay against HePG2 and Huh-7 cell line which are representative of two main cancer problems present in Egypt (Table 1). IC_{50} and R fraction were calculated using Emax model as previously described. CH_2Cl_2 exhibited higher potency against Huh-7 (IC_{50} 0.58 ± 0.065 mg/ml) followed by BuOH (IC_{50} 1.31 ± 0.05 mg/ml). RA extract didn't show any effect against Huh-7 at the used concentrations. The resistant fraction of Huh-7 was (0%) which denoted the potency on liver cell carcinoma. Cytotoxic Effect against A549 revealed BuOH and EtOAc with similar activity (IC_{50} 1.04 ± 0.65, 1.04 ± 0.56 mg/ml respectively). Substantial R-fraction of A549 was also 0%. Comparable results were obtained with different Artemisia species where the IC_{50} was higher than 500 µg/ml [32].

Table 2: Phenolic and flavonoid contents of *A.judaica* extracts identified by HPLC

R_t*	Compound	EtAc	BuOH
3.4	Quercetin	4.9%	5.11
14.9	Isoferulic acid	0.96	0.56
22.3	Caffeic acid	1.35	0.1
26.7	Vitexin	1	10.32
27.25	Isovitexin	-	1.5
32.9	Luteolin 7 glucoside	0.78	-
37.1	Orientin	11.86	21.16

R_t: Retention time in minute*

A.judaica growing in Egypt has slight differences in its microscopical characters compared to other closely related Artemisia species, in addition, it is a very rich source of phenolic and flavonoid that may provide a promising source of natural antioxidant.

CONCLUSION

The present study provides information about the microscopical features in relation to pharmacognostical identification of *A.judaica* leaves. Furthermore, *A.judaica* demonstrated powerful radical scavenger activity compared with ascorbic acid due to high phenolic and flavonoid contents. Further studies are needed to evaluate the in vivo potential of these bioactive extracts in animal models.

REFERENCES

1. Bora KS, Sharma A, The genus Artemisia: A comprehensive review. *Pharmaceutical Biology* 2011; 49: 101–109.
2. Tackholm V, Students' Flora of Egypt. Cooperative Printing Co., Beirut, Lebanon, 1974.
3. El-Massry KF, EL-chorab AH, Farouh A. Antioxidant activity and volatile components of Egyptian *Artemisia judaica*. *Food Chemistry* 2002; 79 (3): 331 - 6.
4. Abdalla SS, Abu-Zagra MH. Effects of cirsimaritin, a flavone isolated from *Artemisia judaica*, on isolate guinea-pig ileum. *Planta Medica* 1987; 53: 322–324.
5. Saleh MA, El-Ghazooly SI, Abou-Zaid MM. Flavonoid of *Artemisia judaica*, *A. monosperma* and *A. herba-alba*. *Phytochemistry* 1987; 26: 3059–3064.
6. Khafagy SM, El-Din AA, Jakupovic J, Zdero C, Bohlmann F. Glaucolide-like sesquiterpene lactones from Artemisia judaica. *Phytochemistry* 1988; 27: 1125–1128.
7. Saleh MA. Volatile components of *Artemisia monosperma* and *Artemisia judaica* L. growing in the Egyptian deserts. *Biochemical Systematics and Ecology* 1985; 13: 265–269.
8. Abd ElHady, HK, Insecticidal activity and chemical composition of essential oil from *Artemisia judaica* L. against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)
9. Batanouny KH, Aboutabl E, Shabana M, Soliman F, Wild medicinal plants of Egypt: an inventory for conservation and sustainable use. Academy of Scientific Research and Technology, National Committee of IUCN (International Union for Conservation), Cairo,1999 ; 117-119.
10. Tan RX, Zheng WF, Tang HQ, Biologically active substances from the genus *Artemisia*. *Planta Medica* 1998; 64: 295-302.
11. Sellappan S, Akoh CC, Krewer G, Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry* 2002; 50 (8): 2432-38.
12. Kosalec H, Bakmaz M, Pepeljnjak S, Vladimir-kne S, Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharmaceutica* 2004; 54**:** 65-72.
13. Mattila P, Astola J, Kumpulainen J, Determination of flavonoids in plant material by HPLC with Diode array and electroarray detections. *Journal of Agricultural and Food Chemistry* 2000; 48: 5834-5841.
14. Shimada K, Fujikawa K, Yahara K, Nakamura T, Antioxidative properties of xanthone on the autooxidation of soybean in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry* 1992; 40: 945-48.
15. Oktay M, Gulcin I, Kufrevioglu OI, Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensm. Wiss. Technol. Ð *Food Science and Technology* 2003, 36: 263-71.
16. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR, New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute* 1990; 82**:** 1107-12.
17. Al-Abd AM, Lee JH, Kim SY, Kun N, Kuh HJ, Novel application of multicellular layers culture for in situ evaluation of cytotoxicity and penetration of paclitaxel. *Cancer Science* 2008; 99: 423-31.
18. Singh S, Manchawal L, Chauhan MG. Pharmacognostic study of male leaves of Trichosanthes dioica Roxb. with special emphasis on microscopic technique. *Journal of Pharmacognosy and Phytotherapy* 2010; 2(5): 71-75.
19. Rajmohanan TP, Sudhakaran Nair CR, Padmaja V, Pharmacognostical and Phytochemical Studies on *Cayratia pedata* (Lam). *International Journal of Pharmacognosy and Phytochemical Research* 2014; 6(2); 227-233.
20. Noorbakhsh SN, Ghahreman A, Attar F, Mahdigholi K, Leaf anatomy of artemisia (asteraceae) in Iran and

its Taxonomic implications. *Iranian journal of botany* 2008; 14 (1): 54-69.

21. Suresh J, Ahuja J, Paramakrishnan N, Pharmacognostical investigation of *Artemisia parviflora* Roxb *Asian Pacific Journal of Tropical Biomedicine* 2012, S532-S535.
22. Ascensão L, Salomé Pais M, Ultrastructure and histochemistry of secretory ducts in *Artemisia campestris* ssp. maritima (Compositae). *Nordic Journal of Botany* 1988; 8 (3): 283-292.
23. Kelsey RG, Shafizadeh F, Glandular trichomes and sesquiterpene lactones of *Artemisia nova* (Asteraceae). *Biochemical Systematics and Ecology* 1980; 8 (4):371-377.
24. Hayat MQ, Ashraf M, Khan MA, Yasmin G, Shaheen N, Jabeen S, Diversity of foliar trichomes and their systematic implications in the genus Artemisia (Asteraceae). *International journal of agriculture & biology* 2009; 11: 542-546.
25. Council of Europe, European pharmacopeia, 5th edition. Strasbourg: Council of Europe; 2005.Wormwood; 2710
26. Bercu R, Broască L, Histoanatomical features of *Artemisia alba* subsp. Saxatilis (willd.) P. Fourn. *Annals of RSCB* 2012; 17:278-283.
27. Lee YJ, Thiruvengadam M, Chung IM, Nagella P, Polyphenol composition and antioxidant activity from the vegetable plant *Artemisia absinthium L. Australian Journal of Crop Science* 2013; 7(12): 1921-1926.
28. Rashid ACh, Qureshi MZ, Raza SA, William J, Arshad M, Quantitative determination of antioxidant potential of *Artemisia persica. Analele UniversităŃii din Bucuresti – Chimie (serie nouă)* 2010; 19:23-30
29. Yang GE, Bao L, Zhang XQ, Wang Y, Li Q, Zhang WK, Ye WC, Studies on flavonoids and their antioxidant activities of *Artemisia annua. Zhong Yao Cai* 2009; 32(11):1683-1686.
30. El-Sayed MA, BaAbbad R, Balash A, Al-Hemdan NA and Softah A, The Potential Anti *Helicobacter pylori* and antioxidant effects of *Artemisia Judaica*. *Functional Foods in Health and Disease* 2013; 3(9):332-340.
31. Leopoldini M, Marino T, Russo N, Toscano M, Antioxidant properties of phenolic compounds: H-Atom versus electron transfer mechanism. *The Journal of Physical Chemistry A* 2004; 108: 4916-4922
32. Gordanian B, Behbahani M, Carapetian J, Fazilati M, In vitro evaluation of cytotoxic activity of flower, leaf, stem and root extracts of five Artemisia species. *Research in Pharmaceutical Sciences* 2014; 9(2): 91-99.