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Evaluation of Antioxidant, Anti-Tyrosinase, and Anti-Melanoma Activities of *Phlomis Rigida*

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Abstract:

The aim of this study is to evaluate *in vitro* cytotoxicity of *Phlomis rigida* (*P. rigida*) aerial parts on the malignant melanoma cells as well as assess its antioxidant and anti-tyrosinase activities. The total phenolic and flavonoid contents along with *in vitro* antioxidant and anti-tyrosinase activities of *P. rigida* MeOH (80%) extract were determined using Folin-Ciocalteu, aluminum chloride, DPPH radical scavenging, and mushroom tyrosinase assays, respectively. The cytotoxicity of the extract was investigated by determination of the cell viability using MTT assay on the normal fibroblast (NIH3T3) and malignant melanoma (SKMEL-3) cells. The extract showed a weak scavenging activity with SC_{50} value higher than 5 mg/mL and the tyrosinase inhibitory activity with an IC_{50} value of 1.092 mg/mL. Interestingly, the extract was not toxic on NIH3T3 cells at all tested concentrations (0.0001-0.1 mg/mL), however, it could significantly reduce cell viability on SKMEL-3 cells, particularly at higher concentrations than 0.01 mg/mL (the $IC_{50} \approx 0.148$ mg/mL). Based on our results, a selective cytotoxic effect against SKMEL-3 cells was found for the extract of *P. rigida* compared with NIH3T3 cells. Therefore, it is recommended as a good candidate for further investigation to discover bioactive natural agents in melanoma treatment.

Keywords: Antioxidant, Anti-tyrosinase, Cell viability, NIH3T3, *Phlomis rigida,* SKMEL-3.

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1. Introduction

Melanin is the main component responsible for the color of hair, eyes, and skin. The main role of melanin is skin protection against ultraviolet (UV) light damage through absorbing UV radiation and eliminating reactive oxygen species (ROS) [1]. Biosynthesis of melanin comprises a series of oxidative and enzymatic reactions and tyrosinase is the key enzyme in the melanogenesis pathway. In this context, tyrosinase overactivity increases melanin production, which leads to several disorders and diseases such as ageing, melasma and melanoma skin cancers and in contrast, underactivity of tyrosinase leads to hypopigmentation disorders such as vitiligo and whitening of hair. Tyrosinase as a polyphenol oxidase, is a copper containing monooxygenase mainly involving in two different reactions of melanin synthesis: hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) *via* monophenolase action and oxidation of DOPA to DOPA-quinone *via* diphenolase action [2]. A large number of plants are traditionally used for treatment of various skin problems caused by pigmentation disorders such as freckles, melisma, aging spots, malignant melanoma, and vitiligo. Numerous studies confirmed that secondary metabolites such as phenolics, flavonoids, and phenylethanoids are able to inhibit

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reactive oxygen species (ROS), tyrosinase activity, and tumour cell proliferation [3,4].
The genus *Phlomis* L. (Lamiaceae) has been estimated about 100 species. The greatest diversity of *Phlomis* species is in the northwestern regions of Iran and southern and eastern Anatolia [5]. This genus is represented by 28 species, containing four hybrids in the Flora Iranica area [6]. *Phlomis* species are traditionally used as tonics, stimulants, and diuretics and for the treatment of wounds, ulcers, inflammatory disorders, and gastrointestinal problems [7]. Previous pharmacological studies have described cytotoxicity, anti-tumor, anti-pigmentation, antibacterial, and antioxidant activity of several *Phlomis* species [8-12]. *Phlomis rigida* Labill. is a perennial herb that grows mainly in Iran, Iraq, Turkey, Lebanon, and Syria [13,14]. Previous studies showed that *P. rigida* is rich in phytochemicals such as phenolics, flavonoids, iridoid glucosides, and essential oils [7,8,15]. In the recent study in Iran, the phytochemical constituents and inhibitory effect of essential oils and methanol extract from *P. rigida* on DPPH radicals and cancer cells (MCF-7, MDBK, HT-29 and A-549) were considered by Heydari *et al.* [8]. Based on the study, (Z)-*β*-ocimene, isobornyl acetate, *trans*-verbenol and *α*-pinene were major compounds in *P. rigida* essential oil and luteolin, luteolin-7-O-glucoside and rosmarinic acid were identified in methanol extract from the leaves. They also found both the essential oil and methanol extract of *P. rigida* have mild antioxidant and cytotoxic activities. Okur *et al.* [14] investigated *in vitro* and *in vivo* anti-inflammatory and wound healing effects of *P. rigida* aerial parts and reported that these effects probably due to the presence of antioxidant compounds such as luteolin and apigenin.
To our knowledge, no research has been done on the anti-melanoma activity of Iranian *P. rigida*. Therefore, for the first time, the present study was carried out to evaluate *in vitro* cytotoxicity of *P. rigida* methanol extract on the malignant melanoma cells. We further considered the antioxidant and anti-tyrosinase activity of the extract in order to find a possible mechanism for such an effect.

2. Materials & Methods

2.1. Chemicals

Mushroom tyrosinase, kojic acid, quercetin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), potassium dihydrogen phosphate, di-potassium hydrogen phosphate, dimethyl sulfoxide (DMSO), and the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin and trypsin-EDTA were obtained from GibcoBRL (Grand Island, USA). All other solvents and chemicals were purchased from Merck (Germany).

2.2. Preparation of Plant Extract

The aerial parts of *Phlomis rigida* Labill. were collected from the west of Iran (Kordestan province) and identified by Dr. Yousef Ajani. A voucher specimen (No: 1557) was deposited at Herbarium of Medicinal Plant Institute, Academic Center for Education, Culture and Research (ACECR) Karaj, Iran. Thirty grams of dried powder of plant were extracted using 80% aqueous MeOH (1700 mL) by percolation apparatus for 72h at room temperature. The solvent extract was filtered using Whatman paper No.1 and then concentrated by a rotary evaporator and freeze-dryer. The yield of *P. rigida* dried extract was calculated (20.5%) and the extract was kept at 4 ˚C.

2.3. Determination of Total Phenolic Content

The total phenolic content of the extract was quantified according to the Folin-Ciocalteu method with some modifications using 96-well microplates. In brief, 20 µL of the sample was mixed with 40 µL of Folin-Ciocalteu reagent (diluted 10 times). Following 5 min, 80 µL of sodium carbonate 10% (w/v) was added. After incubation for 30 min, the absorbance was measured at 760 nm using microplate reader (Synergy HT, BIO-TEK, USA). The results were expressed as milligrams of gallic acid equivalents per gram of the extract (mg of GA/g of extract) [11].

2.4. Dtermination of Total Flavonoid Content

The total flavonoid content of the extract was estimated through the aluminum chloride colorimetric method using a 96-well microplates. Briefly, the sample (20 µL) was mixed with water (100 µL) followed by addition of $NaNO_2$ (5%, 5µL). After shaking the mixture for 5 min, 20 µL of $AlCl_3$ (10 %) and 50 µL NaOH (4%) were added. The final volume reached 250 mL using distilled water. After 30 min, absorbance of the mixtures was measured at 510 nm using microplate reader. Total

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flavonoid content was calculated as mg quercetin (Q) equivalents per gram of the extract [11].

2.5. DPPH Radicals Scavenging Assay

Antioxidant activity of the methanol extract was determined by DPPH radical scavenging method using 96-well microplates. 160 µL of different concentrations of the extract (0.625-5 mg/mL) was mixed with 40 µL of DPPH solution (150 µM). The plate was incubated at 25 °C for 30 min in a dark place. The absorbance of mixture was then measured at 517 nm using a microplate reader. The ability of the extract to DPPH radical scavenging was calculated using the following formula: DPPH scavenging activity (%) = $[(A-B)/A]\times100$; where A is the absorbance without test sample and B is the absorbance with the test sample. BHT was used as a positive control. The results were reported as SC_{50} values (concentration required for a 50% inhibition of DPPH) [11].

2.6. Mushroom Tyrosinase Inhibitory Assay

The tyrosinase inhibition activity of the methanol extract was determined in 96-well microplates according to the method described by Sarkhail *et al.* [11]. Different concentrations (0.625-5 mg/mL) of the extract were prepared in DMSO. 20 µL of tyrosinase solution (125 units/mL in 50 mM phosphate buffer, pH 6.8), 80 µL phosphate buffer (50 mM), and 40 µL of sample solution were loaded into the wells of a 96-well plate at room temperature. The reaction was completed after 10 minutes by adding 40 µL of L-tyrosine (2 mM). After incubation for 15 min, the amount of produced dopachrome in the reaction mixture was measured at 475 nm using the microplate reader. Kojic acid was used as the positive control. Percentage of tyrosinase inhibition was calculated as follows: tyrosinase inhibition (%) = $[(A-B)/A]\times100$ where A = absorbance at 475 nm without test sample and B = absorbance at 475 nm with test sample. The concentration at which half the tyrosinase activity is inhibited (IC_{50}) was determined for each sample.

2.7. Cell Culture

Mouse embryonic fibroblast cells (ATCC number of NIH3T3 cell line: CRL-1658) and human malignant melanoma cells (ATCC number of SKMEL-3 ce
line: HTB-69) were obtained from Pasteur Institute of Iran (NCBI). Cells were cultured in DMEM containing 10% fetal bovine serum, 100 U/mL of penicillin G, and 100 mg/mL streptomycin. Then the cells were maintained at 37 °C with 5% carbon dioxide in 95% humidity. The cells were subcultured in a ratio of 1:3 on every third or fourth day. For anti-proliferation studies, NIH3T3 and SKMEL-3 cells were plated at a density of 5×10^3 cells/well in 96-well plates. After 24 hours of incubation, the extract were added at the concentrations ranging from 0.0001 to 0.1 mg/mL to the
NIH3T3 cells and from 0.001 to 0.25 mg/mL to the SKMEL-3 cells and then further incubated for 72 hours [16].

2.8. Cell Viability (MTT Assay)

In vitro cell viability of *P. rigida* methanol extract was determined by MTT assay on NIH3T3 and SKMEL-3 cell lines [11]. Following treating the cells, MTT (0.5 mg/mL in phosphate buffered saline) was added and incubated for 4 h at 37 °C. The ability of the cells to reduce MTT to a blue formazan product was measured as cell viability. The formazan crystals were dissolved in DMSO and the color absorbance was measured at 545 nm using a microplate reader. The % of cell viability for NIH3T3 cells and the 50% inhibitory concentration on cell proliferation (IC_{50}) for SKMEL-3 cells were calculated from concentration-response curves after 72 hours exposure period.

2.9. Statistical Analysis

All bioassays were tested with three replications. The results were presented as mean ± S.E.M. The group means were compared using the ANOVA test followed by Tukey post hoc test. P value < 0.05 was considered as statistically significant. All analysis performed using Graph Pad Prism 6.

3. Results & Discussion

The use of medicinal plants in treatment of various cancer diseases has always been a favorite subject for research. Skin cancers are the most common cancer that divided into two major groups, including non-melanoma and melanoma skin cancers. The basal cell carcinoma and squamous cell carcinoma are known as the two main types of non-melanoma skin cancer. Melanoma is caused by malignancy of human melanocytes and representing only about 1% of all skin cancers. Malignant melanoma is the most aggressive form of skin cancer, which is almost resistant to many available treatments with a death rate of over 80% [17,18]. Therefore, research for novel natural sources that are capable to inhibit the melanoma cells proliferation is an important target for cancer therapy. Previous studies indicated that some extracts or fractions from *Phlomis* species have anti-tyrosinase activity as well as cytotoxic effect against different tumor cell lines including, HepG2, MCF7, HT29, A549, Caco2, T47D, and SKMEL-3 cells [8,9,10,19]. *Phlomis* species

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contain large amounts of terpenes, phenolics, flavonoids, phenyethanoids and iridoid glycosides, which reveal multiple biological activities such as antioxidant, antimicrobial, anti-tyrosinase, anti-diabetic and anti-cancer effects [7]. Given the above data, in the present study, we investigated *in vitro* cell viability of *P. rigida* methanol extract on the normal fibroblast and the malignant melanoma cell lines as long with its antioxidant, anti-tyrosinase activities. Besides, we determined the total phenolic and flavonoid contents to find a correlation between with these contents and antioxidant and anti-tyrosinase activity. The total phenol level of *P. rigida* methanol extract in terms of gallic acid equivalent (standard curve equation: y=0.287x+0.375, R^2=0.99) was 12.41±0.22 mg GA/g extract. The flavonoid content of the methanol extract was measured in terms of quercetin equivalent (the standard curve equation: y=0.124x+0.129, R^2=0.998) and was 12.75±1.36 mg Q/g extract. Of note, in the study of Saeidnia *et al.* [20] the total phenol content of *P. rigida* methanol (80%) extract was 101.8±3.3 mg GAE/g extract, which was higher than our result. As many studies have described, the phenolic and flavonoid contents are influenced by many factors, including geographical and climate conditions, harvest time and the extraction methods. However, our findings were far lower than many previous reports on *Phlomis* species [11,20-22].

Our findings from the DPPH assay exhibited a weak effect on the scavenging DPPH radicals for *P. rigida*, which was concentration-dependent with SC_{50} value higher than 5 mg/mL (data not shown). BHT as a positive control showed a SC_{50} value of 0.072 mg/mL. Recently, Heydari *et al.* [8] reported that SC_{50} of the methanol extract of *P. rigida* through DPPH radical scavenging assay was 0.2 mg/mL. Okur *et al.* [14] investigated the antioxidant activity of *P. rigida* methanol extract by ABTS and DPPH assays. They obtained a SC_{50} value of 0.89 mg/mL and 0.99 mg/mL, using DPPH radical scavenging and ABTS assays, respectively. In another study, the SC_{50} value for the methanol extract of *P. rigida* measured by DPPH assay was 58.7 µg/mL [20]. The difference in the type and level of phenolic compounds can affect the level of antioxidant activity. In our study, outcomes displayed a very weak DPPH radical scavenging activity that may be related to the low level of phenolics and flavonoids with antioxidant properties. In addition, in this context, our findings uncovered an inhibitory effect of the extract on mushroom tyrosinase activity, which is augmented with the increasing concentration of the extract. The IC_{50} value of the extract (1.092 mg/mL) that was far higher than that of kojic acid, a well-known tyrosinase inhibitor (0.049 mg/mL). In agreement with our results, previous studies indicated that the IC_{50} values for the anti-tyrosinase activity of some *Phlomis* species ranged from 0.99 to 1.56 mg/mL [10,11,23]. According to our literature survey, the anti-tyrosinase activity was reported for some phenolic and flavonoid compounds [3,24]. Therefore, we suggest that the anti-tyrosinase activity that has been observed for *P. rigida* methanol extract in the current study may be correlated with its phenolic and/or non-phenolic profiles. On the other hand, as the scavenging radical mechanism is one of the important pathways of tyrosinase activity inhibition, it can be proposed the weak anti-tyrosinase activity of the extract is likely connected with its low radical scavenging capacity.

Furthermore, in order to investigate the anti-melanoma activity of *P. rigida* MeOH (80%) extract, an MTT test was performed using malignant SKMEL-3 cells. In addition, to determine the selectivity of toxicity, the effect of the extract on the cell viability in NIH3T3 normal cells was examined by MTT assay. Interestingly, the extract not only showed no cytotoxic effect at all tested concentrations (0.0001-0.1 mg/mL) on NIH3T3 cells, but also exhibited a proliferative effect on the cells, particularly at low concentrations. As shown in Fig. 1, the cells viability of *P. rigida* extract significantly increased at the concentrations 0.0001 to 0.1 mg/mL compared to the control group after 72 hours of treatment.

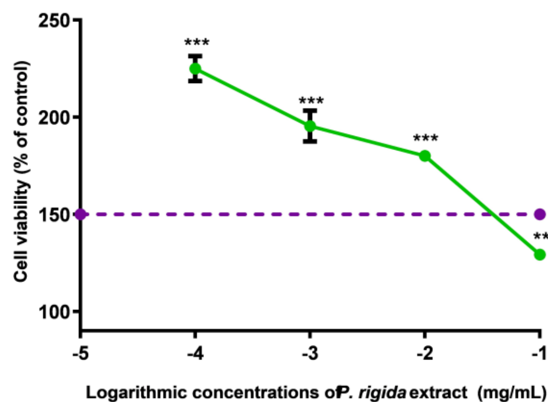

Fig 1. Effect of different logarithmic concentrations of *P. rigida* extract on viability of NIH3T3 cell line was determined by MTT assay after 72 hours. Results were expressed as percentages relative to the control, and are presented as mean ± S.E.M of at least three independent experiments. **, and *** significant increment from the untreated control group at P< 0.01, and P< 0.001, respectively.

Inversely, all tested concentrations of *P. rigida* MeOH extract (0.001-0.25 mg/mL) had a significant reduction effect on the viability of SKMEL-3 cells compared to the

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control group (Fig. 2) and with increasing the extract concentration, more cell death occurs.

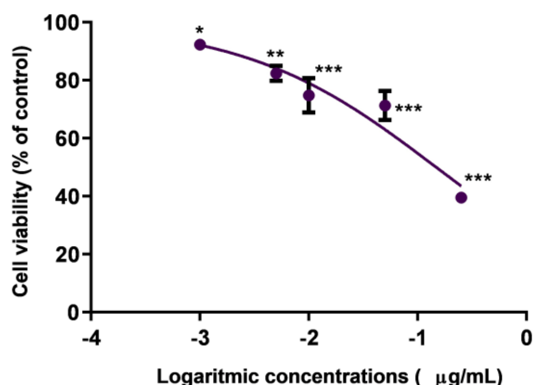

Fig 2. Effect of different logarithmic concentrations of *P. rigida* extract on viability of SKMEL-3 cell line was determined by MTT assay after 72 hours. The results were expressed as percentages relative to the control, and are presented as mean ± S.E.M of at least three independent experiments. *, **, and *** significant increment from the untreated control group at $P<0.05$, $P< 0.01$, and $P< 0.001$, respectively.

The extract showed cytotoxicity against SKMEL-3 cells with an IC_{50} value about 0.148 mg/mL (95% confidence limit, 0.09345 to 0.2362) after 72 hours of treatment. The results indicated that the methanol extract was not toxic on the normal cells at concentrations between 0.0001 and 0.1 mg/mL, but significantly reduced cell viability in the malignant melanoma cells, especially at higher concentrations than 0.01 mg/mL (with $P<0.01$ and $P<0.001$). Previous studies have shown that some *Phlomis* species have cytotoxic properties against malignant SKMEL-3 cells [10,11,23]. In the earlier study, the extract of *P. persica*, *P. bruguieri*, *P. anisodontea*, *P. olivieri*, and *P. kurdica* revealed the cytotoxic effect around 50% against SKMEL-3 cells at concentration of 0.5 mg/mL [10,23]. In another study, *P. caucasica* methanol extract has shown the cytotoxic effect on SKMEL-3 cells with the IC_{50} value of 0.143 mg/mL [10], which is closely similar to our result. Resently, cytotoxicity of the methanol extract from aerial parts of *P. rigida* against four cancer cell lines including, MCF-7, MDBK, HT-29 and A-549 were considered using the MTT method. The results showed the relatively mild toxicity of extracts on these cells (IC_{50} > 100 µg /mL) [8], which was almost similar to our results on SKMEL-3 cells (IC_{50} ≈ 148 µg/mL). Some isolated phenolics, flavonoids, phenylethanoids, and iridoid glycosides from *P. rigida* and other species were known as cytotoxic and anti-melanoma compounds such as luteolin-7-O-glucoside, shanzhiside methyl ester, and verbascoside [4,7,8,24,25]. For example, luteolin-7-O-glucoside has an Inhibiting human lactate dehydrogenase activity, which is known as an essential enzyme for survival and proliferation of cancer cells [25] On the other hands, verbascoside is one of the major phenylethanoid glycosides that found in the most species of *Phlomis* [7,26]. Some reported studies indicated that verbascoside has evidenced selectivity toward some tumor cells such as A5 cells (multistage cancer of mouse skin) [27]. These studies supported the idea that *P. rigida* methanol extract contains compounds with cytotoxic effect on malignant SKMEL-3 cells.

4. Conclusion

In conclusion *P. rigida* methanol (80%) extract showed very weak DPPH radical scavenging effect. In addition, it had weaker anti-tyrosinase activity than kojic acid. These effects are closely correlated with its phenolic and flavonoid contents that were not remarkable in the extract. In addition, *P. rigida* methanol extract showed a selective cytotoxic effect against malignant melanoma SKMEL-3 cells compared with the normal NIH3T3 cells that may be due to the presence of different chemical composition such as polyphenolics and iridoid glycosides. This is the first report about the effect of this species on mushroom tyrosinase and malignant melanoma cells. Therefore, based on our results, *P. rigida* methanol extract can be used as a good candidate for further investigation to discover bioactive natural agents in melanoma treatment.

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Conflict of interest

The authors of this article announce that we have no conflict of interest.

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References

[1] Chang TS. An updated review of tyrosinase inhibitors. Int J Mol Sci. 2009; 10(6): 2440-2475.
[2] Solano F, Briganti S, Picardo M, Ghanem GH. Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. Pigment Cell Res. 2006; 19(6): 550-571.
[3] Qian W, Liu W, Zhu D, Cao Y, Tang A, Gong G, Su H. Natural skin whitening compounds for the treatment of melanogenesis (Review). Exp Ther Med. 2020; 20, 173-185.
[4] Khalaf H, Jasim R, Ibrahim I. Verbascoside-A review of its antitumor activities. Pharmacol Pharm. 2021; 12(6): 109-126.
[5] Azizizn D, Cutler DF. Anatomical, cytological and phytochemical studies on *Phlomis* L. and Eremostachys Bunge (Labiatae). Bot J Linn Soc. 1982; 85(4): 249-281.
[6] Rechinger KH. In: Rechinger K.H. (ed.), Flora Iranica, Labiatae, Vol. 150, pp. 292-317. Academische Druck and Verlagsanstalt, Graz, Austria, 1982.
[7] Limem-Ben Amor I, Boubaker J, Ben Sgaier M, I. Skandrani, Bhouri W, et al. Phytochemistry and biological activities of *Phlomis species*. J Ethnopharmacol. 2009; 125(2): 183-202.
[8] Heydari F, Ghafarzadegan R, Mofasseri M, Ghasemi S V, Kashefi M, Hajiaghaee R et al. Phytochemical analysis and biological activities of essential oil and extract of *Phlomis rigida* Labill. J. Med. Plants 2021; 20 (80) :13-22.
[9] Soltani-Nasab F, Asgarpanah J, Majdzadeh M, Ostad SN. Investigating the effect of *Phlomis lanceolata* Boiss and hohen on cancer cell lines. Acta Med Iran. 2014; 52(5): 333-336.
[10] Salimi M, Sarkhail P, Tahmasvand R, Baeeri M. Determination of anti-melanogenic activity of *Phlomis kurdica* in human melanoma SKMEL-3 cells. Iran J Pharm Res. 2016; 12(1): 1-10.
[11] Sarkhail P, Salimi M, Sarkheil P, Heidarnezhad F, Saeidnia S. Evaluation of anti-Melanogenic and cytotoxic Activities of *Phlomis caucasica* on human melanoma SKMEL-3 cells. Int J Cancer Management. 2017; 10(3):e4633.
[12] Turker AU, Yıldırım AB. Evaluation of Antibacterial and Antitumor Activities of Some Turkish Endemic Plants. Trop J Pharm Res. 2014; 12(6): 1003.
[13] Demirci B, Baser KHC, Dadandi M. Composition of the Essential Oils of *Phlomis rigida* Labill. and *P. samia* L. J Essent Oil Res. 2006; 18. 328-331.
[14] Okur ME, Karadağ A, Özhan Y, Sipahi H, Ayla Ş, Daylan B, Kültür Ş, Demirci B, Demirci F. Anti-inflammatory, analgesic and *in vivo-in vitro* wound healing potential of the *Phlomis rigida* Labill. extract. J Ethnopharmacol. 2020; 113408.
[15] Takeda Y, Matsumura H, Masuda T, Honda G, Otsuka H,, Takaishi Y, Sezik E, Eşilada E. Phlorigidosides A-C, iridoid glucosides from *Phlomis rigida*. Phytochem 2000; 53, 931-935.
[16] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65(1-2):55–63.
[17] Eskolaky EB, Ardjmand M, Akbarzadeh A. Evaluation of anti-cancer properties of pegylated ethosomal Paclitaxel on human melanoma Cell Line SK-MEL-3. Trop J Pharm Res. 2015; 14(8):1421.
[18] Saginala K, Barsouk A, Aluru JS, Rawla P, Barsouk A. Epidemiology of Melanoma. Med Sci (Basel). 2021; 9(4):63.
[19] Sarkhail P, Sahranavard S, Nikan M, Gafari S, Tehrani Ba. Evaluation of the cytotoxic activity of extracts from six species of *Phlomis* genus. J Appl Pharm Sci. 2017; 7(20): 180-184.
[20] Saeidnia S, Nikan M, Mirnezami T, Gohari AR, Manayi A. Micromorphological characterizations and phytochemical contents of some *Phlomis* species from Iran. Int J Pharm Pharm Sci. 2016; 8(1):157-161.
[21] Sarikurkcu C, Sabih Ozer M, Cakir A, Eskici M, Mete E. GC/MS Evaluation and *in vitro* antioxidant activity of essential oil and solvent extracts of an endemic plant used as folk remedy in Turkey: *Phlomis* bourgaei Boiss. Evid Based Complement Alternat Med. 2013; 2013: 1-7.
[22] Al-Qudah MA, Obeidat SM, Saleh AM, El-Oqlah AA, Al-Masaeed E, Al-Jaber HI, et al. Volatile components analysis, total phenolic, flavonoid contents, and antioxidant activity of *Phlomis* species collected from Jordan. Essent Oil-Bear Plants. 2018; 21(3): 583-599.
[23] Sarkhail P, Salimi M, Saeidnia S, Nikan M, Kurpaz-mahmoodabadi M. Influence of four *Phlomis* species on melanogenesis in human malignant melanoma (SKMEL-3) *cells. Res* J Pharmacogn. 2019; 6(2): 19-24.
[24] Sun L, Guo YQ, Zhang Y, Zhuang Y. Antioxidant and anti-tyrosinase activities of phenolic extracts from rape bee pollen and inhibitory melanogenesis by cAMP/MITF/TYR pathway in B16 mouse melanoma cells. Front Pharmacol. 2017; 8. 10.3389/fphar.2017.00104.
[25] Bader A, Tuccinardi T, Granchi C, Martinelli A, Macchia M, et al. Phenylpropanoids and flavonoids from *Phlomis kurdica* as inhibitors of human lactate dehydrogenase. Phytochem. 2015; 116, 262-268.
[26] Sarkhail P, Nikan M, Sarkheil P, Gohari AR, Ajani Y, Hosseini R, Hadjiakhoondi A, Saeidnia S. Quantification of verbascoside in medicinal species of *Phlomis* and their genetic relationships. Daru J Pharm Sci. 2014; 22(1): 22-32.
[27] Cheimonidi C, Samara P, Polychronopoulos P, Tsakiri EN, Nikou T, Myrianthopoulos V, SakellaropoulosT, Zoumpourlis V, Mikros E, Papassideri I, Argyropoulou A. Selective cytotoxicity of the herbal substance acteoside against tumor cells and Its mechanistic insights. Redox Biol. 2018; 16, 169-178.

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