Prayogik Rasayan

In vitro evaluation of UV-filtering and anti-tyrosinase potential in selected medicinal plants in Sri Lanka for the development of herbal cosmetics

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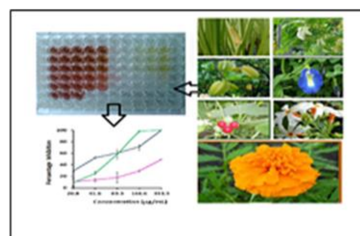
Abstract

This study was conducted to evaluate the *in vitro* Ultra-Violet(UV) filtering and anti-tyrosinase potential in several medicinal plants. The methanolic extract of *Tagetes erecta* has displayed a sun protection factor (SPF) of 40 and was found to be photostable. Furthermore, an extreamly potent tyrosinase inhibition (IC_{50} = 48.27 µg/mL) as well as antioxidant activity (EC_{50} = 14.82 µg/mL) were observed for the same extract revealing the high potential for the development of herbal cosmetics.



Keywords: ***Antityrosinase; herbal cosmetics; Medicinal plants; UV-filtering***

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1. Introduction

The application of herbal formulations to enhance appearance and to treat dermatological diseases is a common practice among many cultures around the world. The phytochemistry and the biological activities of some of these herbal remedies have been investigated in the quest for developing more effective and safer topical skin care agents. These modern scientific studies have revealed the presence of diverse array of phytochemicals that not only calm, restore and heal the skin, but also stand up to the scrutiny of clinical trial and pharmacological testing[1].

Despite the wide utility of plant species in the indigenous systems of medicine in Sri Lanka as skin care agents[2], there is a dearth of scientific information to rationalize these traditional claims. In order to fulfill this knowledge gap, the present study has focused on the *in vitro* evaluation of UV-filtering and skin whitening potential of seven medicinal plants that have been widely used in Sri Lanka to enhance complexion and as dermatological therapeutics. Thereby the ethnopharmacological significance of these plant species could be rationalized while the feasibility to formulate herbal cosmetics could also be assessed.

UV-filtering effect: The intense or excessive exposure to solar ultraviolet (UV) radiation has a variety of harmful effects from mild inflammatory effects to melanoma skin cancers, thus,various photoprotection measures have been introduced in the recent past. Among those different approaches, topical application of sunscreen products has turned to be the most popular strategy in the present day[3-5]. Sunscreens are incorporated in several cosmetic products such as creams, gels, oils and lotions and the active molecules in sunscreens could be inorganic or organic agents. Inorganic sunscreens reflect and scatter the UV radiation, while organic sunscreens absorb UV radiation and then re-emit energy as heat or light[6]. Octylmethoxycinnamate, mexenone, provatene, avobenzone, benzophenone-3 are a few examples of active ingredients found in most of the synthetic sunscreen products widely popular among a large segment of world population. However, with the realization of adverse side effects associated with these synthetic skin care formulations, over recent years, there is a continuous search for alternative formulations of plant origin with low side effect profiles[7]. Thus, natural/herbal sunscreens rich with phytochemicals capable of UV-filtering potential have been introduced to substitute for or to reduce the quantity of synthetic sunscreen agents[8].

Tyrosinase inhibition: Melanin is responsible for the pigmentation of human skin, eye and hair and the whole process involved in the synthesis of melanin is known as “melanogenesis”. Despite its role in the protection against UV damage, the over production and accumulation of melanin is harmful as it could lead to skin disorders such as solar melanosis, ephelides, melasma, and postinflammatory hyperpigmentation[9-10]. As tyrosinase is the key enzyme involved in melanogenesis, the inhibitors of this enzyme have become progressively significant as depigmenting agents in hyperpigmentation disorders. Several types of tyrosinase inhibitors have been reported, from both natural and semisynthetic/synthetic sources, however, due to various safety concerns, only a few of them are marketed as skin-whitening agents. Since several skin and health problems, from mild cases such as irritation and rashes to serious adverse effects such as ochronosis, corneal degeneration and genotoxicity could be linked with the usage of synthetic skin-whitening agents, both physicians and dermatology

patients are searching for long-term topical skin care solutions to address problems presented by skin hyperpigmentation. In this respect, plant based natural skin lightning ingredients would be safer and cost effective alternatives[11].

2. Experimental

2.1 Plant Material

Rhizomes of *Acorus calamus* (sweet flag; Family Acoraceae), fruits of *Averrhoa carambola* (star fruit; Family Oxalidaceae), flowers of *Clitoria ternatea* (butterfly pea; Family Fabaceae), *Sesbania grandiflora* (vegetable hummingbird; Family Fabaceae), *Nyctanthes arbor-tristis* (night-flowering jasmine; Family Oleaceae), *Tagetes erecta* (Mexican marigold; Family Asteraceae), and *Mukia maderaspatana* (Heen kekiri; Family Cucurbitaceae) were collected from local cultivations in North Western and Sabaragamuwa provinces of Sri Lanka in 2016. Based on the application in traditional medicine, the plant parts were selected for the present study. The plants were authenticated by comparison with the herbarium specimens at the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. A voucher specimen of each plant is deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka.

2.2 Preparation of crude extracts

The plant materials were washed with running water and dried in shade (30 °C) for six days. Dried plants were powdered using a domestic grinder. The powdered materials (10-15 g) were extracted in 300 mL of 70% methanol-water. In addition, 12 g of the flowers of *Tagetes erecta* was extracted in 100% methanol separately (extraction yield = 15.5%). The extracts were evaporated into complete dryness using a rotary evaporator.

2.3 Evaluation of UV-filtering potential

UV-filtering potential of the extracts was determined following the method described by Napagoda et al (2016)[12] and the sun protection factor (SPF) was calculated according the Mansur equation[13].

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where, EE(λ) – erythemal effect spectrum; I(λ) – solar intensity spectrum; Abs(λ) – absorbance of sunscreen product; CF – correction factor (=10)

The photostability of the extracts were determined by exposing the extracts to direct sunlight for 21 days and measuring the UV absorbance at 7th, 14th and 21st day.

A commercially available sunscreen product (SC) (containing benzophenone- 3 and TiO_2 as active ingredients) was used as the reference substance.

2.4 Anti-tyrosinase assay

The plant extracts were dissolved in 50 mM potassium phosphate buffer (pH 6.5) and initially tested for the tyrosinase inhibition at a concentration of 333.3 μg/mL at the 96-microwell plate as described by Curto et al (1999)[14] and modified by Napagoda et al (2018)[15]. Briefly, 70 μL of each extract (1000 μg/mL) was mixed with 30 μL of tyrosinase (333 units/mL in phosphate buffer). The mixture was incubated at room temperature (37 °C) for 10 minutes. Then 110 μL of 2 mM L-tyrosine was added to each well. The reaction mixture was incubated again at room temperature (37 °C) for 30 minutes. The absorbance was measured at 492 nm (Thermo Scientific-Multiskan Go Microplate spectrometer). The percentage inhibition of tyrosinase activity was calculated as follows[15,16].

$$\% \text{ Inhibition} = (A - B) / A \times 100$$

where, A = absorbance without the test sample (control), B = absorbance with the test sample.

Based on the preliminary observations, the extracts that have displayed an inhibition ≥ 50% at 333.3 μg/mL were identified and were further subjected to dose-response studies.

Ascorbic acid was used as a positive control. The experiments were carried out in triplicate and the IC_{50} values were calculated using Graph-Pad Prism version 6.01.

2.5 Radical scavenging capability

The radical scavenging activity of the extracts was determined by measuring the reduction of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) following the method described by Blois, 1958[17] with minor modifications. The test extracts and DPPH solution were incubated under gentle shaking in the dark for 30 min and the absorbance was recorded at 517 nm. The percentage antioxidant activity (AA) was calculated using the following formula and the EC_{50} was determined using Graph-Pad Prism version 6.01[18].

$$AA\% = (Ab_{control} - Ab_{sample}) / Ab_{control} \times 100$$

Butylated hydroxyanisole (BHA) and ascorbic acid were used as positive controls and all the measurements were carried out in triplicate.

2.6 Statistical analysis

All the above experiments were performed in triplicate and the values were given as mean ± S.D

2.7 Preparation of topical formulation

The most potent extracts were incorporated into the commercially available aqueous cream-base at different ratios. Additional preservatives were not incorporated into the formulation. The extracts were miscible in aqueous cream-base and the ideal ratio of the extract and the cream-base was determined based on the homogeneity, spreadability and grittiness of the formulation[15].

3. Result and discussion:

3.1 UV- filtering potential

Among the tested extracts, *C. ternatea, M. maderaspatana, N. arbor-tristis* and *T. erecta* (methanol) have displayed SPF ≥ 15 (Table 1). Similar to our observations in the previous study, the actual SPF value of

the commercial photoprotective cream (reference substance) was found to be much lower than the labelled SPF value[12].

Table 1: Measured SPF values of the plant extracts and the commercial sunscreen

Extract	Measured SPF
Acorus calamus (M-W)	5.6 ± 0.03
Averrhoa carambola (M-W)	10.6 ± 0.4
Clitoria ternatea (M-W)	40 ± 0
Mukia maderaspatana (M-W)	15.4 ± 0
Nyctanthes arbor-tristis (M-W)	18.7 ± 0
Sesbania grandiflora (M-W)	8.6 ± 0.09
Tagetes erecta (M-W)	11.9 ± 0
Tagetes erecta (M)	40 ± 0
SC1 (Standard reference)	28.8 ± 0.11 (labeled SPF : 45+)

*M-W= methanol-water extract, M=methanol extract

Furthermore, *T. erecta* (methanol) and *M. maderaspatana* did not display a reduction in the SPF after exposure to direct solar radiation for 21 days. The high SPF value determined for *C.ternatea* at the beginning of the experiment has drastically reduced with time, indicating that the extract is not photostable. The SPF value of *N. arbor-tristis* has also decreased with time (Figure 1).

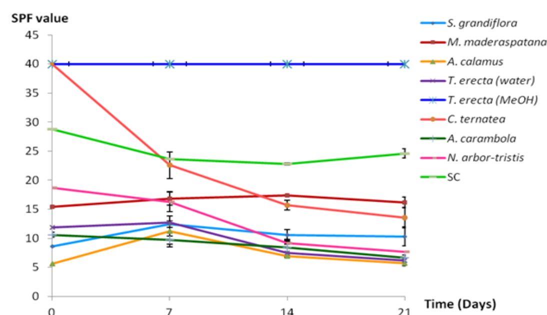

Figure 1: Variation of SPF in plant extracts and standard reference after exposure to direct sunlight

Moreover, methanol extract of *T. erecta* has exhibited high UV absorbance throughout the range of 260-400 nm (Figure 2) while a high UV absorbance was also observed in the range of 260-340 nm for *C. ternate* extract. In addition, the maximum absorbance of *N. arbor-tristis* lies within the UV-B range while most of the other extracts have displayed the maximum absorption in the UV-C range.

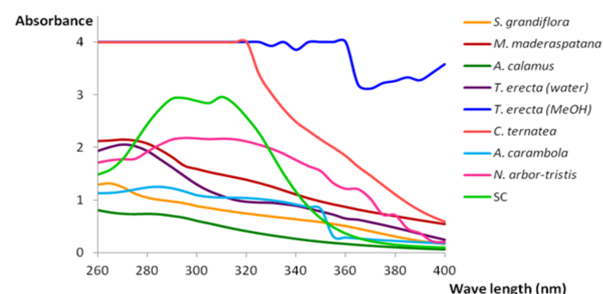

Figure 2: Absorption of UV radiation between 260-400 nm

3.2 Tyrosinase inhibition

The preliminary observations have indicated that only *T. erecta* (methanol) and *N. arbor-tristis* were capable of inhibiting the enzyme ~ 50% or higher at the initial concentration of 333.3 µg/mL in a micro-well (Table 2). Thus the dose-response studies were conducted on these two extracts (Figure 3).

Table 2: Percentage inhibition of the tyrosinase enzyme at the initial concentration of 333.3 µg/mL

Extract	% inhibition at 333.3 µg/mL in a micro-well
Acorus calamus (M-W)	Not active
Averrhoa carambola (M-W)	10.66 ± 2.51
Clitoria ternatea (M-W)	26 ± 2.82
Mukia maderaspatana (M-W)	Not active
Nyctanthes arbor-tristis (M-W)	49.17 ± 4.59
Sesbania grandiflora (M-W)	23.95 ± 1.42
Tagetes erecta (M-W)	22.5 ± 0.71
Tagetes erecta (M)	100 ± 0
Ascorbic acid (positive control)	100 ± 0.58

*M-W= methanol-water extract, M=methanol extract

The IC_{50} value of the most potent extract, *T. erecta* (methanol) was determined as 48.27 µg/mL which is comparable with that of the positive control, ascorbic acid (IC_{50}= 33.46 µg/mL).

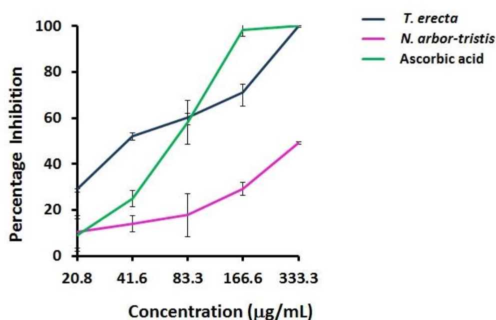

Figure 3: Dose-response studies on tyrosinase inhibition for most potent extracts

3.3 Radical scavenging capability

The radical scavenging potential was extremely high in *T. erecta* extracts according to the DPPH assay (Table 3). The EC_{50} value for the methanol extract of *T. erecta* was better than those of the two positive controls, BHA and ascorbic acid.

Table 3: Antioxidant activity of the plant extracts

Extract	EC_{50} for DPPH assay (µg/mL)
Acorus calamus (M-W)	85.68
Averrhoa carambola (M-W)	75.68
Clitoria ternatea (M-W)	108.9
Mukia maderaspatana (M-W)	83.31
Nyctanthes arbor-tristis (M-W)	227.8
Sesbania grandiflora (M-W)	97.72
Tagetes erecta (M-W)	22.66
Tagetes erecta (M)	14.82
BHA (positive control)	23.12
Ascorbic acid (positive control)	18.03

*M-W= methanol-water extract, M=methanol extract

3.4 Development of herbal formulations

The most potent extracts, i.e. methanolic extract of *T. erecta* and hydro-alcoholic extract of *N. arbor-tristis* were incorporated into the aqueous cream base at different ratios to achieve a good spreadability of the formulation (Figure 4).

Figure 4: Appearance of the formulation developed from (A) *N. arbor-tristis* (B) *T. erecta*

Further optimization of these herbal formulations is in progress and it will be followed by the comprehensive evaluation of bioactivities, cytotoxicity and *in vivo* experiments that are aimed at evaluating the deposition and absorption of the prepared formulations after application on skin etc.

The application of herbal formulations to enhance complexion and to treat dermatological disorders has been practicing in Sri Lanka for over thousand years. A large number of plant species have presumably been selected in indigenous medicine for the above purposes by a process of “trial and error”. This suggests that Sri Lankan flora could be a potential source for the development of herbal pharmaceuticals and cosmetics which are safe, efficient and devoid of undesirable side effects. However, the scientific evidences on bioactivity studies of medicinal plants in Sri Lanka that could lead towards the development of herbal cosmetics are extremely scarce. For example, only a limited number of studies have been conducted on photoprotective potential of Sri Lankan plants[19-21] as well as on anti-tyrosinase activity[15,22]. Therefore, a significant contribution was made from the current study to fill up the knowledge gap in the field of herbal cosmetics in Sri Lanka.

Due to its geographical location in the equatorial belt, Sri Lanka receives high amount of solar radiation throughout the year and the people are highly vulnerable to get UV-induced skin damages due to the extensive exposure to the intense solar radiation. Thus the formulation of herbal sunscreen products with high SPF value would be highly beneficial. In this respect, the UV-filtering potential exhibited by the methanol extract of *T. erecta* is particularly important, not merely due to the high SPF value, but also due to its photostability and broad spectrum sunscreening activity. On the other hand, the high SPF value determined for the extract of *C. ternatea* has drastically reduced with time probably due to the fact that the phytochemicals responsible for UV-filtering activity may get degraded or destroyed over the time or when get exposed to sunlight. Moreover, the extracts of *M. maderaspatana* and *N. arbor-tristis* have also displayed moderate SPF values (SPF 12-30)[23], however, the photostability was observed in *M. maderaspatana* only.

Furthermore, our investigations revealed that *T. erecta* and *N. arbor-tristis* possess anti-tyrosinase activity. However, this activity is not prominent in other plant extracts tested in the study except the moderate activity observed for *C. ternatea*. The extracts prepared from the plants with historical claims as enhancers of complexion such as *A. calamus* and *M. maderaspatana*, did not display any inhibition of the enzyme even at the highest concentration used. The tyrosinase inhibitory potential in *T. erecta* has been already documented by researchers in Thailand with IC_{50} value of 1078 and 1467 µg/mL for ethanol and ethyl acetate extracts respectively[24]. These observations suggested the possible anti-tyrosinase activity in methanol extract of *T. erecta*, thus a special emphasis was given in our study to investigate this extract in addition to the methanol-water extract. As expected, the methanol extract of *T. erecta* has inhibited the enzyme with an IC_{50} of 48.27 µg/mL, which is extremely

lower than the IC_{50} values obtained for other extracts prepared from the same plant in Thailand. This indicates an extremely potent enzyme inhibition by the methanol extract of *T . erecta* of Sri Lankan origin. However, the inhibitory potential in the *T . erecta* extract prepared from methanol-water was not prominent, thus suggests that the variation in phytochemical composition would have great impact in the bioactivity of these two extracts. Based on our preliminary experiments and literature findings, anti-tyrosinase activity was negligible in organic extracts of other plant species used in this study, therefore, here we have not focused on the evaluation of tyrosinase inhibitory activity in methanolic extracts of the other six plant species.

Several studies have reported the ability of polar substances of plant extracts to inhibit tyrosinase and it has been documented that the activities are partly contributory by antioxidant potentials of the extracts[23]. Therefore, the DPPH assay was employed to investigate the potential antioxidant activity of the plant extracts where all the extracts except *N. arbor-tristis* and *C. ternatea* have displayed EC_{50} < 100 µg/mL. Interestingly the EC_{50} value was highest in *N. arbor-tristis* although it possesses considerable anti-tyrosinase activity. This suggested that the anti-tyrosinase activity of this extract might not be due to its reducing capability. Even though, the extracts of *A. calamus* and *M. maderaspatana* were incapable of the inhibition of tyrosinase enzyme, a potent antioxidant activity could be observed. Furthermore, both methanol-water and methanol extracts of *T. erecta* possess strong antioxidant activities which are comparable with the positive controls, irrespective of their difference in tyrosinase inhibitory capacity. These results suggest the requirement of further experiments to determine the correlation between anti-tyrosinase and antioxidant activities of the above plant extracts as well as the evaluation of the mechanism of action of the inhibition of tyrosinase enzyme by the most potent extracts.

4. Conclusions

The preliminary findings of this study reveals that Sri Lankan medicinal plant preparations have a high potential to be developed into herbal cosmetics. Especially the methanol extract prepared from the flowers of *Tagetes erecta* possess strong anti-tyrosinase as well as antioxidant activities, thus suitable as a skin lightening agent. Moreover, the same extract displayed strong UV-filtering activity, hence, could be developed into a herbal cosmetic for the treatment of hyperpigmentation disorders caused specially due to the excessive exposure to the UV radiation.

5. Acknowledgements

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6. Notes and References

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Notes

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Ms. Maduwanthi Kumari and Ms. Udari Samaranayake: Undergraduate students following B.Sc (Pharmacy) Degree at the Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka and they conducted the above research under the supervision of Dr. Napagoda.