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

Running Title: Biological Screening of the plants in Akkuş Distrinct

Abstract

In the present study, ethnomedicinal uses of the plants naturally growing in 35 villages of Akkuş District have been determined. Informations about the uses of the plants have been obtained from villagers using a questionnaire. At the end of the identification of the plants collected from villages, 58 taxa belong to 32 families have been determined. Furthermore, tyrosinase enzyme activity studies of methanolic extracts of all the species carried out spectroscopicallly. Eventually, the plants has been demonstrated to be used as folk medicine, food animal feed, ornamental, stuff, dyeing, odour, insecticide and fishing. According to the biological screening studies, some species have been indicated to have inhibitör effect, but the others have activator effect on tyrosinase enzyme. Among the species, *Chaerophyllum byzantinum* have been showed the highest tyrosinase inhibitör effect.

Key Words: Akkuş, Biological Screning, Ethnomedicinal, Folk Medicine, Tyrosinase Enzyme

1. INTRODUCTION

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

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

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Pehlivan M, Sevindik M. Antioxidant and Antimicrobial Activities of Salvia multicaulis. Turkish Journal of Agriculture-Food Science and Technology, 2018; 6(5), 628-631.

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

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

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

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

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

Besides, our country which hosted many civilizations has a rich cultural heritage and a wealth of ethnobotanical datas (13). The traditional knowledge on plants and their uses has been disappearing in recent years because of urbanization, increased migration to urban areas, developments in technology, and health services easier accessibility (14-16). Therefore, any information about uses of the plant in our daily life should be recorded as soon as possible.

Ordu province has been located (40°18'-41°08' N, 36°52'-38°12' E) in Euro-Siberian phytogeographic region (~~15~~17). However There are the plenty of studies on the folk medicine in the Black Sea region, some of which is ethnobotanical research about Ordu, any study in Akkus district has not been found (9, 14, ~~16~~18-~~24~~26). Also, the effects of tyrosinase enzyme of the plants naturally growing in 35 villages of Akkuş District and used as folk medicine have been explored for the first time in this study.

2. MATERİAL and METHODS

2.1. Field Trips

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

2.2. Study Area

Akkus is located in the inner parts of the Middle Black Sea in Turkey. The height of the sea is 1340 meters and has a rough land (Fig 1). The Akkus region includes 6 towns, 35 villages and 15 neighborhoods. Continental climate prevails in the region (~~25~~27).

2.3. Identification of Plants

The plant samples collected from the research area were identified using references, i.e. Flora of Turkey and the East Aegean Islands (11, ~~26~~28, ~~27~~29). Identifications were performed by two of the authors (MB and UO). The dried plant samples are deposited at the Herbarium of the Faculty of Pharmacy of Ankara University.

2.4. Studies on the Tyrosinase Enzyme

2.4.1. Chemical Materials Used in Experiments

Methanol (CH_3OH-Merck, 106009), Tyrosinase (Sigma, T3824-25KU), kojic acid (Sigma, K3125-5G), potassium dihydrogen phosphate (NaH_2PO_4-Sigma Aldrich, 7558-80-7), disodium hydrogen phosphate anhydride (Na_2HPO_4-Sigma Aldrich, 10039-32-4), L-DOPA (Sigma, D9628-5G) and 8-Methoxsalen (Roth, 5497.2) were used in the experiments.

2.4.2. Preparation of the Extract

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

2.4.3. Tyrosinase Enzyme Inhibition

Tyrosinase inhibitory activity was determined employing various concentrations of kojic acid solutions as standard (~~28~~30). Tyrosinase solution (46 U/mL), methonolic plant extract (500, 100, 50 and 25 µg/mL) were prepared. 120 µL of 0.2 M phosphate buffer (pH 7.0), and 40 µL tyrosinase solution for A wells; 160 µL of 0.2 M phosphate buffer (pH 6.8) for B wells; 80 µL of 0.2 M phosphate buffer (pH 6.8), 40 µL tyrosinase solution and 40 µL sample solution for C wells; 120 µL of 0.2 M phosphate buffer (pH 7.0) and 40 µL sample solution for D wells were added and mixed in a 96-well plate and incubated for 10 min at 23 °C. Then, 2,5 mM L-DOPA solution (40 µL) was added to all wells and incubated for 10 min at 23 °C. The absorbance of the reaction mixture was determined at 490 nm using the spectrophotometric method in a microplate reader. The percentage of tyrosinase inhibitory activity was calculated using the formula follows:

% Inhibition = [[(A-B)-(C-D)] / (A-B)] x 100

The results were given as IC_{50} levels.

2.4.4. Tyrosinase Enzyme Activation

Tyrosinase Enzyme Activation was determined employing various concentrations of 8-Methoxsalen (8-MOP) solutions as standard (~~29~~31). Tyrosinase solution (46 U/mL), methonolic plant extract (500, 100, 50 and 25 µg/mL) were prepared. 120 µL of 0.2 M phosphate buffer (pH 7.0), and 40 µL tyrosinase solution for A wells; 160 µL of 0.2 M phosphate buffer (pH 6.8) for B wells; 80 µL of 0.2 M phosphate buffer (pH 6.8), 40 µL tyrosinase solution and 40 µL sample solution for C wells; 120 µL of 0.2 M phosphate buffer (pH 7.0) and 40 µL sample solution for D wells were added and mixed in a 96-well plate and incubated for 10 min at 23 °C. Then, 2.5 mM L-DOPA solution (40 µL) was added to all wells and incubated for 10 min at 23 °C. The absorbance of the reaction mixture was determined at 490 nm using the spectrophotometric method in a microplate reader. The percentage of tyrosinase enzyme activation was calculated using the formula follows:

% Activation = [[(A-B)-(C-D)] / (A-B)] x 100

The results were given as AC_{50} levels.

3. RESULTS AND DISCUSSION

3.1.The Results of Ethnobotanical Studies

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

Table 1. Species used in Akkuş villages

Family, Plant Species, Herbarium Nunmer	Local name	Plant Part(S) Used	Uses	Administration Ways
Asteraceae				
Anthemis cotula (AEF 26978)	Papatya	Aerial Parts	Asthma, Cold, and Alopecia	Boiled in water and used up as hot drink, also used as vapour
~~*Anthemis*~~ *A. tinctoria* var. *tinctoria* (AEF 26972)	Sarı papatya	Aerial Parts	Dyeing Food	Dyes wools to yellow for wool Consumed as tea

Bellis perennis (AEF 26979)	Küçük papatya	Aerial Parts	Stomach ache Sinusitis	Boiled in water and drunk Used as vapour
Cirsium arvense (AEF 26981)	Köygöçüren		Food	Consumed as meal
Tanacetum parthenium (AEF 26977)	Papatya	Aerial Parts	Alopecia	Boiled in water and drunk
Tussilago farfara (AEF 26884)	Öksürük otu	Flowers Leaf	Breathless Bronchitis	Boiled and used as vapour
Helichrysum compactum (AEF 26823)	Yayla çiçeği	Herba	Earache Ornamental	Boiled and the water is dropped to ear Used as odour
Amaranthaceae				
Amaranthus albus (AEF 26904)	Hoşkıran, hoşberin	Herba	Food	Consumed as meal Roasted and mixed to yoghurt or consumed as soup
Boraginaceae				
Trachystemon orientalis (AEF 26966)	Kaldırık	Leaf	Food	Consumed as meal and pickle
Caprifoliaceae				
Sambucus ebulus (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				
Dianthus carmelitarum (AEF 26822)			Ornamental	Used as Ornamental plant
Silene compacta (AEF 26888)			Ornamental	Used as ornamental plant
~~*Silene*~~ *S. vulgaris var. vulgaris (AEF 26891)*	Gıcırık otu, gırşılık, düdüklük otu	Leaf Herba	food Stuff	Consumed as meal, Roasted and mixed to yoghurt Used to make caval
Chenopodiaceae				
Chenopodium album (AEF 26902)	Küllüce, sirken	Herba	Food	Roasted and eaten
Convolvulaceae				
Convolvulus arvensis (AEF 26817)	Sarmaşık	Herba	Farming	Used as animal feed
Cruciferae				
Capsella bursa-pastoris (AEF 26895)	Kuş pancarı	Herba	Food	Roasted and eaten
Cornaceae				
Cornus mas (AEF 26897)	Kızılcık	Fruit	Food	Bolied in water and drunk, Consumed as marmalade
Euphorbiaceae				
Euphorbia sp. (AEF 26903)	Sütotu, akkapla	Latex Herba	Alopecia Farming	Latex is applied to alopesic area Used as animal feed
Ericaceae				
Vaccinium arctostaphylos (AEF 26969)	Yaban gülü		Stuff	Used to make whisk
Fabaceae				
Coronilla cretica (AEF 26982)	Fiğ otu	Herba	Farming	Used as animal feed

Coronilla varia (AEF 26886)	Yabani korunga	Herba	Farming	Used as animal feed
Lathyrus aureus (AEF26819)	Karanfil		Mouth sore	
Lathyrus sp. (AEF 26974)	Eşek palası	Leaf	Food	Consumed as meal
Vicia sativa (AEF 26973)	Yabancı fiğ	Herba	Farming	Used as animal feed
Fagaceae				
Fagus orientalis (AEF 266892)	Kayın	Seed	Anxiolytic	
Quercus cerris var. cerris (AEF 26879)	Meşe	Shoot	Stuff	Used to make hamper
Quercus infectoria (AEF 26881)	Meşe	Shoot	Stuff	Used to make hamper
Hypericaceae				
Hypericum perforatum (AEF 26820)	Kantaron, bitki otu	Herba	Sedative Antitussive	Drunk as tea Drunk as tea freshly
Hypolepidaceae				
Pteridium aquilinum (AEF 26975)	Kızılot, mayasıl, eğrelti	Herba	Antihemorrhoid	Boiled and wrapped up waist, also sit on vapour
Juglandaceae				
Juglans regia (AEF 26880)	Ceviz	Fruit	Dyeing	Used to dye rugs
Lamiaceae				
Mentha longifolia (AEF 26887)	Narpus	Herba	Cold	Consumed as tea
Mentha sp (AEF 26951)	Nane, narpus	Leaf	Food Dyspepsia	Consumed as spice Consumed as tea
Origanum vulgare (AEF 26907)	Kekik, kaba topağı	Fruit Herba	Food Dyspepsia Food	Consumed as spice Consumed as tea
Prunella orientalis (AEF 26824)	Çay otu, karabaş otu	Herba	Cold Antitussive	Consumed as tea
Salvia verticillata var. verticillata (AEF 26900)	Karacaabla	Herba Flower	Farming Dyeing	Used as animal feed Used to dye purple
Stachys byzantina (AEF 26883)		Leaf	Stuff	Used as hanky
Loranthaceae				
Viscum album var. album (AEF 26906)	Çam gökçesi, gökçe otu	Leaf	Treatment of renal disorders Farming	Boiled and eaten Used as animal feed
Malvaceae				
Malva neglecta (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				
Papaver rhoeas (AEF 26967)	Gelincik		Ornamental	Used as ornamental plant
Pinaceae				

Pinus sylvestris *(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				
Plantago major var. major *(AEF 26885)*	Sinir otu, siğil otu, kesik otu, sinirli yaprak, yara otu	Leaf	Antihemorragic Antitussive Antidiabetic Antirheumatic Anti-inflamatuar	Dried, powdered and apllied to wound and incision by wrapping Consumed as tea Haeted slightly and wrapped Boiled in water and consumed as tea
Platanaceae				
Platanus orientalis *(AEF 26894)*	Çınar	Leaf	antirheumatic	Consumed as tea
Polygonaceae				
Polygonum convolvulus *(AEF 26976)*	Perzi	Leaf	Food	Consumed as meal
~~*Polygonum*~~ *P. persicaria* *(AEF 26984)*	Biber otu	Leaf Herba	Fishing Farming	Used to fishing Used as animal feed
Rumex acetosella *(AEF 26968)*	Acumuk, kuzukulağı	Leaf Herba	Food Insecticide	Eaten freshly Used for fleas
~~*Rumex*~~ *R. patientia* *(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				
Lysimachia verticillaris *(AEF 26821)*			Ornamental	Used as ornamental plant
Rhamnaceae				
Frangula alnus *(AEF 26965)*	Çeti ağacı	Shoot	To treatment rash, wound, and burn Antiinflamatory	Consumed as tea, applied to wound, infected area
Rosaceae				
Agrimonia eupatoria *(AEF 26905)*	Mikbaşı	Leaf	Dyspepsia	Roasted and eaten
Crataegus stevenii *(AEF 26897)*	Kuş dikeni	Fruit	Food	Consumed as jam
Mespilus germanica *(AEF 26889)*	Töngel, muşmula	Stem Shoot And Stem	Dyspesia and cold Antitussive	Boiled and consumed as tea Boiled and consumed as tea
Pyrus sp. *(AEF 26899)*	Armut ağacı	Herba	Stuff	Used to make spoon
Rubus ideaus *(AEF 26983)*	Böğürtlen	Stem Fruit	To treatment stomachaches and antiinflamatory Food	 Consumed as jam

Scrophulariaceae				

Verbascum pyramidatum (AEF 26980)	Sığır kuyruğu, küçük kabalak	Herba	Food	Eaten after peelinf off
Tiliaceae				
Tilia platyphllos (AEF 26896)	Ihlamur	Flowers	Antitussive and to treatment stomachache	Consumed as tea
Umbelliferae				
Chaerophyllum byzantinum (AEF 26970)	Baldıran	Herba	Food	Boiled and the water is drunk
Urticaceae				
Urtica dioica (AEF 26882)	Sırgan otu, ısırgan	Herba	Food To treatment knee ache	Consumed as meal and soup Heated and wrapped up aching area
Vitaceae				
Vitis vinifera (AEF 26893)	Asma yaprağı, üzüm yaprağı	Leaf	Food	Consumed as meal

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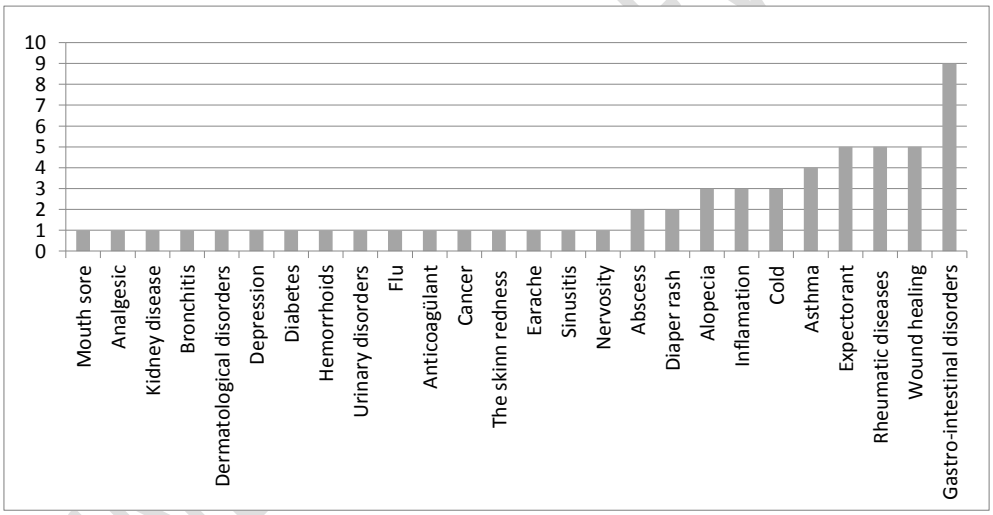

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142 **Fig 1. Ethnomedicinal usages of the plants in Akkuş**

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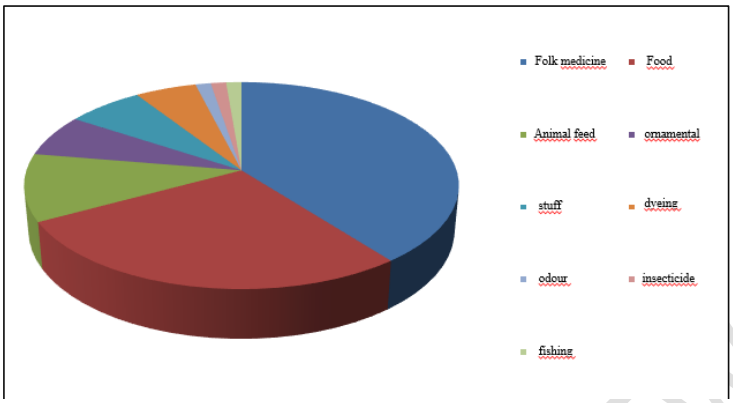

Fig 2. Classification of plants collected from Akkuş according to their usage

3.2. The Results of Biological Screening Studies

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

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

Tyrosinase Inhibitor Effective species IC_{50} (µg/mL)	
Chaerophyllum byzantinum	25±.60
Vaccinium arctostaphylos	103±.75
Trachystemon orientalis	133±.98
Hypericum perforatum	148±.70
Rumex acetosella	270±.62
Capsella bursa-pastoris	425±.37
Kojic acid	3±.482
Tyrosinase Activator Effective Species AC_{50} (µg/mL)	
8-MOP	17±.162

There are the plenty of studies on the folk medicine in the Black Sea region, some of which is ethnobotanical research about Ordu (~~16~~18-~~24~~26), but any study in Akkus district has not been found. Also, the effects of tyrosinase enzyme of the

plants naturally growing in 35 villages of Akkuş District and used as folk medicine have been explored for the first time in this study.

200 plants have been collected from Akkuş and its villages for the present study. At the end of the identification of the plants collected from villages, 58 taxa belong to 32 families have been determined [Asteraceae (7 taxa), Amaranthaceae (1 taxa), Boraginaceae (1 taxa), Caprifoliaceae (1 taxa), Caryophyllaceae (3), Chenopodiaceae (1), Convolvulaceae (1), Cruciferae (1 taxa), Cornaceae (1 taxa), Euphorbiaceae (1 taxa), Ericaceae (1 taxa), Fabaceae (5 taxa), Fagaceae (3 taxa), Hypericaceae (1 taxa), Hypolepidaceae (1 taxa), Juglandaceae (1 taxa), Lamiaceae (6 taxa), Loranthaceae (1 taxa), Malvaceae (1 taxa), Papaveraceae (1 taxa), Pinaceae (1 taxa), Plantaginaceae (1 taxa), Platanaceae (1 taxa), Polygonaceae (4 taxa), Primulaceae (1 taxa), Rhamnaceae (1 taxa), Rosaceae (5 taxa), Scrophulariaceae (1 taxa), Tiliceae (1 taxa), Umbelliferae (1 taxa), Urticaceae (1 taxa), Vitaceae (1 taxa)].

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

C. cretica, *V. pyramidatum*, *P. convolvulus*, *C. album, T. farfara*, *A. albus*, *S. verticillata* var. *verticillata*, *P. major* var. *major*, *P. orientalis*, *P. persicaria*, *M. longifolia*, *S. byzantina*, *A. eupatoria*, *O. vulgare*, *D. carmelitarum*, *F. Alnus*, *C. varia*, *P. orientalis*, *Q. infectoria*, *U. dioica*, *H. compactum*, *M. neglecta*, *T. parthenium, S. ebulus*, *R. ideaus*, *V. album var. album*, *B. perennis*, *A. tinctoria* var. *tinctoria*, and *A. cotula* from the collected species have been showed to have tyrosinase inhibitory effect; but the results were not significant. Because the IC_{50} values of them haven't been our detection range.

As shown in Table 2; *T. orientalis*, *C. bursa-pastoris*, *V. arctostaphylos*, *R. acetosella*, *C. byzantinum*, and *H. perforatum* have been caused to inhibition on tyrosinase enzyme. In view of values of IC_{50} of the methanolic extracts of the plants, *C. byzantinum* have been indicated to have the best effect on the enzyme. Besides, *P. aquilinum, C. arvense, S. compacta, F. orientalis, T. platyphllos, Mentha* sp., *S. vulgaris* var. *vulgaris, C. arvensis, P. rhoeas, R. patientia, P. Sylvestris* and *C. mas*

have been detected to have tyrosinase activator effect; however the values of IC_{50} of them were greater than 1000 µg/mL so the results were not significant.

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

Conflict of Interest

The authors declare that there are no conflicts of interest

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