

	<p>International Journal of</p> <h1>Innovative Drug Discovery</h1> <p>e ISSN 2249 - 7609 Print ISSN 2249 - 7617</p> <p><a href="http://www.ijidd.com">www.ijidd.com</a></p>
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## SCREENING OF ANTIOXIDANT AND ANTITYROSINASE ACTIVITIES OF A HERBAL FORMULATION

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### ABSTRACT

The present study was designed to examine the antityrosinase and antioxidant activities of a herbal formulation. The skin whitening ability was assessed by anti tyrosinase assay. The antioxidant activity of different extracts of herbal formulation was investigated by DPPH, super oxide scavenging, nitric oxide scavenging, total antioxidant capacity and ferric reducing antioxidant power (FRAP). Of all the extracts petroleum ether extract exhibited maximum radical scavenging activity than others. The highest inhibition of tyrosinase (84.6%) was observed with petroleum ether extract. The results led to the conclusion that the formulated herbal plant mixture exhibited antityrosinase and antioxidant activities.

**KEY WORDS:** Antityrosinase. skin depigmentation. melanin. cosmetics.

### INTRODUCTION

The skin is an important barrier which protects our body from the damage due to direct contact with the outside environment. UV irradiation is a harmful environmental factor that damages the skin and is involved in the formation and delivery of melanin within melanosomes [1]. Melanin is the major pigment for color of the human. It is secreted by melanocyte cells in the basal layer of the epidermis [2]. The melanin may be overproduced with chronic sun exposure resulting in malignant melanoma or other hyper pigmentation diseases [3]. In recent years, a number of depigmenting agents have been developed for undesirable skin discolorization [4].

Oxidative stress due to UV radiation results in the depletion of antioxidant defense system and was correlated with melanogenesis [5]. To prevent hyper melanosis antioxidant defense system must be promoted. Medicinal plant with antioxidant properties are used in skin whitening products as ultraviolet radiation dependent stress is believed to aggravate hyper pigmentation [6]. Tyrosinase is a copper containing mono oxygenase and is used in the melanin biosynthetic pathway. Tyrosinase catalyses the oxidation of L-tyrosine to 3,4 Dihydroxy phenyl L-alanine (L-DOPA), followed by the oxidation of L-DOPA to dopaquinone derivatives which yield melanin. Tyrosinase

inhibitors are increasingly used in the cosmetic industry due to their skin whitening effect and for the treatment of hyperpigmentation by UV irradiation [7].

The synthetic substance in cosmetic products for long term use can cause side effects, including carcinogenesis, atrophy, ochronosis [8]. Cosmetics from natural sources are considered safer, better and Eco friendly [9]. Herbal and pharmaceutical agents are traditionally used to treat hyper pigmentation [10]. Different species of plants are used in India for skin lightening purposes and in removing marks or pigmentation on the face. The objective of study was to test the effect of a herbal formulation on tyrosinase enzyme and to determine the effect so that it can be used as a possible skin lightening agent [11].

### MATERIAL AND METHODS

#### Plant material collection and Extraction

Herbal plants were purchased from the local market in Coimbatore and pulverized in a grinder. Table 1 provides the different components in the formulation.

#### Extraction of tyrosinase from potato [12]

One hundred grams of peeled potato was homogenized in a blender with 100 ml of sodium fluoride.

This was homogenized for about one minute at high speed. The homogenate was filtered through several layers of cheesecloth. An equal volume of saturated ammonium sulphate was added to the filtrate. A flocculent white precipitate was formed. The ammonium sulphate treated homogenate is divided into chilled centrifuge tubes and centrifuged at 1, 500 x g for 5 minutes at 4°C. Supernatant was carefully discarded and the pellet was collected. All of the pellets were combined into 60 ml of citrate buffer, pH 4.8. Again the solution is divided into centrifuge tubes and recentrifuged at 300 x g for 5 minutes at 4°C. The supernatant (enzyme) was collected and saved for further use.

#### Determination of Tyrosinase inhibitor activity [13]

The mixture was prepared by adding 10µl tyrosinase 20µl 1.5mM L-tyrosine, 10µl 1.5mM herbal extract and 110µl of 0.1 M sodium phosphate buffer (pH 6.5). The resulting mixture (150µl) was incubated for 10 min at 37°C and absorbance at 490 nm was measured. The percent inhibition of tyrosinase activity was calculated as given in the formula:

$$\text{Inhibition}(\%) = \frac{\text{Abs}(\text{control}) - \text{Abs}(\text{extract})}{\text{Abs}(\text{control})} \times 100$$

The IC<sub>50</sub> values were determined from plots of percent inhibition. Arbutin was used as the reference Tyrosinase inhibitor. The test was performed in triplicate.

#### ANTIOXIDANT ASSAY

The antioxidant activity of various solvent extracts of the herbal formulation was determined by employing the following methods.

##### DPPH radical scavenging assay [14]

DPPH (2, 2-diphenyl -1-picryl hydrazyl) is the most commonly used stable free radical, which is purple in colour. Antioxidant molecules when incubated, reacts with DPPH and converts it into 2,2 -diphenyl-1-picryl hydrazine, which is yellow in colour. The degree of discoloration of purple to yellow was measured at 520 nm, which is a measure of the scavenging potential of plant extracts. 5 µl of plant extract was added to 195 µl of DPPH solution (0.1mM DPPH in methanol) in a test tube. The reaction mixture was incubated at 25 °C for 5 minutes, after that the absorbance was measured at 520 nm. The DPPH with corresponding solvents (without plant material) serves as control.

$$\text{Inhibition}(\%) = \frac{\text{Abs}(\text{control}) - \text{Abs}(\text{extract})}{\text{Abs}(\text{control})} \times 100$$

##### Nitric Oxide Scavenging Activity [15]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat. The reaction mixture (3ml) containing 2

ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25°C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P<sup>H</sup> spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm. The nitric oxide radical scavenging activity was calculated from the formulae:

$$\text{Inhibition}(\%) = \frac{\text{Abs}(\text{control}) - \text{Abs}(\text{extract})}{\text{Abs}(\text{control})} \times 100$$

#### Total antioxidant capacity (TAC) by Phosphomolybdenum method

The total antioxidant capacity of the ethanol extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al* [16]. The method is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. 0.3 ml of the extract (100-500µg) was combined with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction was measured at 695 nm using a spectrophotometer. Ethanol (0.3 ml) was used as the blank. The calibration curve was prepared by using ascorbic acid at different concentration (100 to 500µg/ml).

#### Reducing power

Reducing power was determined according to the method of Oyaizu [17]. Herbal extract (2.5ml) was mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide and the mixture was incubated at 50°C for 20 min. Then 2.5ml of 10% trichloroacetic acid was added, the mixture was centrifuged at 3000 X g for 10 min. The upper layer (2.5ml) was mixed with 2.5ml of deionized water and 0.5ml of 0.1% ferric chloride. Finally, the absorbance was measured at 700nm against a blank.

#### Ferric reducing/antioxidant power

The ferric reducing/antioxidant power (FRAP) assay was carried out according to the method described by Okonogi [18]. This assay measures the reducing properties of antioxidants based on the reduction of ferric ion. Therefore, ferrous sulfate (FeSO<sub>4</sub>) was used for calibration. A freshly prepared FRAP solution contained 50 ml of 0.3 M acetate buffer (pH 3.6) was added to 5 ml of 10 mM TPTZ solution in 40 mM HCl (previously prepared) and 5 ml of 20 mM ferric chloride. After mixing 2.85 ml of FRAP solution with 20 µL of each sample, the ferric reducing

ability was measured at the end of 30 min at an absorbance of 590nm.

## RESULT

### Determination of antityrosinase activity

The antityrosinase activity of the formulation with different solvents was summarized in Fig 1. The herbal formulation showed tyrosinase inhibitory activity in a concentration dependent manner (100-500 $\mu$ g). The petroleum ether extract of the formulation exhibited the maximum inhibition (84.6%) of tyrosinase activity when compared to the aqueous (66.66%), DMSO (77.77%) and hydroethanolic extract (44.44%). The tyrosinase inhibitory activity of the standard arbutin was found to be 76.6%.

### DPPH radical scavenging assay

In the DPPH radical scavenging assay, the colour change from purple to yellow was due to scavenging of DPPH radical by antioxidants. The lowest absorbance of the reaction mixture indicates highest anti-oxidant activity. Herbal formulation showed the highest inhibition of DPPH radical scavenging (84.1%) at a concentration of 500 $\mu$ g which was higher than standard rutin (76.60%) (Fig 2).

### Nitric oxide radical scavenging assay

The nitric oxide scavenging assay showed the scavenging of nitric oxide radical by the herbal formulation and the standard ascorbate. The inhibition of nitric oxide was in a dose dependent manner and in the formulation it was found to be 80% and 78.3% for ascorbate at 500  $\mu$ g/ml (Fig-3)

### Total antioxidant capacity (TAC)

The total antioxidant capacity was evaluated by the phosphomolybdate method. The basic principle to assess the antioxidant capacity through phosphomolybdenum assay which includes the reduction of Mo (VI) to Mo (V) by the herbal formulation possessing antioxidant properties. The total antioxidant capacity of herbal formulation was estimated to be 87% at a concentration of 500 $\mu$ g and for the standard ascorbate it was found to be 75% (Fig 4)

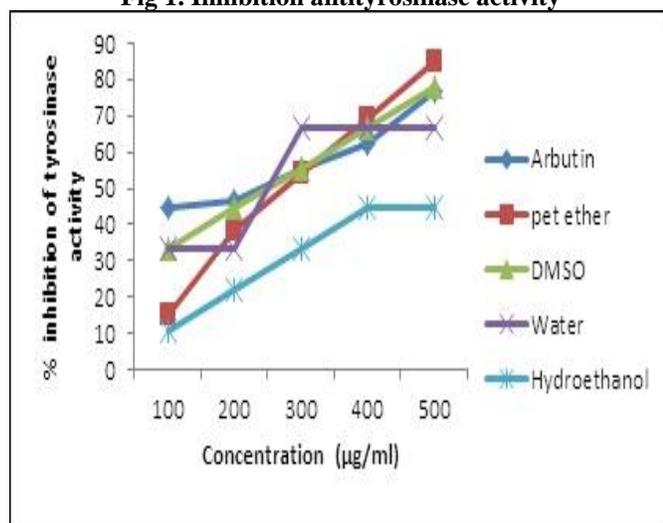
### Ferric reducing antioxidant power (FRAP)

The ferric reducing capacity of the compound may serve as a significant role in its potential antioxidant capacity. This method is based on the ability of the test sample to reduce Fe (III) to Fe (II). In the presence of TPTZ complex, the reduction is accompanied by the formation of a colored complex with Fe (II). The herbal formulation showed a lower FRAP value (44%) than ascorbate 68% (Fig 5).

**Table 1. Composition of the formulation**

PLANT	PART USED
<i>Curcuma longa</i>	Rhizome
<i>Ocimum sanctum</i>	Leaves
<i>Prunus dulcis</i>	Seed
<i>Cucumis sativus</i>	Seed
<i>Papaver somniferum</i>	Bark
<i>Vigna radiata</i>	Seed
<i>Cympopogan zizanioides</i>	Leaves

**Fig 1. Inhibition antityrosinase activity**



**Fig 2. DPPH radical scavenging activity**

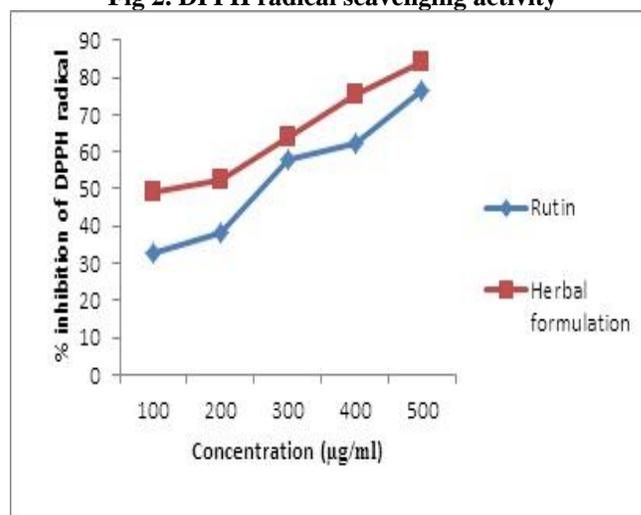


Fig 3. Nitric oxide scavenging assay

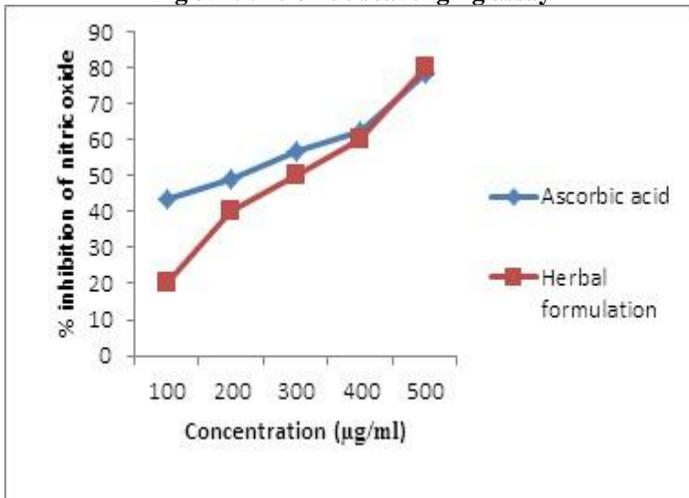


Fig 4. Total antioxidant capacity

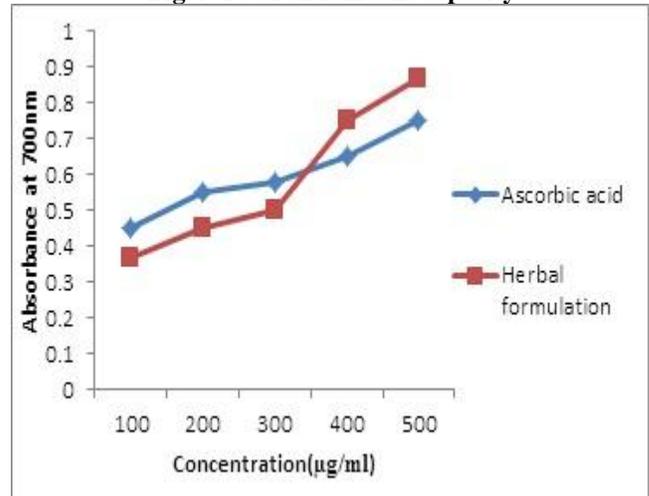
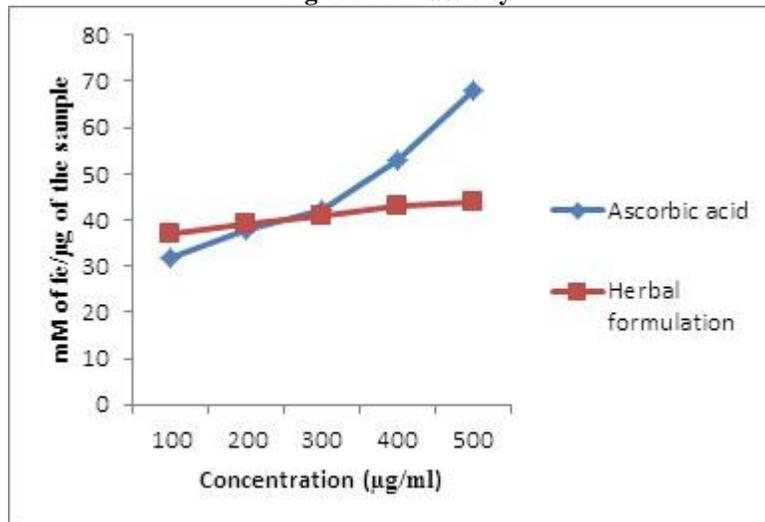


Fig 5. FRAP activity



## DISCUSSION

In recent years there is an increased demand for natural substances which can be used for depigmenting, anti wrinkle, and other cosmeceutical purposes [19,20]. Moreover, plant extracts with an inhibitory effect on melanin formation and elastase activity may be good choices for cosmetic purposes because of their relatively low incidence of side effects. The cosmetic preparations have been developed using plant extracts such as *Areca catechu* [21] and *Morus alba* [22,23] as anti wrinkle and whitening agents.

Inhibition of tyrosinase by a variety of compounds has been studied, with the result that several inhibitors are now used as cosmetic additives or as medicinal products for hyper pigmentation [24]. Traditional Indian herbal medicines have been used in clinical practice for centuries; they are often used to maintain good health or used to treat various diseases. In the present study, a herbal formulation was selected based on compiling ethno botanical data that

revealed the agents are commonly used in skin applications. The antityrosinase activity of the formulation was evaluated and the percentage inhibition was calculated [25]. The result of DPPH scavenging activity in this study indicated that the plant was potently active. This suggested that the herbal formulation contained compounds that were capable of donating hydrogen to a free radical in order to remove an odd electron which is responsible for oxidative stress. The DPPH radical scavenging activity of the formulation was found to be effective for treating radical related pathological damages, especially at higher concentration [26]. Nitric oxide inhibitors have been shown to have beneficial effects on inflammation and tissue damage seen in inflammatory diseases. It is a radical produced in mammalian cells and it is involved in neuro transmission, Vascular homeostasis [27]. The basic principle to assess the antioxidant capacity through phosphomolybdenum assay includes the reduction of Mo (VI) to Mo (V) by the plant extract possessing antioxidant compounds. The results of the study clearly

indicate that the herbal formulation possess antioxidant activity with respect to mechanisms of both free radical scavenging and reducing activities. The ferric reducing antioxidant potential (FRAP) assay is a simple and inexpensive method for the detection of total antioxidant levels in plants. FRAP assay is used to analyze the antioxidant status in humans after hyperbaric oxygen therapy [28]. It has also been used to compare antioxidant activity in plants and mammals [29]. The higher the FRAP value the greater is the antioxidant activity.

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## CONCLUSION

The herbal formulation exhibited anti-tyrosinase and antioxidant activities and this may be suggested as a source of skin whitening agent in cosmetics.

## ACKNOWLEDMENT

Authors wish to acknowledge the management of PSG College of Arts and Science, Tamil Nadu, India, for providing the necessary laboratory facilities.

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