

# In vitro Study of *Ixora coccinea* Fruit Extracts for their Antioxidant and Tyrosinase Inhibitory Activities

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## ABSTRACT

**Aim:** The aim of the study is to investigate the antioxidant and antityrosinase properties of *Ixora coccinea* Linn. fruit extracts from various solvents. **Materials and Methods:** The antioxidant property of the extract was tested by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, antityrosinase activity by mushroom tyrosinase inhibitory method, and phenol estimation by Folin-Ciocalteu method. **Results:** The qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenols, and glycosides, in the fruit extracts. *In vitro*, antioxidant analysis showed that the methanolic fruit extract has good free radical scavenging activity when compared with standard Ascorbic acid and also exhibited potent tyrosinase inhibition using Kojic acid as a standard tyrosinase inhibitor. **Conclusion:** The present study proved that the methanolic fruit extract of *Ixora coccinea* has significant antioxidant and skin-whitening properties, and can be used for making skin care formulations that are safe and cost effective.

**Keywords:** *Ixora coccinea* Fruits, Antioxidant activity, DPPH radical scavenging, Inhibitory potency of tyrosinase, Total phenols.

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## INTRODUCTION

Medicinal plants, also known as medicinal herbs, have been discovered and used in traditional medical systems since prehistoric times, and it's likely that products made from natural oils and herbs will continue to be sold on the market in the coming years. Numerous medicinal plants and other herbs contain chemical constituents that have pharmacological effects like hepatoprotective, anti-inflammatory, antimicrobial, and antityrosinase effects, as well as the ability to inhibit lipid peroxidation. As they avoid the negative effects of synthetic products, herbal cosmetics are growing in popularity.<sup>[1]</sup> Herbs with these functional properties have been mentioned in the literature, and when used topically, the cosmetic

industry has a sizable potential market for them.<sup>[2,3]</sup> One of the primary factors in the aging of the skin is oxidative stress as well as skin conditions. The most common exogenous factor that harms the skin is ultraviolet radiation from the sun. Reactive oxygen species, lipid peroxides, and enzyme activity alter connective tissue as a result of ongoing environmental exposure, which leads to a variety of skin conditions.<sup>[4]</sup> The main cause of several dermatological conditions like age spots and hyperpigmentation is melanin hyperpigmentation. Cosmetics with their complexion-improving properties typically inhibit the tyrosinase enzyme in the melanin pathway. Antioxidants are active in skin cells and can improve skin health. The chemical bleaching agent kojic acid, which is present in facial creams, makes skin more sensitive to the sun, increases the risk of allergic contact dermatitis, and causes unfavourable skin reactions.<sup>[5]</sup> In light of these adverse effects, researchers are looking for tyrosinase inhibitors that are safer, non-toxic,

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**Figure 1: Fruits of *Ixora coccinea*.**

and reasonably priced to treat some dermatological conditions linked to melanin hyperpigmentation.

*Ixora coccinea* is a species of flowering plant with Asian origins that belongs to the Rubiaceae family. It is also known as jungle flame, Vecchi, Thechi, jungle germanium, etc. Both Hindu rituals and Indian folk medicine frequently employ the use of red *Ixora* flowers. The dense, multi-branched evergreen shrub *Ixora coccinea* is typically 4-6 feet tall, but it can grow as high as 12 feet. The stems bear opposite pairs or whorls of the 4-inch long, glossy, leathery, oblong leaves, which have entire margins. Fruits are two-seeded, palatable, fleshy berries<sup>[6]</sup> (Figure 1).

*Ixora coccinea* exhibited hepatoprotective,<sup>[7,8]</sup> chemoprotective,<sup>[9,10]</sup> antimicrobial,<sup>[11]</sup> antioxidant,<sup>[12-14]</sup> antinociceptive,<sup>[15]</sup> anti-mitotic, anti-inflammatory, cardioprotective, anti-ulcer, anthelmintic, antiasthmatic, hypolipidemic, and hypoglycemic activities.<sup>[16]</sup> Diarrhea, dysentery, leucorrhea, dysmenorrhea, hemoptysis, and catarrhal bronchitis have all been treated with various plant parts since the dawn of time.<sup>[15]</sup> *Ixora coccinea* displayed significant reducing power as well as overall antioxidant capacity.<sup>[17]</sup> The leaves also contain kaempferol, quercetin, anthocyanidins, phenolic acids, ferulic acid, and flavonoids. According to phytochemical studies, Lupeol, oleic acid, linoleic acid, ursolic acid, oleanolic acid, stearic acid, and sitosterol are the main compounds found in *Ixora coccinea*.<sup>[18]</sup> The objective of the current study is to assess the anti-oxidant and anti-tyrosinase properties of fruits from the *Ixora coccinea* genus in various solvent extracts. The antioxidant activity was determined using DPPH radical scavenging, tyrosinase inhibitory activity by the mushroom tyrosinase assay, and phenol estimation.

## MATERIALS AND METHODS

### Preparation of Plant extracts

The fruits of *Ixora coccinea* Linn. were collected from Kerala lands. Identification and authentication were carried out by Dr. Geetha R Nair, Assistant Professor and Head of the Department of Botany, NSS College, Nilamel. The fruit berries were dried in the shadow, rinsed under running water, and ground into a finely powdered material. The final consistent powder was used for separating the plant material's active components. Soxhlet extraction instruments were used to prepare extracts of methanol and ethyl acetate.

### Preparation of aqueous extract from Fruits

A cold maceration process was used to prepare the aqueous extracts. Three extracts viz; Methanol Extract (MeOH), Aqueous Extract (AQ), and Ethyl Acetate (EA) of *Ixora coccinea* fruits were prepared. The samples were stockpiled in bottles and preserved under refrigeration.

### Preliminary Phytochemical screening

The qualitative analysis of phytochemicals was carried out in freshly prepared extracts of *Ixora coccinea* fruits using standard Procedures.<sup>[19]</sup>

### Determination of total phenolics

Folin-Ciocalteu colorimetric method<sup>[20]</sup> was used for the estimation of total phenolic content. Gallic acid was taken as the reference standard. An extract solution containing 10 mg/10 mL was taken, from this solution, and 1 mL was added to 10 mL using the respective solvents. In various test tubes, different concentrations were pipetted. The standard Gallic acid was also treated in the same manner. After adding 1 ml of Folin-Ciocalteu reagent, the contents were thoroughly mixed. 4 mL of Na<sub>2</sub>CO<sub>3</sub>(20%) was added after 5 min. Keep it for 30 min with repeated shaking. The blue color appeared was read at 765 nm. The results were denoted as mg/g equivalents of Gallic acid.

### Antioxidant assay

#### DPPH radical scavenging assay

The assay was carried out according to the protocol.<sup>[21]</sup> A freshly made 75  $\mu$ L (1.3 mg/mL) DPPH solution was added to test tubes containing various concentrations of fruit extracts. After 30 min of dark incubation, the mixture was measured for absorbance at 517 nm. The calculation of the IC<sub>50</sub> value was from % inhibition. Ascorbic acid was the reference standard used. The

control was created using the same volume of methanol but no extract, reference standard, or blank. Using the equation,

$$\% \text{ inhibition} = (A_0 - A_1 / A_0) \times 100$$

$A_0$  = Absorbance of the control,  
 $A_1$  sample = Absorbance of the Sample.

The ability of % scavenging of the DPPH was calculated.

### Antityrosinase assay or Skin whitening assay

Utilizing tyrosinase from mushrooms, an inhibitory assay was performed.<sup>[22]</sup> All of the extracts utilized for the assay were first dissolved in a solution of DMSO (0.87 mL, 4.5 mM) and phosphate buffer (0.9 mL, 0.1 M, pH 6.8). For 5 min, the solution was held at 30°C. The mixture was added with extracts in varying concentrations and an aqueous solution of tyrosinase from mushrooms (4000 units). The difference in absorbance at 475 nm was measured over a period of 25 min at 1 min intervals. Without inhibitors, the absorbance of controls was determined. Inhibition (%) = [(A control-A sample)] / [A control] × 100 was the formula employed to determine the percent inhibition. ANOVA was applied for the statistical study with the SPSS 20.

## RESULTS

### Preliminary phytochemical screening

Methanolic extracts of *Ixora coccinea* fruits showed the presence of alkaloids, phenols, phytosterols, flavonoids, glycosides, tannins, saponins, sterols, and quinones (Table 1).

### Yield and Total Phenolic Content (TPC) from various Fruit extracts of *I. coccinea*.

**Table 1: Phytochemical analysis of fruit extracts of *Ixora coccinea*.**

Compounds	Extracts		
	MeOH	AQ	EA
Alkaloids	+	+	-
Glycosides	+++	++	++
Flavonoids	+++	++	+
Phytosterols	++	-	+
Phenols	+++	++	+
Saponins	+	-	-
Tannins	+++	++	+
Sterols	+	-	+
Quinones	++	+	-

+++ = large quantity; ++ = medium quantity; + = small quantity; - = absent.

**Table 2: Yield and Total Phenolic Content from Various Fruit Extracts of *Ixora coccinea*.**

Extracts	Yield (g/kg dry weight)	Total Phenolic Content (mg/g dry weight)
MeOH	39.71±0.38 <sup>a</sup>	31.38±0.58 <sup>a</sup>
AQ	24.50±0.78	20.21±0.14
EA	12.30±0.08	17.30±0.13

Results are expressed as mean± SEM. (n= 6). a p<0.05.

**Table 3: Inhibition of DPPH by various Fruit extracts of *Ixora coccinea*.**

Concentration (µg/mL)	% Inhibition			
	MeOH	AQ	EA	Ascorbic acid
10	22.68±0.18	18.11±0.02	6.48±0.06	52.6±0.16
20	31.19±0.32	29.15±0.36	10.93±0.09	62.8±0.08
30	44.93±0.24	36.32±0.18	18.64±0.23	76.44±0.21
40	62.89±0.01	48.23±0.16	24.81±0.27	84.26±0.11
50	80.66±0.23	56.24±0.72	33.92±0.29	98.82±0.30
60	94.87±0.45	68.35±0.13	42.80±0.39	99.96±0.25

The yield and total phenolic content of the prepared fruit extracts of *Ixora coccinea* namely; Methanol (MeOH), Aqueous (AQ), and Ethyl acetate (EA) were shown in Table 2. The total phenolic contents were reported as mg gallic acid equivalent per gram dry weight. The total phenolic content values of MeOH, AQ, and EA were 31.38±0.13 mg/g, 20.21±0.24 mg/g, and 17.30±0.06 mg/g respectively. When compared with aqueous and ethyl acetate extracts, *Ixora coccinea* methanolic fruit extracts had significantly higher yields and higher phenolic levels.

### Antioxidant assay

#### DPPH assay

The DPPH method was used to evaluate the free radical scavenging activity of methanol, aqueous, and ethyl acetate extracts of *Ixora coccinea* fruits. Ascorbic acid at a concentration of 60 µg/mL exhibited a percentage inhibition of 99.96±0.25%. At a concentration of 60 µg/mL methanolic extract showed a percentage inhibition of 94.87±0.45%. As the concentration of the sample increases percentage inhibition of the test extract also increases as shown in Table 3 and Figure 2. The IC<sub>50</sub> values of ascorbic acid and methanolic extract are 7.08 µg/mL and 35.71 µg/mL respectively, Figures 3 and 4. From the results, it is observed that compared with aqueous and ethyl acetate extracts

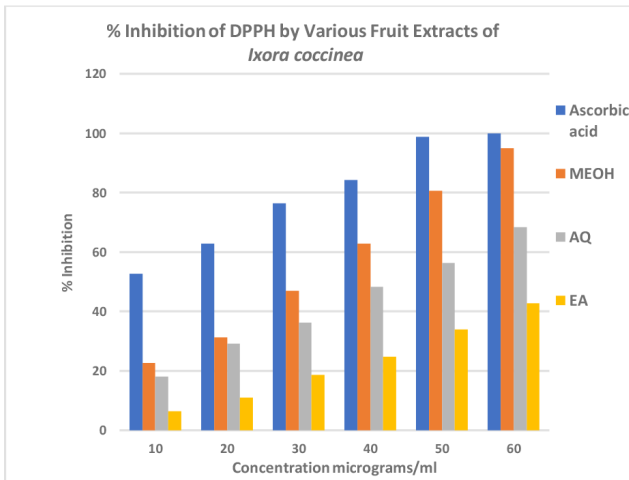


Figure 2: Bar graph showing % Inhibition of DPPH by various Fruit Extracts of *Ixora coccinea*.

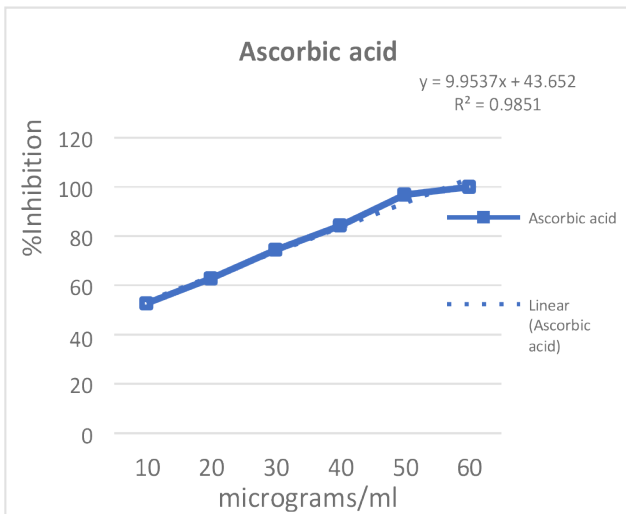


Figure 3: Concentration dependent inhibition of DPPH by ascorbic acid.

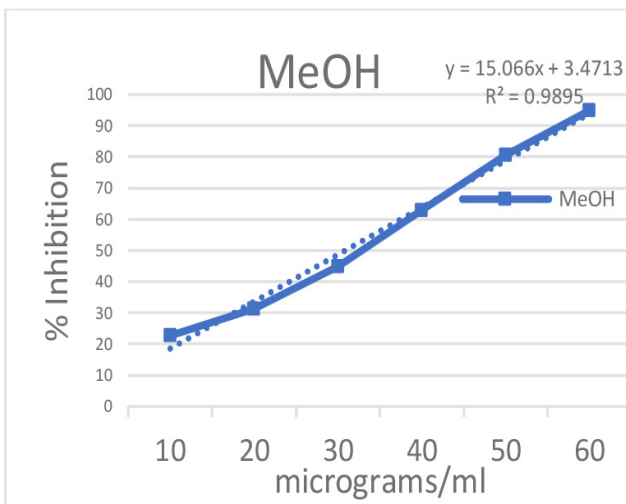


Figure 4: Concentration dependent inhibition of DPPH by methanolic extracts of *Ixora coccinea* fruit.

methanolic extracts of *Ixora coccinea* fruits had the maximum DPPH radical scavenging activity.

### Concentration dependent Inhibition of DPPH

#### Antityrosinase assay

The antityrosinase activity of methanolic and aqueous extracts of *Ixora coccinea* fruits was assessed using the inhibitory activity of mushroom tyrosinase. As shown in Table 4 and Figure 5, the percentage inhibition for the methanolic extract at a concentration of 250 µg/mL was 96.99±0.35% and for the aqueous extract, the percentage inhibition was 50.48±0.28%. The IC<sub>50</sub> values of Kojic acid and methanolic extract are 7 µg/mL and 125 µg/mL respectively, Figures 6 and 7. The methanolic extracts of *Ixora coccinea* fruits showed tyrosinase inhibition in a dose-dependent manner compared with

Table 4: Inhibition of tyrosinase by extracts of *Ixora coccinea*.

Concentration (µg/ml)	Plant extracts		Kojic acid	
	MeOH	AQ	Concentration (µg/ml)	%Inhibition
50	20.62±0.23	12.58±0.18	1.56	26.49±0.18
100	34.69±0.26	18.44±0.19	3.12	36.21±0.19
150	60.44±0.29	28.14±0.19	6.25	50.53±0.24
200	70.45±0.31	34.48±0.20	12.5	71.66±0.28
250	96.99±0.35	50.48±0.28	25	88.48±0.41

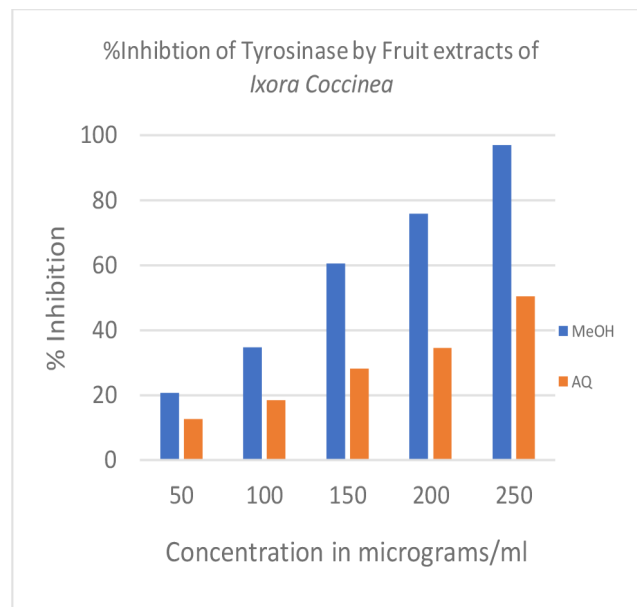
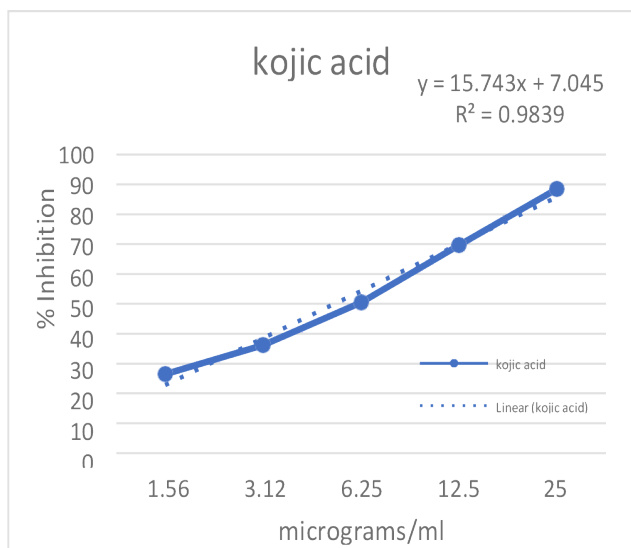
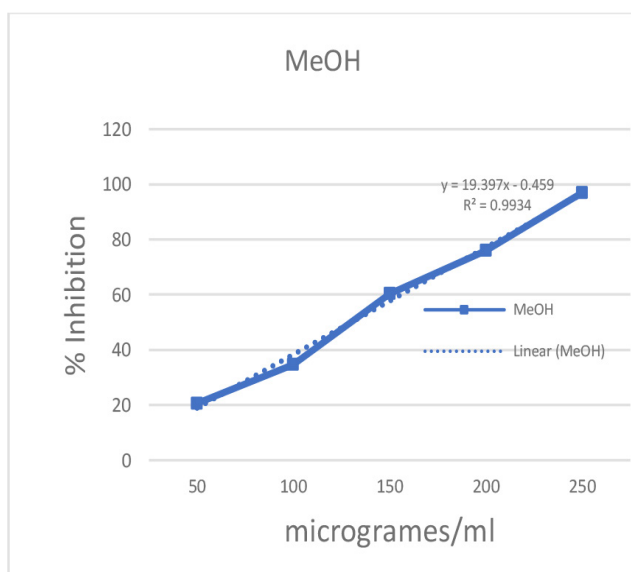


Figure 5: Bar graph showing % Inhibition of tyrosinase by the Fruit Extracts of *Ixora coccinea*.

Kojic acid, a synthetic reference standard. Compared to



**Figure 6: Concentration dependent inhibition of tyrosinase by Kojic acid.**



**Figure 7: Concentration dependent inhibition of tyrosinase by methanolic extracts of *Ixora coccinea* fruits.**

aqueous extracts, methanolic extracts of *Ixora coccinea* fruits exhibited greater tyrosinase inhibitory activity.

### Concentration dependent inhibition of Tyrosinase

## DISCUSSION

Traditional plant-based products have been getting more and more attention in the treatment of a variety of dermatological conditions.<sup>[23,24]</sup> *Ixora coccinea*, a member of the Rubiaceae family, is a traditional medicinal herb that possesses a wide range of therapeutic properties.<sup>[8]</sup>

The current study identified the presence of various phytochemicals such as alkaloids, phenols, flavonoids, glycosides, tannins, saponins, sterols, and quinones in the fruits of *Ixora coccinea*. This is in agreement with the phytochemical screening of bioactive compounds in *Ixora coccinea* fruits by Shreelakshmi *et al.*<sup>[25]</sup> and the study of phytochemical parameters in *Ixora coccinea* plant by Sneha *et al.*<sup>[6]</sup>

Natural antioxidants are gaining increasing importance in treating disorders associated with oxidative stress, since synthetic antioxidants are harmful to melanocytes and produce adverse effects.<sup>[5]</sup> In this study, the methanolic extracts of *Ixora coccinea* fruits exhibited the highest DPPH radical scavenging activity when compared to aqueous and ethyl acetate extracts. Here ascorbic acid was used as the reference standard. Fruit extracts of *Ixora coccinea* had significantly higher yields and phenolic content, which was supported by studies undertaken by Shreelakshmi *et al.*<sup>[25]</sup> and Nithya *et al.*<sup>[28]</sup> in *Ixora coccinea* extracts. The total phenolic contents of *Ixora coccinea* fruit extracts are in the order methanol > water > ethyl acetate, and the variations are equally significant as the antioxidant activities. The results indicate that methanolic extracts of *Ixora coccinea* fruits have significant antioxidant activity and that active ingredient is highly soluble in methanol. Similar results were reported by Sreelekshmi *et al.*<sup>[25]</sup> and Muhammed *et al.*<sup>[13]</sup> According to Masaki *et al.*,<sup>[26]</sup> the lipid peroxidation of the skin is the major reason for dermatological disorders and aging. Karim *et al.*<sup>[27]</sup> demonstrated that plants with a high total phenolic content exhibited remarkable antioxidant properties. Antioxidants inhibit the formation of free radicals by scavenging or accelerating their decomposition.<sup>[4]</sup> Thus UV-induced DNA damage in keratinocytes is protected by phenolic compounds.<sup>[29]</sup> Antioxidants perform this function by the following mechanisms: Scavenging reactive oxygen species, lowering reactive oxygen species reactivity, absorbing UV rays, preventing oxidation by donating either hydrogen or electrons, and by inhibiting the catalytic activity of tyrosinase.<sup>[28]</sup> This reduces the possibility of wrinkle development and shields the skin from ageing.<sup>[30]</sup> According to our findings, *Ixora coccinea* methanolic fruit extract has a high phenolic content and strong anti-free radical properties.

In this study, methanolic extracts derived from the fruits of *Ixora coccinea* exhibited the most significant tyrosinase inhibitory activity when compared to aqueous extracts. The tyrosinase inhibitory activity of the methanolic extracts obtained from the fruits of *Ixora coccinea* was observed to be dose-dependent, as evidenced by the comparison with Kojic acid, a synthetic reference

standard. This is supported by the tyrosinase inhibitory activity of *Ixora coccinea* extracts, Nithya *et al.*<sup>[28]</sup> Melanogenesis is an important biological phenomenon occurring in the melanocytes to protect the skin from free radical attacks, which cause potential cellular injury to the skin. Pérez-Bernal *et al.*,<sup>[29]</sup> reported that the excess secretion of melanin from melanocytes results in skin darkening or hyperpigmentation disorders. This can be prevented by antityrosinase activity of medicinal plant extracts. Kojic acid, a widely used synthetic tyrosinase inhibitor, has been observed to induce severe cutaneous reactions by causing allergic contact dermatitis, erythema and enhancing skin photosensitivity.<sup>[4]</sup> The mechanism of tyrosinase inhibition is mediated by disrupting copper's chelating activity in the enzyme's active site and preventing copper ions from binding to oxygen.<sup>[30]</sup> The introduction of a potent antioxidant serves to hinder the activation of oxygen by tyrosinase.<sup>[31,32]</sup> Another mechanism involves the use of a free radical scavenging agent to impede the formation of melanin.<sup>[33]</sup> Lastly, competitive inhibitors are employed as a means to interfere with the enzymatic activity.<sup>[34]</sup> Based on previous studies by Chatatikun *et al.* and Phonmakham *et al.*,<sup>[35,36]</sup> it has been observed that compounds possessing potent antioxidant and free radical scavenging abilities also exhibit significant antityrosinase properties, rendering them appropriate for the production of skin-whitening and cosmetic formulations. The maximum tyrosinase inhibitory potential of methanolic extracts of *Ixora coccinea* fruits is due to the presence of a high concentration of phenolic compounds as explained by Fatiha *et al.*<sup>[37,38]</sup> Thus methanolic extracts of *Ixora coccinea* which block the rate-limiting step in melanin synthesis, have a potent inhibitory effect on melanogenesis and the extract is an effective ingredient in the treatment of skin discoloration and a safer alternative for depigmenting chemicals.

## CONCLUSION

The present study revealed a significant free radical scavenging potential and tyrosinase inhibitory activity of methanolic extracts from *Ixora coccinea* fruits. This research offers a new perspective that will contribute to the marketable application of *Ixora coccinea* as a cost-effective herbal source with strong antioxidant and antityrosinase activities. Future research will focus on identifying and isolating antioxidant components from *Ixora coccinea* fruits that can be used as natural skin-whitening ingredients in cosmetic products.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**MeOH:** Methanol extracts of *Ixora coccinea* fruits; **AQ:** Aqueous extracts of *I. coccinea* fruits; **EA:** Ethyl acetate extracts of *I. coccinea* fruits; **DPPH:** 2,2 Diphenyl-1-picryl hydrazyl; **TPC:** Total phenolics; **DMSO:** Dimethyl Sulfoxide; **mg:** Milligram; **g:** Gram; **µg:** Micrograms; **mL:** Millilitres; **µL:** Microliters.

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