Investigation of Environment-Friendly Skin Whitening Agents from the Plant *Polyscias balfouriana* L.H. Bailey

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Abstract - Skin is the most visible organ in the body. The use of whitening agents can help skin to glow and lighten the colour by reducing the melanin concentration. Despite using synthetic whitening agents which contain heavy metals like mercury that are toxic and not environment-friendly, it is always better to consume environment-friendly whitening agents which are produced using natural products and to achieve sustainable development goals. The *Polyscias balfouriana* L.H.Bailey belonging to family Araliaceae, leaves were used to find activities which inhibit the melanin synthesis that leads to whitening the skin. The leaves were extracted using hexane, ethanol, methanol and ethyl acetate. The obtained extracts were used to determine the antityrosinase activity. Inhibitory activity of the mushroom tyrosinase method was used to determine the antityrosinase activity. The most effective activity (48% inhibition) was observed for the ethanol extract with the concentration of 0.25 mg/mL. The standard (kojic acid) showed only 22% inhibition at the same concentration. However, when the ethanol extract concentration was raised the antityrosinase activity declined. This observation was opposite to the behaviour of standard. The other two extracts followed a similar trend to that of ethanol. Therefore, according to the results *Polyscias balfouriana* L.H.Bailey leaves show some positive antityrosinase activity.

Keywords: Melanin, Tyrosinase, Antityrosinaes, Enzyme, Polyscias balfouriana L.H.Bailey

I. INTRODUCTION

Skin is the most visible organ in the human body. So, most of the individuals including the youth are interested in improving their skin complexion by applying commercially available cream/lotions which contain harmful chemical substances. These products may help to lighten the skin or to provide an even skin colour mainly by reducing the melanin concentration in the skin. These agents can be synthetic or non – synthetic. But most of the skin whitening agents found today are synthetic which contain heavy metals like nickel, mercury and zinc [1]. They are toxic and not environment-friendly, and they can cause health issues such as neurological problems and kidney problems. Therefore, in the view of sustainable development goals, the development of nontoxic and environment-friendly whitening agents is desirable.

Tyrosinase enzyme can be found in plants and animal tissues. It is an enzyme which contains copper. The enzyme functions as a catalyst in the melanin synthesis during melanogenesis. This biological reaction converts tyrosine into a polymer of melanin. Tyrosinase enzyme limits the rate of the melanogenesis stage [2]. In fact, the first step of the melanin synthesis is controlled by the tyrosinase enzyme. *Polyscias balfouriana* L.H.Bailey is native to Australia and Papua New Guinea. It belongs to the family Araliaceae and it is commonly cultivated in South-eastern Asia. This plant is not famous in Sri Lanka however, it is widely available in urban areas. It is a small tree with many branches and has broad leaves. Reported data shows that plants belonging to the Araliaceae family have bioactivities including antitoxin, antibacterial, and anti-inflammatory effects which are helpful in the treatment of asthma [3]. Parts of this plant are used as anti-dysentery, febrifuge, diuretic and for neuralgia and rheumatic pains. Therefore, as a part of an ongoing effort to develop environment-friendly skin lightening agents, it is interesting to investigate the potential antityrosinase activity of *Polyscias balfouriana* L.H.Bailey.

II. MATERIALS AND METHODS

Polyscias balfouriana L.H.Bailey leaves were collected from Gampaha district. They were first dried and homogenized using a blender. Powdered leaves (50 g) were soaked in 350 mL of hexane and it was kept on a shaker for 8 hours at room temperature at a speed of 200 rpm. Then it was kept it in a refrigerator for 2-3 days. The residue was soaked in 350 mL of 100% ethanol and kept on a shaker for 4 hours at room temperature at a speed of 200 rpm. Then it was kept in a refrigerator for a day. Ethanol solution was then filtered using a cheese cloth and the solvent was removed by rotary evaporation. Finally, it was collected into an evaporating dish and purged nitrogen to obtain the final crude product. A similar procedure was followed to get methanol and ethyl acetate extracts.

A. Tyrosinase inhibition assay

A weight of 1.70 g of KH_2PO_4 was dissolved in 250 mL of deionized water to prepare a phosphate buffer solution (50 mM, pH 6.5). A weight of 61.2 mg of the L-DOPA solid was dissolved in 25 mL of the phosphate buffer to prepare L-DOPA solution (12 mM). Extraction of the tyrosinase enzyme was done by torning and squeezing 200 g of fresh oyster mushrooms and filtering it using a cheesecloth into a beaker which was kept in an ice bath. The standard/sample was dissolved in the phosphate buffer (50 mM) to a final concentration of 4 mg/mL. For the solution preparation, 0.6 mL of tyrosinase enzyme was mixed with 1.2 mL of each standard/sample of different concentration (2.0-0.12 mg/mL). The control was prepared in a similar way by adding 1.2 mL of phosphate buffer (50 mM) in the place of standard/sample. First, samples were incubated at room temperature for 5 min and 2.2 mL of 12 mM L-DOPA

solutions was added. Then the mixture was incubated at 15 ^{0}C for 30 min. In this procedure, Kojic acid was used as the standard. Absorbance of the samples was measured at 475 nm [4] using UV-VIS spectrometer and plate reader.

III. RESULTS AND DISCUSSION

Percentage tyrosinase inhibition was calculated using the formula given below.

$$\%\, Tyrosinase\ inhibition = \left[\frac{(A_{control} - A_{standard/sample})}{A_{control}} \times\right] 100 \qquad (1)$$

Polyscias balfouriana L.H.Bailey leaves were extracted using ethanol, methanol and ethyl acetate solvents. First, the extracts at 0.25 mg/mL concentration were analyzed for the antityrosinase activity. All the extracts showed some antityrosinase activity and the most effective activity of 48% inhibition was observed for the ethanol extract. At this concentration, the standard (kojic acid) only showed 22% inhibition.

On the basis of these results, different concentrations of the extracted solutions were analyzed. When the concentration was lowered to 0.125 mg/mL, ethanol extract continued to show the best activity among the other extract. However, the effective activity was low compared to the activity at 0.25 mg/mL due to the low concentration. Thus, the extract concentration was increased to 0.5 mg/mL and ethanol extract showed an effective activity of 42 %. The standard (kojic acid) showed only 27 % inhibition at that same concentrations. This shows that the inhibitory activity of ethanol extract concentrations at 0.125 mg/mL and 0.5 mg/mL was lower than that at 0.25 mg/mL concentration. However, it is still greater than the inhibitory activity of the standard at the same respective concentrations. Later, the extract concentration was increased to 1.00 mg/mL. At this concentration the inhibitory activity of ethanol extract decreased compared to that of the standard. The variations of the % tyrosinase inhibitions with the different extracts are shown in Figure 1.

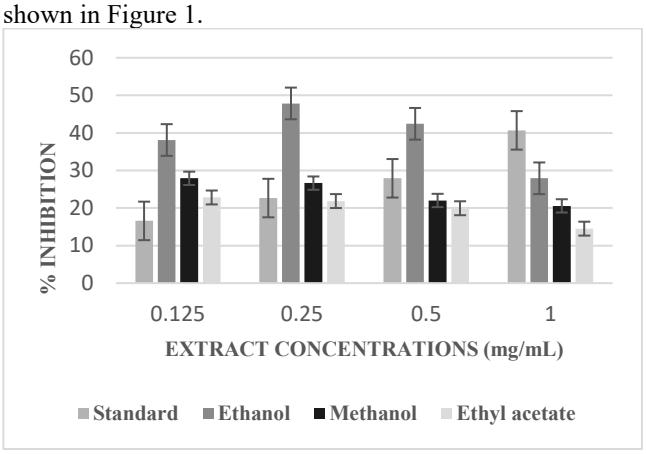

Fig. 1. Tyrosinase Inhibition % Vs Extract Concentrations

Therefore, the ethanol extracts showed better tyrosinase inhibitory activity at low concentrations and the activity declined at high concentrations. Both methanol and ethyl acetate extracts showed some inhibitory activity at all concentrations, but it was always low compared to the ethanol extract activity till 1.00 mg/mL concentration. After 0.5 mg/mL inhibition activity of the ethanol extract started to declined. But when the concentration was further increased ethanol extract had no inhibitory activity compared to other two extracts.

IV. CONCLUSION

Polyscias balfouriana L.H.Bailey leaves were extracted using ethanol, methanol and ethyl acetate and all the extracts showed some positivity towards the inhibitory activity. Ethanol extract showed the most effective tyrosinase inhibition activity at 0.25 mg/mL concentration which was greater than the standard's inhibition activity at the same concentration. Even at 0.125 mg/mL and 0.5 mg/mL concentrations the highest inhibitory activity was shown in the ethanol extract. But when the concertation was increased, the highest inhibition percentage was shown in the standard. Therefore, according to these results *Polyscias balfouriana* L.H.Bailey leaves show some positive antityrosinase activity and better inhibition at lower concentrations. Therefore, it is believed that these inhibitory activities can be further improved by purifying these ethanol extracts. Future studies will focus on isolating the active compounds responsible for the inhibitory activity and using them in making potential skin whitening agents which are environmentally friendly.

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