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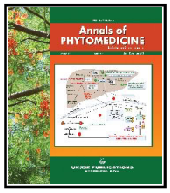

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Development of hydroquinone free herbal gel for the management of melasma

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Abstract

A frequent and acquired hyperpigmented condition of the face is melasma. Although, there are numerous treatment options for melasma, their effectiveness is only moderate. They may result in serious adverse responses in some circumstances. For instance, prolonged use of the drug hydroquinone, which is frequently prescribed to treat hyperpigmentation, can result in hazardous side effects such as depigmentation, hypochromia that resembles vitiligo, or leukoderma. Hence, the hydroalcoholic extract (70%v/v) of *Glycyrrhiza glabra* root, *Vitis vinifera* fruits, *Vaccinium myrtillus* fruits, *Citrullus lanatus* seed, *Phyllanthus emblica* fruits, *Butea monosperma* flower, *Nelumbo nucifera* leaves, *Senna auriculata* leaves, *Acalypha indica* leaves tested for the tyrosinase enzyme inhibitory activity. All the tested extracts demonstrated comparable inhibition of tyrosinase enzyme in a dose-dependent manner when compared with standard kojic acid and a herbal gel containing the extracts of above plants was formulated and evaluated for its product performance. The herbal gel product displayed acceptable pharmacological behavior in addition to strong anti-melanogenic effects. The developed topical gel product would be a viable hydroquinone-free natural formulation candidate for clinical investigations on hyperpigmentation.

1. Introduction

Melasma, which is an overproduction of melanin in the skin, gets its name from the Greek word for black - "Melas". One of the most common and treatment-resistant forms of acquired hyperpigmentation is melasma (Handel *et al*., 2014). Women are 9 times more likely than men to get melasma, and those with darker complexion between the ages of 20 and 45 are more likely to get melasma. Previous studies showed that at least four pathogenesis are involved in the development of melasma such as melanogenesis, inflammation, and vascularization. Although, melasma is not life threatening, it greatly impacts the quality of the life of patients. Management of melasma can be challenging and requires long-term treatment with topical agents (Bandyopadhyay, 2009). They may result in serious adverse responses in some circumstances. For instance, repeated applications of hydroquinone, a substance frequently used to treat hyperpigmentation, can result in toxic responses, depigmentation, ochronosis, hypochromia that resembles vitiligo, and skin cancer (Zhang *et al*., 2019). Melasma can be expensive to cure, and herbal therapies typically cost less, which is another factor driving interest in them for the treatment of melasma.

Therefore, it is a challenging skin disorder. Hence, development of effective and safe products for melasma is becoming emergent, several of them from natural sources. Topical polyherbal preparations are the most recent additions to the list of melasma treatment options, as they reduce and divert melanin synthesis. When antioxidant, arbutin, anti-inflammatory ingredients were used in cosmetic formulations, the therapeutic impact of such a therapy was increased (Rashmi Sarkar *et al*., 2019). The development of numerous novel agents, many of which are derived from natural sources, has been recommended because of safer effect in the treatment of melasma. The Ayurvedic philosophy promotes the use of several herbs in a certain ratio to achieve greater potency. The toxicity of individual herbs is significantly reduced when they are used in a certain combination. The following herbs were used for the formulation of herbal gel, *viz., G. glabra* root, *V. vinifera* seeds, *V. myrtillus* fruits, *C. lanatus* seed, *P. emblica* fruits, *B. monosperma* flower, *N. nucifera* leaves, *S. auriculata* leaves, *A. indica* leaves are shown in Figure 1.

G. glabra (Fabaceae) extract is one of many natural compounds that act as skin brightener when applied topically. By spreading the melanin, preventing melanin biosynthesis, and suppressing cyclooxygenase activity, liquorice extract reduces hyperpigmentation by lowering the formation of free radicals (Yokota *et al*., 1998). *V. vinifera* (Vitaceae) fruits have a beneficial effect on melasma and it has tyrosinase inhibitor also (Salma Akbar Bagwan *et al*., 2022). *V. myrtillus* (Ericaceae) fruit extract is proven to fortify skin against redness and increase environmental defenses against UVA rays when applied topically to the skin (Wing-Kwan Chu *et al*., 2011). *C. lanatus* (Cucurbitaceae) seed contains vitamin A and other minerals that helps improve hyperpigmentation and overall skin appearances (Maria Sorokina *et al*., 2021). *P. emblica* (Phyllanthaceae) fruit affects skin changes such as wrinkles, dryness, and uneven pigmentation. (Tamanna Malik *et al*., 2020).

B. monosperma (Fabaceae) flower has antioxidant properties and also have free radical scavenger (Anuradha Sehrawat and Vijay Kumar,

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2012). *N. nucifera* (Nelumbonaceae) leaves regenerates new skin cells with regulated melanin and eliminates dead skin cells to remove hyperpigmentation efficiently. It used as a skin whitening and anti-wrinkle agents (Su-Yeon Kim and Gap-Soon Moon, 2015). *S. auriculata* (Fabaceae) is good for external application and helps treat many skin disorders. It prevent black spot, treats uneven skin tone and improves skin complexion (Vijayakumar Rajendran *et al*., 2017). *A. indica* (Euphorbiaceae) leaves is widely used for treatment of skin disease and ailments (Sudhakar Chekuri *et al*., 2020). The proposed study intends to produce a hydroquinone free safe topical gel for the treatment of melasma because herbal medicines are more widely accepted due to their less adverse effects and lower prices.

Figure 1: Plants selected for the formulation of herbal gel.

2. Materials and Methods

All the crude drugs were purchased from commercial store - Ansi Siddha and Ayurvedha, Zamin Pallavaram, Chennai. Kojic acid (K3125), tyrosinase lyophlized powder (T3824) and L-DOPA (D9628) was obtained from Sigma Aldrich (K3125). All chemicals and reagents used were of analytical grade.

2.1 Extraction of plant samples

The plants *G. glabra* root, *V. vinifera* seed (Grape seed), *C. lanatus* seed (Water melon), *V. myrtillus* fruits (Blue berry), *P. emblica* fruit, *B. monosperma* flower, *N. nucifera* leaves (Lotus), *S. auriculata* leaves was purchased from the commercial Siddha store. The shade dried crude drug was powdered and extracted by maceration with 70% v/v ethanol into a conical flask in the proportion of 1:10. The conical flask was kept in an orbital shaker for 24 h and filtered with gauze cloth. Thus, the obtained extract was stored for further use.

2.2 Qualitative analysis for phytochemicals

Hydroalcoholic (70% v/v) extract of selected plants were subjected for qualitative phytochemical analysis of secondary metabolites as per standard procedure (Khandelwal, 2004).

2.3 *In vitro* antimelanogenic effect of herbal extract (Tyrosinase inhibitory assay)

In the assay, each sample was dissolved in DMSO at a concentration of 100^{-1} g/ml (or M for pure chemicals). The enzyme solution (15 ml in 0.1 M phosphate buffer pH 6.8) and 1900 µl of test solution were combined immediately to obtain the assay mixtures. The reaction was started by adding 1000 µl of the substrate solution (1.5 mM L-DOPA in 0.1 M phosphate buffer pH 6.8) after a 30 min preincubation period at room temperature. A Shimadzu UV-1800 spectrophotometer was used to detect the absorbance at 475 nm after the assay mixture had been incubated at room temperature for 7 min. As a positive control, kojic acid, a well-known tyrosinase inhibitor, was used. In the aforementioned assay technique, tyrosinase inhibitory activity was expressed as a percentage inhibitory of the enzyme tyrosinase (Daclé Juliani Macrini *et al*., 2009).

$$\text{Percentage inhibition} = (1 - B/A) \times 100$$

where, A was the activities of the enzyme without test material and B was the activities of the enzyme with test material.

2.4 Formulation of herbal gel

The topical gel was prepared using ethanol (70%) v/v) extract of selected plants as per the formula given in the Table 1. The water

required for the formulations were separated into two parts. Propylene glycol was added to an exact amount of extracts that have been individually dissolved in 15 ml of water in one part. In another part, 35 ml of water were used to dissolve xanthan gum. To get the requisite skin pH (6.8-7) and to give the gel the desired viscosity, both of these solutions were combined in a beaker. Triethanolamine was then added dropwise to the mixture. After that, it was swirled for 2 h at 500 rpm using a propeller. The produced gel seemed homogeneous and bubble-free after stirring. The prepared gel was kept for 24 h at room temperature.

Table 1: Formulation of herbal gel using various herbal extract

S.No	Ingredients	Quantity taken per 100 gm (in gram)	Role of ingredients
1.	*G. glabra* root	0.3	Antioxidant (glabridin)
2.	*V. vinifera* seed	0.3	Antioxidant (resveratrol)
3.	*V. myrtillus* fruit	0.3	Source of arbutin.
4.	*C. lanatus* seed	0.3	Treats skin pigmentation
5.	*P. emblica* fruit	0.3	Treats skin pigmentation
6.	*B. monosperma* flower	0.3	Flavonoids
7.	*N. nucifera* leaves	0.3	flavonoids
8.	*S. auriculata* leaves	0.3	antibacterial
9.	*A. indica* leaves	0.3	Antiinflammatory
10.	Xanthan gum	1	Gelling agent/thickener
11.	Propylene glycol	2	Humectants/solvent
12.	Triethanolamine	0.5	Stabilizer or neutralizer
13.	Distilled water	q.s	q.s

Figure 2: Formulated herbal gel.

2.5 Evaluation of gel

Using a conventional approach, herbal formulations were assessed for their pH, gel appearance, spreadability, washability and skin irritation test (Amal *et al*., 2020). .

2.5.1 Physical stability

Checked visually for spreadability, consistency and appearance of gel.

pH measurement: pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter

2.5.2 Spreadability

Excess sample was placed between two glass slides for the purpose of determining spreadability, and it was compressed to a uniform thickness by placing 1 kg of weight on top of it for 5 mins. Weight (50 g) was then added to the pan. The spreadability was measured by the amount of time it takes for the upper glass slide moves over to the lower plate.

$$S = M \times L / T$$

M- Weight placed over the upper slide

L - Length moved on the glass

T - Time taken

2.5.3 Grittiness

The existence of particles in each formulation was examined under a microscope. If no particulate is seen under a light microscope, the gel preparation satisfies the condition of being free of specific matter and from grittiness as suitable for a topical application.

2.5.4 Skin irritation

Skin irritation tests on healthy male and female volunteers were conducted. On the inside surface of the hand, a 2 cm^2 area of 100 mg gel was administered for 6 h. After 6 h, the area was cleansed with acetone, and readings were taken using Draize's scale. No irritation; less irritation; irritation.

3. Results

3.1 Extraction

The percentage yield of *G. glabra* root (Liquorize), *V. vinifera* seed (Grape seed), *C. lanatus* seed (Water melon), *V. myrtillus* fruits (Blue berry), *P. emblica* fruit, *B. monosperma* flower, *N. nucifera* leaves (Lotus), *S. auriculata* leaves were tabulated in Table 2.

3.2 Qualitative phytochemical analysis

The phytochemical analysis was performed to identify the presence of phytoconstituents in the hydroalcoholic extract of selected plants.

Table 2: Percentage yield of the hydroalcoholic extract of selected plants

Name of the plant	Parts used	Percentage yield (%w/w)	Colour
G. glabra	Root	3.04	Reddish brown
V. vinifera	Seed	3.0	Reddish brown
V. myrtillus	Fruit	6.096	Brown
C. lanatus	Seed	4.08	Yellowish brown
P. emblica	Fruit	13.16	Blackish brown
B. monosperma	Flower	7.412	Reddish brown
S. auriculata	Leaves	5.24	Reddish brown
A. indica	Leaves	4.95	Reddish brown
N. nucifera	Leaves	4.964	Blackish brown

Table 3: Qualitative phytochemical analysis of the hydroalcohol extract of selected plants

Plants	Alkaloids	Steroids	Glycoside	Tannins	Saponins	Terpenoids	Flavonoids
G. glabra rhizome	-	+	-	-	+	+	+
V. vinifera seed	-	+	+	+	-	+	+
V. myrtillus fruit	+	+	-	+	-	-	+
C. lanatus seed	+	-	+	-	-	+	-
P. emblica fruit	+	-	-	+	-	-	+
B. monosperma flower	+	+	-	-	+	+	+
N. nucifera leaves	+	+	-	+	+	_	+
S. auriculata leaves	+	+	+	_	-	-	-
A. indica leaves	+	+	+	+	+	-	+

Note: +ve indicates presence, whereas –ve indicates absent.

The beneficial physiological and therapeutic effects of plant materials is due to the presence of secondary metabolites and due to the combinations of these secondary products present in the plant. The study revealed the presence of several secondary metabolites, *viz.*, alkaloids, flavonoids, tannins, steroids, terpenoids and glycosides which are tabulated in Table 3.

3.3 Anti tyrosinase assay of herbal extract

The tyrosinase inhibitory effect of plant extracts were assessed using a spectrophotometric method. Kojic acid was used as a positive control because the drug is an effective inhibitor of tyrosinase (Wang *et al.*, 2022). The inhibitory activities on melanogenesis of the extracts were calculated based on the comparison between data obtained from tests extracts and from negative controls. The hydroalcoholic extract of *G. glabra, V. vinifera, V. myrtillus, C. lanatus, P. emblica, B. monosperma, N. nucifera, S. auriculata* and *A. indica* showed comparable inhibitory activity in a dose dependent manner when compared to standard Kojic acid against the concentration of tyrosinase of 475 U/ml are shown in Table 4.

Table 4: Antityrosinase assay of herbal extract

Test extract	Percentage inhibition at various concentrations				
	25 mg	50 mg	100 mg	200 mg	400 mg
G. glabra	25 ± 0.05	30 ± 0.15	39 ± 0.71	41 ± 0.18	41 ± 0.91
S. auriculata	14 ± 0.71	16 ± 0.91	19 ± 0.12	25 ± 0.05	31 ± 0.62
V. vinifera	22 ± 0.79	24 ± 0.26	25 ± 0.14	34 ± 0.56	35 ± 0.29
V. myrtillus	11 ± 0.03	16 ± 0.18	17 ± 0.65	20 ± 0.59	25 ± 0.74
C. lanatus	11 ± 0.76	12 ± 0.50	25 ± 0.74	27 ± 0.21	27 ± 0.94
P. emblica	7 ± 0.4	14 ± 0.7	36 ± 0.8	53 ± 0.5	70 ± 0.9
B. monosperma	30 ± 0.88	38 ± 0.97	44 ± 0.85	51 ± 0.47	57 ± 0.35
A. indica	11 ± 0.03	21 ± 0.32	24 ± 0.26	26 ± 0.47	36 ± 0.76
N. nucifera	9 ± 0.12	10 ± 0.36	18 ± 0.78	21 ± 0.39	28 ± 0.14
Kojic acid	23 ± 0.53	34 ± 0.56	36 ± 0.03	40 ± 0.44	44 ± 0.12

All the tested extracts show significant percentage of inhibition of tyrosinase enzyme at higher concentration which inhibit the melanin production that help to remove the skin hyperpigmentation.

3.4 Evaluation of formulated gel

The developed herbal gel was assessed for its grittiness profile, pH, consistency, spreadability and physical appearance. The specifics of the study's results, which fell within acceptable ICH criteria, are listed in Table 5. The prepared formulation exhibited good product performance.

Table 5: Physical evaluation of herbal gel

S.No.	Parameters	Observation
1.	Gel appearance	Brown
2.	Odour	Characteristic odour
3.	pH	6.4
4.	Consistency	Consistent
5.	Skin irritation	None observed
6.	Spreadability (gm.cm/sec)	3.8 ± 0.36 cm
7.	Washability	Easy washable
8.	Grittiness	Absent

4. Discussion

The pigment melanin, which is found in skin, gives plants and mammals their color. Hyperpigmentation of the skin results from an increase in melanin levels in the skin (Rathee *et al*., 2021). Tyrosinase enzyme is primarily responsible for melanin synthesis. It causes the production of melanin in the skin's epidermal layer, which affects the color of the skin, by converting L-tyrosine into L-DOPA and L-DOPA into dopaquinone. There are many medicinal plants and phytochemicals with tyrosinase inhibitory effect. As a result, there is an increase in both industrial and clinical demand for tyrosinase inhibitors, leading to the development of numerous *in vitro* assay and screening techniques for tyrosinase inhibition and other skin-whitening agents (Mohammad *et al*., 2019). Hence, herbal extract of selected plants were tested for the tyrosinase enzyme inhibitory activity. All the tested extracts demonstrated comparable inhibition of tyrosinase enzyme in a dose-dependent manner when compared with standard kojic acid.

In general, gel formulation is preferred over other topical semisolid preparations because it has better application characteristics, a longer residence time on the skin, a higher viscosity, a moisturizing effect on flaky skin due to their occlusive properties, more bio-adhesiveness, less irritation, and independence from the water solubility of active ingredient's and better release characters (Rajasekaran Aiyalu *et al*., 2016). Hence in the present study, topical gel was prepared using ethanol (70%) v/v) extract of nine plants as per the formula given in the Table 1 and evaluated for their pH, gel appearance, spreadability, washability, skin irritation test. The prepared gels were found to be uniform, attractive, and consistent. The formulation's pH value was within a narrow range of neutral pH (7.42-7.88), therefore, it did not irritate the skin. Spreadability values showed that gel compositions are easily spreadable. When the prepared herbal gel was tested for its irritant effect on the skin, no erythema or edema was seen for the formulation even after 6 hrs of testing (Table 5), indicating that it was deemed to be safe.

5. Conclusion

Treatment for melasma can be difficult and time-consuming, involving the use of topical medications over an extended period of time. Tyrosinase enzyme inhibition helps to reduce skin hyperpigmentation by preventing the formation of melanin. Hence herbal extract of selected plants were tested for the tyrosinase enzyme inhibitory activity. All the tested extracts demonstrated comparable inhibition of tyrosinase enzyme in a dose-dependent manner when compared with standard kojic acid. With the therapeutic effects of the selected plants containing antioxidant and anti tyrosinase active secondary metabolites a safe herbal gel was formulated and evaluated for its product performance for the treatment of melasma. Since synthetic cosmetic ingredients can have potential side effects, the developed herbal gel containing anti oxidant and anti melanogenic active metabolites are considered to be effective and safe herbal products for the management of melasma.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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