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Ixora coccinea: A Phytochemical Treasure Trove - Unveiling Secondary Metabolites and Biological Activity for Ecuadorian Biotechnology

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Introduction

The tropical dry forest is a biodiverse ecosystem characterized by a tropical climate, with prolonged periods of drought and shorter wet seasons that harbor many plant and animal species (Siyum, 2020). This unique ecosystem is home to numerous botanical families, among which Rubiaceae (Rosalba & Heidy Paola, 2020) stands out. Within this family, Ixora coccinea is a plant adapted to warm climates that flourishes under intense sun conditions, and its aesthetic value makes it frequent in gardens, nurseries, parks, and streets, where it is admired for its bright flowers (Mex et al., 2019).

In addition to being eye-catching, this species is also interesting to the scientific community for its biological diversity and impact on various industries. An article from the Federal Urdu University highlights the multiple medicinal effects of I. *coccinea*, including its ability to treat asthma, reduce lipid and glucose levels, prevent abnormal cell growth, and be effective against ulcers and parasites. It also possesses antioxidant activities and protective proper-

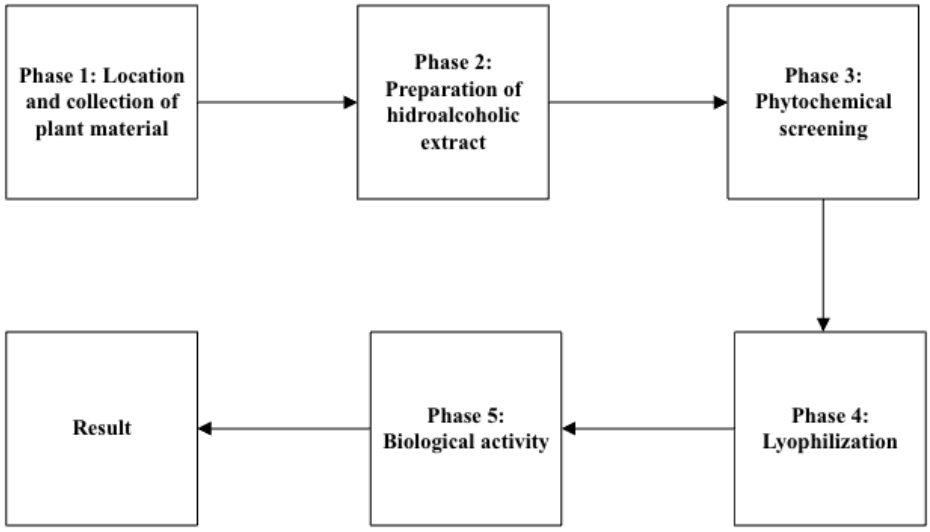
ties against liver and harmful chemicals (Muhammad et al., 2020).

Moreover, a study conducted at the Central Food Technology Research Institute in India found that I. *coccinea* fruits contain high levels of phenols, flavonoids, and anthocyanins, known for their beneficial health effects, thus proving their antioxidant activity and cytotoxicity against human prostate carcinoma cells, suggesting their potential for the development of new treatments for prostate cancer (Shreelakshmi et al., 2021).

Materials and Methods

This research uses the following experimental methodology to identify the plant's secondary metabolites and determine its antioxidant activity:

Figure 1
Phases for obtaining the extract, identifying secondary metabolites and evaluating antioxidant activity



Phase 1: Location and collection of plant material

Ixora coccinea leaves were collected at the Maria Auxiliadora campus of the Salesian Polytechnic University, located at kilometer 19.5 of the Via a la Costa in Guayaquil, Ecuador. Samples of I. *coccinea* were collected, identified, and analyzed. These leaves were used to extract hydroalcoholic compounds, which will be subjected to phytochemical analysis and antioxidant activity tests.

Phase 2: Preparation of hydroalcoholic extract

The plant material was separated and cut, and then a washing process was carried out with distilled water to eliminate any impurities in the sample (Da Silva et al., 2018). Subsequently, the plant material was dried at room temperature for 1 hour to eliminate surface moisture and then transferred to a Binder oven, which was kept at 40 °C for 96 hours (Babu et al., 2018). This controlled drying process allowed the obtaining of completely dehydrated samples that were subsequently pulverized.

The Soxhlet method was employed to obtain the hydroalcoholic solution, using a mixture of 96% ethanol and distilled water in a 90:10 ratio (Al Jitan et al., 2018). Before phytochemical analysis, the hydroalcoholic extract was concentrated and purified using a rotary evaporator at 40 °C (Aronés Jara et al., 2022).

Phase 3: Phytochemical screening.

Different photochemical tests were performed to identify secondary metabolites in *I. coccinea* (see Table 1). These tests were based on the methodology of the National Polytechnic Institute of Mexico GSL Medical College and General Hospital of India (García-Granados et al., 2019; Kancherla et al., 2019).

Table 1
Assays performed for the identification of secondary metabolites

Test	Secondary metabolites
Mayer test	Alkaloids
Dragendorff test	
Hager test	
Wagner test	
Shinoda test	Flavonoids
Zinc test	
Sodium hydroxide test	Coumarins
Keller-Kilani test	Cardiotonic glycosides
Benedict test	Carbohydrates
Fehling test	
Terpenoid test	Proteins
Salkowski test	Triterpenes
Peroxide test	Saponins
Molish test	
Baljet test	Lactones sesquiterpenes

Phase 4: Lyophilization

A Christ Alpha 1-4 LSC freeze dryer was used, adjusting the conditions to a condenser freezing temperature of -50 °C and a vacuum pressure of 0.04 millibar. The process included a central drying for 4 hours and a final drying of 3 hours, after which the extracts were flash-frozen at -80 °C (Cheaib et al., 2018).

Phase 5: Biological activity.

The DPPH free radical technique was applied to perform the antioxidant activity assay, starting with preparing the leading solution composed of 5 milligrams of DPPH, gauged in 100 milliliters of 96% ethanol. In parallel, as a standard solution, 5 milligrams of ascorbic acid gauged in 50 milliliters of 96% ethanol was prepared (Castañeda Castañeda et al., 2008). To prepare the sample solution, 5 milligrams of the hydroalcoholic extract was dissolved in 50 milliliters of 96% ethanol. For completing homogenization, the solution was agitated in an iSonic P4860 ultrasonic bath for 30 minutes (Das et al., 2014). Finally, to remove impurities or residues in the solution, the extract was filtered using 0.45-micrometer CHMLAB Group syringe filters (Ding & Scheer, 2004). To analyze the antioxidant activity of the hydroalcoholic extract of *I. coccinea* leaves, the amounts of extract, ethanol, and DPPH were specifically prepared (see Table 2).

After preparation, the vials were shaken at 300 rpm for 3-5 min at room temperature and were allowed to stand for 30 min before reading (La et al., 2011).

Table 2
Preparation of solutions for antioxidant activity testing

Flask	Amount of DPPH (milliliters)	Amount of ethanol 96 % (microliters)	Sample quantity (microliters)
White	2.9	100	0
1	2.9	95	5
2	2.9	90	10
3	2.9	80	20
4	2.9	50	50
5	2.9	20	80
6	2.9	0	100

Results

Healthy and mature leaves were selected, avoiding those affected by pests or physical damage (see Fig. 2). The samples were placed in sealed plastic bags and transferred to the Instrumental Laboratory of the Salesian Polytechnic University.

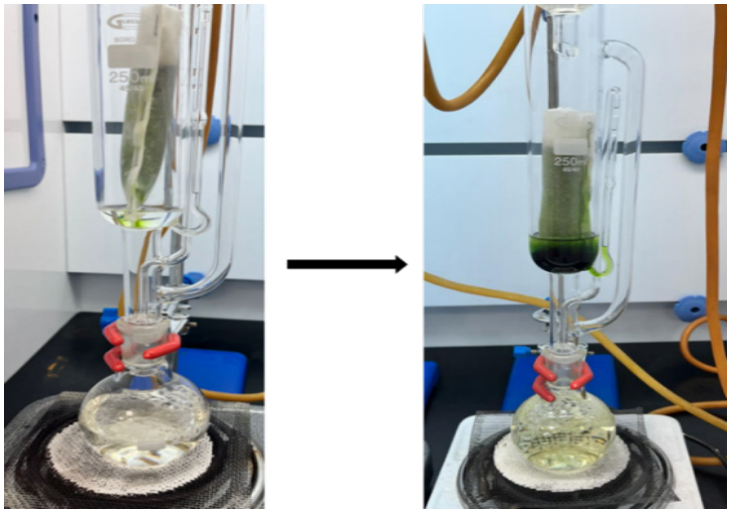
Figure 2
Collection of I. coccinea species

The morphological characteristics of the leaves of *I. coccinea* that were collected were verified (see Table 3). The hydroalcoholic extract was obtained with a final volume of 750 milliliters, presenting a dark green color and a pH of 5.40 (see Fig. 3). The green coloration of the hydroalcoholic extract is due to pigments such as chlorophyll and carotenoids; this tonality intensifies when exposed to air or light due to the oxidation of these pigments, which can affect the properties of the extract; this shows the importance of properly handling and storing the extract to maintain its quality (Tena & Asuero, 2020).

Table 3
General description of the morphological characteristics of I. coccinea leaves

Morphological characteristics of the leaves of I. coccinea						
Image	**Form**	**Size**	**Color**	**Odor**	**Texture**	**Consistency**
	Simple and lanceolate	Length: 4 centimeters Width: 2 centimeters	Dark green	No odor	Smooth	Rigid

Figure 3
Preparation of hydroalcoholic extract



The secondary metabolites found in the hydroalcoholic extract of *I. coccinea* were qualitatively expressed with the following symbols: '+++' for abundant presence, '++' for moderate presence, '+' for low presence, and '-' for absence (see Table 4) [18]. (Loja et al., 2017).

Table 4
Phytochemical screening of the hydroalcoholic extract of I. coccinea leaves

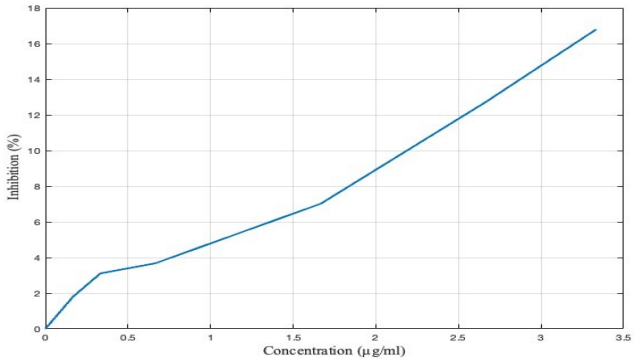
Secondary metabolites	Essay	Results
Alkaloids	Mayer	+++
	Dragendorff	+++
	Hager	+++
	Wagner	+++
Flavonoids	Shinoda	+
	Zinc	+++
Coumarins	NaOH	++
Cardiotonic glycosides	Keller-Kilani	-
Carbohydrates	Benedict	+++
	Fehling	+
Terpenoids	Terpenoids	+++
Triterpenes	Salkowski	++
Saponins	Peroxide	-
	Molish	-
Sesquiterpene Lactones	Baljet	+++

The DPPH technique indicates that the hydroalcoholic extract presents 1.79% inhibition with five microliters and 16.78% with 100 microliters, showing that the inhibition of free radicals increases with the concentration of the extract. In addition, it is demonstrated that *I. coccinea* has an IC50 of 10.65 micrograms over milliliters (see Table 5). The antioxidant activity of the hydroalcoholic extract was compared with that of ascorbic acid, a pure chemical reference with an antioxidant value of 5.38 micrograms per milliliter, demonstrating the strong antioxidant capacity of I. *coccinea* (see Fig. 4). This finding highlights the antioxidant capacity of I. *coccinea* in comparison with other species, such as *Cinchona pubescens Vahl*, belonging to the Rubiaceae family, which has an IC50 of 46.66 micrograms per milliliter, according to a study conducted at the Salesian Polytechnic University, Quito (Barukcic Revelo & Sola Montero, 2015).

Table 5
Results of the DPPH antioxidant test

Ixora coccinea (µl)	Concentration (µg/ml)	Absorbance at 517 nanometers		Average absorbance	Inhibition %
		Repetition 1	Repetition 2		
White	0	1.480	1.481	1.481	0
5	0.167	1.454	1.454	1.454	1.80
10	0.333	1.435	1.434	1.435	3.11
20	0.667	1.426	1.426	1.426	3.68
50	1.667	1.376	1.377	1.377	7.02
80	2.667	1.292	1.292	1.292	12.73
100	3.333	1.232	1.232	1.232	16.78
				IC50	10.65

Figure 4
Inhibition of the DPPH radical according to its concentration



The hydroalcoholic extract of *I. coccinea*, rich in flavonoids, terpenoids, and triterpenoids, has a strong antioxidant potential, supportingsupports its application in medicine, agriculture, food, and the cosmetic industry, as well as in developing various sustainable products and practices (Castaño Amores & Hernández Benavides, 2018)

Conclusions

A diversity of secondary metabolites was determined through phytochemical screening, including alkaloids, flavonoids, coumarins, carbohydrates, terpenoids, and sesquiterpene lactones. These compounds were analyzed to explore their potential in biotechnological applications, such as developing drugs with therapeutic properties and formulating compounds for the food and cosmetic industries. The quantification of the antioxidant activity by DPPH obtained an IC50 of 10.65 micrograms over milliliters, indicating a significant antioxidant capacity. This result suggests a potential use of *I. coccinea* extracts in creating antioxidant products, which is interesting to the biotechnological industry, especially in health and cosmetics. Confirming the presence of secondary metabolites and quantifying antioxidant activity in *I. coccinea* promotes their study and sustainable use, benefiting society and the environment.

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