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Promotion and Computation of Inhibitory Effect on Tyrosimase Activity of Herbal Cream by Incorporatmg Indigenous Medicinal Plants

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Abstract: Herbal cream imparts a chief role in regulating melamin production of skin. The phytoconstituents present in herbal cream impact biological functions of skin and contribute nutrients required for the healthy skin. In the present study, it was envisaged to prepare three batches of herbal cream (HC1, HC2 and HC3) containing ethanol extracts of *Emblica officinalis* (fruits), *Daucus carota* (root), *Mangifera indica* (leaves), *Mentha arvensis* (leaves), *Terminalia arjuna* (bark) and *Cucumis sativus* (fruits) and investigated the prepared cream for inhibitory effect on tyrosinase activity. The herbal cream was formulated by incorporating different ratio of extracts, by using cream base. Each formulation HC1, HC2 and HC3 were segregated into three different formulations (HC1.1, HC1.2, HC1.3, HC2.1, HC2.2, HC2.3, HC3.1, HC3.2 and HC3.3) by incorporating increasing ratio of extract in formulation. The HC3.2 cream produces highest tyrosinase inhibitory effect 65.23±0.07%, while the HC2.1 exhibited minimum tyrosinase inhibitory effect 26.19±0.08% compared to other prepared cream. Comparison of the inhibitory activity of the formulations demonstrated that the rank order was HC3.2>HC3.3>HC1.2>HC1.3>HC3.1>HC1.1>HC2.3>HC2.2>HC2.1. It has been observed from the result that the formulations of antityrosinase activity were not concentrate dependent. This finding suggests that decrease in antityrosinase activity of HC1 and HC3 might be considering that the incompatibility of the higher extract content with the base of cream. The HC3 produce the maximum inhibitory effects on tyrosinase activity might be due to higher level of polyphenol and flavonoids present in extracts.

Key words: Herbal cream, antityrosinase activity, polyphenol, flavonoids

INTRODUCTION

The melamin is present in human skin, leading a prominent role for color production over skin (Lin and Fisher, 2007). Melanin is the main defensive pigment in arresting of UV induced skin damage (Hanamura *et al*., 2008). It is generated by melanocyte cells found in the stratum basale of the skin epidermis (Wang *et al*., 2006). In the human body melanocyte cells produced two patterns of melanin particularly pheomelanin and eumelanin (Pilawa *et al*., 2007). The pheomelanin originates the yellow to reddish-brown color in the body while eumelamin yield light brown to black color in the body (Sugumaran, 2002). Over disclosure to UV radiation can induce the creation of melanin in the skin. Additionally, the tyrosinase enzyme induces the production of melanin, which leads to hyperpigmentation in skin and resulted in various skin disorders. Tyrosinase oxidize the conversion of L-tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and of DOPA to DOPA quinone, both reactions being the first two rate-limiting steps in the melanin synthesis pathway (Song *et al*., 2009). UV radiation intensifies the formation ROS in the skin and these ROS boost melanin biosynthesis, damage DNA and may induce proliferation of melanocytes (Yamakoshi *et al*., 2003). The over production and accumulation of melamin pigment in the skin produces a change in skin color, skin wrinkling and skin aging (Herrling *et al*., 2007). This hyper pigmentation could develop serious problem in skin resulting in a large number of skin diseases, including chloasoma dermatitis, freckles and geriatric pigment spots. The hyperpigmentation of skin can be controlled by avoiding UV exposure, by obstruction of melanocyte metabolism and proliferation, by inhibition of tyrosinase. Moreover, the hyperpigmentation can also be reduced by ROS scavengers or inhibitors such as antioxidants might suppress melanogenesis in the epidermal layer of skin

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Table 1: Phytoconstituents and cosmeceuticals uses of medicinal plants

Plants	Phytoconstituents	Cosmeceuticals uses	References
T. arjuna (Combretaceae)	Tannins like catechol, gallocatechin, epigallocatechin and flavonoids and also phenolics like gallic acid and ellagic acid	Antioxidant activity, hyaluronidase, elastase inhibition and collagen synthesis	Satardekar and Deodhar (2010) Rahman *et al.* (2004)
C. sativus (Cucurbitaceae)	Ascorbic acid	Antioxidant activity and inhibitory effect on hyaluronidase, and elastase activity	Nema *et al.* (2011)
E. officinalis (Euphorbiaceae)	Ascorbic acid , tannins and polyphenolic componnds	Antioxidant activity, promoting procollagen production and inhibiting MMP-1 in human skin fibroblasts	Scartezzini *et al.* (2006), Fujii *et al.* (2008), Adil *et al.* (2010) and Mukherjee *et al.* (2011)
D. carota (Apiaceae)	Anthocyanins, caffeic acid, carotenoids, ferulic acid and cinnamic acid derivatives.	Free radical scavenging activity	Kim *et al.* (2007)
M. indica (Anacardiaceae)	Citric and ascorbic acids, carotenoids, phenolic componnds, flavonoids, β-amyrins, gallotannin, glucogallin, indicol, taraxerol, friedelin, lupeol	Anti-oxidant activity, emollient and antibiotics	Reddy *et al.* (2011)
M. arveusis (Labiatae)	Menthol, menthone α and β-pinene, α-thujene, l-limonene, flavonoids	Anti-oxidant activity and reduces free radical damage	Reddy *et al.* (2011)

(Wang *et al.*, 2006f). Therefore, various whitening cosmetics and medicines are being developed to control melanogenesis.

At present, herbal whitening cosmetic products of demand have been increased in comparison to synthetic skin care products. Several plants extracts have been scientifically validated for antioxidant activity. In many research, paper reported that the antioxidant substances could inhibit the tyrosinase enzyme (Kubo *et al.*, 2000; Meda *et al.*, 2005; Lee *et al.*, 2012). Consequently, the natural antioxidant components are used in herbal cream to prevent hyperpigmentation. Now these reported antioxidant plant extracts are required to develop in the form of herbal cream. Once unconventional herbal product will fully develop, hope this product will be cost effective compared to other marketed herbal creams.

According to traditional system and scientific justification with modern uses, medicinal plants were selected for formulation of herbal cream (Table 1). *Emblica officinalis*, *Daucus carota*, *Mangifera indica*, *Mentha arvensis*, *Terminalia arjuna* and *Cucumis sativus* have been selected for formulation of Herbal Cream (HC) to produce the inhibitory effects on tyrosinase activity. The objective of the present study was to develop herbal cream composining of different ratio of plant extracts and evaluate the inhibitory effect on tyrosinase activity of formulated herbal cream.

MATERIALS AND METHODS

Plant materials *Emblica officinalis* (fruits), *Daucus carota* (root), *Mangifera indica* (leaves), *Mentha arvensis* (leaves), *Terminalia arjuna* (bark) and *Cucumis sativus* (fruits) were selected because of their reported physiological action and were from local market in Bhopal, Madhya Pradesh. The species was identified by the local people during the time of collection and later on authentication was made by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai, India. The plant parts were washed with distilled water to remove dirt and soil and shade dried. The dried materials were powdered and passed through a 10-mesh sieve. All the other chemicals and reagents used in the study were of analytical grade.

Preparation of extracts: One kilogram of each powdered drug was packed in soxhlet apparatus separately and extracted with petroleum ether to defat the drug. Defatted powdered drug was then extracted with ethanol (90%). The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure to get crude ethanol extract.

Preparation of herbal cream: Various ingredients were weighed accurately. Oil in water (O/W) emulsion-based cream was formulated. The formulations were divided into three batches (HC1, HC2 and HC3) and each batch consisting three plants. Total nine formulations were prepared from three batches. The emulsifier and other oil soluble components namely stearic acid (9%), Cetyl alcohol (3%), starch (1.5%), SLS (1%) and almond oil (15%) were melted in a beaker and heated to 75°C. The different concentration of extract (2.25, 4.5 and 9%) was dissolved in required amount of water and filtered. To the filtrate methyl paraban (0.02%), glycerol (3%) were added and maintained at 75°C in beaker. When the temperature of both the phases reached 75°C. The aqueous phase was added gradually into oily phase with continuous stirring until cooling of emulsifier took place and left at room temperature to obtain the required product. The flavoring agent was added when it is hot to obtain herbal cream (Sahu *et al.*, 2012a, b;

Table 2: Composition of herbal extract based various creams

	Formula (%) w/w								
Ingredients	HC1.1	HC1.2	HC1.3	HC2.1	HC2.2	HC2.3	HC3.1	HC3.2	HC3.3
Stearic acid	9	9	9	9	9	9	9	9	9
Cetyl alcohol	3	3	3	3	3	3	3	3	3
Starch	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
SLS	1	1	1	1	1	1	1	1	1
Almond oil	15	15	15	15	15	15	15	15	15
Glycerol	3	3	3	3	3	3	3	3	3
E. officinalis	1	2	4	-	-	-	-	-	-
D. carota	0.5	1	2	0.5	1	2	0.5	1	2
C. sativus	0.75	1.5	3	-	-	-	0.75	1.5	3
M. indica	-	-	-	1	2	4	-	-	-
M. arveusis	-	-	-	0.75	1.5	3	-	-	-
T. arjuna	-	-	-	-	-	-	1	2	4
Methyl paraban	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Rose oil (drops)	10	10	10	10	10	10	10	10	10
Triethanolamine	qs	qs	qs	qs	qs	qs	qs	qs	qs
Water, qs, 100	qs	qs	qs	qs	qs	qs	qs	qs	qs

HC: Herbal cream, SLS: Sodium lauryl sulfate, qs: Sufficient quantity

Rajvanshi *et al*., 2011). The compositions of the different herbal cream are given in Table 2.

Skin whitening assay: Using a previously described method, with minor modifications, a spectrophotometric assay was performed using L-tyrosine as the substrate for determination of tyrosinase inhibitory activity. Herbal cream was extracted three times by obtaining 1.0 g of the product and 10 mL of the extractant, a 70% (v/v) ethanol. The resulting extracts were mixed and the extractant was added to reach 10 mL volume. A 10 μL sample of herbal cream was added to an assay mixture containing with 1 mM L-tyrosine solution, 100 mM sodium phosphate buffer, pH 6.5 and 20 μL of the aqueous solution of mushroom tyrosinase (1,000 units) was added to a 96-well microplate. Following incubation of the assay mixture at 37°C for 20 min, the amount of dopachrome produced in the reaction mixture was determined as the optical density at 490 nm in a microplate reader and the inhibition percent of tyrosinase activity was calculated according to the following formula (Hapsari *et al*., 2012; Kamkaen *et al*., 2007; No *et al*., 1999):

$$\text{Inhibition (\%)} = \left[1-\frac{(\text{Asample (490 nm)})}{\text{Acontrol (490 nm)}}\right]\times 100$$

Statistical analysis: The experimental results obtained were expressed as mean±standard error of mean (SEM). All measurements were carried out in triplicate.

RESULTS AND DISCUSSION

Six plants extract namely *D. carota*, *E. officinalis*, *M. indica*, *T. arjuna*, *C. sativu* and *M. arvensis* were selected for study. The herbal cream was formulated by incorporating different ratio of extracts, by using cream base to exhibit inhibitory effect on tyrosinase activity. In present study, the ratio of extracts in different proportion was selected on the basis of reported antioxidant and other cosmeceuticals property for formulation of herbal cream.

The creams were categorized in three distinct batches, i.e., HC1, HC2 and HC3. The extracts of *E. officinalis*, *D. carota* and *C. sativus* were included in preparation of HC1 cream. The HC2 cream containing extracts of *M. indica*, *D. carota* and *M. arvensis*. The extracts of *T. arjuna*, *D. carota* and *C. sativus* were incorporated in HC3 cream. Each formulation HC1, HC2 and HC3 were segregated into three different formulations (HC1.1, HC1.2, HC1.3, HC2.1, HC2.2, HC2.3, HC3.1, HC3.2 and HC3.3) and incorporating, increasing ratio of extract in formulation.

From Table 3 it was observed that the HC3.2 cream produces highest tyrosinase inhibitory effect 65.23±0.07%, while the HC2.1 exhibited minimum tyrosinase inhibitory effect 26.19±0.08% compared to other prepared cream. Comparison of the inhibitory activity of the formulations demonstrated that the rank order was HC3.2>HC3.3>HC1.2>HC1.3>HC3.1>HC1.1> HC2.3>HC2.2>HC2.1. The intention of using increasing ratio of extracts in formulation was to check the effect of concentration of extracts on efficacy of cream. It has been observed from the result that the formulations of antityrosinase activity were not concentrate dependent. This finding suggests that decrease in antityrosinase activity of HC1 and HC3 might be considering that the incompatibility of the higher extract content with the base of cream. The inhibitory effect on tyrosinase activity increases from HC1.1 to HC1.2, but HC1.3 exhibits reduction in antityrosinase activity from HC1.2. The similar observation has been recorded in series of HC3

Table 3: Tyrosinase inhibitory effect of various herbal creams

Formulation	Tyrosinase inhibition (%)
HC1.1	45.32±0.09
HC1.2	61.81±0.05
HC1.3	57.46±0.04
HC2.1	26.19±0.08
HC2.2	35.73±0.03
HC2.3	41.67±0.06
HC3.1	51.82±0.04
HC3.2	65.23±0.07
HC3.3	63.18±0.05

Values are means±SEM of triplicate determinations

formulations. In contrast, HC2 formulation revealed that antityrosinase activity had been increased from HC2.1 to HC2.3. The antityrosinase activity of HC2 formulation reveals that on increasing the concentration of extracts it enhances the inhibitory effect on tyrosinase activity. However, the inhibitory effect on tyrosinase activity of HC2 formulation is lower than the antityrosinase activity of HC1 and HC3.

The obtained value of inhibitory effect on tyrosinase activity of all formulations is very appreciating when compare with that of other herbal extracts as reported in various research paper. Narayanaswamy *et al.* (2011) chosen five plants to assessment tyrosinase inhibitory effect and reported that aqueous extract of *Asparagus racemosa* was greater in its inhibiting potential (43.29%). *Asparagus racemosa* had the highest level of phenolics among the five medicinal plants tested. Fawole *et al*., (2012) documented that the pomegranate methanol peels extracts expressed strong tyrosinase inhibition activities. The pomegranate fruit peel is rich in polar substances such as polyphenol, flavonoids and tannins. In solution, the informative characteristic of tannins is the capability to precipitate mainly proteins, and the structure of flavonoid is analogous with the roles of both substrates and inhibitors of tyrosinase. The conclusion was that tannin content in pomegranate peel could precipitate the tyrosinase enzyme, thereby inhibiting enzymatic activity in the reaction medium (Kubo *et al*., 2000; Meda *et al*., 2005). Lee *et al*. (2012) studied that nuruk extract possessed significant tyrosinase inhibitory effect. The study referred to that this activity was due to its antioxidant property. The methanolic extract of *Crocus sativus* flowers showed a significant inhibitory effect on tyrosinase activity of 28.22% (Sariri *et al*., 2011). The author determined that presence of phenolic compound in extract; make it to produce tyrosinase inhibitory activity (Chang, 2009).

It has been proclaimed that tyrosinase, also known as a polyphenol oxidase, plays a decisive role in catalyzing the melanogenesis and elevating the reactive metabolites produced in the process of melanin formation. Polyphenols are also the largest groups in tyrosinase inhibitors until now (Chang, 2009). Polyphenolic compounds, such as flavonoids, could form complexes with metal ions and exhibit antioxidative action. During antioxidant study of each plant extract, it was observed that the level of total flavonoids and polyphenol in extracts of *T. arjuna* and *C. sativus* were superior to other extracts. The extract of *T. arjuna* and *C. sativus* were present in HC3 formulation and in this study, HC3.2 showed greater inhibitory effects on tyrosinase activity. These findings suggest that HC3 exhibited the inhibition of tyrosinase could be associated with their total flavonoids and polyphenol. Moreover, some extracts represent moderate amount of total flavonoids and total polyphenol. It is possible that the synergistic effects may exist between total flavonoids and polyphenol of extracts of herbal cream in tyrosinase inhibitory effects. Other unknown active components present in extracts of cream could also play critical roles in their biological effects.

CONCLUSION

In conclusion, the findings revealed that each batch of formulated herbal cream exerted antityrosinase activity. The HC3 produce the maximum inhibitory effects on tyrosinase activity might be due to higher level of polyphenol and flavonoids present in extracts. Further investigations are in progress in the laboratory for fully development of this herbal cream, because it is interesting material for cosmetics manufacturers.

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