Research Article

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Psoralea corylifolia (babchi) seeds enhance proliferation of normal human cultured melanocytes: GC–MS profiling and biological investigation

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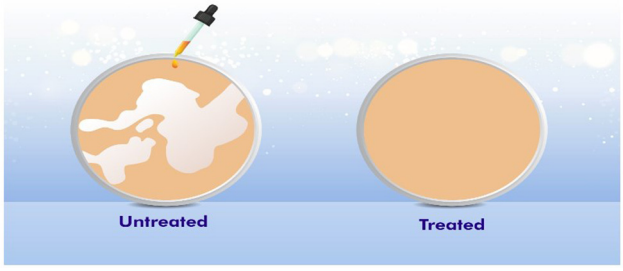
Abstract: Plants are considered as a source of medicine to treat skin-related disorders since decades. Seeds of *Psoralea corylifolia* were used thousands of years ago for re-pigmentation in vitiligo patients especially in South Asia. It is a well-known fact that melanocytes, which are the specialized cells in the skin causes vitiligo through a series of chemical and enzymatic reactions, mainly due to the copper containing enzyme tyrosinase. Conditions associated with overproduction of melanin are termed hyperpigmentation. The treatment options are generally limited due to the complicated and slow pathogenesis of the disease. Therefore, therapeutic repigmentation modalities for the treatment of vitiligo are highly desirable. The aim of this study was to investigate the effects of various doses of ethanol and water extracts of *P. corylifolia* seeds on proliferation of normal human cultured melanocytes and its gas chromatography–mass spectrometry profiling.



Graphical abstract

Keywords: melanocytes, babchi, pigmentation, tyrosinase, vitiligo

1 Introduction

Natural products isolated from plants are an important component of modern-day health care system. Wide ranges of substances that can be used to treat chronic, autoimmune disease like vitiligo are derived from plants used for traditional medicine. Currently, 1% of the world's population is diagnosed with vitiligo, which is a depigmentation disorder [1]. It appears as white patches on the skin due to the loss of functioning melanocytes. These white patches may affect cosmetic appearance and psychosocial functions [2]. This disorder is primarily treated by several medical therapies. Medicinal plants have been evaluated for their effect against skin disorders including vitiligo as well as for their chemical properties [3,4].

It has been proved that pharmacological investigations and *in vitro* screening methods of crude plant extracts with potentially useful properties could provide the needed observations necessary to elect for further investigation. A plant-based medicine is often prepared from crude plant extracts consisting of roots and stems

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and includes mixture of various phytochemicals ranging from polar to non-polar. Treatment of prolonged as well as contagious diseases can be done by these phytochemicals having complex structures. A large number of biologically active secondary metabolites exist in various plant species, but only few of them have been examined and sustained to be significant source of bioactive agents. Important information on chemical and pharmacological activities can be provided by spectrometric and chromatographic methods, which therefore helps to select the most potent constituents. To our knowledge, this is the first time, human cultured melanocytes proliferation and gas chromatography–mass spectrometry (GC–MS) studies were examined together.

2 Materials and methods

2.1 Preparation of extract

A portion of dried seeds (100 g) of *P. corylifolia* was crushed and soaked in distilled water/ethanol (200 mL) at 45°C. Extracts were filtered through a paper filter and the resulting solutions were concentrated in vacuum to dryness to give ethanol and water extracts (12 g approx) simultaneously. Both extracts were stored in a refrigerator for further use at 4°C.

2.2 GC–MS analysis

For GC–MS analysis, Agilent GC 7890A combined with a triple axis detector 5975C (single quadrupole mass spectrometer) was used. Agilent HP 5MS chromatographic column (30 m × 0.25 mm × 0.25 µm film thickness) was used, with high-purity helium as the gas carrier, at a flow rate of 1 mL/min. The temperature recorded for the injector was 280°C and it was equipped with a splitless injector at 20:1. The MS source temperature was set at 230°C and the Quad temperature was at 150°C. The oven temperature was initially at 40°C which was held for 1 min and then increased to 150°C at 5°C/min for another 1 min, then increased further to 300°C at 5°C/min for 1 min. For the MS ion source the temperature was set at 150°C, while the inlet line temperature was set to 280°C. The scan range was set at 40–600 mass ranges at 70 eV (electron energy) and the solvent delay of 3 min. Finally, after scanning the unknown compounds were identified by comparing the spectra with that provided in the NIST 2008 library (National Institute of Standard and Technology library).

2.3 Melanocyte isolation and culturing

This study has been approved by the ethical committee, College of Medicine, King Saud University. Normal human foreskin was used in this study to obtain melanocytes, which was used in our previous communication as well. Skin biopsy samples were washed three times each with sterile phosphate-buffered saline (PBS) and then cut into small pieces (5 mm × 5 mm) for further processing. The obtained tissue samples were kept in PBS containing 2.5% trypsin solution followed by incubation at 37°C for 1 h. Dermal face was used to obtain epidermal sheets, and epidermal cells were then released by vigorous pipetting. Centrifugation method (200*g*, 5 min, at room temperature) is used to harvest suspended cells and were seeded in two 25 cm^2 tissue culture flasks with a melanocyte growth-promoting medium (GIBCO, Grand Island, NY, USA). The obtained medium is incubated at 37°C in a humidified atmosphere containing 5% CO_2 as described earlier with slight modifications [5]. After every 24 h of seeding to remove the free-floating cells, the melanocyte growth-promoting medium was changed. Subsequently, the culture medium was also changed regularly after every 48 h. About 7–10 days after primary seeding, harvesting of semi-confluent cells were done by incubation with 0.25% trypsin and 0.01% ethylenediamine tetraacetic acid solution (Sigma, St Louis, MO, USA) at 37°C for 3–5 min. Soybean trypsin inhibitor solution (Sigma) was used to terminate the reaction. Cells were centrifuged at 500*g* for 5 min, and the cell pellet obtained was re-suspended and further incubated in the melanocyte growth-promoting medium. The third- to sixth-passage cells were used in the current experimental work.

2.4 Exposure to plant extract

For seed melanocytes 96-well plates were used at a density of 2×10^4 cells per well and overnight incubation. The medium was replaced with the different concentrations of plant extract containing melanocyte growth-promoting medium. Melanocyte growth-promoting medium was added to the control melanocytes without plant extract. Different concentrations of plant extract exposed cells and control cells were then incubated for 72 h.

2.5 Cell proliferation assessment

The plant extract was evaluated on normal human cultured melanocytes by cell proliferation assay for dose–response

curve. Capacity of the reducing enzymes present in viable cells to convert 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to formazan crystals provides information about the evaluation of cell proliferative effect. Control (without any plant extracts) and experimental wells were replaced after incubation at 37°C for 72 h by 100 µL/well of MTT (0.5 mg/mL) in PBS and were incubated at 37°C for further 3 h. Purple formazan crystals were formed at the bottom of the wells after MTT solution was removed and the crystals were dissolved using isopropanol (100 µL/well) with shaking for 1 h at room temperature. Microplate reader (ELx800 counter, Universal Microplate Reader, BioTek Instruments, USA) confirms the absorbance at 549 nm. All the measurements were recorded in triplicates. The dose–response curve of the plant extract was established utilizing concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.95 µg/mL [6].

2.6 Statistical analysis

Mean values of quantitative variables across the categorical variables were compared by unpaired student *t*-test. The analysis was used to confirm or identify any significant differences between the different treated groups and the controls. A *P*-value <0.05 was considered as statistically significant. The data were analyzed using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA) statistical software.

3 Results

3.1 Plant extracts enhanced proliferation of cultured melanocytes

Based on mitochondrial activity, the MTT assay showed a significant increase in the proliferation of melanocytes treated with ethanol and water plant extracts. As illustrated in Figure 1(a), the ethanol extracts enhanced proliferation by 1.41-, 1.5-, and 1.60-fold at doses of 1.95, 3.91, and 7.81 µg/mL, respectively, compared with controls, with a maximal and significant effect obtained following 7.81 µg/mL ($P = 0.0003$). On the other hand, ethanol extract (Figure 1(a)) significantly decreased melanocytes proliferation rate by 0.25-, 0.27-, 0.30-, 0.33-, 0.42-, and 0.39-fold at doses of 15.63, 31.25, 62.5, 125, 250, and 500 µg/mL, respectively, compared to control, with a minimum and significant obtained following 15.63 µg/mL ($P < 0.0001$).

The water extracts enhanced proliferation by 1.21-, 1.24-, 1.26-, 1.27-, 1.32-, 1.23-, and 1.23-fold at doses of 7.81, 15.63, 31.25, 62.5, 125, 250, and 500 µg/mL, respectively, compared with controls, with a maximal and significant effect obtained following 125 µg/mL ($P = 0.0009$). In addition, 1.95 and 3.91 µg/mL doses did not show any significant effect on melanocytes proliferation, although there was 1.08- and 1.17-fold proliferation (Figure 1(b)).

4 Discussion

P. corylifolia is from family Fabaceae. It is called babchi or bakuchi in local language and an important medicinal plant. It is mainly found in South Asia [7]. Medicinal values and uses of *P. corylifolia* have been reported in British, Chinese, Indian, and American pharmacopeias as well as in the traditional system of medicines of Ayurveda and Unani [8]. Seeds of *P. corylifolia* contain furocoumarins, which is a source of psoralens [9]. These products have been used in the treatment of vitiligo, leprosy, and psoriasis from ages [10].

In the current study, the ethanol and water extract of seeds of *P. corylifolia* were used for proliferation studies on normal human cultured melanocytes. GC–MS studies further confirm the presence of various pharmacophores that are believed to be involved in the process.

A dark pigment responsible for the darkening of the skin and are found in the skin of humans is called as melanin. This condition is called vitiligo due to the activity of specialized cells in the skin called melanocytes following a series of biological reactions, mainly involving the enzyme tyrosinase [11,12]. Melanosomes are the packets of melanin pigment migrated to the outer layer of the skin and cause the darkening of the skin. The degree of darkening is associated with certain dermatological conditions like skin type and/or sun exposure. There are several dermatological conditions associated with unwanted or excessive production of melanin, which results in the overproduction of melanin, a condition termed as hyperpigmentation. Due to the difficult nature of the disease, the treatment options are often difficult due to the risk for relapse in the patients who do respond. Therefore, the need for new therapeutic repigmentation modalities for the treatment of vitiligo is necessary [13].

Proliferation is essential biological parameters for any cells, including melanocytes. To determine the proliferation of melanocytes, MTT assay used reported that

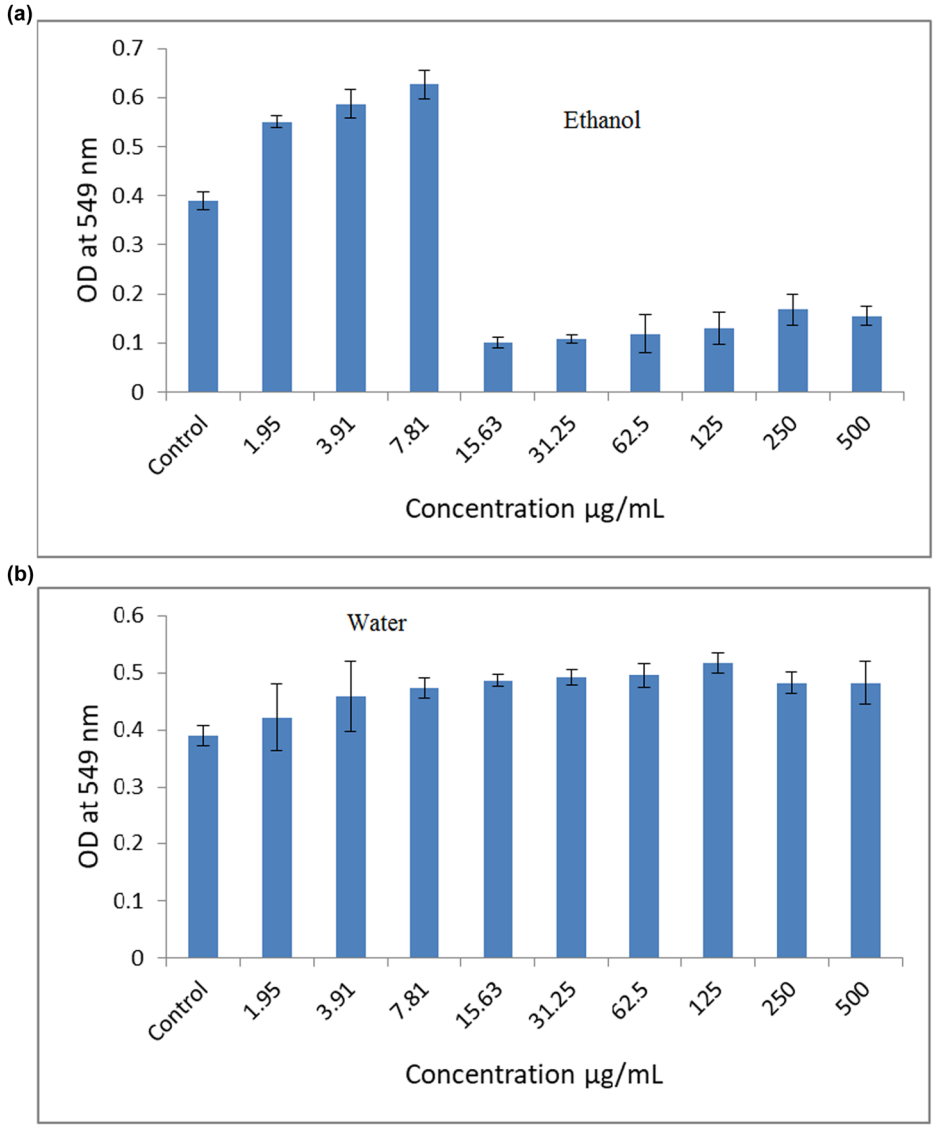

Figure 1: (a) Effect of different doses of ethanol plant extract on the proliferation of normal human cultured melanocytes as measured by MTT 72 h following exposure. (b) Effect of different doses of water plant extract on the proliferation of normal human cultured melanocytes as measured by MTT 72 h following exposure; OD: optical density.

the seed powder of *P. corylifolia* shows confirmed effectiveness in comparison to control in pigmenting the vitiligo skin on the patient's forearm and face in *in vivo* study [14]. Therefore, in the present study, melanocytes were treated with ethanol and water extracts of seeds of *P. corylifolia*, as the objectives were to stimulate the proliferation of melanocytes in *in vitro* study.

Our results showed that ethanol plant extract significantly increased the proliferation of melanocytes at lower doses (1.95–7.81 µg/mL), whereas higher doses (15.63–500 µgmL) significantly decreased melanocytes proliferation. Water-based plant extract was enhanced proliferation of melanocytes as dose-dependent manner. However, lower doses (1.95 and 3.91 µg/mL) of water-based extracts did not enhance proliferation significantly. Nevertheless, higher doses (7.81–500 µg/mL) significantly increased melanocytes proliferation. The above results indicate induction of proliferation in ethanol and water extracts treated melanocytes compared with the controls. The graphs support these results (Figure 1(a) and (b)).

4.1 Phytochemical screening

In order to further support our findings and to identify the most potent molecules present, we analyzed both ethanol and water extracts with gas chromatography coupled

Table 1: GC–MS data of ethanol extract

Hit name	Mol weight (amu)	RT (min)	Area (Ab*s)	Area (%)
Caryophyllene oxide	220.183	10.284	1,168,253	2.1
2*H*-Furo[2,3-*H*]-1-benzopyran-2-one	186.032	11.643	900,441	1.62
Hexadecanoic acid, ethyl ester	284.272	12.532	1,999,187	3.6
2(1*H*)-Quinolinone, 3,4-dimethyl-	173.084	13.254	24,101,472	43.43
Linoleic acid ethyl ester	308.272	13.38	5,127,603	9.24
Octadecanoic acid, ethyl ester	312.303	13.497	1,886,557	3.39
1,2-Dimethyl-4-quinolone	173.084	13.657	4,998,491	9
1,2,3,7-Tetramethylindole	173.12	13.791	1,454,192	2.62
2-Quinolinone, 3,4-dimethyl-	173.084	14.017	2,786,775	5.02
1,2-Dimethyl-4-quinolone	173.084	14.076	7,151,418	12.88
Tricosane	324.376	14.244	3,914,572	7.05

with mass spectroscopy (Tables 1 and 2). GC–MS has commonly been employed from the last many years for detection and identification of various bioactive therapeutic compounds present in medicinal plants [15]. Moreover, GC–MS is one of the rapid techniques to identify various compounds, including alcohols, nitro compounds, long chain hydrocarbons, alkaloids, steroids, esters, organic, and amino acids. Therefore, in the present study, GC–MS technique was adopted for detection and identification of phytochemicals present utilizing the library present. Since we are aiming for the polar components ethanol and water extract were used during this study. The results of preliminary investigation on the bioactive natural products present in water extract of *P. corylifolia* are presented in Tables 1 and 2. It is evident from the data that the GC–MS spectrum of ethanol and water confirmed the presence of various phytochemicals with varied retention times. Compounds were analyzed by mass spectrometer eluted at different retention times to identify the compounds on the basis of their structure and polarity. These mass spectra of the eluted compound were identified from the data library.

Sesquiterpenes, quinolinone, long chain alkanes, and alkaloids were noticed in all solvent extracts used. Ethanol extract showed the presence of hexadecanoic acid, a major phytoconstituent known to possess strong antimicrobial activity [16]. Moreover, caryophyllene oxide, which is a sesquiterpene is also found in the extract which has known anti-inflammatory activity. Dimethyl quinolinone and its various derivatives, which are present in large amount, are known for its antidepressant activity [17]. Tricosane, which is a long chain alkane and have known biological activity, is also present in significant amount

Table 2: GC–MS data of water extract

Hit name	Mol weight (amu)	RT (min)	Area (Ab*s)	Area (%)
2-Pentanone	86.073	4.035	4,471,787	2.68
Acetic acid	60.021	4.436	1,777,642	1.06
Phenol, 2-methoxy-	124.052	12.681	693,004	0.42
Benzaldehyde, 3-methyl-	120.058	17.058	1,614,881	0.968
Phenol, 2,6-dimethoxy-	154.063	20.003	1,911,844	1.14
Benzaldehyde, 4-hydroxy-	122.037	21.657	1,101,137	0.66
2,7-Naphthalenediol	160.052	23.929	1,059,783	0.63
Phthalazin-1-one	146.048	29.184	3,523,988	2.11
4-Acetylisocoumarin	188.047	30.106	2,374,616	1.42
2*H*-Furo[2,3-*H*]-1-benzopyran-2-one	186.032	32.085	98,539,382	59.11
2-(1-Hydroxycycloheptyl)-furan	180.115	40.807	1,606,987	0.96
6-Aza-*B*-homo-5.alpha-cholestano[6,7-*d*]tetrazole	426.372	44.586	2,835,331	1.7
2(1*H*)naphthalenone, 3,5,6,7,8,8*a*-hexahydro-4,8*a*-dimethyl-6-(1-methylethenyl)-	218.167	45.636	6,448,549	3.86
Stigmast-4-en-3-one	412.371	48.301	4,912,337	2.94
Friedelan-3-one	426.386	50.961	30,599,697	18.35
Citrost-7-en-3-ol	428.402	53.48	3,228,693	1.93

along with 1,2,3,7-tetramethylindole. Phytochemicals which are identified by data library like sesquiterpenes, quinolinone, hexadecanoic acid, and tricosane are not reported earlier and are known to have a wide range of pharmacological properties including antibacterial, antifungal, antiviral, and antioxidant. Coumarin, isocoumarin, and triterpene are the plant secondary metabolites occurring in water extract and in several other medicinally important plants known to possess many biological activities like antimicrobial, antidepressant, antitumor, and anti-inflammatory. Moreover, these compounds are also reported for the first time in the present communication. Some phenolic compounds are also found in the water extract, which provide defense against various pathogens, regulate cell division and growth, and help in pigmentation and many other metabolic pathways as mentioned in the literature [18]. Benzopyranone, which is a furocoumarin, is found in more than 59% quantity and have never reported before in the water extract of *P. corylifolia*. Since many decades, it is used as a universal treatment for many types of disease specially vitiligo in Middle East and South Asia. GC–MS profiling was performed for water and ethanol extracts of seeds due to the fact that they enhance the proliferation of normal human cultured melanocytes. The present study identified few other compounds which are not reported to be present in the extract of the seeds of *P. corylifolia* are citrost-7-en-3-ol, friedelan-3-one, stigmast-4-en-3-one, 2-pentanone, and acetic acid. Many of these identified constituents are known to possess several pharmacological activities [19].

5 Results

Different doses of plant extracts significantly increased the proliferation of melanocytes. Ethanol extract significantly increased proliferation from dose 1.95 to 7.81 µg/mL and significantly decreased proliferation dose from 15.63 to 500 µg/mL compared to the control. Water extracts significantly increased proliferation from 7.81 to 500 µg/mL compared to the control.

6 Conclusions

These results encourage the potential implementation of *P. corylifolia* seeds extracts as application in vitiligo treatment by enhancing the proliferation of normal human cultured melanocytes. Therefore, ethanol and water extracts of seeds of *P. corylifolia* might be considered as a potential agent for a possible therapy for skin hypo-pigmentation disorders such as vitiligo. Moreover, the potential efficacy and safety of high doses of ethanol and water extracts of seeds of *P. corylifolia* should also be critically assessed by further larger *in vivo*/human studies.

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval: This study has been approved by the ethical committee, College of Medicine, King Saud University.

Data availability statement: All data are incorporated in the manuscript.

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