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ANTITYROSINASE ACTIVITY AND ANTIOXIDANT ACTIVITY OF *SANTALUM ALBUM* & *ROSA BRACTEATAE* PLANT EXTRACTS FOR SKIN WHITENING ASSAY

Hemalakshmi Mohanarangan* and Dr. D. Chandra Prabha

*Associate Professor, Department of Biochemistry, Sri Ramakrishna College of Arts & Science for Women, Coimbatore, Tamilnadu.

***Corresponding Author: Hemalakshmi Mohanarangan**
Associate Professor, Department of Biochemistry, Sri Ramakrishna College of Arts & Science for Women, Coimbatore, Tamilnadu.

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ABSTRACT
Natural products containing melanin synthesis inhibitory activity are of interest with their potential cosmetic applications and anti-ageing, for example skin-whitening or anti-browning preparations. Various sources of anti-tryosinase compound have been reported but plants source needs to be safe and easily available. The present study attempts to find tyrosinase inhibitors from the selected medicinal plants extracts in three different solvents. The objective of study was to test the effect of herbal formulation on tyrosinase enzyme and to determine the effect so that it can be used as a possible skin lightening agent. The sandalwood and rose petals extract are used for the study to determine its tyrosinsae inhibitory effects at 517nm and 470nm respectively. High invitro quenching effect was observed in ethyl acetate extract along with high tyrosinase inhibition activity, thereby creating a correspondence of anti-oxidant and anti-tyrosinase activity.

KEYWORDS: Anti-tyrosinase, anti-oxidative properties, melanin, skin-lightening assay, skin-whitening assay.

INTRODUCTION
The skin pigmentation varies among populations, and skin type can range from dry to oily. Such skin variety provides a rich and diverse habitat for bacteria that number roughly 1000 species from 19 phyla, present on the human skin.[1] Excessive melanin accumulation leads to human skin disorders, such as melasma, freckles, age spots and malignant melanomas. Furthermore, formation of neuromelanin in the mammalian brain may be related to neuro-degeneration associated with Parkinson's disease. Therefore, the development of safe and effective tyrosinase inhibitors has become important for improving food quality and preventing pigmentation disorders and other melanin related human health issues.[2]

In the skin, melanogenesis occurs after exposure to UV radiation, causing the skin to visibly tan. Melanin is an effective absorber of light; the pigment is able to dissipate over 99.9% of absorbed UV radiation. Because of this property, melanin is thought to protect skin cells from UVB radiation damage, reducing the risk of cancer. Furthermore, though exposure to UV radiation is associated with increased risk of malignant melanoma, a cancer of the melanocytes, studies have shown a lower incidence for skin cancer in individuals with more concentrated melanin, i.e. darker skin tone. Nonetheless, the relationship between skin pigmentation and photo protection is still being clarified.[3] Much of the research work regarding the biological effects of antioxidant nutrients has concentrated on their potential role in inhibiting or preventing tissue damage induced by free radical species produced during metabolism.[4]

Additionally, tyrosinase inhibitors are supposed to have broad applications as cosmetics whitening agents. As plants are a rich source of bioactive chemicals that are mostly free from harmful side effects, interest in finding natural tyrosinase inhibitors in bioactive chemicals is also increasing. Some potent tyrosinase inhibitors, such as anisaldehyde, quercetin and recently dalenin have been isolated from various plants.[5] When there is an escalation of endogenous and exogenous oxidative stressors, the surplus of reactive oxygen species (ROS) may have a degenerative effect on the body (and skin).

Plant extracts having an inhibitory effect on melanogenesis, may be a good choice for cosmetic applications because of their natural origin and with very low side effects.[6] Hence, in this work, tyrosinase inhibitors from the *Santalum album* & *Rosa bracteatae* plants extracts are estimated and correlated with their anti-oxidative properties for the benefits of skin whitening process. The objective of study was to test the effect of herbal formulation on tyrosinase enzyme and to determine the effect so that it can be used as a possible skin lightening agent.

MATERIALS AND METHOD

Preparation of plant extracts

Plant extracts were prepared from the powder of sandalwood and dried rose petals (complete absence of chlorophyll). For a quantity of about 50mg different solvents such as Hexane, Ethyl acetate and Ethyl alcohol were added sequentially to the plant powders in the ratio of 1:3.

They were left undisturbed for dissolving for about 48 hours and then transferred carefully into petri dishes. They were condensed completely in a condenser until the extracts become extremely thick. They were saved in an Eppendorf container for further use.

DPPH free radical scavenging assay

Procedure

The ability of the extracts to annihilate the DPPH radical (1,1-diphenyl-2-picryl hydrazyl) was investigated by the method described by **Blois** (1958). Stock solution of extracts was prepared to the concentration of 10 mg/ml. Different concentration of the extract (200, 600 and 1000 µg) of extracts were added, at an equal volume, to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula,

% of Inhibition = (Abs of control – Abs of Test)/Abs of control * 100

Extraction of Tyrosinase From Potato

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 sulfate was added to the filtrate. A flocculent white precipitate was formed. The ammonium sulfate 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 re centrifuged 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

The method from **Tomita** (1993) was performed with slight modifications, 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 absorption at 490 nm was measured. The percent inhibition of tyrosinase activity was calculated as given in the formula:

Inhibition (%) = Abs (control) – Abs (extract) / Abs (control) * 100

RESULTS AND DISCUSSION

Invitro Scavenging Activity

Antioxidants quenches the free radicals generated in living system. The antioxidant activity of the extracts was evaluated by employing the DPPH radical scavenging assay.

Table 1: Scavenging activity of sandalwood (hexane extract).

Sample	Concentration (µg)	Scavenging activity	
		% of Inhibition DPPH	% of Inhibition Ascorbic acid
Sandalwood (Hexane extract)	200	79.49	57.04
	600	82.63	75.87
	1000	89.30	90.46

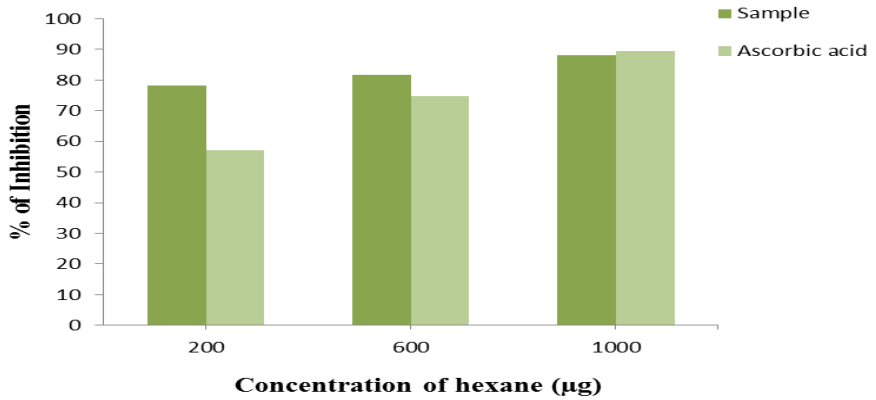


Fig. 1: Graph showing scavenging activity of sandalwood.

Table 2: Scavenging activity of sandalwood (ethyl acetate extract).

Sample	Concentration (µg)	Scavenging activity	
		% of Inhibition DPPH	% of Inhibition Ascorbic acid
Sandalwood (Ethyl acetate extract)	200	20.49	57.04
	600	25.63	74.87
	1000	33.30	89.46

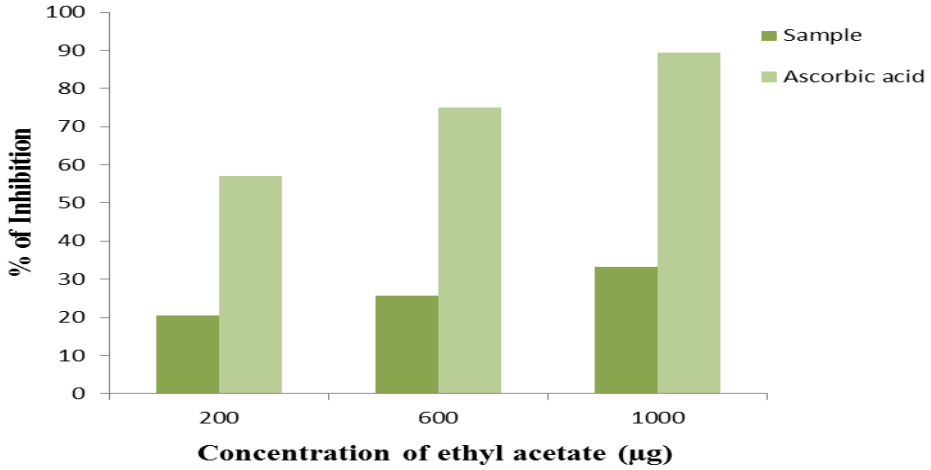


Fig. 2: Graph showing scavenging activity of sandalwood.

Table 3: Scavenging activity of sandalwood (ethyl alcohol extract).

Sample	Concentration (µg)	Scavenging activity	
		% of Inhibition DPPH	% of Inhibition Ascorbic acid
Sandalwood (Ethyl alcohol extract)	200	62.62	57.04
	600	74.60	74.87
	1000	76.61	89.46

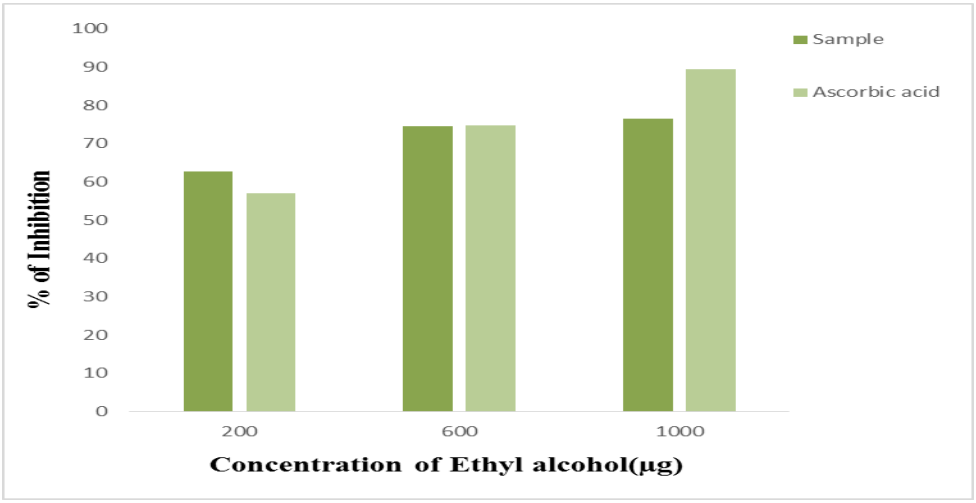


Fig. 3: Graph showing Scavenging activity of sandalwood.

Table 4: Scavenging activity of rose petals (hexane extract).

Sample	Concentration (µg)	Scavenging activity	
		% of Inhibition DPPH	% of Inhibition Ascorbic acid
Rose Petals (Hexane extract)	200	23.41	57.04
	600	30.52	74.87
	1000	40.19	89.46

DPPH is a relatively stable nitrogen centered free radical that easily accepts an electron by reacting with suitable reducing agents. As a result, the electrons become paired off and the DPPH solution losses its violet color depending on the number of electrons taken up. The decrease in the absorbance of DPPH radical after the addition of plant extract was measured at 520 nm.

The results obtained from the DPPH assay shown that the Ethyl acetate extract for the Rose petals produced highest scavenging activity of the free radicals. While the Sandalwood of Hexane extract showed the lowest scavenging activity. The percentage of ethyl alcohol extract for both the samples was similar in range.

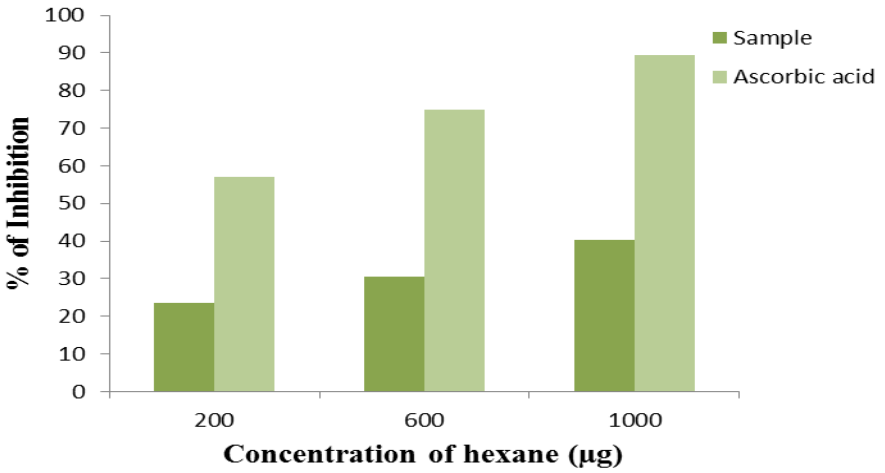
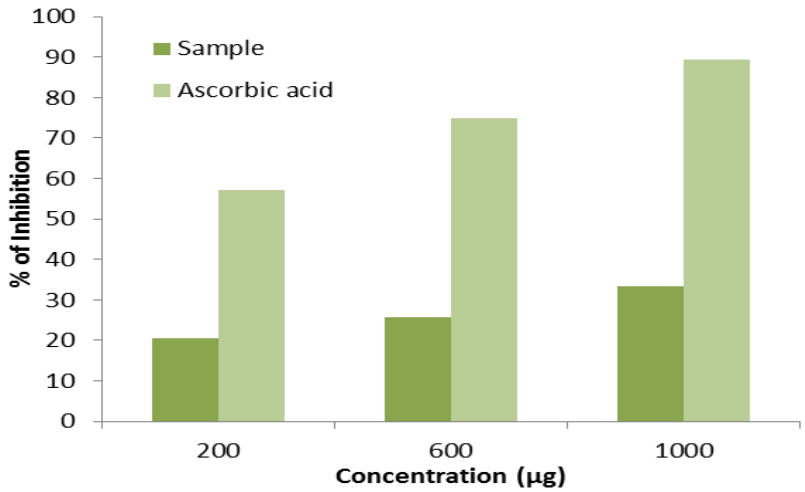


Fig. 4: Graph showing scavenging activity of rose petals.

Table 5: Scavenging activity of rose petals (ethyl acetate extract).

Sample	Concentration (µg)	Scavenging activity	
		% of Inhibition DPPH	% of Inhibition Ascorbic acid
Rose Petals (Ethyl acetate extract)	200	62.62	57.04
	600	74.60	74.87
	1000	76.61	89.46



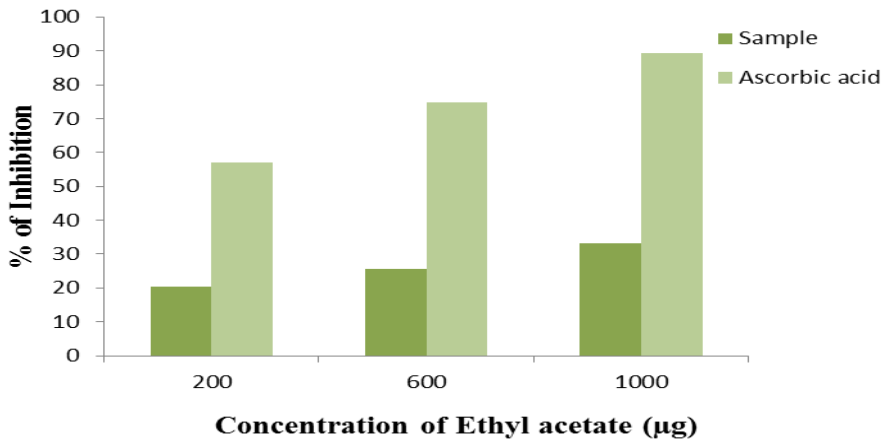


Fig. 5: Graph showing scavenging activity of rose petals.

Table 6: Scavenging activity of rose petals in ethyl alcohol extract.

Sample	Concentration (µg)	Scavenging activity	
		% of Inhibition DPPH	% of Inhibition Ascorbic acid
Rose Petals (Ethyl alcohol extract)	200	42.41	57.04
	600	59.34	74.87
	1000	75.10	89.46

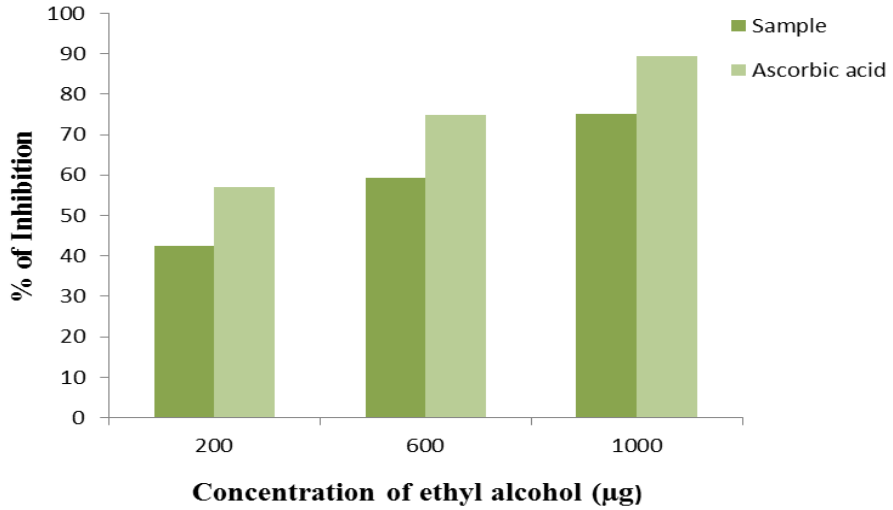


Fig. 6: Graph showing Scavenging activity of rose petals in ethyl alcohol extract.

Thus the Rosa bracteatae showed the high quenching of DPPH radical. 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.

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 at a concentration of 500µg which was higher than standard rutin as revealed from the work of.[7]

According to the work of[8], the DPPH scavenging efficacy of five herbs in different solvents was examined. Among the various solvent extracts of medicinal plants tested, aqueous and ethanolic extracts of the herbs were found to possess good antioxidant activity. Out of five medicinal plants investigated for their radical quenching activity, Holarrhena antidysentrica extract was found to have superior radical scavenging efficacy in all the solvent systems. The percentage inhibition of DPPH radical by aqueous and ethanolic extracts of Holarrhena

antidysentrica was almost in similar range which are in accordance with the work of.[9]

Antityrosinase Activity

Skin pigmentation depends on the melanin content. Tyrosinase activity prevents over production of melanin. Thus tyrosinase activity directly reflects on the synthesis of melanin. The anti-tyrosinase activity was determined by performing the assay with the tyrosinase extraction obtained from potato. The antityrosinase activity of the extracts with different solvents is given in table 5.5.1. The herbal extracts showed tyrosinase inhibitory activity in a concentration dependent manner (100-500µg).

Table 7: Tyrosinase inhibition activity of sandalwood from various solvents.

Sample	Tyrosinase inhibition (%)
Sandalwood (Hexane extract)	67.39
Sandalwood (Ethyl acetate extract)	39.90
Sandalwood (Ethyl alcohol extract)	58.83

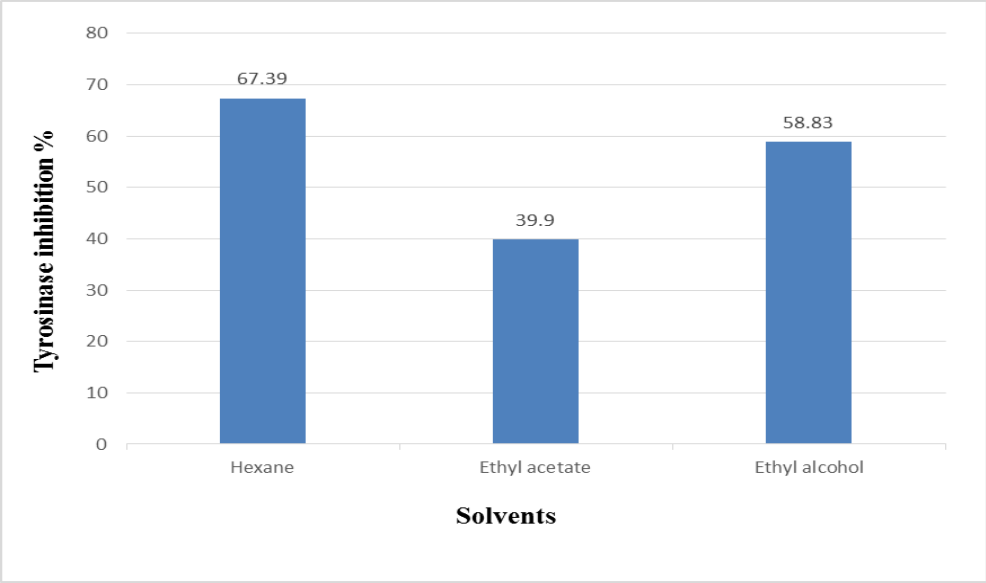


Fig. 7: Graph Sowing Anti-tyrosinase activity of sandalwood.

Table 8: Tyrosinase inhibition activity of rose petals from various solvents.

Sample	Tyrosinase inhibition (%)
Rose petals (Hexane extract)	54.88
Rose petals (Ethyl acetate extract)	68.56
Rose petals(Ethyl alcohol extract)	53.41

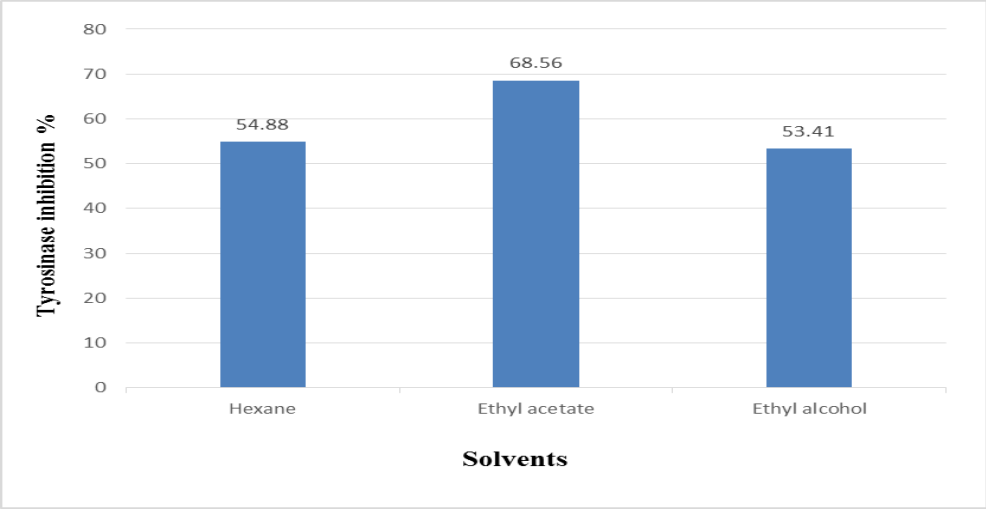


Fig. 8: Graph showing anti-tyrosinase activity of rose petals.

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 hyperpigmentation. Recently, natural substances such as green-plant products have been in increased demand in the global market for new agents for de-pigmenting, cosmeceutical, and skin-lightening purposes. 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 this study of[10] these materials were selected based on compiled ethno botanical data that revealed the agents are usually used clinically as skin applications. Therefore, effects on tyrosinase enzyme are evaluated as its percentage inhibition activities. The selected traditional Indian herbal medicines were extracted with methanol, with extract yields ranging from 2.1 to 33.5%. In the present study, tyrosinase enzyme is used as an in vitro model because of the need to measure % inhibition.

In this current study of *Santalum album* and *Rosa bracteatae*, *R.bracteatae* extract in the ethyl acetate solvent yielded highest tyrosinase inhibition activity than the other extracts. The lowest tyrosinase inhibitory activity was seen with ethyl acetate extract of *S.album*. The other solvent extracts almost ranged in similar values, it is inferred that the existence of correlation between the antioxidant activity and anti tyrosinase activity of the plant extracts. The radical scavenging activity of *Rosa bracteatae* in the ethyl acetate extract is the highest just as the anti tyrosinase activity of the same. Thus inhibiting the melanin biosynthesis more that ultimately causes skin lightening.

CONCLUSION

High *invitro* quenching effect were observed in ethyl acetate extracts of *S.album* and *R.bracteatae*. This implies that the extract is highly active in quenching the free radicals by donating the protons thereby reducing the oxidative stress and ageing related mechanism.

Also it was observed that the ethyl acetate extract of *S.album* and *R.bracteatae* possess a high tyrosinase inhibition activity. This property is highly beneficial leading to the inhibition of melanin biosynthesis and thus plays a major role in the depigmentation process. These are vitally important for anti-cancerous activities in addition to skin whitening process and delayed ageing.

Future Recommendations

Further investigations are in line to extend the study to *in vivo* models to explore the potential activities of antioxidant activities which play a major role in exerting the tyrosinase inhibition. Also the formulations of various combinatorial extracts for skin beneficiaries.

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