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Research Article

In Vitro Antioxidant and Enzyme Inhibition Activity of *Hesperis İsatidea* (Boiss.) D.A. German & Al-Shehbaz (Brassicaceae) and its Anatomy

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ABSTRACT

Hesperis isatidea (Brassicaceae) is a perennial herbaceous endemic plant known as "Allıgelin". It has traditional use concerning the cough, wound, cold and vulnerary in the form of decoction and poultice in Eastern Turkey. In this study, the *in vitro* antioxidant activity (DPPH, ABTS, FRAP, and metal chelating) and enzyme inhibitory activities (anticholinesterase, anti-tyrosinase) of *H. isatideae* were evaluated. Besides, the transverse sections were taken from the leaf, stem and fruit to determine the morphological and anatomical features of *H. isatidea*. The characteristic elements of the preparations were determined by Leica CME stereomicroscope and the distinctive features were shown in photographs. The obtained results of *in vitro* antioxidant and enzyme inhibitory assays suggest that *H. isatidea* may useful to develop bioactive compounds rich nutraceuticals for health care.

Keywords: *Phenolics, anatomy, DPPH, ABTS, FRAP, Metal chelating, Anticholinesterase and anti-tyrosinase*

Hesperis Isatidea (Boiss.) D.A. German & Al-Shehbaz (Brassicaceae) Türünün *in vitro* Antioksidan ve Enzim Aktivitesi ve Anatomisi

Öz

Endemik bir tür olan *Hesperis isatidea* (Brassicaceae) "Allıgelin" olarak bilinen çok yıllık ve otsu bir bitkidir. Doğu Anadolu'da öksürükte, yara iyileştirmede, soğuk algınlığında dekoksisyon ya da lapa olarak geleneksel kullanımlarına rastlanmaktadır. Bu çalışmada, *H. isatidea* türünün *in vitro* antioksidan (DPPH, ABTS, FRAP ve metal bağlama) ve enzim inhibe edici aktiviteleri (antikolinesteraz, anti-tirozinaz) değerlendirilmiştir. Ayrıca *H. isatidea* türünün morfolojik ve anatomik özelliklerini aydınlatmak üzere yaprak, gövde ve meyveden enine kesit alınarak türün karakteristik unsurları Leica CME stereomikroskop ile belirlenmiş ve ayırt edici özellikleri fotoğraflanmıştır. *In vitro* antioksidan ve enzim inhibe edici deneylerin sonuçlarına göre, *H. isatidea* türünün sağlığa faydalı biyoaktif bileşikler açısından zengin olduğu ve nutrasötik geliştirme çalışmalarına katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: *Fenolik bileşikler, Anatomy, DPPH, ABTS, FRAP, Metal bağlama,* Antikolinesteraz ve anti-tirozinaz

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I. INTRODUCTION

The Brassicaceae is one of the large and important plant families. *Hesperis* L. is distributed in the warm climate zone of Eurasia and in the mountainous regions in Europe, Southwest Asia, Caucasus and Transcaucasia and in Northern and Central Asia. The genus comprises around 56 species in the world-wide [1]–[4] half of which are naturally distributed in Turkey, where the transition zone of the Mediterranean, Irano-Turanian and Euro-Siberian phytogeographical regions, with the endemism ratio of 82% shows Turkey is the possible diversity center for the genus [4]–[7]. The genus consists of biennial or perennial herbs. Trichomes are unicellular, furcate-stellate, glandular or eglandular. Leaves often deeply dentate. Inflorescence is a racemose. Flowers are often striking. Petals are purple, pink, violet, white, greenish, yellowish or brown. Fruit type is a siliqua. Stigma is with 2 decurrent carpidiallobes [4]–[6]. *Hesperis isatidea* (Boiss.) D.A. German & Al-Shehbaz is an endemic species, which has distribution in Eastern Turkey in Elazığ, Erzincan, Erzurum, Gümüşhane, Giresun, Sivas, Tunceli provinces. It has important diagnostic features such as the indumentum of a mixture of long simple setose and smaller stalked branched trichomes, large fragrant flowers with closed bisaccate calyx and long-unguiculate petals, flattened filaments of inner stamens, deeply bilobed stigmas, non-margined seeds [6]–[8]. The recent molecular phylogenetic studies showed that *Tchihatchewia* Boiss. is synonym of *Hesperis* [9]–[10]. Turkey is rich in medicinal plants and *Hesperis* is one of the therapeutic species used in folk medicine for wound healing [11] cough, sinusitis, bronchitis, skin diseases [12] and has use as well as ornamental purpose [13]. According to recent studies, the genus exhibits several bioactivities such as antimicrobial and anticholinesterase activities [14]. In this work, *in vitro* antioxidant activity (DPPH, ABTS, FRAP and metal chelating,) and enzyme inhibitory activities (anticholinesterase and anti-tyrosinase) of *H. isatidea* were evaluated as well as its morphological and anatomical features determination. Besides, the total phenolic and flavonoid contents of the extract were investigated by Folin-Ciucalteu and aluminum chloride colorimetric methods, respectively.

II. MATERIAL AND METHODS

A. PLANT MATERIAL AND EXTRACTION PROCEDURE

Hesperis isatidea (Boiss.) D.A. German & Al-Shehbaz in Phytotaxa 334(1): 95-98, 2018. **Lectotype:** [TURKEY, Erzincan], Armenia ad orientum Urbis Erzindjan. Aia minor, OEst. 1858. M. de Tchihatchef s.n. Designated by Al-Shehbaz in Phytotaxa 334(1): 95-98, 2018. **Synonym:** *Tchihatchewia isatidea* Boiss. in Asie Min., Bot. 1: 292, 1866. *Neotchihatchewia isatidea* (Boiss.) Rauschert in Syst. Verz. 31: 558 1982.

The plant materials were collected by Assoc. Prof. Dr. Gülderen Yılmaz at flowering stage in Geçitli Village in Tunceli province in Turkey. Voucher specimen (AEF 28861) was deposited Ankara University, Faculty of Pharmacy Herbarium (AEF). 50 g of aerial parts were air-dried, grounded by a laboratory mill into a fine powder and macerated with methanol at room temperature for 24 hours. Rotary evaporator was used for concentrate the extracts at 40°C and the extracts (5g) were stored at at 4°C.

B. ANATOMY

The research materials of the species were preserved in 70% of alcohol. The Cross-sections of stem, leaf and fruit are examined in Sartur and chloral hydrate solutions. An automatic camera adapted on a Leica CME microscope was used to take photographs of the sections.

C. EXTRACTION PROCEDURE

C. 1. *In-Vitro* Antioxidant Activity

C.1.1. Total Phenolic Content

Folin-Ciocalteu method was performed for determination of the total phenolic content of the extracts [15]. Ten times diluted100 µL (1/10, v/v) Folin-Ciocalteu reagent and 10 µL (2mg/mL) of each extract were mixed. After 5 min of reaction, 100 µL (7.5%) sodium carbonate was added to each tube. After that the mixture placed in incubation for 60 min. The samples absorbance was measured at 650 nm. The experiment was conducted three times and the standard curve was prepared by 1-1000 µg/mL solutions of gallic acid in DMSO. The total phenol content was showed as the gallic acid (mg) in per g of dry weight of the extract (mg GAE/g DW).

C.1.2. Total Favonoid Content

According to the literature, we used aluminium chloride colorimetric method to determine the total flavonoid content [16]. Serial diluted solutions of 0.0625, 0.125, 0.25 and 0.5 mg/mL were prepared using quercetin stock solution (1 mg/mL) for calibration. The test solution (150 µL, 0.3 mg / mL) was prepared with ethanol which is mixed on an aliquot of 96 well plates with an equal volume of 2% AlCl3. It was incubated for 15 min at room temperature. Then, the absorbance was read at 435 nm. The quantities of total flavonoids were calculated mg equivalence of quercetin over g dry weight of extract.

C.1.3. DPPH Radical Scavenging Activity

2-2′-diphenyl-1-picryhydrazyl (DPPH) radical scavenging potential of the extracts was determined with spectrophotometric method [15]. 20 µL of diluted with DMSO test solutions were mixed in a 96-well plate with 180 µL of DPPH solution at a concentration of 40 µg/mL prepared in methanol. After the plates are left in the dark for 15 min, the absorbance was read at 540 nm with an Elisa reader. DMSO was used as blank instead of the test sample, and a standard solution of gallic acid was run in parallel. The following equation was used for express the results as % DPPH scavenging effect. % DPPH Scavenging Effect = [(Control Absorbance – Sample Absorbance)] / Control Absorbance) x 100 The control absorbances are all solutions that do not contain the test substances, and the sample absorbance is the absorbance of extract/quercetin.

C.1.4. ABTS Radical Scavenging Activity

Re et al. (1999) method was used for determine ABTS scavenging activities of the plant extracts [17]. Sample preparation method was like in the DPPH method. ABTS + radical stock solution was prepared by allowing 264 µL of 140 mM potassium persulfate and 15 mL of 7 mM ABTS solution to stand in the dark for 16h at room temperature. The ABTS working solution was adjusted to give the absorbance as 0.70 ± 0.02 at 734 nm by diluting with methanol just before the experiment. 50 µL of sample solution and 100 µL of ABTS working solution were mixed on a 96-well plate. The mixture was left for 10 min at room temperature. After that, the absorbance was read at 734 nm. The ABTS + scavenger activities of the plant extracts were compared with Trolox, and the formula below used for calculation of the percent inhibition:

ABTS + radical scavenging activity (%) = [(Control - Sample) / Control × 100

C.1.5. Iron Chelating Activity

Ferrozine spectrophotometric method was used for determine the metal chelating activity [14],[15]. Briefly, a mixture of 0.4 mL of 0.25 mM ferrozine and 0.2 mL of 0.10 mM FeSO4, 0.2 mL of extract reacted for 10 min at room temperature. Then, absorbance was read at 562 nm. A blank was created for each measurement by substituting water for FeSO4 and ferrozine to account for background absorbance. As the positive control disodium salt of EDTA was used.

C. 2. Enzyme Inhibition Activity

C.2.1. Tyrosinase Inhibition Assay

The spectrophotometric method used for measure the inhibitory effect on tyrosinase with minor modifications [18–19]. Briefly, (100 mM, pH 6.8, 100 µl) phosphate buffer, (250 U/mL of tyrosinase, 20 µL) enzyme solution and (test compounds in different concentrations, 20 µL) a total volume of 140 µL of assay mixture containing sample solution were added to each well of a 96-well plate before preincubated for 10 min at 25 °C. After that, (3 mM of L-tyrosine, 20 µL) substrate solution was added. After incubation for 30 min at 25 °C, microplate reader was used for determination of the mixture absorbance at 492 nm. As the positive control kojic acid was used. The following equation used for calculation of the percentage inhibition of tyrosinase activity: Inhibition % = [1 × (Aa - Ab) / (Ac - Ad)] × 100, in which Aa is the absorbance with both enzyme and test sample, Ab is the absorbance without enzyme but test samples, Ac is the absorbance without test sample but enzyme, and Ad is the absorbance without both enzyme and test samples at 492 nm.

C.2.2. Acetylcholinesterase Inhibition Assay

Ellman's method was used for test the plant extracts against the acetylcholinesterase enzyme with slight modifications [20]. Briefly, 20 µL (0.22 U/mL) of acetylcholinesterase, 140 µL of 0.1 mM (pH 6.8) phosphate buffer and various concentrations of 20 µL of the extract solution were added in microplate. After that, it was incubated with mixing for 30 min. The reaction was started by the addition of 10 µL of 0.71 mM acetylthiocholine after adding 10 µL of 0.5 mM 5, 5′-dithiobis [2-nitrobenzoic acid] (DTNB). The acetylthiocholine hydrolysis-induced thiocholine was released and 5-thio-2-nitrobenzoate dianion (TNB), the yellow compound, formed by the reaction of thiocholine with the chromogenic DNTB. A microplate reader at 412 nm used for measure the absorbance. The positive control was galantamine and the negative control was methanol.

III. RESULTS AND DISCUSSION

A. ANTIOXIDANT ACTIVITY

Phenolic compounds are known for their potential antioxidant effects, therefore used widely in the healthcare products. In this context, investigation of antioxidant potential of different medicinal plants is very important for providing basic research data. In this study, Folin-Ciocalteu colorimetric method is used for determination of the total phenolic content (TPC) and expressed as gallic acid equivalent on dry weight basis of extract. TPC of *H. isatidea* extract was found as 109.66 mg GAE/g and total flavonoid content (TFC) was determined as 15.42 mg QE/g by aluminium chloride colorimetric method. Similarly, TFC was found as 31.3 mg RE/g by Zengin et al., but according to the same study, TPC was found as 30.18 mgGAE/g which is lower compare to our value of TPC [21].

DPPH and ABTS discoloration assays are used for determining radical scavenging potential of the extract. The DPPH and ABTS radical scavenging activity of the extract was detected as 905.08 and 534.58 µg/mL, respectively. According to Zengin et al. DPPH and ABTS radical scavenging activity

of the methanol extract was 78.69 and 167.81 µg/mL, respectively [21]. This strong radical scavenging activity in our results may be explained with the correlation between total phenolic contents and radical scavenging ability. The metal ions such as iron involved in the metabolic reactions, but they accelerate oxidations of important cellular compounds. Therefore, chelating of metal ions is also important for antioxidant effects. The iron chelating activity of the *H. isatidea* extract was found as 413.8 µg/mL.

Tablo 1. *Antioxidant activity of the extract-in vitro*

Taxa	Radical scavenging activities		Iron chelating activity (IC_{50})	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
	DPPH (IC_{50})	ABTS (IC_{50})			
H. isatidea	905.08 ± 1.70	534.58 ± 2.23	413.8 ± 0.69	109.66 ± 2.89	15.42 ± 0.27
Reference standard					
Gallic acid	53.87 ± 4.93				
Trolox		248.17 ± 0.66			
EDTA			425.3 ± 0.14		

B. ENZYME INHIBITORY ACTIVITY

Enzyme inhibitors have become therapeutic strategy for treatment of some disease such as diabetes mellitus and Alzheimer's diseases. Therefore, enzyme inhibitory evaluation assays are more popular due to assessment of the potential health benefits of plants, nutraceuticals as well as developing natural products or functional foods. Among these enzymes, the cholinesterase is related with pathology of Alzheimer diseases, and tyrosinase is related with Parkinson's disease and skin whitening. In this work, we investigated anti-acetylcholinesterase and anti-butyrylcholinesterase activity and tyrosinase inhibitory activity and the results are shown in Table 2 and Table 3. In this study, it is found that the methanol extract of *H. isatidea* possessed moderate antityrosinase activity with mean value of 16.67 % inhibition at 2 mg/ml concentration, while the reference standard kojic acid showed 82% inhibitory activity against tyrosinase.

Table 2. *Tyrosinase inhibitory activity of the extract (% inhibition)*

Sample	0.5(mg/ml)	1(mg/ml)	2(mg/ml)
H. isatidea	11.89±2.58	17.08±2.77	16.67±3.06
Reference standard			
Kojic acid	71.09±7.43	78.12±3.32	82.07±2.83

In addition, it was demonstrated that the extract of *H. isatidea* had anticholinesterase activity, and the acetylcholinesterase inhibitory activity (69.09%) was higher than butyrylcholinesterase inhibitory activity (43.38%) at 2 mg/ml concentration.

Table 3. *Anticholinesterase inhibitory activity of the extract (% inhibition)*

Sample	Antiacetylcholinesterase activity (mg/ml)			Antibutyrylcholinesterase activity (mg/ml)		
	0.5	1	2	0.5	1	2

H. isatidea	28.92±0.13	50.27±2.02	69.09±1.60	30.88±8.94	38.97±1.04	43.38±7.68
Reference standard						
Galantamine	70.58±5.96	97.43±2.48	98.86±0.72	69.32±1.82	70.83±3.88	91.92±7.47

C. ANATOMY

In this study, the leaf, stem and fruit anatomy of *H. isatidea* were examined. The stem is cylindrical. In the cross-section, uniseriate, thickened wall epidermis layer in the outermost of the stem. After the epidermis, the collenchyma cells are 2-3 layered. Sclerenchyma cells lie next to the phloem in the cortex. The cambium is made up of cells in a regular row. Tracheal elements are arranged in the radial direction and surrounded by sclerenchyma including parenchyma cells between them. Parenchyma tissue is 5-6 layered. Between vascular fascicles, there are 7-12 layered sclerenchyma cells. Different-sized parenchymatic cells fill the pith region (Figure 1).

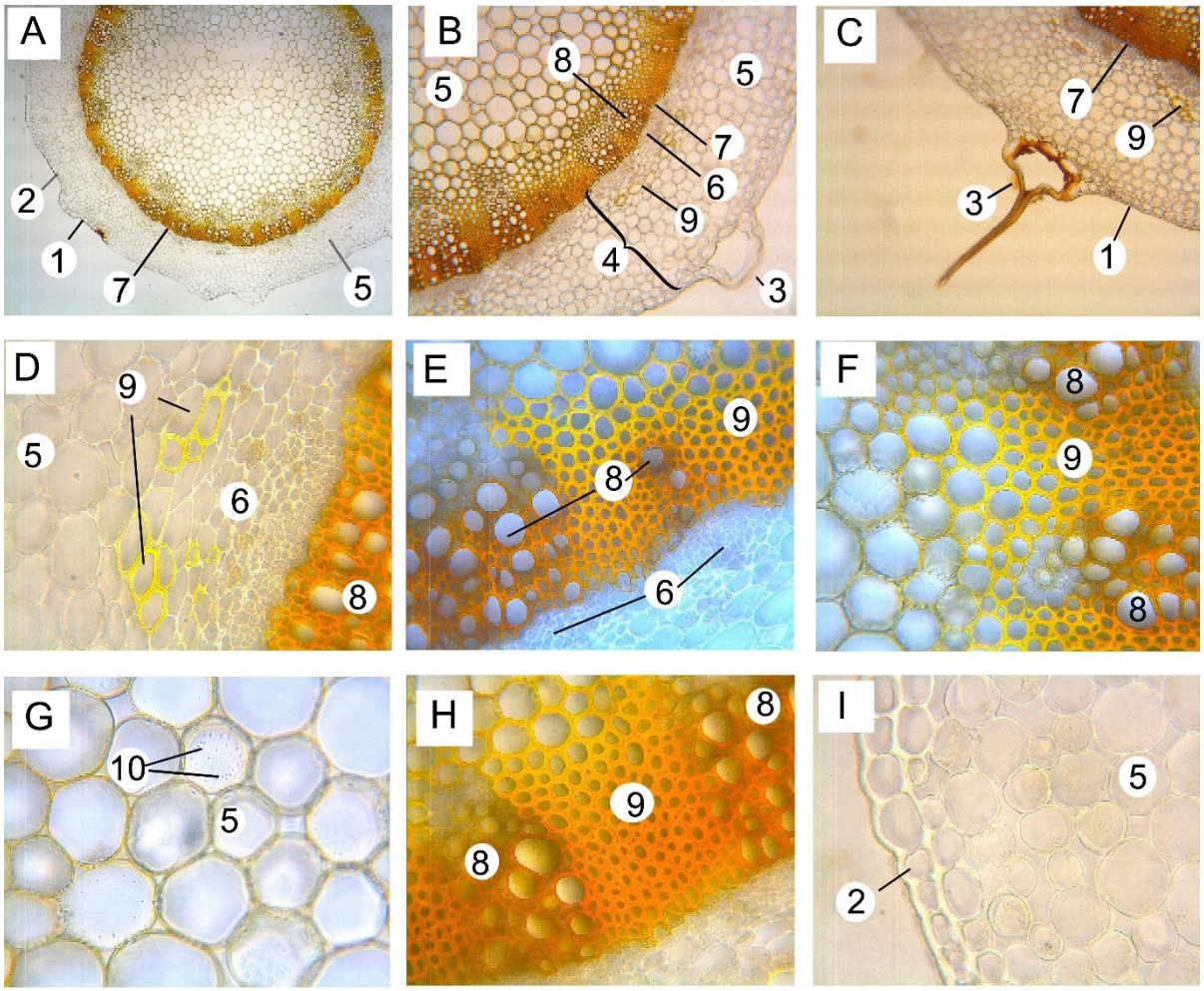

Figure 1. *(A-I) stem cross-section. 1) cuticle, 2) epidermis, 3) trichome, 4) cortex, 5) parenchyma, 6) phloem, 7) cambium, 8) xylem, 9) sclerenchyma cells, 10) crystals*

In the leaf cross-section, cuticle located on the lower and upper epidermis. There is a single-layered epidermis on the lower and upper surface of the leaf with simple and bifurcated trichomes on the leaf surfaces. Vascular bundles are surrounded by collenchyma cells. The mesophyll is composed of different shaped palisade parenchyma cells. The leaf is isobilateral with 2-3 layered palisade

parenchyma cells on both surfaces. Spongy parenchyma cells are 2-3 layered. The vascular bundle type is collateral. In leaf, superficial section epidermis cells are located at upper and lower leaf surfaces and display similar structures on both sides including stomata. The leaf type is amphistomatic. Stomata are anisocytic, surrounded by 3-4 neighbour cells. One of the neighbouring cells is much smaller than the others and reflects the characteristics of the Cruciferae type stoma (Figure 2).

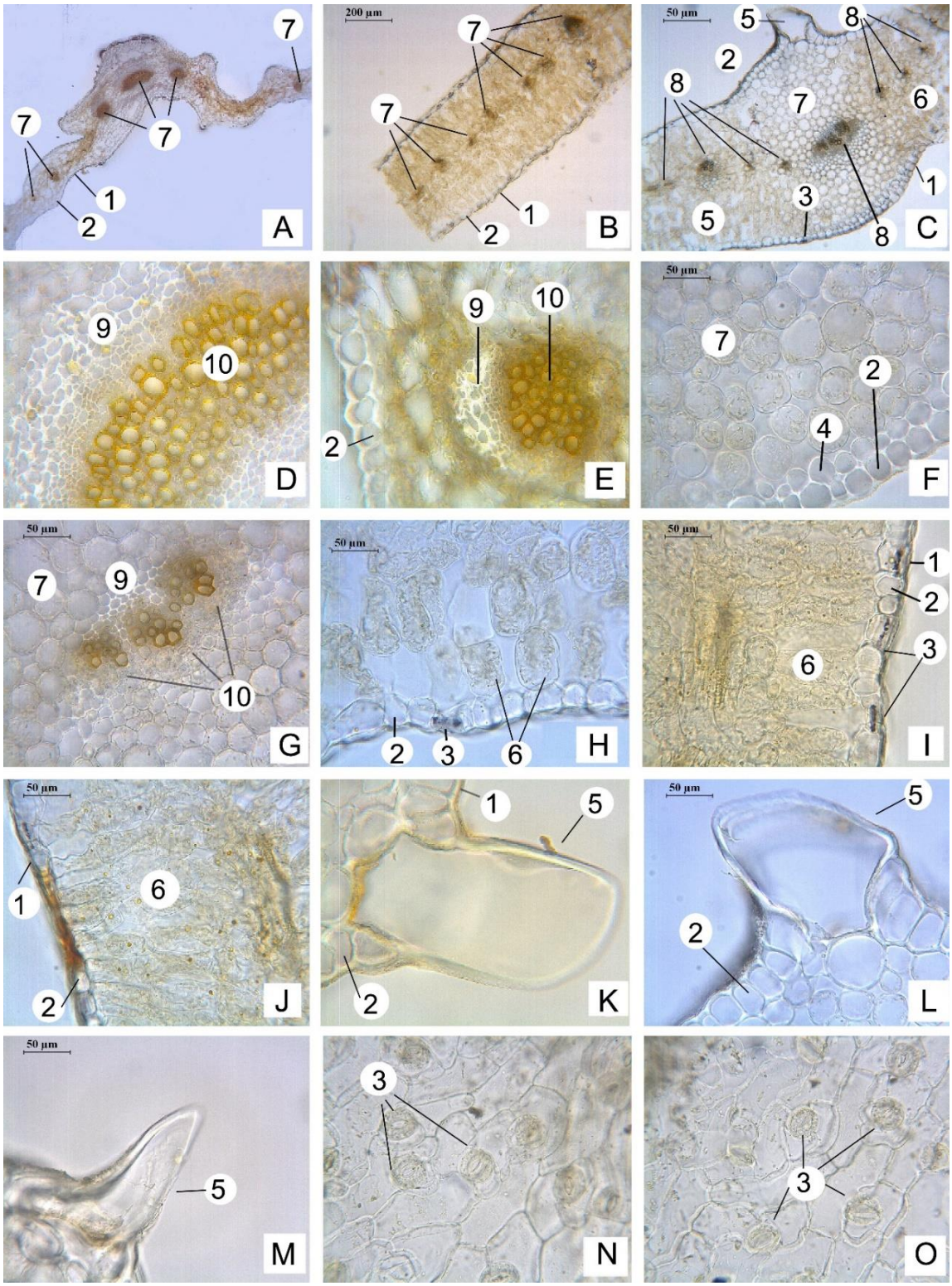

Figure 2. *(A-O) leaf transversal section. 1) cuticle, 2) epidermis, 3) stomata, 4) collenchyma, 5) simple trichome, 6) palisade parenchyma, 7) spongy parenchyma, 8) vascular bundle, 9) phloem, 10) xylem*

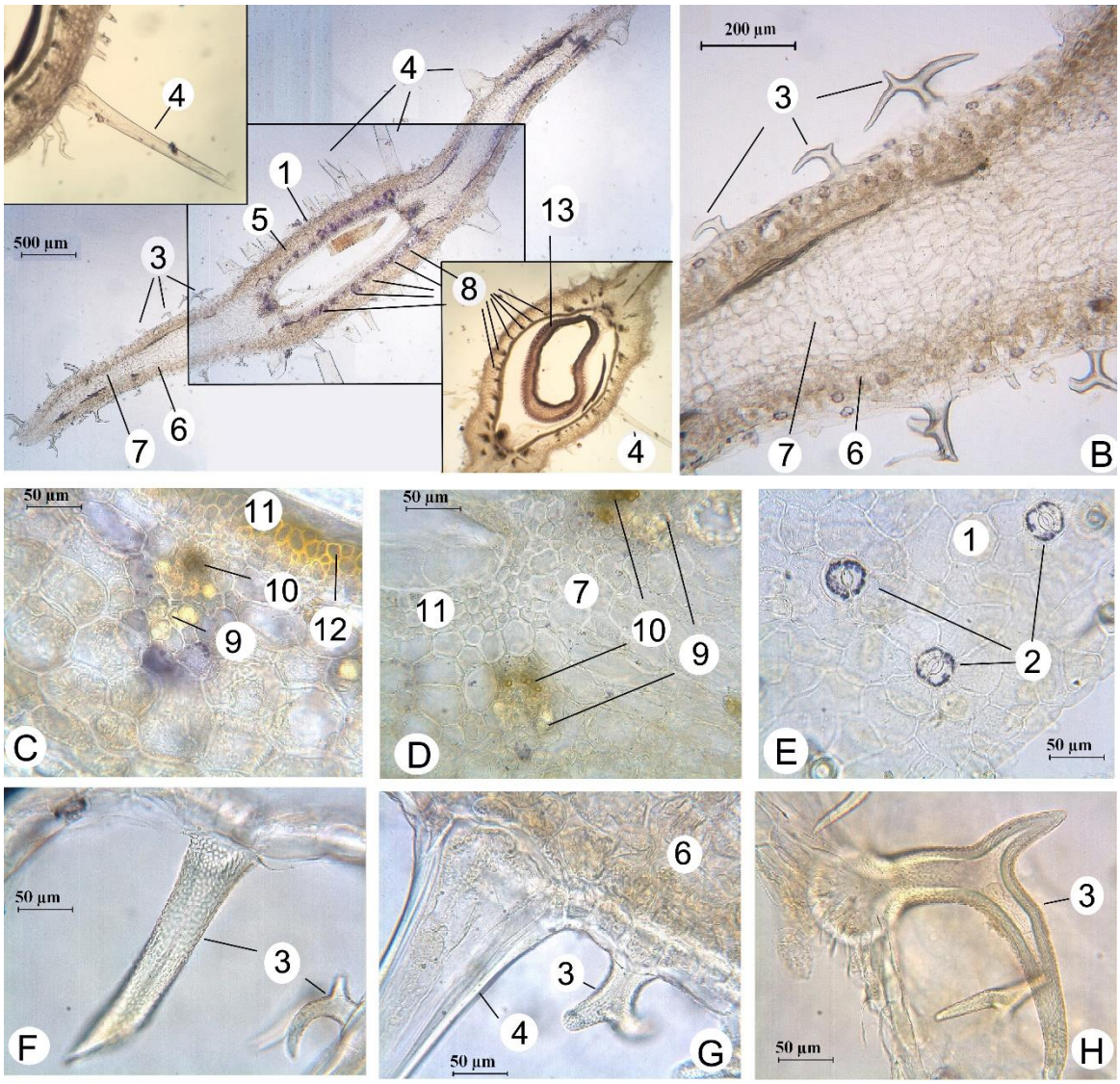

Figure 3. *(A-H) fruit cross-section. 1) exocarp, 2) stomata, 3) bifurcated trichome, 4) simple trichome, 5) mesocarp, 6) palisade parenchyma, 7) spongy parenchyma, 8) vascular bundle, 9) phloem, 10) xylem, 11) endocarp, 12) sclerenchyma, 13) seed*

In the fruit cross-section, fruit surrounded by one layered, thick walled exocarp. After exocarp lie 3-5 layered palisade parenchyma cells at both side. 5-10 layered spongy parenchyma inside. The fruit is flat and winged. The entire surface is covered with simple and bifurcated trichomes. Simple trichomes numbers increase towards the centre of the fruit. Simple trichomes are much larger than bifurcate trichomes. Many vascular bundles are surrounding the endocarp from the inside. There are 12-14 vascular bundles on both side of the fruit, and one relatively large vascular bundles on both side facing the fruit wings. Before endocarp sclerenchyma cells are 3-4 layers. Sclerenchyma cells are located between vascular bundles and endocarp. There are 1-2 seeds in the middle, one of them is well developed. At superficial section, stomatas have 3-5 neighbour cells (Figure 3).

IV. CONCLUSION

In this study, the stem, leaf and fruit anatomy of *Hesperis isatidea* are studied. *H. isatidea* represents by distinctive anatomical characters like bifurcated trichomes on the fruit surface. Our results have

contributed to the taxonomy of the genus *Hesperis* and confirmed the importance of anatomical structures. Besides, TPC of *H. isatidea* extract was found as 109.66 mg GAE/g. The DPPH and ABTS radical scavenging activity of the extract was found as considerable high with 905.08 µg/mL and 534.58 µg/mL, respectively. The acetylcholinesterase inhibitory activity of the extract was found higher than butyrylcholinesterase inhibitory activity. In addition, the extract possessed moderate antityrosinase activity with mean value of 16.67 % at the 2 mg/ml concentration. The obtained results of enzyme inhibitory and *in vitro* antioxidant assays suggest that this plant may be important source for the development of new formulations with natural constituents for pharmaceutical preparations.

IV. REFERENCES

[1] S. Aras, A. Duran, G. Yenilmez, and D. C. Duman, "Genetic relationships among some *Hesperis* L. (Brassicaceae) species from Turkey assessed by RAPD analysis," *African Journal of Biotechnology*, vol. 8, pp. 3128–3134, 2009.

[2] A. Duran, "Two New Species With Pendulous Fruits in *Hesperis* (Brassicaceae) from South Anatolia, Turkey," *A Journal for Botanical Nomenclature*. vol. 18, pp. 453–463, 2008.

[3] A. E. Farouji, H. Khodayari, and M. Assadi, "Taxonomic significance of pollen and seed micromorphology in the genus *Hesperis* L. (Brassicaceae)," *Iranian Journal of Botany*. vol. 24, pp. 91–104, 2018.

[4] A. E. Farouji, H. Khodayari, M. Assadi, B. Özüdoğru, Ö. Çetin, K. Mummenhoff, and S. Bhattacharya," Numerical taxonomy contributes to delimitation of Iranian and Turkish *Hesperis* L. (brassicaceae) species," *Phytotaxa*, vol. 367, pp. 101–119, 2018.

[5] A. Duran and Ö. Çetin, "New species and a synonym of the genus *Hesperis* (Brassicaceae) from Turkey," *Turkish Journal of Botany*, vol. 40, pp. 87–96, 2016.

[6] J. Cullen, "*Hesperis* L.," in *Flora Turkey East Aegean Islands*, P.H. Davis,ed., Edinburgh, UK: Edinburgh University Press, 1965, vol. 1, pp. 452–460.

[7] A. Duran and A. Ocak, "*Hesperis turkmendaghensis* (sect. *Hesperis*) (Cruciferae/Brassicaceae), a new species from the Central Anatolia region, Turkey," *Botanical Journal of the Linnean Society*, vol. 147, pp. 239–247, 2005.

[8] O. Appel and I. A. Al-Shehbaz, Cruciferae. *Flowering Plants Dicotyledons*, Heidelberg, Berlin, Springer, 2003, pp. 75–174.

[9] D. A. German and I. A. Al-Shehbaz, "A reconsideration of *Pseudofortuynia* and *Tchihatchewia* as synonyms of sisymbrium and hesperis, respectively (Brassicaceae)," *Phytotaxa*, vol. 334, pp. 95–98, 2018.

[10] S. I. Warwick, K. Mummenhoff, C. A. Sauder, M. A. Koch, and I. A. Al-Shehbaz, "Closing the gaps: Phylogenetic relationships in the Brassicaceae based on DNA sequence data of nuclear ribosomal ITS," *Plant Systematics and Evolution*, vol. 285, pp. 209–232, 2010.

[11] E. Tuzlacı and A. Doğan, "Turkish folk medicinal plants, IX: Ovacık (Tunceli) IX," *Marmara Pharmaceutical Journal*, vol. 14, pp. 136–143, 2010.

[12] M. Korkmaz and E. Karakurt, "An ethnobotanical ınvestigation to determine plants used as folk medicine in Kelkit (Gümüşhane/Turkey) district," *Biological Diversity and Conservation*, vol. 8, pp. 290–303, 2015.

[13] N. Kirimer, S. Gökmen, B. Demirci, K. H. C. Baser, and A. Duran, "Volatile compounds of seeds of *Hesperis bicuspidata*, *H. bottae* and *H. podocarpa*,"*Journal of Essential Oil Research*, vol.

22, pp. 230–231, 2010.

[14] S. Ozlem Sener, M. Badem, S. Kanbolat, N. Korkmaz, U. Ozgen, R. Aliyazicioglu, S. A. Karaoglu, and S. Terzioglu, " The Therapeutic Potency of *Hesperis matronalis* subsp. *matronalis* for Alzheimer's and Microbial Diseases," *Süleyman Demirel Üniversitesi Sağlık Bilimleri Dergisi*, vol. 12, pp. 38–43, 2021.

[15] G. Clarke, K. N. Ting, C. Wiart, and J. Fry, "High correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the m," *Antioxidants*, vol. 2, pp. 1–10, 2013.

[16] H. Yang, Y. Dong, H. Du, H. Shi, Y. Peng, and X. Li, "Antioxidant compounds from propolis collected in Anhui, China," *Molecules*, vol. 16, pp. 3444–3455, 2011.

[17] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Free Radical Biology and Medicine*, vol. 26, pp. 1231–1237, 1999.

[18] S. H. Jeong, Y. B. Ryu, M. J. Curtis-Long, H. W. Ryu, Y. S. Baek, J. E. Kang, W. S. Lee, and K. H. Park, "Tyrosinase inhibitory polyphenols from roots of *Morus Ihou*," *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 1195–1203, 2009.

[19] Z. Yang, Y. Wang, Y. Weng, and Y. Zhang, "Bioassay-guided screening and isolation of α-glucosidase and tyrosinase inhibitors from leaves of *Morus alba*," *Food Chemistry*, vol. 131, pp. 617–625, 2012.

[20] G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, "A new and rapid colorimetric determination of acetylcholinesterase activity," *Biochemical Pharmacology*, vol. 7, pp. 88–95, 1961.

[21] G. Zengin, H. H. Abdallah, A. Dogan, A. Mollica, Z. Aumeeruddy-Elalfi, and M. F. Mahomoodally, "Phenolic components and assessment of biological properties of *Tchihatchewia isatidea* Boiss. extracts: Docking and functional approaches for designing novel products," *Food and Chemical Toxicology*, vol. 111, pp. 423–431, 2018.