Screening of commonly used plant extracts in Jordanian skin lightening folkloric recipes for their tyrosinase inhibitory activity: An *in vitro* study

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ABSTRACT

In Jordanian folkloric medicine, several medicinal plants–based recipes are used for skin lightening. Local recipes for skin lightening were collected and the tyrosinase inhibitory activity of the plants reported in these recipes, as a potential depigmentation mechanism was evaluated *in vitro* on both, mushroom and murine melanoma tyrosinase. The surveyed recipes included a total of 25 traditional medicinal plants belonging to 19 families. Kojic acid and licorice (*Glycyrrhiza glabra*) extract were used as positive controls. Thirteen extracts exhibited good mushroom tyrosinase inhibitory potential (>70%), and 7 extracts showed moderate tyrosinase inhibition activity (30-70%) while 5 extracts showed poor mushroom tyrosinase inhibitory activity (<30%).

Four of the tested extracts; *Juniperus communis* L. (Juniper), *Rosa indica* L. (Rose), *Amygdalus communis* var. *amara* L. (Bitter almond), and *Carthamus tinctorius* L. (Safflower) showed good inhibitory activity (>70%) against both, mushroom and melanoma tyrosinase enzymes that was similar or better than that of kojic acid. While, 6 tested extracts, obtained from *Raphanus sativus* L. (radish), *Juniperus communis* L. (juniper), *Petroselinum sativum* Hoffm. (parsely), *Salvia triloba* L. (sage), *Viola odorata* L. (garden violet), and *Mentha piperita* L. (mint), showed almost similar mushroom tyrosinase inhibitory activity as licorice extract (73.4%).

Tyrosinase inhibitory activities observed in many of the tested plant extracts validate their traditional use.

Keywords: Tyrosinase, Melanoma, Folkloric medicine, Skin lightening, Melasma.

1 INTRODUCTION

Skin hyperpigmentary disorders, such as melasma, freckles, and post inflammatory hyperpigmentation, are characterized by overproduction and accumulation of melanin [1]. They can have negative impact on subjects' psychosocial status since they are common on exposed areas of the face and the neck [1].The synthesis of skin pigment; melanin, takes place inside the melanocytes which reside in the basal layer of the epidermis. Melanin is formed by a complex pathway that initially involves two major reactions catalyzed by tyrosinase (a copper containing monooxygenase) [2]. Tyrosinase catalyses the

Received on 7/9/2020 and Accepted for Publication on 8/12/2020.

hydroxylation of L-tyrosine and the oxidation of the o-diphenol product L-DOPA (3,4-dihydroxyphenylalanine) to give rise to o-dopaquinone that is transformed into melanin through a series of reactions [2]. As a result of the key role, played by tyrosinase in melanin biosynthesis, most marketed skin lightening products use a tyrosinase inhibitor as the active ingredient (e.g., hydroquinone, kojic acid and arbutin). However, the use of these products is marred by safety and/or effectiveness concerns [3-5].

Thus, identification of new depigmenting agents, especially of plant origin, is an active research area that is reinforced by the belief that plant extracts have a superior safety profile to synthetic chemicals [6-8]. In cosmetic preparations many plant extracts such as *Morus alba* and *Glycyrrhiza glabra* have been used as depigmenting agents [9].

The use of skin-lightening products is a common practice among women living in Jordan [10]. In addition to its application in the treatment of hyperpigmentary disorders, the use of such products is reinforced by the beliefs that lighter skin tone plays a role in self-esteem, perception of beauty and youth as well as marriage and employment opportunities [10]. This is reflected in the fact that several plant-based skin lightening recipes are used in the Jordanian folkloric medicine.

In the present study, to accomplish the list of plants used in skin lightening recipes, the authors have interviewed major local herbalists in Amman and surveyed local folkloric medicine books. Then, the tyrosinase inhibitory activity of their aqueous extracts was evaluated to provide an evidence based justification for their folkloric use and to identify high potency extract(s) of local plants that might lead to the development of an effective skin lightening product for various hyperpigmentary disorders.

2. Materials and Methods:

2.1. Surveying and collecting folkloric skin lightening recipes:

Major herbalists (Attarins) in downtown Amman were interviewed and local folkloric medicine books were searched for local recipes used for skin lightening and for the treatment of hyperpigmentation. Some of these recipes are reported in Table 1. The plants reported in these recipes were purchased from the local market and identified by one of the authors (F. Afifi) using descriptive references and by comparison with the herbarium specimens of the Department of Biology, School of Science, University of Jordan. Voucher specimen were kept at the Faculty of Pharmaceutical Sciences, Hashemite University (FMSL1-FMSL26).

The scientific and common name of the reported plants, their families as well as the parts used in the recipes were determined and are summarized in Table 2.

2.2. Preparation of plant extracts:

Aqueous extracts of the plant parts specified in folkloric recipes were prepared by extracting each 5 grams of the powdered dried plant parts in 100 mL of distilled water at 60 ^{o}C for 2 hours. The resulting aqueous extracts obtained were filtered, stored in 50 ml centrifuge tubes (Jet-Biofil, Canada) and refrigerated at 4 ^{o}C until they were used in the *in vitro* tyrosinase inhibition assay.

2.3. Enzymatic assay of mushroom tyrosinase:

The effect of the prepared aqueous plant extracts on mushroom tyrosinase activity was determined spectrophotometrically using a previously published methodology after modifications and validation using both Kojic acid and licorice (*G. glabra*) extract as positive controls [9]. Mushroom tyrosinase (50 KU, Sigma, Aldrich) was aliquoted in potassium phosphate buffer (50 mM, pH=6.5) at final concentration of 500 U/ml and stored in -20 ^{o}C freezer until use. Twenty microliters (20 µl) of mushroom tyrosinase aliquot, and 60 µl of L-tyrosine (0.1 mg/ml) were incubated with different volumes (60, 120 µl) of the prepared aqueous plant extracts using 96-well plates. The final volume of each well was made to 220 µl with potassium phosphate buffer (50 mM, pH=6.5) and the plates were incubated at 37 ^{o}C for 20 minutes. The absorbance (Abs) at 490 nm was then measured using

microplate reader (680XR, Biorad, Bio-Rad Lab. Inc. USA). The same mixture without the plant extract was used as a control. Wells containing the plant extracts and mushroom tyrosinase without the substrate (tyrosine) were used as a blank to omit the effect of plant extract on absorbance. Licorice aqueous extract and Kojic acid (100µg/ml) in Phosphate Buffered Saline pH 7.2 (PBS, Euroclone, Italy) were used as positive controls.

The percent inhibition of tyrosinase activity was calculated as follows:

% Inhibition = (C - E) × 100/C ……. Equation (1)

Where C is the absorbance at 490 nm without plant extract, and E is the absorbance at 490 nm with plant extract. Results are shown in Table 3.

2.4. Cell Culture of murine melanoma:

The mouse (murine) melanoma, producing melanin; B16-F1 (ECACC 92101203) cells, were purchased from the ECACC (European Collection of Cell Culture) and cultured in DMEM (Euroclone, Italy) supplemented with 10% (v/v) fetal bovine serum (Euroclone, Italy), 1% L-glutamine (Euroclone, Italy) and 1% (v/v) antibiotic/antimycotic (100 units/ml, Sigma, Aldrich) at 37^{o}C in a humidified atmosphere with 5% CO_2. Cells were fed every other day until 80-90% confluency, cells were then harvested with 1X trypsin/EDTA (Euroclone, Italy) and lysed using 1% Triton X-100 (Promega, USA). Lysates were clarified by centrifugation at 13,000 rpm for 20 minutes and used in enzymatic inhibition assay after determining its protein content using Bicinchonic Protein Assay Kit (BCA) (Euroclone, Italy).

2.5. Enzymatic assay of murine melanoma tyrosinase:

The inhibition of melanoma tyrosinase in mouse melanoma cell lysate was performed as described previously with modifications [11-13]. Fifty microliters (50µl) of aqueous plant extract were added to a 96-well flat-bottom plate containing 50 µl of 4mM L-Tyrosine (Sigma, Aldrich) and 50 µl of 4 mM L-DOPA (Sigma, Aldrich). The plates were incubated at 37 ^{o}C for 10 minutes after which 50 µl of protein lysate containing equal amounts of protein (30 µg) in Phosphate Buffered Saline pH 7.2 (PBS, Euroclone, Italy) were added in each well. The final volume of each well was 200µl. The plate was then incubated at 37 ^{o}C for 60 minutes. The absorbance (Abs) at 490 nm was then measured using microplate reader (680XR, Biorad, Bio-Rad Lab. Inc. USA). The same mixture without the plant extract served as a control. Blank wells containing the extracts without protein lysate were used to omit the effect of plant extract on absorbance. Licorice aqueous extract and Kojic acid (100µg/ml) in PBS were used as positive controls. The percent inhibition of tyrosinase activity was calculated using Equation 1.

The enzymatic assay was repeated for each plant extract using only 100 µl of 2mM L-DOPA as the substrate without L-Tyrosine. The absorbance at 490 nm was then measured using plate reader. The same mixture without the plant extract served as a control. The percent inhibition of tyrosinase activity was calculated using Equation 1.

2.6. Stability study of selected plant extracts:

Mushroom tyrosinase inhibition assay was performed using freshly prepared plant extracts of *Crocus sativus, Lepidium sativum and Petroselinum sativum* and repeated after one month storage in the refrigerator (4 ^{o}C) in 50 ml centrifuge tubes (Jet-Biofil, Canada).

2.7. Data Analysis:

Enzymatic inhibition assays (mushroom and melanoma tyrosinase) were performed at least in triplicate at a minimum of 3 independent times. The percentage inhibition results were summarized in Table 3 as the Mean ± SD.

3. Results and Discussion:

A total of 25 traditional medicinal plants, (Table 2), belonging to 19 families were found in Jordanian folkloric recipes recommended to ameliorate skin hyperpigmentation or cause skin lightening.

The reported plants and plants found in the recipes mentioned in Table 1 were evaluated for their potential skin depigmenting effect by testing the tyrosinase

inhibitory activities of their aqueous extracts *in vitro* using both mushroom and murine melanoma tyrosinase.

Tyrosinase was targeted in the screening process as it is the rate-limiting enzyme in melanin production and its inhibition is one of the major strategies in developing new skin depigmenting agents [6]. Tyrosinase catalyses the first two steps of melanin production. It hydroxylates the amino acid L-tyrosine to L-dihydroxyphenylalanine (L-DOPA), then oxidizes L-DOPA to produce L-DOPA quinone which is processed through several elaborate steps to produce melanin.

The aqueous extracts were used to imitate the traditional used practices in the folk medicines. The plant extracts that caused enzyme inhibition percentage of less than 30% were considered poor inhibitors and the ones that caused 30-70% inhibition of enzyme activity were considered moderate enzyme inhibitors, while good enzyme inhibitors were those extracts that caused more than 70% inhibition of the enzyme activity.

Table 3 summarizes the results of mushroom tyrosinase inhibition assay presented as percentage (%) of tyrosinase inhibition caused by two different volumes (120µl and 60µl) of the prepared aqueous plant extracts corresponding to the concentrations of 27.3 and 13.6 mg dry plant /ml. Out of the 25 plant extracts, only five extracts showed poor mushroom tyrosinase inhibitory activity (<30%) while seven extracts showed moderate tyrosinase inhibition activity (30-70%). The remaining thirteen extracts showed good mushroom tyrosinase inhibitory activity (>70%).

Table 3 also shows the results of murine melanoma tyrosinase inhibition assay, presented as percentage (%) of tyrosinase inhibition caused by 50µl of the prepared aqueous plant extracts corresponding to a concentration of 12.5 mg dry plant /ml. The murine melanoma tyrosinase inhibitory activity of the plant extracts was carried out under two substrate conditions, namely using L-tyrosine as a substrate and L-DOPA as a cofactor and using L-DOPA alone without the L-tyrosine. Out of the 25 plant extracts, nine extracts exhibited poor melanoma tyrosinase inhibition activity (<30%), and eight moderate inhibition activity (30-70%) while with six extracts good inhibitory activity (>70%) was observed.

On the other hand, the positive control *G. glabra* aqueous extract displayed 70-73% inhibition of mushroom tyrosinase and 43.9-62% inhibition of melanoma tyrosinase. The second positive control, Kojic acid (100µg/ml) caused 91% inhibition of the mushroom tyrosinase and 78% inhibition of melanoma tyrsoinase. Interestingly, four of the screened plant extracts; *Juniperus communis* (Juniper), *Rosa indica* (Rose), *Amygdalus communis* L. var. *amara* (Bitter almond), and *Carthamus tinctorius* (Safflower), exhibited inhibitory activities (>70%) against both mushroom and melanoma tyrosinase enzymes that were almost similar or even better than kojic acid inhibition (Table 3).

Most of the plant extracts had shown moderate to good mushroom tyrosinase inhibitory activities while only the listed first five plant extracts in Table 3 showed poor mushroom tyrosinase inhibition. Nevertheless, two of these five plants that showed poor mushroom tyrosinase inhibition potential exerted moderate to good inhibition of melanoma tyrosinase. The aqueous extract of *Nigella sativa* (black cumin) and *Rosmarinus officinalis* (Rosemary) showed 49.7% & 86.5% melanoma tyrosinase inhibition, respectively, and poor inhibition (< 30%) of the mushroom tyrosinase. Earlier, Subramanian and Sahithya (2016) revealed for the alcohol extract of *N. sativa* moderate inhibition (49.6%) of mushroom tyrosinase [14]. Similarly, researchers detected for the essential oil of *R. officinalis* poor mushroom tyrosinase inhibitory potential [15].

The aqueous extract of the seeds of sweet almond *A. communis* var. *dulcis* showed poor inhibition of the mushroom and melanoma tyrosinase. The aqueous extract of the hull of *A. communis* var. *dulcis* was previously studied and showed also poor tyrosinase inhibition potential [16]. However, the alcohol extract of the leaves of

the same plant was shown to possess moderate mushroom tyrosinase inhibition potential [17]. On the contrary, the aqueous extract of the seeds of bitter almond *A. communis* var. *amara* showed in the present study 100% inhibition of mushroom tyrosinase and 78.9% inhibition of murine melanoma tyrosinase. This was not reported previously for bitter almond based on literature search.

The aqueous extract of the peel of *Cucumis melo* (Muskmelon) showed in our study an inhibition of mushroom tyrosinase activity of 24.5-39.2% that agreed well with a previous report [18].

The aqueous extract of leaves of *Thymus vulgaris* (Thyme) resulted in a moderate inhibition of mushroom tyrosinase that was similar to the effect of its alcoholic extract reported in the literature [19]. The same extract showed an even higher inhibition of the murine melanoma tyrosinase (67.8-84%). It has been reported that *T. vulgaris* contain carvacrol; a monoterpenoid compound, that is shown previously to inhibit tyrosinase enzymatic activity in B16F10 mouse melanoma cells better than the kojic acid [20].

The aqueous extract of the leaves of *Origanum vulgare* (Origano) showed moderate inhibition of mushroom tyrosinase and good inhibition to murine melanoma tyrosinase. A novel phenolic glucoside; origanoside, has been previously isolated from *O. vulgare* aerial parts and its depigmenting potential was confirmed in both melanoma B16 cells and in animal study [21].

The aqueous extracts of *Curcuma longa* (Tumeric) roots and *C. sativus* (Saffron) stigmas/styles showed moderate inhibition of mushroom tyrosinase similar to that previously reported using the methanolic extracts of the same plants [22, 23].

The aqueous extract of the flower of chamomile, *Matricaria aurea,* is considered a commonly used herbal drink among Jordanians for various ailments [24]. The aqueous extract of this plant showed moderate inhibition to both, mushroom (31.9-53.8%) and murine melanoma tyrosinase (23.7-70.1%). This inhibition potential was shown previously for another chamomile species, *M. recutita* but not for *M. aurea* species [25].

Six tested plants showed almost similar mushroom tyrosinase inhibition potential as the positive control *G. glabra* (73.4%). Those were the aqueous extracts of *Raphanus sativus* (73.8%), *J. communis* (74%), *P. sativum* (75.3%), *Salvia triloba* (76.4%), *Viola odorata* (81.6%), and *Mentha piperita* (82.2%).

The propylene glycol extract of the roots of *R. sativus* showed previously 88% inhibition of mushroom tyrosinase comparable to the inhibition obtained in this study for the aqueous extract of the seed parts of the same plants [26].

For the ethanolic extract of the fruits of *J. communis* Jagal et al. (2017) have previously reported that this extract inhibits tyrosinase activity and lighten the UV-radiated skin of HRM-2 mice [27].

Petroselinum sativum, known as garden parsley and *Salvia triloba,* known as the East Mediterranean sage [28] are two of the most popular herbs in Middle Eastern kitchen have exhibited good inhibition of the mushroom tyrosinase and moderate inhibition of the murine melanoma tyrosinase (Table 3). Literature survey revealed no previous studies on the antityrosinase inhibitory activity for both commonly used culinary herbs.

The aqueous extract of garden violet (*Viola adorata*) showed 81.6% inhibition of the mushroom tyrosinase and 54.3% inhibition of the murine melanoma tyrosinase. Although similar inhibitory activities for the organic extract was reported, this is the first report on the inhibitory activity of the water extract of garden violet [29 30].

Mentha piperita (mint) is widely used as a common herbal tea among the Jordanians and classified as a culinary herb for many dishes. Its aqueous extract showed 82.2% inhibition of the mushroom tyrosinase. The plant essential oil was also previously reported to inhibit mushroom tyrosinase [31].

Rosa indica (Rose) showed 91.8 % inhibition of mushroom tyrosinase and 100% inhibition of murine melanoma tyrosinase. Again, no similar findings could be

retrieved from the literature survey.

The mushroom tyrosinase inhibitory efficacy of the methanolic extract of the seeds of nutmeg, *Myristica fragrans* (69%) was reported previously [9]. However, more potent inhibitory activity of the aqueous extract of the seeds (96.3%) was detected in the present study.

Although in an earlier study the methanolic extract of the seeds of *L. sativum* (Garden cress) did not exhibit any tyrosinase inhibitory potential, in the present study, for the aqueous extract of the same plant 99.1% inhibition of mushroom tyrosinase was observed [32].

The aqueous extract of the flowers of *C. tinctorius* showed 100% inhibition of mushroom tyrosinase and 84.9% inhibition of murine melanoma tyrosinse. The methanolic extract of another part of this plant; seeds, was previously reported to inhibit mushroom tyrosinase as well as reduce melanin content of B16 melanoma cells [33]. In addition, Carthamus yellow; the major pigment component extracted from *C. tinctorius*, showed both, tyrosinase inhibition potential and melanin reduction potential in B16F10 melanoma cells [34].

Fenugreek (*Trigonella foenum-graecum*) seeds are commonly used as a medicinal plant in Jordan for lactation deficiency and general weakness [24]. The findings of the current study indicated 100% inhibition of mushroom tyrosinase of the aqueous extract of its seeds. This depigmenting potential has been reported previously for the alcoholic extracts of the seeds of this plants which caused more than 50% inhibition of the mushroom tyrosinase [14]. Furthermore, its alcoholic extracts reported to possess anti-inflammatory properties as well as caused reduction in melanin synthesis in murine melanoma B16F1 cells [35].

Cicer arietinum (Chickpea) seeds is a key ingredient in hummus; a popular dip in Jordanian kitchens. Interestingly, Chickpea has been mentioned by the great traditional Persian scientists; Avicenna and Razi who mentioned the cutaneous benefits of this legume [36]. Although no previous studies were carried out for the antityrosinase inhibitory activity of chickpea, the results of the present study revealed 100% inhibition of the mushroom tyrosinase activity.

In addition to the evaluation of the antityrosinase inhibitory potential of the selected 25 plant species, the effect of storage in the refrigerator on the mushroom tyrosinase inhibition potential of the extracts was studied using three aqueous extracts as examples. Mushroom tyrosinase inhibition potential was tested at baseline for the freshly prepared extracts of these selected three plants and again thirty days after storage in the refrigerator. Results are shown in Table 4. Apparently the three tested plant extracts; *C. sativus*, *L. sativum* and *P. sativum* have preserved their mushroom tyrosinase inhibition potential. This finding supports the use of these aqueous plant extracts as potential stable and effective component in skin depigmenting formulations in the Jordanian traditional medicine.

4. Conclusion:

The use of most of the plant components in the collected recipes in ameliorating hyperpigmentary disorders and skin lightening appears to be substantiated by the enzyme inhibition studies. More than two third of the tested plant extracts exhibited moderate to good tyrosinase inhibition efficacy. Of the 25 extracts examined, five showed more than 70% inhibition of both mushroom and murine tyrosinase and only three plant extracts; *Cyperus esculentu* (Earth almond), *A. communis L. var. dulcis* (Sweet almond) and *Sambucus niger* (Elderberry) were poor inhibitors of both mushroom and murine tyrosinase.

Four of the screened plant extracts; *J. communis, R. indica, A. communis* L. var. *amara*, and *C. tinctorius* L., inhibited both mushroom and melanoma tyrosinase enzymes almost similar or even better than kojic acid.

Interestingly, the tyrosinase inhibition potential of the of *P. sativum* (parsley), *S. triloba* (sage), *A. communis* var. *amara* (bitter almond) and *R. indica* (rose), which exhibited good tyrosinase inhibition, has not been reported

previously based on the comprehensive literature survey.

The aqueous extracts of *C. sativus* (saffron), *L. sativum* (garden cress) and *P. sativum* (parsley) preserved their tyrosinase inhibitory properties for one month at 4 ^{o}C which indicates the stability of the active constituents and their potential to be incorporated into cosmeceutical formulations. Further *in vitro* and *in vivo* evaluation of the promising plant extracts are recommended.

Acknowledgment:

This project was funded by Abdul Hameed Shoman Scientific Research Support Fund, Amman, Jordan.

Conflicts of Interest:

The authors declare that they have no conflicts of interest.

Table 1: A number of Jordanian skin lightening folk recipes and direction for use. Plants in recipes are written in **Bold**.

No.	Components	Direction for use
1.	**Sage** extract and honey	Morning and evening
2.	**Common violet** and **elderberry** extracts	Morning and evening
3.	Boiled seeds of **radish**	Twice daily
4.	Extract of **parsley**	Apply at night and wash in the morning
5.	Extracts of **chickpeas**	Use as mask for 30 min and wash
6.	Peel of the **Muskmelon** and honey or olive oil	Apply to face for 20 minutes and wash with water
7.	Castor oil (*Ricinus communis*)	Twice daily for one month
8.	Powder of **Black cumin** and vinegar	Mix to form a paste, apply to skin for 30 min and wash
9.	Cucumber, **sweet almond, garden cress, Parsley**, Chicory	Orally and topically
10.	Juice of the **Muskmelon**	Once daily
11.	**Safflower** extract	Once daily
12.	Extract of **Fenugreek** seeds	Use cotton wool to apply to the skin twice daily
13.	**Garden cress** juice and honey	Twice daily
14.	Starch and **Saffron**	Mix together and apply to melasma
15.	Extract of **Origano**	Apply at night and wash in the morning
16.	Powdered **Muskmelon** peel and honey	Once daily for one week
17.	Oil of **sweet and bitter almonds**	Once evening for 3 days
18.	Castor oil	Once evening for 4 weeks
19.	Ground seeds of **nutmeg** mixed with honey	Mask for 1 hour
20.	Ground **earth almond** and honey	Mask for 1 hour
21.	**Mint** leaves extract	Apply and leave for overnight
22.	Juice of the **Muskmelon**	Mask for 1 hour
23.	Castor oil, **ground tumeric**, Vaseline and beeswax	Once in the evening for at least 4 weeks
24.	Castor oil, **earth almond**, Vaseline, beeswax	Once in the evening for at least 4 weeks

Table 2: The plants screened, their scientific names, families, Arabic names and parts used.

No.	Scientific name	Family	Common name	Arabic name	Parts used
1*	***Amygdalus communis* L. var. *dulcis***	Rosaceae	Sweet almond	Loz hilo	Seeds
2	***A. communis* L. var. *amara***	Rosaceae	Bitter almond	Loz mur	Seeds
3	***Carthamus tinctorius* L.**	Asteraceae	Safflower	O'sfur	Flowers
4	***Cicer arietinum* L.**	Fabaceae	Chickpea	Hummus	Seeds
5	***Crocus sativus* L.**	Iridaceae	Saffron	Z'afaran	Stigmas/Styles
6	***Cucumis melo* L.**	Cucurbitaceae	Muskmelon	Shomam	Peels
7	***Curcuma longa* L.**	Zingiberaceae	Turmeric	Curcum	Roots
8	***Cyperus esculentus* L.**	Cyperaceae	Earth almond	Hab alaziz	Fruits
9	***Glycyrrhiza glabra* L.**	Leguminosae	Licorice (positive control)	Aerq alsos	Roots
10	***Jasminum officinale* L.**	Oleaceae	Jasmin	Yasmin	Flowers
11	***Juniperus communis* L.**	Cuprassaceae	Juniper	Ar'ar	Aerial parts
12	***Lepidium sativum* L.**	Cruciferae	Garden cress	Rashad	Seeds
13	***Matricaria aurea* (Loeffl) Sch Bip**	Asteraceae	Chamomile	Babonej	Flowering heads, leaves
14	***Mentha piperita* L.**	Lamiaceae	Mint	Na'na	Leaves
15	***Myristica fragrans* L. Hout**	Myristicaceae	Nutmeg	Jozettib	Seeds
16	***Nigella sativa* L.**	Ranunculaceae	Black cumin	Alhaba alsouda	Seeds
17	***Origanum vulgare* L.**	Lamiaceae	Origano	Mardaqush	Leaves
18	***Petroselinum sativum* Hoffm.**	Umbelliferae	Parsley	Bakdonis	Leaves, stem
19	***Raphanus sativus* L.**	Brassicaceae	Radish	Fejil	Seeds
20	***Rosa indica* L.**	Rosaceae	Rose	Ward	Petals, flowers
21	***Rosmarinus officinalis* L.**	Lamiaceae	Rosemary	Iklil aljabal	Leaves
22	***Salvia triloba* L.**	Lamiaceae	Sage	Meramia	Leaves
23	***Sambucus nigra* L**	Caprifoliaceae	Elderberry	Bailasan	Flowers
24	***Thymus vulgaris* L.**	Lamiaceae	Thyme	Z'atar	Leaves
25	***Trigonella foenum-graecum* L.**	Leguminosae	Fenugreek	Holbe	Seeds
26	***Viola odorata* L.**	Violaceae	Garden violet	Banafsaj	Flowers

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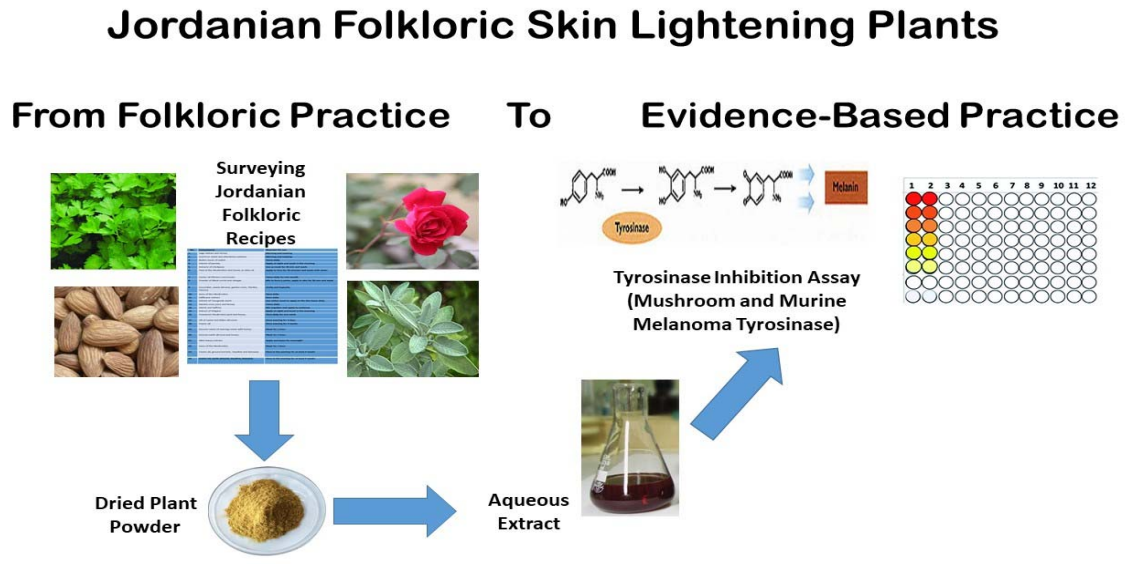
Table 3: Percentage mushroom tyrosinase inhibition at two different volume/concentrations of aqueous plant extracts (60 & 120 µl) and percentage of murine melanoma tyrosinase inhibition (using either L-tyrosine with L-dopa as substrate or L-dopa alone) by 50 µl of aqueous plant extracts found in folkloric recipes. Kojic acid (100µg/ml) & *Glycyrrhiza glabra L.* served as positive controls.

No.	Scientific name	Inhibition of mushroom tyrosinase %inhibition (± SD)		Inhibition of melanoma tyrosinase %inhibition (± SD) 50µl (12.5mg/ml)	
		120 µl (27.3 mg/ml)	60µl (13.6 mg/ml)	Tyrosine + DOPA	DOPA
1	*Cyperus esculentus* L.	0.4 (4.9)	6.5 (5.1)	11.1 (10.7)	10.2 (5.6)
2	*Nigella sativa* L.	12.4 (4.8)	16.8 (9.4)	49.7 (7.1)	1.1 (1.5)
3	*Rosmarinus officinalis* L.	19.4 (5.2)	28 (1.0)	86.5 (1.3)	93.0 (1.3)
4	*Amygdalus communis* L. var. *dulcis*	19.7 (4.6)	3.7 (9.1)	1.9 (3.2)	5.9 (5.3)
5	*Sambucus nigra* L.	21.6 (0.7)	0.1 (0.2)	ND	ND
6	*Jasminum officinale* L.	32.3 (14.9)	35.3 (9.1)	33.3 (3.9)	31.1 (7.0)
7	*Cucumis melo* L.	39.2 (10.6)	24.5 (3.8)	ND	ND
8	*Thymus vulgaris* L.	39.4 (5.6)	37.6 (2.6)	67.8 (3.7)	84.0 (3.6)
9	*Origanum vulgare* L.	41.4 (7.0)	16.5 (1.8)	71.9 (1.9)	83.2 (6.2)
10	*Curcuma longa* L.	42.7 (1.2)	44 (3.5)	17.3 (3.0)	13.9 (3.0)
11	*Crocus sativus* L.	50.1 (10.8)	7.8 (2.3)	9.9 (7.1)	4.9 (9.1)
12	*Matricaria aurea* (Loeffl) Sch Bip.	53.8 (1.1)	31.9 (9.4)	23.7 (13.8)	70.1 (4.9)
13	*Glycyrrhiza glabra* L.	70.4 (17.4)	73.4 (7.4)	62.3 (13.2)	43.9 (3.7)
14	*Raphanus sativus* L.	73.8 (8.0)	46.2 (3.7)	0.5 (0.2)	6.5 (5.5)
15	*Juniperus communis* L.	74 (0.7)	74.4 (7.2)	71.3 (39.8)	ND
16	*Petroselinum sativum* Hoffm.	75.3 (2.6)	35.5 (6.3)	2.6 (4.8)	56.4 (7.9)
17	*Salvia triloba* L.	76.4 (13.2)	59.6 (7.5)	2.5 (4.6)	61.7 (4.5)
18	*Viola odorata* L.	81.6 (9.3)	54.7 (5.5)	12.2 (2.7)	54.3 (18.6)
19	*Mentha piperita* L.	82.2 (16.4)	77.7 (12.2)	0.5 (0.3)	7.8 (4.7)
20	*Rosa indica* L.	91.8 (8.2)	82.8 (5.0)	100.0 (14.1)	80.5 (34.6)
21	*Amygdalus communis* L. var. amara	95.1 (7.0)	100 (6.3)	78.9 (15.8)	67.7 (6.5)
22	*Myristica fragrans* L. Hout	96.3 (1.1)	92.2 (3.1)	33.9 (5.9)	27.4 (4.3)
23	*Lepidium sativum* L.	98.5 (0.5)	99.1 (1.2)	6.5 (7.4)	10.4 (6.9)
24	*Carthamus tinctorius* L.	100 (25)	79 (11)	84.9 (13.3)	85.1 (15.1)

No.	Scientific name	Inhibition of mushroom tyrosinase %inhibition (± SD)		Inhibition of melanoma tyrosinase %inhibition (± SD) 50µl (12.5mg/ml)	
		120 µl (27.3 mg/ml)	60µl (13.6 mg/ml)	Tyrosine + DOPA	DOPA
25	*Trigonella foenum-graecum* L.	100 (13.5)	64.6 (9.1)	0.6 (0.3)	15.1 (1.4)
26	*Cicer arietinum* L.	100.5 (15.4)	71.8 (13.2)	0.62 (0.4)	0.33 (0.23)
27	Kojic acid (100µg/ml)	91.2 (1.6)		78.4 (0.9)	

Table 4: The stability of selected plant extracts (120µl) stored at 4ºC for one month.

No.	Scientific name	%Inhibition of mushroom tyrosinase (±SD) at two time points	
		0 time (freshly prepared)	After 1 month
1.	*Crocus sativus* L.	44.6±6.0	54.5±16.7
2.	*Lepidium sativum* L.	103.6±0.8	100.7±4.2
3.	*Petroselinum sativum* Hoffm.	62.9±0.5	53.3±11.7



REFERENCES

(1) Briganti S., Camera E., Picardo M. Chemical and Instrumental Approaches to Treat Hyperpigmentation. Pigment Cell Res. 2003;16(2):101-10.

(2) Slominski A., Tobin D. J., Shibahara S. and Wortsman J. Melanin Pigmentation in Mammalian Skin and Its Hormonal Regulation. Physiol Rev. 2004;84(4):1155-228.

(3) Kooyers T., Westerhof W. Toxicology and health risks of hydroquinone in skin lightening formulations. J. Eur. Acad. Dermatol. Venereol. 2006;20(7):777-80.

(4) Nakagawa M., Kawai K., Kawai K. Contact allergy to kojic acid in skin care products. Contact Dermatitis. 1995;32(1):9-13.

(5) Parvez S., Kang M., Chung H-S., Cho C., Hong M-C., Shin M-K. and Bae H. Survey and mechanism of skin depigmenting and lightening agents. Phytother Res. 2006;20(11):921-34.

(6) Chang T-S. An Updated Review of Tyrosinase Inhibitors. Int. J. Mol. Sci. 2009;10(6):2440-75.

(7) Hamed S. H., Sriwiriyanont P., deLong M. A., Visscher M. O., Wickett R. R. and Boissy R. E.Comparative efficacy and safety of deoxyarbutin, a new tyrosinase-inhibiting agent. J. Cosmet. Sci. 2006;57(4):291-308.

(8) Baurin N., Arnoult E., Scior T., Do Q. T. and Bernard P. Preliminary screening of some tropical plants for anti-tyrosinase activity. J. Ethnopharmacol. 2002;82(2-3):155-8.

(9) LEE K. T., KIM B. J., KIM J. H., HEO M. Y. and KIM H. P. Biological screening of 100 plant extracts for cosmetic use (I): inhibitory activities of tyrosinase and DOPA auto-oxidation. Int. J. Cosmet. Sci. 1997;19(6):291-98.

(10) Hamed S. H., Tayyem R., Nimer N. and Alkhatib H. S. Skin-lightening practice among women living in Jordan: prevalence, determinants, and user's awareness. Int. J. Dermatol. 2010;49(4):414-20.

(11) Fujii T., Saito M. Inhibitory Effect of Quercetin Isolated from Rose Hip (Rosa canina L.) against Melanogenesis by Mouse Melanoma Cells. Biosci. Biotechnol. Biochem. 2009;73(9):1989-93.

(12) Hwang J.H., Lee B.M. Inhibitory effects of plant extracts on tyrosinase, L-DOPA oxidation, and melanin synthesis. J. Toxicol. Environ. Health A 2007;70(5):393-407.

(13) Chawla S., DeLong M. A., Visscher M. O., Wickett R. R., Manga P. and Boissy R. E. Mechanism of tyrosinase inhibition by deoxyArbutin and its second-generation derivatives. Br. J. Dermatol. 2008;159(6):1267-74.

(14) Subramanian V., Sahithya D. Preliminary Screening of Selected Plant Extracts for Anti Tyrosinase Activity. Journal of Natural Remedies 2016;16:18.

(15) Satyal P., Jones T. H., Lopez E. M., McFeeters R. L., Ali N. A. A., Mansi I., Al-kaf A. G. and Setzer W. N. Chemotypic Characterization and Biological Activity of Rosmarinus officinalis. Foods 2017;6(3):20.

(16) Tlili N., Kirkan B., Sarikurkcu C. LC–ESI–MS/MS characterization, antioxidant power and inhibitory effects on α-amylase and tyrosinase of bioactive compounds from hulls of Amygdalus communis: The influence of the extracting solvents. Ind. Crops. Prod. 2019;128:147-52.

(17) Ghafari S., Fahimi S., Sahranavard S. Plants used to treat hyperpigmentation in Iranian traditional medicine: a review. Res. J. Pharmacog. 2017;4(4):71-85.

(18) Shin Y-S., Lee J-E., Yeon I-K., Do H-W., Cheung J-D., Kang C-K. et al. Antioxidant Effects and Tyrosinase Inhibition Activity of Oriental Melon (Cucumis melo L. var makuwa Makino) Extracts J. Life Sci. 2008; 18.

(19) Chiocchio I., Mandrone M., Sanna C., Maxia A., Tacchini M. and Poli F. Screening of a hundred plant extracts as tyrosinase and elastase inhibitors, two enzymatic targets of cosmetic interest. Ind. Crops. Prod. 2018;122:498-505.

(20) Jeon N-J., Kim Y-S., Kim E-K., Dong X., Lee J-W., Park J-S. et al. Inhibitory effect of carvacrol on melanin synthesis via suppression of tyrosinase expression. J. Funct. Foods 2018;45:199-205.

(21) Liang C-H., Chou T-H., Ding H-Y. Inhibition of

melanogensis by a novel origanoside from Origanum vulgare. J. Dermatol. Sci. 2010;57(3):170-77.

(22) Kim J. A., Son J. K., Chang H. W., Jahng Y., Kim Y. Na M. and Lee S. H.. Inhibition of Mushroom Tyrosinase and Melanogenesis B16 Mouse Melanoma Cells by Components Isolated from Curcuma longa. Nat. Prod. Commun. 2008;3(10):1934578X0800301014.

(23) Sariri R., Sabbaghzadeh R., Poumohamad F. In-vitro antioxidant and anti-tyrosinase Activity of methanol extracts from Crocus sativus flowers. Pharmacologyonline 2011;3:1-11.

(24) Abu-Irmaileh B.E., Afifi F.U. Herbal medicine in Jordan with special emphasis on commonly used herbs. J. Ethnopharmacol. 2003;89(2):193-97.

(25) Cvetanović A., Švarc-Gajić J., Zeković Z., Jerković J., Zengin G., Gašić U., Tešić Ž. et al. The influence of the extraction temperature on polyphenolic profiles and bioactivity of chamomile (Matricaria chamomilla L.) subcritical water extracts. Food Chem. 2019;271:328-37.

(26) Kamkaen N., Mulsri N., Treesak C. Screening of Some Tropical Vegetables for Antityrosinase Activity. Thail. Pharm. Health Sci. J. 2007;2

(27) Jegal J., Chung K. W., Chung H. Y., Jeong E. J. and Yang M. H. The Standardized Extract of Juniperus communis Alleviates Hyperpigmentation in Vivo HRM-2 Hairless Mice and in Vitro Murine B16 Melanoma Cells. Biol. Pharm. Bull. 2017;40(9):1381-88.

(28) Gali-Muhtasib H. Anticancer and medicinal properties of essential oil and extracts of East Mediterranean sage (salvia triloba). Adv. Phytomed. 2006;2.

(29) Erdogan Orhan. I., Senol F., Aslan Erdem S., Tatli I., Kartal M. and Alp Ş. Tyrosinase and Cholinesterase Inhibitory Potential and Flavonoid Characterization of Viola odorata L. (Sweet Violet): Enzyme Inhibitiory Effect of Viola odorata. Phytother. Res.: PTR 2015;29.

(30) Rahimi V. B., Askari V. R., Emami S. A. and Tayarani-Najaran Z. Anti-melanogenic activity of Viola odorata different extracts on B16F10 murine melanoma cells. Iran. J. Basic. Med. Sci. 2017;20(3):242-49.

(31) Fiocco D., Fiorentino D., Frabboni L., Benvenuti S., Orlandini G., Pellati F. et al. Lavender and peppermint essential oils as effective mushroom tyrosinase inhibitors: a basic study. Flavour Fragr. J. 2011;26(6):441-46.

(32) Muddathir A. M., Yamauchi K., Batubara I., Mohieldin E. A. M. and Mitsunaga T. Anti-tyrosinase, total phenolic content and antioxidant activity of selected Sudanese medicinal plants. S. Afr. J. Bot. 2017;109:9-15.

(33) Roh J. S., Han J. Y., Kim J. H. and Hwang J. K. Inhibitory Effects of Active Compounds Isolated from Safflower (Carthamus tinctorius L.) Seeds for Melanogenesis. Biol. Pharm. Bull. 2004;27(12):1976-78.

(34) Chen Y-S., Lee S-M., Lin C-C., Liu C-Y. Wu M-C. and Shi W-L. Kinetic study on the tyrosinase and melanin formation inhibitory activities of carthamus yellow isolated from Carthamus tinctorius L. J. Biosci. Bioeng. 2013;115(3):242-45.

(35) Kawabata T., Cui M-Y., Hasegawa T., Takano F. and Ohta T. Anti-Inflammatory and Anti-Melanogenic Steroidal Saponin Glycosides from Fenugreek (Trigonella foenum-graecum L.) Seeds. Planta Med. 2011;77(07):705-10.

(36) Mahjour M., Khoushabi A., Noras M. and Hamedi S. Effectiveness of Cicer arietinum in Cutaneous Problems: Viewpoint of Avicenna and Razi. Curr. Drug Discov. Technol. 2018;15 (3):243-50.

(37) Issa R., Khattabi A., Alkarem T.A., Altameemi O. The Use of Antidiabetic Herbal Remedies by Jordanian Herbalist: A Comparison of Folkloric Practice vs. Evidence-Based Pharmacology. Jord. J. Pharmac. Sci. 2019;12(3): 23-37.

(38) Wan Salleh W.M.N.H., Ahmad F., Abdul Azziz S.S.S., Ahmad M.S. In Vitro Pharmacological Evaluation of the Leaves and Stem Bark Extracts of Beilschmiedia penangiana Gamble. Jord. J. Pharmac. Sci. 2019;12(1):47-57.

فحص المستخلصات النباتية الشائعة الاستخدام في وصفات تفتيح البشرة الشعبية الأردنية لنشاطها المثبط لإنزيم التايروسينيز (دراسة مخبرية)

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ملخص

في الطب الشعبي الأردني، تستخدم العديد من الوصفات القائمة على النباتات لتفتيح البشرة. تم جمع الوصفات المحلية لتفتيح البشرة وتم تقييم النشاط المثبط لمستخلصات النباتات المتواجدة في هذه الوصفات على كل من انزيم المشروم تايروسينيز وانزيم التايروسينيز من خلايا الميلانوما من الفئران كآلية عمل محتملة لتفتيح البشرة وازالة التصبغات.

شملت الوصفات الشعبية ما مجموعه **25** من النباتات الطبية والتي تعود الى 19 عائلة نباتية وتم استخدام حمض الكوجيك ومستخلص عرق السوس كضوابط إيجابية معروفة في تثبيط انزيم التايروسينيز.

أظهر ثلاثة عشر مستخلص نباتي تثبيط جيد لإنزيم المشروم تايروسينيز (تثبيط أكثر من 70% من نشاط الإنزيم)، وأظهرت 7 مستخلصات نباتية تثبيط معتدل لإنزيم المشروم تايروسينيز (تثبيط 30–70٪ من نشاط الإنزيم) في حين أظهرت 5 مستخلصات تثبيط ضعيف لإنزيم المشروم تايروسينيز (تثبيط اقل من 30٪ من نشاط الإنزيم) .

أربعة من المستخلصات المختبرة وهي **Juniperus communis (Juniper), Rosa. indica (Rose) Amygdalus communis var. amara (Bitter almond), and Carthamus tinctorius (Safflower)** أظهرت نشاطا مثبطا جيدا (أكثر من 70٪) لكل من إنزيم المشروم تايروسينيز وإنزيم التايروسينيز من خلايا الميلانوما من الفئران وكان تثبيطهم مماثلا أو أفضل من تثبيط حمض الكوجيك (الضابط الايجابي). وأظهرت ستة من المستخلصات النباتية نشاطا مثبطا لإنزيم المشروم تايروسينيز تقريبا مماثل لمستخلص عرق السوس (الضابط الايجابي) وهي

Raphanus sativus (radish), Juniperus communis (juniper), Petroselinum sativum (parsely), Salvia triloba (sage), Viola odorata (garden violet), and Mentha piperita (mint).

من الملاحظ ان نشاط العديد من المستخلصات النباتية في تثبيط كل من إنزيم المشروم تايروسينيز وإنزيم التايروسينيز من خلايا الميلانوما يدعم صحة استخدامها التقليدي في وصفات تفتيح البشرة وعلاج التصبغات.

الكلمات الدالة: التايروسينيز، الميلانوما، الطب الفولكلوري، تفتيح البشرة، الكلف .

تاريخ استلام البحث 2020/9/7 وتاريخ قبوله للنشر 2020/12/8.