

1 **Biological Screening of Traditional Medicinal Plants from Villages of**
2 **Akkuş (Ordu) in Turkey on the Effects of Tyrosinase**

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5 **Running Title:** Biological Screening of the plants in Akkuş District
6

7 **Abstract**

8 In the present study, ethnomedicinal uses of the plants naturally growing in 35
9 villages of Akkuş District have been determined. Informations about the uses of the
10 plants have been obtained from villagers using a questionnaire. At the end of the
11 identification of the plants collected from villages, 58 taxa belong to 32 families have
12 been determined. Furthermore, tyrosinase enzyme activity studies of methanolic
13 extracts of all the species carried out spectroscopically. Eventually, the plants has
14 been demonstrated to be used as folk medicine, food animal feed, ornamental, stuff,
15 dyeing, odour, insecticide and fishing. According to the biological screening studies,
16 some species have been indicated to have inhibitör effect, but the others have
17 activator effect on tyrosinase enzyme. Among the species, *Chaerophyllum*
18 *byzantinum* have been showed the highest tyrosinase inhibitör effect.
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21 **Key Words:** Akkuş, Biological Screening, Ethnomedicinal, Folk Medicine, Tyrosinase
22 Enzyme

23 **1. INTRODUCTION**

24 Melanin is known to be one of the major pigments for skin and hair color of
25 mammals. Melanin have been synthesized in differentiated cells such as
26 melanocytes in the skin, retinal epithelium, and central nervous system in mammals
27 (1-3).

28 Tyrosinase, being copper-containing in active site, is known to be a member of
29 the polyphenol oxidase enzyme family, which is an key enzyme in charge of melanin
30 biosynthesis. The tyrosinase enzyme inhibitors from natural resources have been

31 investigated by many researchers but there is not been enough studies on the
32 tyrosinase enzyme activators (3).

33 Defect of melanin synthesis have been associated with pigmentation disorders.
34 Decreasing of melanin level have been caused various skin diseases such as
35 psoriasis, vitiligo, because of hypopigmentation (4). Using the agents having
36 tyrosinase activator effect is an efficient approach for treatment of hypopigmentation
37 disorders. Hyperpigmentation knowned increasing production of melanin have been
38 induced some disorders like actinic damage, melasma, freckle and age-related stains
39 The tyrosinase inhibitors can be used as a remedy for these diseases (5).

40 Also, Tyrosinase inhibitors have been believed to be used to cure ailments
41 related to neurotoxicity like Parkinson. Tyrosinase have been give rise to
42 accumulating of oxide-dopamine derivatizations due to neuronal damage (6).

43 Ethnobotany is described as “the use of plants by the local population” by John
44 W. Harsberger and ethnobotanical studies are important to determine the relationship
45 between ethnobotany and plants (7). In our country, the interest in ethnobotanical
46 studies beginning in the early 19th century has been observed to be higher in recent
47 years (8).

48 Turkey, located at the junction of three different phytogeographic regions,
49 namely Mediterranean, Irano-Turanian, Euro-Siberian and has a rich flora (8). Turkey
50 is a leading country in terms of medicinal and endemic plants in the World (Lamond,
51 1978). It is known that about 10000 flowering and fern plant species has naturally
52 grown in Turkey, and 30% of them are endemic (10-12).

53 Besides, our country which hosted many civilizations has a rich cultural heritage
54 and a wealth of ethnobotanical datas (13). The traditional knowledge on plants and
55 their uses has been disappearing in recent years because of urbanization, increased
56 migration to urban areas, developments in technology, and health services easier
57 accessibility (14) Therefore, any information about uses of the plant in our daily life
58 should be recorded as soon as possible.

59 Ordu province has been located (40°18'-41°08' N, 36°52'-38°12' E) in Euro-
60 Siberian phytogeographic region (15). However There are the plenty of studies on
61 the folk medicine in the Black Sea region, some of which is ethnobotanical research
62 about Ordu, any study in Akkus district has not been found (9, 14, 16-24). Also, the
63 effects of tyrosinase enzyme of the plants naturally growing in 35 villages of Akkuş
64 District and used as folk medicine have been explored for the first time in this study.

65 **2. MATERIAL and METHODS**

66 **2.1. Field Trips**

67 The materials of our research includes plant samples collected from 35 villages
68 of Akkus District of Ordu province in Turkey. Plant samples were obtained by visiting
69 the research area twice between July and September 2016. A questionnaire form
70 (name, surname, age, telephone numbers, local names of plants, parts used,
71 preparation methods and the purpose of using plants) was prepared for the
72 participants. The participants were visited at their homes and asked to them to fill out
73 the questionnaire form. After each interview, plant samples were collected with
74 assistance from the participants.

75 **2.2. Study Area**

76 Akkus is located in the inner parts of the Middle Black Sea in Turkey. The height
77 of the sea is 1340 meters and has a rough land (Fig 1). The Akkus region includes 6
78 towns, 35 villages and 15 neighborhoods. Continental climate prevails in the region
79 (25).

80 **2.3. Identification of Plants**

81 The plant samples collected from the research area were identified using
82 references, i.e. Flora of Turkey and the East Aegean Islands (11, 26, 27).
83 Identifications were performed by two of the authors (MB and UO). The dried plant
84 samples are deposited at the Herbarium of the Faculty of Pharmacy of Ankara
85 University.

86 **2.4. Studies on the Tyrosinase Enzyme**

87 **2.4.1. Chemical Materials Used in Experiments**

88 Methanol (CH₃OH-Merck, 106009), Tyrosinase (Sigma, T3824-25KU), kojic
89 acid (Sigma, K3125-5G), potassium dihydrogen phosphate (NaH₂PO₄-Sigma Aldrich,
90 7558-80-7), disodium hydrogen phosphate anhydride (Na₂HPO₄-Sigma Aldrich,
91 10039-32-4), L-DOPA (Sigma, D9628-5G) and 8-Methoxsalen (Roth, 5497.2) were
92 used in the experiments.

93 **2.4.2. Preparation of the Extract**

94 Methanol extract (10 mg/mL) of each species from Akkus was prepared. Diluted
95 samples were obtained from methanol extract with potassium phosphate buffer (pH
96 6.8) at concentrations of 25, 50, 100 and 500 µg / mL.

97 **2.4.3. Tyrosinase Enzyme Inhibition**

98 Tyrosinase inhibitory activity was determined employing various concentrations
99 of kojic acid solutions as standard (28). Tyrosinase solution (46 U/mL), methanolic
100 plant extract (500, 100, 50 and 25 µg/mL) were prepared. 120 µL of 0.2 M phosphate
101 buffer (pH 7.0), and 40 µL tyrosinase solution for A wells; 160 µL of 0.2 M phosphate
102 buffer (pH 6.8) for B wells; 80 µL of 0.2 M phosphate buffer (pH 6.8), 40 µL
103 tyrosinase solution and 40 µL sample solution for C wells; 120 µL of 0.2 M phosphate
104 buffer (pH 7.0) and 40 µL sample solution for D wells were added and mixed in a 96-
105 well plate and incubated for 10 min at 23 °C. Then, 2,5 mM L-DOPA solution (40 µL)
106 was added to all wells and incubated for 10 min at 23 °C. The absorbance of the
107 reaction mixture was determined at 490 nm using the spectrophotometric method in
108 a microplate reader. The percentage of tyrosinase inhibitory activity was calculated
109 using the formula follows:

$$110 \quad \% \text{ Inhibition} = \frac{[(A-B)-(C-D)]}{(A-B)} \times 100$$

111 The results were given as IC₅₀ levels.

112 **2.4.4. Tyrosinase Enzyme Activation**

113 Tyrosinase Enzyme Activation was determined employing various
114 concentrations of 8-Methoxsalen (8-MOP) solutions as standard (29). Tyrosinase

115 solution (46 U/mL), methonolic plant extract (500, 100, 50 and 25 µg/mL) were
 116 prepared. 120 µL of 0.2 M phosphate buffer (pH 7.0), and 40 µL tyrosinase solution
 117 for A wells; 160 µL of 0.2 M phosphate buffer (pH 6.8) for B wells; 80 µL of 0.2 M
 118 phosphate buffer (pH 6.8), 40 µL tyrosinase solution and 40 µL sample solution for C
 119 wells; 120 µL of 0.2 M phosphate buffer (pH 7.0) and 40 µL sample solution for D
 120 wells were added and mixed in a 96-well plate and incubated for 10 min at 23 °C.
 121 Then, 2.5 mM L-DOPA solution (40 µL) was added to all wells and incubated for 10
 122 min at 23 °C. The absorbance of the reaction mixture was determined at 490 nm
 123 using the spectrophotometric method in a microplate reader. The percentage of
 124 tyrosinase enzyme activation was calculated using the formula follows:

$$125 \quad \% \text{ Activation} = \frac{[(A-B)-(C-D)]}{(A-B)} \times 100$$

126 The results were given as AC₅₀ levels.

127 3. RESULTS AND DISCUSSION

128 3.1. The Results of Ethnobotanical Studies

129 The plants naturally growing in 35 villages of Akkuş District has been showed to
 130 be used as folk medicine (30 taxa), food (21 taxa), animal feed (8 taxa), ornamental
 131 (5 taxa), stuff (5 taxa), dyeing (4 taxa), odour (1 taxa), insecticide (1 taxa), and
 132 fishing (4 taxa). The informations from the ethnobotanical studies have been included
 133 in Table 1.

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135

136 **Table 1. Species used in Akkuş villages**

Family, Plant Species, Herbarium Nunmer	Local name	Plant Part(S) Used	Uses	Administration Ways
Asteraceae				
<i>Anthemis cotula</i> (AEF 26978)	Papatya	Aerial Parts	Asthma, Cold, and Alopecia	Boiled in water and used up as hot drink, also used as vapour
<i>Anthemis tinctoria</i> var. <i>tinctoria</i> (AEF 26972)	Sarı papatya	Aerial Parts	Dyeing Food	Dyes wools to yellow for wool Consumed as tea
<i>Bellis perennis</i> (AEF 26979)	Küçük papatya	Aerial Parts	Stomach ache Sinusitis	Boiled in water and drunk Used as vapour
<i>Cirsium arvense</i> (AEF 26981)	Köygöçüren		Food	Consumed as meal

<i>Tanacetum parthenium</i> (AEF 26977)	Papatya	Aerial Parts	Alopecia	Boiled in water and drunk
<i>Tussilago farfara</i> (AEF 26884)	Öksürük otu	Flowers Leaf	Breathless Bronchitis	Boiled and used as vapour
<i>Helichrysum compactum</i> (AEF 26823)	Yayla çiçeği	Herba	Earache Ornamental	Boiled and the water is dropped to ear Used as odour
Amaranthaceae				
<i>Amaranthus albus</i> (AEF 26904)	Hoşkırın, hoşberin	Herba	Food	Consumed as meal Roasted and mixed to yoghurt or consumed as soup
Boraginaceae				
<i>Trachystemon orientalis</i> (AEF 26966)	Kaldırık	Leaf	Food	Consumed as meal and pickle
Caprifoliaceae				
<i>Sambucus ebulus</i> (AEF 26890)	Yivdin, mürver	Fruit Leaf And Fruit	Dyeing Food Rheumatic diseases	Boiled and used to dye Eaten as fresh leaf Cooked or crushed and then applied to aching area
Caryophyllaceae				
<i>Dianthus carmelitarum</i> (AEF 26822)			Ornamental	Used as Ornamental plant
<i>Silene compacta</i> (AEF 26888)			Ornamental	Used as ornamental plant
<i>Silene vulgaris var. vulgaris</i> (AEF 26891)	Gıcırık otu, gırşılık, düdüklük otu	Leaf Herba	food Stuff	Consumed as meal, Roasted and mixed to yoghurt Used to make caval
Chenopodiaceae				
<i>Chenopodium album</i> (AEF 26902)	Küllüce, sirken	Herba	Food	Roasted and eaten
Convolvulaceae				
<i>Convolvulus arvensis</i> (AEF 26817)	Sarmaşık	Herba	Farming	Used as animal feed
Cruciferae				
<i>Capsella bursa-pastoris</i> (AEF 26895)	Kuş pancarı	Herba	Food	Roasted and eaten
Cornaceae				
<i>Cornus mas</i> (AEF 26897)	Kızılcık	Fruit	Food	Boiled in water and drunk, Consumed as marmalade
Euphorbiaceae				
<i>Euphorbia sp.</i> (AEF 26903)	Sütotu, akkapla	Latex Herba	Alopecia Farming	Latex is applied to alopesic area Used as animal feed
Ericaceae				
<i>Vaccinium arctostaphylos</i> (AEF 26969)	Yaban gülü		Stuff	Used to make whisk
Fabaceae				
<i>Coronilla cretica</i> (AEF 26982)	Fiğ otu	Herba	Farming	Used as animal feed
<i>Coronilla varia</i> (AEF 26886)	Yabani korunga	Herba	Farming	Used as animal feed
<i>Lathyrus aureus</i> (AEF26819)	Karanfil		Mouth sore	
<i>Lathyrus sp.</i> (AEF 26974)	Eşek palası	Leaf	Food	Consumed as meal

<i>Vicia sativa</i> (AEF 26973)	Yabancı fiğ	Herba	Farming	Used as animal feed
Fagaceae				
<i>Fagus orientalis</i> (AEF 266892)	Kayın	Seed	Anxiolytic	
<i>Quercus cerris</i> var. <i>cerris</i> (AEF 26879)	Meşe	Shoot	Stuff	Used to make hamper
<i>Quercus infectoria</i> (AEF 26881)	Meşe	Shoot	Stuff	Used to make hamper
Hypericaceae				
<i>Hypericum perforatum</i> (AEF 26820)	Kantaron, bitki otu	Herba	Sedative Antitussive	Drunk as tea Drunk as tea freshly
Hypolepidaceae				
<i>Pteridium aquilinum</i> (AEF 26975)	Kızılot, mayasıl, eğrelti	Herba	Antihemorrhoid	Boiled and wrapped up waist, also sit on vapour
Juglandaceae				
<i>Juglans regia</i> (AEF 26880)	Ceviz	Fruit	Dyeing	Used to dye rugs
Lamiaceae				
<i>Mentha longifolia</i> (AEF 26887)	Narpus	Herba	Cold	Consumed as tea
<i>Mentha sp</i> (AEF 26951)	Nane, narpus	Leaf	Food Dyspepsia	Consumed as spice Consumed as tea
<i>Origanum vulgare</i> (AEF 26907)	Kekik, kaba topağı	Fruit Herba	Food Dyspepsia Food	Consumed as spice Consumed as tea
<i>Prunella orientalis</i> (AEF 26824)	Çay otu, karabaş otu	Herba	Cold Antitussive	Consumed as tea
<i>Salvia verticillata</i> var. <i>verticillata</i> (AEF 26900)	Karacaabla	Herba Flower	Farming Dyeing	Used as animal feed Used to dye purple
<i>Stachys byzantina</i> (AEF 26883)		Leaf	Stuff	Used as hanky
Loranthaceae				
<i>Viscum album</i> var. <i>album</i> (AEF 26906)	Çam gökçesi, gökçe otu	Leaf	Treatment of renal disorders Farming	Boiled and eaten Used as animal feed
Malvaceae				
<i>Malva neglecta</i> (AEF 26950)	Kömeç, ebegümeci	Herba	Anti-inflamatuar anticanser Urinary tract infection Laxative Analgesic	Roasted and eaten, also consumed as tea

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Papaveraceae				
<i>Papaver rhoeas</i> (AEF 26967)	Gelincik		Ornamental	Used as ornamental plant
Pinaceae				
<i>Pinus sylvestris</i> (AEF 26901)	Çam	Shoot Fruit Resin	Asthma, breathless, bronschitis, Food Wound healing Stomache ache	Shoots are eaten after peeled off Fruits kept with sugar for a week and boiled in water, consumed as jam Applied to wound Consumed by chewing
Plantaginaceae				

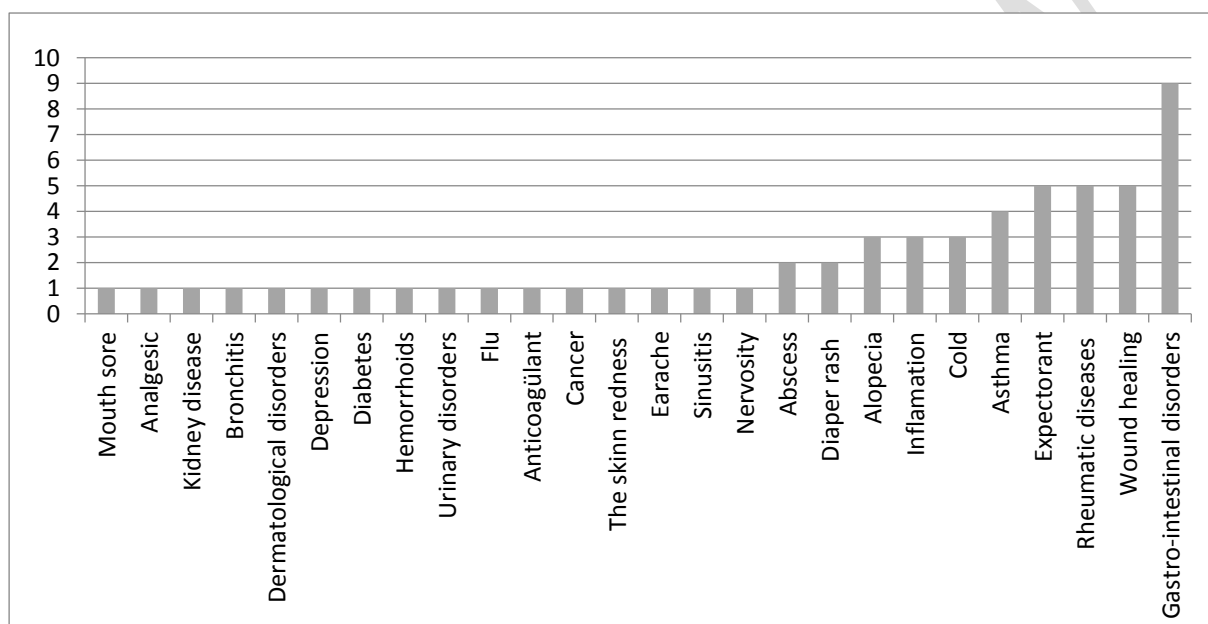
<i>Plantago major</i> var. <i>major</i> (AEF 26885)	Sinir otu, siğil otu, kesik otu, sinirli yaprak, yara otu	Leaf	Antihemorrhagic Antitussive Antidiabetic Antirheumatic Anti-inflamatuar	Dried, powdered and applied to wound and incision by wrapping Consumed as tea Haeted slightly and wrapped Boiled in water and consumed as tea
Platanaceae				
<i>Platanus orientalis</i> (AEF 26894)	Çınar	Leaf	antirheumatic	Consumed as tea
Polygonaceae				
<i>Polygonum convolvulus</i> (AEF 26976)	Perzi	Leaf	Food	Consumed as meal
<i>Polygonum persicaria</i> (AEF 26984)	Biber otu	Leaf Herba	Fishing Farming	Used to fishing Used as animal feed
<i>Rumex acetosella</i> (AEF 26968)	Acumuk, kuzukulağı	Leaf Herba	Food Insecticide	Eaten freshly Used for fleas
<i>Rumex patientia</i> (AEF 26971)	Efelik	Leaf Stem	Analgesic for aching of knee, to cure itching, and skin disorders Food Food	Boiled and wrapped on knee, Kaynatılarak dize sarılır, treated for itching because of nettle Consumed as meal Eaten after peeling off
Primulaceae				
<i>Lysimachia verticillaris</i> (AEF 26821)			Ornamental	Used as ornamental plant
Rhamnaceae				
<i>Frangula alnus</i> (AEF 26965)	Çeti ağacı	Shoot	To treatment rash, wound, and burn Antiinflammatory	Consumed as tea, applied to wound, infected area
Rosaceae				
<i>Agrimonia eupatoria</i> (AEF 26905)	Mikbaşı	Leaf	Dyspepsia	Roasted and eaten
<i>Crataegus stevenii</i> (AEF 26897)	Kuş diken	Fruit	Food	Consumed as jam
<i>Mespilus germanica</i> (AEF 26889)	Töngel, muşmula	Stem Shoot And Stem	Dyspesia and cold Antitussive	Boiled and consumed as tea Boiled and consumed as tea
<i>Pyrus sp.</i> (AEF 26899)	Armut ağacı	Herba	Stuff	Used to make spoon
<i>Rubus ideaus</i> (AEF 26983)	Böğürtlen	Stem Fruit	To treatment stomachaches and antiinflammatory Food	Consumed as jam

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Scrophulariaceae				
<i>Verbascum pyramidatum</i> (AEF 26980)	Siğir kuyruğu, küçük kabalak	Herba	Food	Eaten after peelinf off
Tiliaceae				
<i>Tilia platyphillos</i> (AEF 26896)	İhlamur	Flowers	Antitussive and to treatment stomachache	Consumed as tea
Umbelliferae				

<i>Chaerophyllum byzantinum</i> (AEF 26970)	Baldıran	Herba	Food	Boiled and the water is drunk
Urticaceae				
<i>Urtica dioica</i> (AEF 26882)	Sırgan otu, ısırgan	Herba	Food To treatment knee ache	Consumed as meal and soup Heated and wrapped up aching area
Vitaceae				
<i>Vitis vinifera</i> (AEF 26893)	Asma yaprağı, üzüm yaprağı	Leaf	Food	Consumed as meal

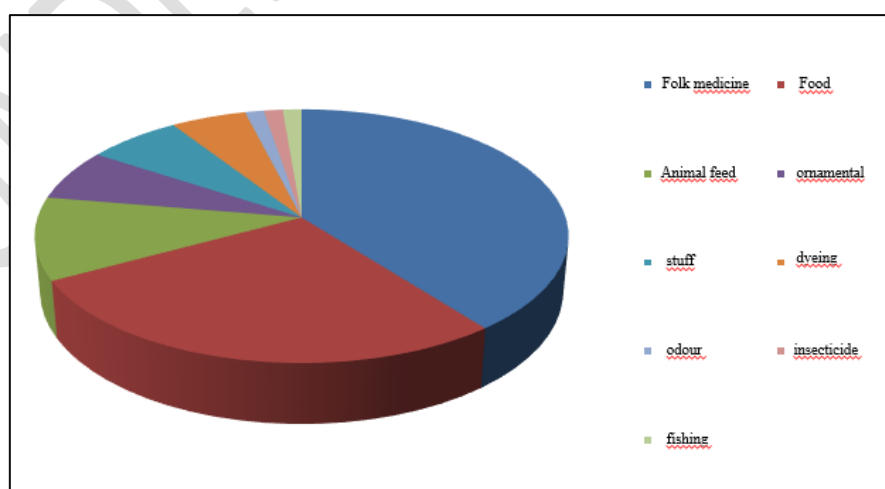
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141 **Fig 1. Ethnomedicinal usages of the plants in Akkuş**

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144 **Fig 2. Classification of plants collected from Akkuş according to their usage**

145 **3.2. The Results of Biological Screening Studies**

146 According to the biological screening studies, some species collected Akkuş
 147 district have been evidenced to have inhibitör effect, and that all the species don't
 148 have activator effect on tyrosinase enzyme. The results of the studies have been
 149 sum up in Table 2. In the tyrosinase enzyme inhibition and activation studies, the
 150 results of the methanol extracts of the species have been compared with kojic acid
 151 for tyrosinase inhibition and 8-MOP for tyrosinase activation used as positive
 152 control. Among the species, *Chaerophyllum byzantinum* have been showed the
 153 highest tyrosinase inhibitor effect.

154 **Table 2. The effects on tyrosinase enzyme of species used in Akkuş villages**

Tyrosinase Inhibitor Effective species	
IC₅₀ (µg/mL)	
<i>Chaerophyllum byzantinum</i>	25,60
<i>Vaccinium arctostaphylos</i>	103,75
<i>Trachystemon orientalis</i>	133,98
<i>Hypericum perforatum</i>	148,70
<i>Rumex acetosella</i>	270,62
<i>Capsella bursa-pastoris</i>	425,37
Kojic acid	3,482
Tyrosinase Activator Effective Species	
AC₅₀ (µg/mL)	
8-MOP	17,162

155 There are the plenty of studies on the folk medicine in the Black Sea region,
 156 some of which is ethnobotanical research about Ordu (16-24), but any study in Akkus
 157 district has not been found. Also, the effects of tyrosinase enzyme of the plants
 158 naturally growing in 35 villages of Akkuş District and used as folk medicine have
 159 been explored for the first time in this study.

160 200 plants have been collected from Akkuş and its villages for the present
 161 study. At the end of the identification of the plants collected from villages, 58 taxa
 162 belong to 32 families have been determined [Asteraceae (7 taxa), Amaranthaceae (1
 163 taxa), Boraginaceae (1 taxa), Caprifoliaceae (1 taxa), Caryophyllaceae (3),
 164 Chenopodiaceae (1), Convolvulaceae (1), Cruciferae (1 taxa), Cornaceae (1 taxa),
 165 Euphorbiaceae (1 taxa), Ericaceae (1 taxa), Fabaceae (5 taxa), Fagaceae (3 taxa),
 166 Hypericaceae (1 taxa), Hypolepidaceae (1 taxa), Juglandaceae (1 taxa), Lamiaceae
 167 (6 taxa), Loranthaceae (1 taxa), Malvaceae (1 taxa), Papaveraceae (1 taxa),
 168 Pinaceae (1 taxa), Plantaginaceae (1 taxa), Platanaceae (1 taxa), Polygonaceae (4
 169 taxa), Primulaceae (1 taxa), Rhamnaceae (1 taxa), Rosaceae (5 taxa),

170 Scrophulariaceae (1 taxa), Tiliceae (1 taxa), Umbelliferae (1 taxa), Urticaceae (1
171 taxa), Vitaceae (1 taxa)].

172 The most popular species in the district for medical uses are *Plantago major*
173 var. *major*, *Pinus sylvestris*, *Malva neglecta*. The species from Akkuş have been
174 used mostly for treatment of cough, rheumatic diseases and wounds, traditionally.

175 *C. cretica*, *V. pyramidatum*, *P. convolvulus*, *C. album*, *T. farfara*, *A. albus*, *S.*
176 *verticillata* var. *verticillata*, *P. major* var. *major*, *P. orientalis*, *P. persicaria*, *M.*
177 *longifolia*, *S. byzantina*, *A. eupatoria*, *O. vulgare*, *D. carmelitarum*, *F. Alnus*, *C. varia*,
178 *P. orientalis*, *Q. infectoria*, *U. dioica*, *H. compactum*, *M. neglecta*, *T. parthenium*, *S.*
179 *ebulus*, *R. ideaus*, *V. album* var. *album*, *B. perennis*, *A. tinctoria* var. *tinctoria*, and *A.*
180 *cotula* from the collected species have been showed to have tyrosinase inhibitory
181 effect; but the results were not significant. Because the IC₅₀ values of them haven't
182 been our detection range.

183 As shown in Table 2; *T. orientalis*, *C. bursa-pastoris*, *V. arctostaphylos*, *R.*
184 *acetosella*, *C. byzantinum*, and *H. perforatum* have been caused to inhibition on
185 tyrosinase enzyme. In view of values of IC₅₀ of the methanolic extracts of the plants,
186 *C. byzantinum* have been indicated to have the best effect on the enzyme. Besides,
187 *P. aquilinum*, *C. arvensis*, *S. compacta*, *F. orientalis*, *T. platyphllos*, *Mentha* sp., *S.*
188 *vulgaris* var. *vulgaris*, *C. arvensis*, *P. rhoeas*, *R. patientia*, *P. Sylvestris* and *C. mas*
189 have been detected to have tyrosinase activator effect; however the values of IC₅₀ of
190 them were greater than 1000 µg/mL so the results were not significant.

191 Compared with previous studies carried in neighborhood, the similar datas
192 have been obtained in terms of ethnomedicinal studies. Through the study, it has
193 been determined the cultural interactions between the human and plant in Akkuş
194 district by identifying the plants used by villagers, the ethnomedicinal properties, their
195 usages and local names for the first time. In addition, the effects of tyrosinase
196 enzyme of collected plant have been carried out a preliminary study in terms of
197 potential usages of the species for skin disorders and the neurodegenerative
198 damages.

199 **Conflict of Interest**

200 The authors declare that there are no conflicts of interest

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