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# Formulation Development and Assessment of Skin Whitening Efficiency of Ethyl Acetate Extract of *Eichhornia crassipes* (Mart.) Solms by *in vitro* Tyrosinase activity

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**Abstract:** Enzymes such as tyrosinase, hyalurodinase etc. contribute to the skin whitening activity of the plants. Tyrosinase inhibition activity is the main factor that determines the skin whitening activity of the plant extracts. Owing to the presence of pharmaceutically significant secondary metabolites in *Eichhornia crassipes* (Mart.) Solms, the ethyl acetate extract was formulated into a cream with ethyl acetate extract, lemon and musk and tested for its skin whitening efficiency by *in vitro* tyrosinase assay. The results of the study revealed 8 to 11% tyrosinase inhibition even at lower concentrations. The plant extract can be formulated into creams with other active ingredients and can be used in cosmeceutical industry.
**Keywords:** *Eichhornia crassipes*, ethyl acetate, skin, whitening, cream, tyrosinase.

## Introduction

Cosmeceutical industry flourished with the advent of the beauty consciousness of the public in the past decade and used synthetic products mostly. With the increasing awareness among the public on the use of synthetic chemicals, the industries have turned their attention towards natural products especially plant derived products. Plant extracts having an inhibitory effect on melanogenesis may be a good choice of cosmetic purposes because of their relatively few side effects. Plants have been used for long as cosmeceuticals such as skin-whitening, antiageing and anti-wrinkle agents, for treatment of skin allergy and many more. *E. crassipes* contains antioxidants like glutathione (1,2), ascorbic acid (3), polyphenols (4) etc., and these compounds show skin whitening and antiageing efficiency (5). Also phytochemical colour tests with the extracts of *E.crassipes* revealed significant secondary metabolites like alkaloids, sterols, terpenoids, phenolics etc (6). Ethyl acetate extract, aqueous extract and methanol fractionate of aqueous extract were found to be non-toxic upto 2000 mg/kg body weight of the Swiss Albino mice (7).

Generally plant extracts are formulated into a cream, to be in line with the wishes of the cosmeceutical industry. The skin cream so prepared should be tested for its efficacy using various assays depending on the purpose for which it is intended, before being marketed.

Skin whitening is often associated with arresting or reversing the effects of actinic light damage associated with time and sun exposure (8). Skin whitening effect of a substance may be tested by assays like tyrosinse assay, melanin inhibition assay (9), DOPA autooxidation inhibition assay (10) and luciferase reporter assay (11) of which tyrosinase assay is most frequently used. Inhibition of tyrosinase results in reducing pigmentation. Tyrosinase is a bifunctional copper protein complex that catalyzes two different reactions: cresolase activity, or hydroxylation of monophenols tyrosine to *o*-diphenols 3,4-dihydroxy phenylalanine (DOPA) and catecholase activity, or oxidation of DOPA to DOPA quinone and oxidation of 5, 6-dihydroxyindole to indolequinone (12,13) (oxidation of *o*-diphenols to *o*-quinone) (14).

Melanogenesis leads to the formation of dark macromolecular pigments, i.e., melanin. Since the accumulation of excessive epidermal pigmentation leads to various dermatological disorders, such as melasma associated with age, freckling, age spots, and sites of actinic damage, the enzyme tyrosinase is the key target for finding out the skin lightening agents either from natural or synthetic origin. Tyrosinase inhibitors have become increasingly important in medication and in cosmetics to prevent hyperpigmentation through the inhibition of enzymatic oxidation (15). Hence, in the present study, tyrosinase inhibition activity for the skin cream prepared with ethyl acetate extract of *E. crassipes* has been carried out.

## Experimental

### Extraction

### Plant collection

Waterhyacinth was collected from a local water body at Coimbatore, Tamil Nadu and the plant sample was identified by Dr.G.V.S.Murthy, Scientist F & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore- 641 002 with the number BSI/SRC/5/23/2011-12/Tech.

### Extraction of the plant material

The root portion was cut off, washed thoroughly to free it from debris and was shade dried for 20 days. The dried plant material was segmented well and ground. Waterhyacinth (100g) was extracted successively with ethyl acetate (2000mL) and water (1000mL) twice for 6 h. The extracts were desolvetised to give ethyl acetate extract and aqueous extract.

### Preparation of skin cream for skin whitening assay

Two skin creams LPR1 and LP3 were prepared for analyzing the skin whitening property of the extract of *E. crassipes*. The weight of the constituents in the skin creams is given in Table 1.

**Table 1. Constituents of the skin creams made with the ethyl acetate extract of *Eichhornia crassipes***

| Sample | Additives/ Extracts | LPR1 | LP3 |
|---|---|---|---|
| Oil phase | Bees wax | 9.71 g | 11.49 g |
| | Emulsifying wax | 35.12 g | 38.48 g |
| Aqueous phase | Glycerol | 30 mL | 30 mL |
| | Water | 97 mL | 30 mL |
| | Ethyl acetate Extract | 3.29 g | 0.192 g |
| | Lemon extract | - | 1.056 g |
| | Musk | - | 0.057 g |

The skin cream was prepared by the addition of aqueous phase to the oily phase with continuous stirring. Paraffin wax and emulsifying wax was heated upto 70±5 °C. Aqueous phase consisting of glycerol and water was heated upto 80±1 °C and was added to the oil phase drop wise with continuous stirring. The ethyl acetate extract (3.29 g) and ethyl acetate extract (0.192 g) together with lemon (1.056 g) and musk (0.057 g) was added to this mixture respectively yielding LPR1 and LP3. The mixture was stirred continuously until homogeneity and preserved in tubes for further use.

***In vitro* skin whitening property of the skin cream by Tyrosinase assay (13)**

Tyrosinase catalyses the transformation of L-tyrosine into L-DOPA by hydroxylation and into *O*-dopaquinone by oxidation. Then, through a series of non-enzymatic reactions, *O*-dopaquinone is rapidly transformed into melanin, which is measured at 492 nm in a spectrophotometer. The skin cream LPR1 and LP3 was assayed for tyrosinase inhibition by measuring its effect on tyrosinase activity using a 96-well reader. The reaction was carried out in a 50 mM potassium phosphate buffer (pH 6.8) containing 20 mM L-tyrosine and 312.5 U/mL mushroom tyrosinase at 30 °C. The reaction mixture was pre-incubated for 10 min before adding the enzyme. The reaction mixture without the enzyme serves as blank. The reaction mixture with the corresponding solvents (without plant material) serves as control. The change of the absorbance at 492 nm was measured. The percent inhibition of tyrosinase was calculated as follows:

$$Tyrosinase inhibition(\%) = \frac{\text{OD of Control - OD of Test}}{\text{OD of Control}} \times 100$$

## Results and Discussion

Skin is the important external defence organ of the body in living organisms. Hence, it is prone to environmental factors including UV light, drugs, pesticides, ozone, industrial waste, chemical solvents and pollutants. The exposure of skin to these environmental factors causes aging, hyperpigmentation, inflammation etc,. Skin aging and hyperpigmentation pose an aesthetic problem in socioeconomic status. Hyperpigmentation is caused by the key enzyme tyrosinase. It is a copper-containing monooxygenase that catalyses melanin synthesis in melanocytes. The accumulation of excessive epidermal pigmentation leads to various dermatological disorders such as freckling, age spots, and sites of actinic damage (13). Hence, it has become essential for any plant extract to inhibit tyrosinase for it to be an effective skin whitening agent.

Skin whitening products are commercially available for cosmetic purposes in order to obtain a lighter skin appearance. They are also utilized for clinical treatment of pigmentary disorders such as melasma or postinflammatory hyperpigmentation. Whitening agents act at various levels of melanin production in the skin. Many of them are known as competitive inhibitors of tyrosinase, the key enzyme in melanogenesis. Others inhibit the maturation of this enzyme or the transport of pigment granules (melanosomes) from melanocytes to surrounding keratinocytes (16).

Skin whitening creams can be prepared as per the standard procedures and any prepared skin cream in India should adhere to the specifications given in IS 6608:2004. In the present study, the ethyl acetate extract was formulated into a cream that adhered to the standard specifications. The results of the percentage inhibition of tyrosinase showed that skin cream containing only ethyl acetate (LPR1) tested for its activity at 0.5 mg to 20 mg showed no inhibition while for the skin cream containing lemon and musk (LP3) at 0.5 and 1.0 mg, 8.25 and 11.10% inhibition respectively was noted. The synergistic effect of lime and musk in LP3 probably might have contributed to the increased tyrosinase inhibitory activity of LP3. Quercetin is reported to be good tyrosinase inhibitor (17-19) which is present in *E. crassipes* (20). In certain cases, this flavone may act as a strong inductor of melanogenesis in normal and malignant human melanocytes and in reconstituted three-dimensional human epidermis models (21). This might also contribute to the decreased tyrosinase inhibition activity of the skin creams. Melanin biosynthesis is a multistep pathway and the extracts may not show tyrosinase inhibition if it had acted on other enzymes in the pathway rather than directly on tyrosinase. Hence, other assays which estimate the skin whitening activity may be carried out for the cream to prove its efficacy as a skin whitening agent.

## Conclusion

The herbal skin cream formulated with the ethyl acetate extract of *E.crassipes*,lemon and musk exhibited tyrosinase inhibition activity. This suggests the potential of the plant for use in cosmeceutical industry. The skin whitening activity of the plant can be further validated with other assays estimating the skin whitening activity.

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