

Extraction efficacy, phytochemical profiling and radical scavenging potential of different parts of *Ixora coccinea* L.

Nivas Desai

nivasdesai88@gmail.com

Dapoli Urban Bank Senior Science College, Dapoli

Research Article

Keywords: *Ixora coccinea*, Phytochemicals, Extraction efficacy, Antioxidant potential, HPLC analysis

Posted Date: December 23rd, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-5623794/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The present work was aimed to evaluate extraction efficiency and quantification of phytochemicals, and antioxidant potential of different parts of *Ixora coccinea* L. Different extraction techniques like Continuous shaking extraction, steam bath assisted extraction, ultrasonic extraction and microwave assisted extraction were used for the extraction of phenolic compounds and antioxidants. Presence of wide range of plant metabolites were observed from the preliminary study. Ultra sound assisted extraction for 20 min was found most suitable for extraction phenolics (44.14 ± 2.25 mg of gallic acid equivalent per gram of dry weight) and flavonoids (23.95 ± 1.15 mg of quercetin equivalent per gram of dry weight). High performance liquid chromatography confirmed the presence of six pharmaceutically important phenolic acids. 1, 1- diphenyl-1-picryl hydrazyl (DPPH) assay, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) scavenging (ABTS) assays were used to determine antioxidant potential. Leaves showed higher yields of illustrated biochemical parameters, however fresh fruit pulp was found a chief source of polyphenols. The current investigation highlighted the variety of phytoconstituents from different parts of *I. coccinea* and its medicinal and nutritional implications were confirmed.

1. Introduction

The use of herbal medicine is quickly becoming more popular as a therapeutic option for a variety of illnesses, perhaps because it is less expensive and has less side effects than synthetic medications. It has become possible to separate different chemical components from therapeutic plants. Over 70% of people in poor nations currently receive their medical care from traditional, complementary, or alternative sources [1]. Red *Ixora* flowers are commonly being used in worship in both Hinduism and Indian traditional medicine. It's claimed that *Ixora* is an Asian native, and her name comes from an Indian goddess. *Ixora coccinea* Linn. (Rubiaceae) is also known as the jungle of geranium and red ixora. It is an evergreen shrub that grows all over India. The Indian traditional medicine known as Ayurveda, along with numerous folk medicines, uses flowers, leaves, roots, and stems to treat a variety of maladies, depending on the condition. After they are fully mature, the fruits are consumed.

Ayurveda and other traditional medical systems have long employed *Ixora coccinea* to treat a wide range of illnesses. The flowers are used in the treatment of leucorrhea, dysentery, dysmenorrhea, haemoptysis, hypertension, irregular menstruation, sprains, bronchitis fever, sores, chronic ulcers, scabies, and skin illnesses in the Ayurvedic medical system [2]. The mixture is made by boiling its flowers with the stem bark of *Madhuca longifolia*, *Centella asiatica*, and *Coldenia procumbens* leaves. It is then combined with coconut oil and applied as a wound healing agent. Dysentery and catarrhal bronchitis are treated with the flowers [3] The blossoms that have been shade-dried are boiled in coconut oil, and the resulting decoction is administered externally to lessen eczema [4].

Phytochemical studies have shown that *Ixora coccinea* has sitosterol, lupeol, stearic acid, oleic acid, linoleic acid, and ursolic acid as its key constituents [5]. Rutin, leucocyanadin glycoside, cyanadin-3-

rutinoside, and delphinidin monoglycoside are all said to be present in the flowers [6]. The root bark has been found to contain octadecadienoic acid, and studies have indicated that the root oil includes methyl esters of oleic, palmitic, stearic, and linoleic acids [7].

From the extensive literature survey it is learnt that no substantial work has been carried out on different extraction techniques for higher yield of phytochemical constituents of *I. coccinea*. Hence an effort was made to investigate Extraction efficiency, phytochemical profiles and antioxidative properties of different parts of *Ixora coccinea*.

2. Material and Methods

1.1 Plant material collection and processing

Plant material (fruit pulp, leaf and seeds of *Ixora coccinea*) were collected from a single population at the Amboli location in the Northern Western Ghats of India (15058'05.6"N; 73059'48.7"E; altitude: 739 m). A specimen was verified as authentic and deposited in the Herbarium of the Department of Botany at SPK College, Sawantwadi (Voch. No. SPK/ND/18). Seasons of vegetative, flowering, and fruiting plants were taken into consideration when collecting them. The material was chopped up into little pieces, given a 15-day shade dry, and then processed in a Wiley mill to a fine powder. The collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

2.2. Reagents and Chemicals

Phenolic acids, ascorbic acid, gallic acid, tannic acid, quercetin, ellagic acid, trolox, and ascorbic acid standards for antioxidant assays, 2,2-diphenyl-1-picryl hydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), 2,2'-azino-bis (3-ethylbenzo-thiazoline-sulphonic acid) (ABTS), and N,N-dimethyl-p-phenylenediamine (DMPD) were obtained from Sigma Aldrich, Bangalore, India. Petroleum ether, acetonitrile, methanol, acetone, and chloroform were all of HPLC grade (Qualigens, India). All the chemicals were analytical grade.

2.3. Extracts preparation and qualitative phytochemicals screening

The leaf, fruit pulp, and seeds were the various plant sections for which a qualitative phytochemical study was performed. One gram of plant powder was introduced into a 250 mL conical flask to begin the extraction process. The flask was filled with one hundred milliliters of each of the following solvents: methanol, acetone, chloroform, petroleum ether, or distilled water separately. It was then shaken for twelve hours at 110 rpm on an orbital shaker manufactured by Rivieratek in Riviera, India. Then, Whatman No. 1 filter paper was used to filter the extracts and the appropriate solvents were used to adjust the volume to 100 ml [8].

2.4. Total phenolics, flavonoids, and antioxidant capacity

With a slight modification, the procedures for the extraction of phytoconstituents from *I. coccinea* were used [9–10]. Extractions were conducted to assess the optimal extraction technique and duration needed to obtain a greater yield of total phenolics, flavonoids, and antioxidants from various *I. coccinea* sections. Since methanol was the best solvent for extracting antioxidants, flavonoids, and phenols from fresh *I. coccinea* fruit pulp in a prior work [11], it was used for these tests. The following extraction methods were used in this investigation.

2.4.1. Ultrasound Extraction (UE)

On an ultrasonic bath (Revotek, India), powdered plant material (leaf, fruit pulp, and seeds) was extracted using ultrasonic waves at a working amplitude of 60 Hz. In a 250 mL beaker, one gram of the powdered substance was added to 100 ml of methanol. Samples were subjected to room temperature sonication for ten or twenty minutes.

2.4.2 Continuous Shaking extraction (CSE)

One gram of powdered plant material (seed, fruit pulp, and leaf) was put on an orbital shaker (Rivotek, Riviera, India) and continuously shaken to extract the material using 100 mL of methanol. The extractions were conducted for 340 or 700 minutes at a constant stirring speed of 110 ± 2 rpm, at a regulated temperature of 25 ± 2 °C.

2.4.3. Microwave assisted extraction (MAE)

In conical flasks, one gram of powdered fruit pulp, seeds, and leaf were extracted separately using 100 mL of methanol. Every flask was placed in a Samsung, India microwave oven set to 180 W for five or ten minutes. To prevent bumping, cooling was applied at regular intervals of two minutes, and multiple exposures were permitted to fulfill the necessary exposure.

2.5. Total phenolic content (TPC)

The Folin-Ciocalteu method was adapted and used to quantify TPC [12]. Using a Thermo Scientific multiskan Go 1510, USA, double beam spectrophotometer, the absorbance of the blue color generated was measured at 760 nm. The data were represented as milligram equivalent per gram dry weight and were compared to standard curves of gallic and tannic acid.

2.6 Total flavonoid content (TFC)

The aluminum chloride calorimetric technique was utilized to quantify the total flavonoid content [13]. At 367 nm, the absorbance of the standard solutions and extracts was measured. Milligrammes of quercetin equivalent per gramme of dry weight were used to express the results.

2.7. HPLC analysis for phenolic compounds

2.7.1. Sample preparation

One gram of powdered plant material (leaf, fruit pulp, and seed separately) was extracted using 100 milliliters of methanol on an orbital shaker over night at 28 degrees Celsius with continuous stirring at 110 rpm. The extracts were placed in an amber vial and kept at 4 °C for less than a week before to HPLC analysis. They were filtered through an Axiva 0.45 mm nylon filter. The extracts' volume was then adjusted to 100 ml using methanol.

2.7.2. HPLC instrument and chromatographic condition

For the reversed phase HPLC analysis, a Shimadzu chromatographic system (model no. LC-20AD) equipped with a dual λ UV absorbance diode array detector (SPD-M20A), quaternary pump, manual injector, and degasser (DGU-20A5) was used. Data processing was done using LC-Solution software (Version 1.25, Shimadzu Corporation, Japan). On a Waters, Nova-Pak C18 column (4 μ m, 4.6 $\times$ 250 mm), chromatographic separation was accomplished. A 20 μ l injection volume was utilized in the mobile phase, which was composed of water, acetonitrile, and glacial acetic acid (90:5:5), for the purpose of separation. With a 60-minute run duration, an isocratic flow rate of 0.9 ml/min was employed. It was detected at 280 nm.

To create a standard stock solution (1 mg/ml), phenolic compounds were accurately measured and dissolved in methanol. Six levels of working concentrations (1, 5, 10, 20, 40, and 100 μ g/ml) were obtained by serially diluting the phenolic compound stock solution using the same solvent. This allowed calibration curves to be plotted. For less than a week, all solutions and analytes were kept in microfuge tubes at 4°C until they were needed.

The test for system compatibility was conducted using three duplicates of standard phenolics at a 40 μ g/ml concentration. The method's repeatability was assessed using the peak regions, which were also examined for resolution and tailing effects. The limits of detection (LOD) and quantification (LOQ) were estimated using signal-to-noise ratios of 3.3 and 10, respectively.

2.8. GC-MS analysis of phytochemicals

2.8. 1. Sample preparation

A Soxhlet extractor was used to extract phytochemicals from ten grammes of dried plant powder. One hundred millilitres of ethyl acetate was used to extract uniform plant material from a thimble. The addition of 1 millilitre of concentrated hydrochloric acid increased the polarity of ethyl acetate. The extraction procedure lasted three hours at a steady temperature of 80°C. Ultimately, 100 millilitres of the filtered extract were used, and it was kept in a refrigerator at 4 degrees Celsius for less than a week before being used again.

2.8.2. Gas Chromatography condition

The Shimadzu model QP-2010 with a nonpolar 60M RTX 5MS column was used for the GC-MS study. The carrier gas used was helium, and the oven's temperature was programmed to start at 40°C and hold

it there for three minutes until reaching 480°C at a rate of 10°C final. In a split-less mode, two microlitres (2 µl) of sample were injected. The electron impact ionisation energy was 70 eV, and mass spectra were acquired spanning the 35–650 amu range. The sample ran for a total of forty-five minutes. The corresponding peak regions were compared to the total ion chromatogram (TIC) to make relative quantitative determinations.

2.8.3. Compound identification

For the molecular identification of compounds, unknown components are compared to known mass spectra from the National Institute of Standards (NIST). A provisional determination was made of the test material's name, molecular weight, retention duration, and peak area %.

2.9. Antioxidant potential

2.9.1. DPPH radical scavenging activity

The antioxidant activity of the plant extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 100 milliliters of cooled methanol as a measure of radical scavengers [14]. A 2.9 ml DPPH solution was added to 100 microliters (100 µl) of plant extract. The absorbance at 517 nm was measured after the reaction suspension was shaken and left in the dark for 30 minutes.

2.9.2. ABTS free radical cation scavenging assay

Free radical scavenging potential of different parts of *I. coccinea* was examined using a modified ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] radical cation decolorization test [15]. The absorbance of the ABTS reagent at 734 nm was 1.1 ± 0.02 when it was diluted 1:10 with methanol. The ABTS antioxidant activity was measured at 734 nm with methanol serving as a blank.

2.3. Statistical analysis

The statistical software GraphPad Prism evaluation version was used to conduct the statistical analysis. The means $\pm$ standard deviation (SD) of the data were presented. Using GraphPad Software, Version 3.06, repeated measure one-way ANOVA at $P < 0.05$ was used to identify significant differences between means. Shimadzu LC Solution software (Version 1.25), which is built-in, was used to examine the chromatographic profiles of each extract.

3. Result and Discussion

3.1. Preliminary phytochemical analysis

The preliminary qualitative phytochemical screening of different *I. coccinea* extracts was investigated, and the results showed the existence of several classes of metabolites, including proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids in Table 1. The fact that these compounds have a variety of therapeutic benefits is widely established [16].

This study discovered that the main source of significant phytochemicals was the methanolic extract of *I. coccinea* pulp and leaves. All parts of *I. coccinea* contain terpenoids, a group of compounds of significant medicinal value, regardless of the solvent system (Table 1).

Table 1
Qualitative phytochemical screening of different parts of *I. coccinea* in various solvents

	Leaves				Fruit pulp				Seeds			
	Solvent system				Solvent system				Solvent system			
	M	A	C	D	M	A	C	D	M	A	C	D
Proteins	+	++	+	+	+++	++	++	++	+	+	+	+
Carbohydra test (Fehling Test)	-	-	-	-	++	++	++	++	-	-	-	-
Phenols	+	+	+	+	+++	++	++	++	+	+	+	+
Tannins	+	+	+	-	+	+	+	+	+	+	+	+
Flavonoids	+++	++	++	++	+++	+++	++	++	+	+	+	+
Saponins	-	-	-	-	+	+	+	+	-	+	-	-
Glycosides Libermanns Test	+	+	+	+	+	+	+	+	+	+	-	-
Salkowaskis Test	++	++	++	+	++	+	++	+	-	-	-	-
Steroids	++	++	+	+	++	++	++	++	+	+	+	-
Terpenoids	+	+	+	+	+	+	+	+	-	-	-	-
Alkaloids	-	+	-	-	+	+	+	+	-	-	-	-
(M) Methanol, (A) Acetone, (C) Chloroform, (D) Distilled water, (+++) Strongly Positive, (++) Moderate Positive, (+) Positive, (-) Negative												

3.2. Total phenolics and flavonoids

The total amounts of flavonoids and phenolic compounds in the fruit, seeds, and leaf of *I. coccinea*, were measured using various extraction methods. It is evident from Table 2 that the extraction conditions had an effect on the extraction efficiency of TPC and TFC. The results showed that of the extraction methods used, MAE (10 min) and CSE (340 min) had the best extractabilities. Thus, it can be seen that the polyphenol yield may be impacted by the extraction conditions. The amounts of polyphenols in the medicinal plant *Clinacanthus nutans* [17] and all other species are also affected by the extraction process. The ranges of phenolic content are 05.18 ± 0.26 to 44.14 ± 2.25 mg GAE/g DW. Leaves > fruit pulp > seeds was found to be the phenolic hierarchy among the various plant parts studied. The current investigation revealed that, in comparison to other plant components, the roots constitute the primary source of polyphenols. Fruit pulp and seeds were discovered to be significant sources of polyphenols.

The leaves also demonstrated the noteworthy quantities. The ranges of flavonoid content are 02.12 ± 0.11 to 23.95 ± 1.19 mg QE/g DW. The levels of phenolic compounds are higher in the leaves than those of the fruits and seeds.

Table 2
Total phenolic and flavonoid content among various parts of *I. coccinea* with respect to different extraction methods and extraction time

Sr.No.	Plant part	Extraction method	Extraction period (Min.)	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
1.	Leaves	MAE	05	19.08 ± 0.95	05.18 ± 0.25
2.			10	18.34 ± 0.91	05.81 ± 0.29
3.		UA	10	17.33 ± 0.86	07.18 ± 0.35
4.			20	19.27 ± 0.96	06.90 ± 0.34
5.		CSE	340	18.62 ± 0.93	10.79 ± 0.53
6.			700	25.72 ± 1.19	02.12 ± 0.11
7.	Fruit pulp	MAE	05	42.44 ± 2.18	21.68 ± 1.08
8.			10	43.77 ± 2.13	19.05 ± 0.95
9.		UA	10	42.32 ± 2.06	19.67 ± 0.98
10.			20	44.14 ± 2.25	23.95 ± 1.15
11.		CSE	340	41.17 ± 2.16	23.05 ± 1.19
12.			700	33.62 ± 1.68	21.23 ± 1.06
13.	Seeds	MAE	05	06.13 ± 0.30	05.49 ± 0.27
14.			10	06.52 ± 0.32	04.61 ± 0.23
15.		UA	10	05.71 ± 0.28	04.41 ± 0.20
16.			20	06.26 ± 0.31	06.18 ± 0.30
17.		CSE	340	05.18 ± 0.26	12.26 ± 0.61
18.			700	05.45 ± 0.27	10.01 ± 0.50
Measurements are mean ± SD of three parallel determinations and expressed as gallic acid and quercetin, equivalent per gram dry weight.					

The most crucial elements in extraction optimization, according to the current study, were the plant part and the extraction technique; the period of extraction had the least impact on variations in TPC and TFC production. Similar findings have recently been reported for *Achyranthes aspera* [18] and *Clinanthus nutans* [17]. It is evident that the amounts of TPC and TFC in the various plant sections under study

varied considerably from one another, with the contents of TPC and TFC being larger in the roots than in the other plant parts.

3.3. Identification and quantification of major phenolic compounds

RP-HPLC/DAD analysis was used to determine which major phenolic acids were present in all parts of *I. coccinea* (Table 3). The principal peaks determined through comparison with actual standards. Various parts of *I. coccinea* were found to contain seven distinct phenolic acids: catechol, hydroxy benzoic acid, caffeic acid, vanilin, ferulic acid, and salicylic acid. (Table 3). The primary source of polyphenol in the current investigation was found to be gallic acid. This naturally occurring phenolic molecule found in plants is well-known for its ability to scavenge free radicals and act as an antioxidant, making it beneficial for consumption [19]. The fruit pulp's primary source, catechol, demonstrates the fruits' nutritional significance. The study verifies the existence of four important phenolic acids in the roots, which may be in charge of their potent antioxidant ability and demonstrate their potential therapeutic uses. Vanilin, gallic acid, and caffeic acid were found in *I. coccinea* leaves.

Table 3
HPLC profile of phenolic acids (mg/g DW) in the different parts of *I. coccinea*.

Sr. No.	Compound name	LOD ($\mu\text{g}/\text{ml}$)	LOQ ($\mu\text{g}/\text{ml}$)	Plant parts		
				Leaves	Fruit pulp	Seeds
1.	Ferulic acid	0.152	0.462	nd	nd	nd
2.	Cumarin	0.092	0.258	nd	nd	nd
3.	Gallic acid	0.158	0.461	0.451 ± 0.023	0.584 ± 0.029	0.443 ± 0.022
4.	Catechol	0.136	0.422	0.317 ± 0.020	0.207 ± 0.020	nd
5.	Vanilin	0.092	0.283	0.053 ± 0.003	0.131 ± 0.007	nd
6.	Hydroxy Benzoic acid	0.087	0.265	nd	nd	nd
7.	Caffeic acid	0.142	0.428	0.182 ± 0.011	0.228 ± 0.014	nd
8.	Syringic acid	0.128	0.380	nd	nd	nd
9.	Total phenolics	-	-	1.003 ± 0.013	1.15 ± 0.028	0.443 ± 0.022
(mean $\pm$ standard deviation; n = 3); nd: not detected						
LOD:- limit of detection; LOQ: limit of Quantification						

3.4. Identification of phytochemicals using GC MS

In the present study 23 phytochemical compounds were detected and tentatively identified that of extracted from various parts of *I. coccinea* (Table 4, Fig. 1). Most of them were reported from Fruit extracts and leaves. The mass spectral analysis of leaves showed presence trans-Geranylgeraniol, n-Hexadecanoic acid, Octadecanoic acid, tetradecyl ester, Ethane, Ethyl Oleate, 2 (2Butoxyethoxy)carbonyl benzoic acid, Hexadecanoic acid, ethyl ester, trans,trans-9,12-Octadecadienoic acid, propyle, 1,1,1-triethoxy, Undec-10-ynoic acid, 17-methyl-, methyl ester.

Table 4
GC-MS identification of phytochemicals in different parts of *I. coccinea* L.

Sr. No.	Name of Compound	Retention Time (min)	Area Peak (%)	Plant part
1.	13-Hexyloxacyclotridec- 10-en-2-one	24.58	2.26	Fruit pulp
2.	1-Decanol, 2-octyl-	23.27	1.38	Fruit pulp
3.	1-Dodecanol, 3,7,11-trimethyl-	18.05	1.66	Fruit pulp
4.	2(2Butoxyethoxy)carbonyl)benzoic acid	16.44	2.66	Leaves
5.	2,4-Dodecadienal	14.19	9.95	Fruit pulp
6.	2,6,10-Trimethyltridecane	14.60	2.16	seed
7.	2-Undecenal	13.30	6.12	Fruit pulp
8.	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	18.77	3.53	Fruit pulp
9.	9-Octadecenoic acid,(E)-	25.97	9.52	Fruit pulp
10.	Diethyl Phthalate	16.41	2.11	Fruit pulp
11.	Ethane, 1,1,1-triethoxy-	6.678	4.15	Leaves
12.	Ethyl Oleate	26.19	23.44	Seed, leaves
13.	Hexadecane	13.77	1.35	seed
14.	Hexadecanoic acid, ethyl ester	23.19	15.48	Leaves
		23.17	19.82	seed
		23.16	1.41	Fruit pulp
17.	Naphthalene, decahydro-1,4a-dimethyl-7-(1-meth	13.11	1.68	seed
18.	n-Hexadecanoic acid	22.75	24.21	Fruit pulp
		22.74	19.67	leaves
20.	Octadecanoic acid, ethyl ester	26.57	8.59	seed
21.	Octadecanoicacid,17-methyl-, methyl ester	26.58	6.31	Leaves
22.	Phytol	25.56	21.89	seed
23.	trans,trans-9,12-Octadecadienoic acid, propyl ester	26.10	21.07	seed
		26.11	16.98	Leaves
25.	trans-Geranylgeraniol	19.21	11.29	Fruit pulp

Sr. No.	Name of Compound	Retention Time (min)	Area Peak (%)	Plant part
		19.23	16.16	Leaves
27.	Undec-10-ynoic acid, tridec-2-yn-1-yl ester	19.06	0.62	Fruit pulp
28.	Undec-10-ynoic acid, tetradecyl ester	19.07	2.93	Leaves

The fruit extract showed presence of 2,4-Decadienal, (E,E)-, 2-Undecenal, 2,4-Dodecadial, Diethyl Phthalate, 1-Dodecanol, 3,7,11-trimethyl-, 3,5-di-tert-Butyl-4-hydroxybenzaldehyde, Undec-10-ynoic acid, tridec-2-yn-1-yl ester, trans-Geranylgeraniol, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 1-Decanol, 2-octyl-, 13-Hexyloxacyclotridec-10-en-2-one, 9-Octadecenoic acid, (E). This study highlights the need for additional research to assess medicinal properties of the compounds.

3.5. Antioxidant potential (AP)

Figure 2 shows the AP of the different *I. coccinea* parts assessed by the DPPH and ABTS assays in the present work. Additionally, trolox and ascorbic acid were used as two reference standards to assess each antioxidant assay. The most used technique for determining a plant's antioxidant potential is the DPPH assay. Figure 2A shows the ability of different *I. coccinea* parts to scavenge DPPH radicals. The various plant parts had DPPH capacities ranging from 175.68 ± 7.18 to 645.41 ± 43.05 μM TEAC and 143.23 ± 6.22 to 644.03 ± 34.21 μM AEAC. The leaves of *I. coccinea* shows a fair quantity of ABTS antioxidant capacity in the current experiment (673.02 ± 43.12 μM TEAC and 631.41 ± 33.07 μM AEAC). Fruit pulp and leaves both have strong antioxidant activity, however seeds have the lowest level of antioxidant capacity. The MAE extraction method was found to be suitable for the study of antioxidant potential.

3.5. Statistical analysis

The significance of the data gathered from the research was assessed using one-way analysis of variance (ANOVA) and the Tukey test for TPC, TFC, and antioxidant capacity of all samples evaluated. Results were considered statistically significant at $p < 0.05$ (Table 5). Comparisons within and between the groups being studied are interpreted by the result. The results from this study have been categorised into three categories for statistical analysis: (i) HPLC analysis; (ii) antioxidant activities (DPPH and ABTS); and (iii) total polyphenols (TPC and TFC).

Table 5: One Way analysis of variance (ANOVA) with Tukey test for the experimental results

Experiments	Degree of freedom		Sum of squares		Men square		F
	Treatment	Residue	Treatment	Residue	Treatment	Residue	
TPC GA	30	55	11303	307.64	468.03	6.03	75.28
TPC TA	30	55	11708	363.03	452.02	6.74	68.04
TFC QE	30	55	6898.9	283.02	128.04	2.86	112.98
TFC EA	30	55	15098.8	302.23	641.01	6.01	109.86
DPPH AEAC	30	55	2303903	98893	89232	2001.12	45.08
DPPH TEAC	30	55	3598622	150465	145658	2901.07	51.09
ABTS AEAC	30	55	4308553	155603	172262	2028.43	83.78
ABTS TEAC	30	55	64098967	159599	270003	3072.05	85.07

Treatment:- Within The Group; Residual:- Between The Group

Conclusion

In conclusion, different *I. coccinea* parts were assessed for the extraction of total phenolics, flavonoids, and antioxidants using a variety of extraction methods. The results showed that, in comparison to other extraction techniques, the microwave aided extraction technique was the most effective. It was found that fruit pulp was an excellent source of secondary metabolites. HPLC methods confirmed the presence of bioactive phenolic acids in different *I. coccinea* parts. The data produced about the antioxidants and secondary metabolites of *I. coccinea* fruits could increase consumer awareness of this underutilized fruit. Further research on the importance (nutritive, medicinal, etc.) and function (biosynthesis, accumulation, etc.) of the identified bioactive chemicals is made possible by this investigation.

Declarations

Author Contribution

NMD carried out experimental work and prepared manuscript

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Azaizeh, H., Saad, B., Cooper, E., & Said, O. (2010). Traditional Arabic and Islamic medicine, a re-emerging health aid. *Evidence-Based Complementary and Alternative Medicine*, 7, 419–424.
2. Sankaranarayanan, S., Bama, P., Ramachandran, J., Kalaichelvan, P. T., Deccaraman, M., Vijayalakshimi, M. & Sathya Bama, S. (2010). Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. *J Med Plants Res*, 4(12), 1089–1101.
3. Sivarajan, V. V., & Balachandran, I. (1994). *Ayurvedic drugs and their plant sources*. Oxford and IBH publishing.
4. Sivaperumal, R., Ramya, S., Ravi, A. V., Rajasekaran, C., & Jayakumararaj, R. (2009). Herbal remedies practiced by Malayali's to treat skin diseases. *Environ We Int J Sci Tech*, 4(1), 35–44.
5. Ayyanar, M., & Ignacimuthu, S. (2009). Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. *International journal of Applied research in Natural products*, 2(3), 29–42.
6. Latha, P. G., Nayar, M. N. S., Singh, O. V., George, V., Panikkar, K. R., & Pushpangadan, P. (2001). Isolation of antigenotoxic ursolic acid from *Ixora coccinea* flowers. *Actualidades Biologicas*, 23(74), 21–24.
7. Deshpande, A., Jadge, D., Dhawale, S., Patrakar, R., & Gadgul, A. (2010). Flower extract of *Ixora coccinea* as a natural indicator in acid base titration. *J Pharm Res*, 3(10), 2512–13.
8. Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*, 3(12).
9. Pai, S. R., Nimbalkar, M. S., Pawar, N. V., & Dixit, G. B. (2011). Optimization of extraction techniques and quantification of Betulinic Acid (BA) by RP-HPLC method from *Ancistrocladus heyneanus* Wall. *Ex Grah. Industrial Crops and Products*, 34(3), 1458–1464.
10. Chavan, J. J., Jagtap, U. B., Gaikwad, N. B., Dixit, G. B., & Bapat, V. A. (2013). Total phenolics, flavonoids and antioxidant activity of Saptarangi (*Salacia chinensis* L.) fruit pulp. *Journal of plant biochemistry and biotechnology*, 22, 409–413.
11. Latha, L. Y., Darah, I., Jain, K., & Sasidharan, S. (2012). Pharmacological screening of methanolic extract of *Ixora* species. *Asian Pacific Journal of Tropical Biomedicine*, 2(2), 149–151.
12. Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of agricultural and food chemistry*, 51(3), 609–614.
13. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of food and drug analysis*, 10(3), 3.
14. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1), 25–30.
15. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9–10), 1231–1237.

16. Julsing, M. K., Koulman, A., Woerdenbag, H. J., Quax, W. J., & Kayser, O. (2006). Combinatorial biosynthesis of medicinal plant secondary metabolites. *Biomolecular engineering*, 23(6), 265–279.
17. Mustapa, A. N., Martín, Á., Mato, R. B., & Cocero, M. J. (2015). Extraction of phytocompounds from the medicinal plant *Clinacanthus nutans* Lindau by microwave-assisted extraction and supercritical carbon dioxide extraction. *Industrial Crops and Products*, 74, 83–94.
18. Upadhy, V., Pai, S. R., & Hegde, H. V. (2015). Effect of method and time of extraction on total phenolic content in comparison with antioxidant activities in different parts of *Achyranthes aspera*. *Journal of King Saud University-Science*, 27(3), 204–208.
19. Paixão, N., Pereira, V., Marques, J. C., & Câmara, J. S. (2008). Quantification of polyphenols with potential antioxidant properties in wines using reverse phase HPLC. *Journal of separation science*, 31(12), 2189–2198.

Figures

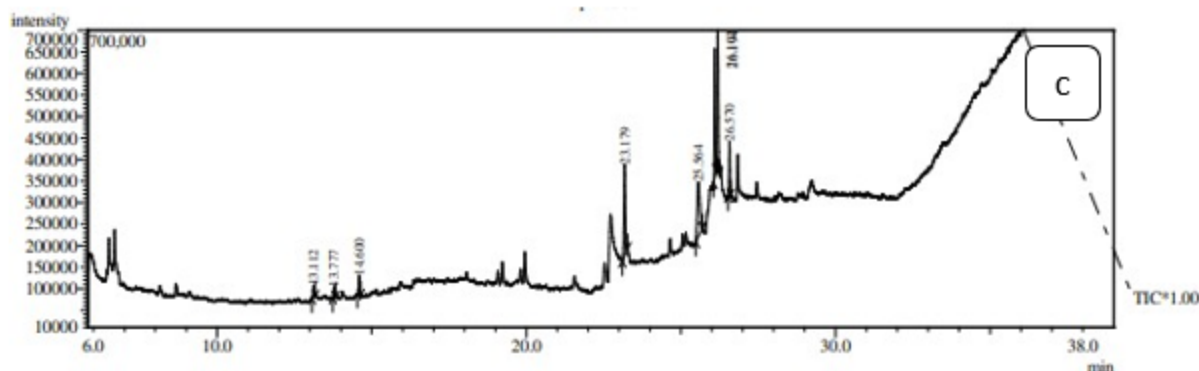
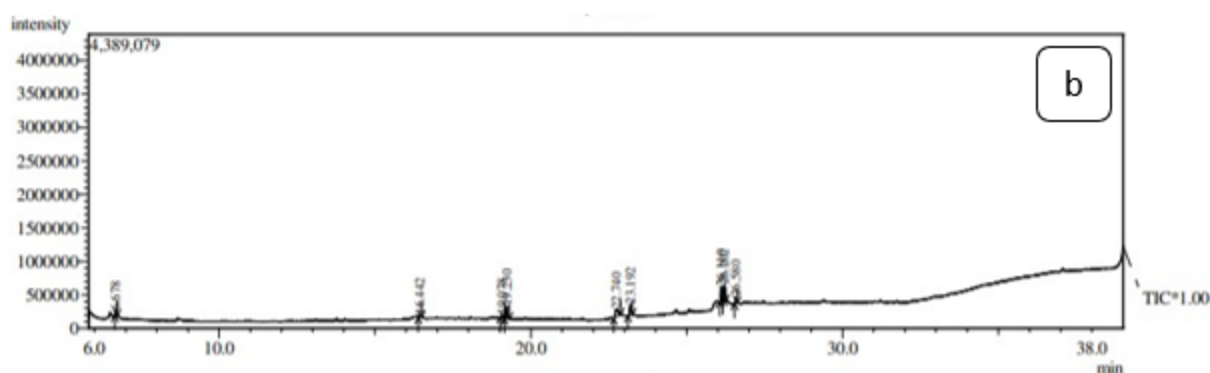
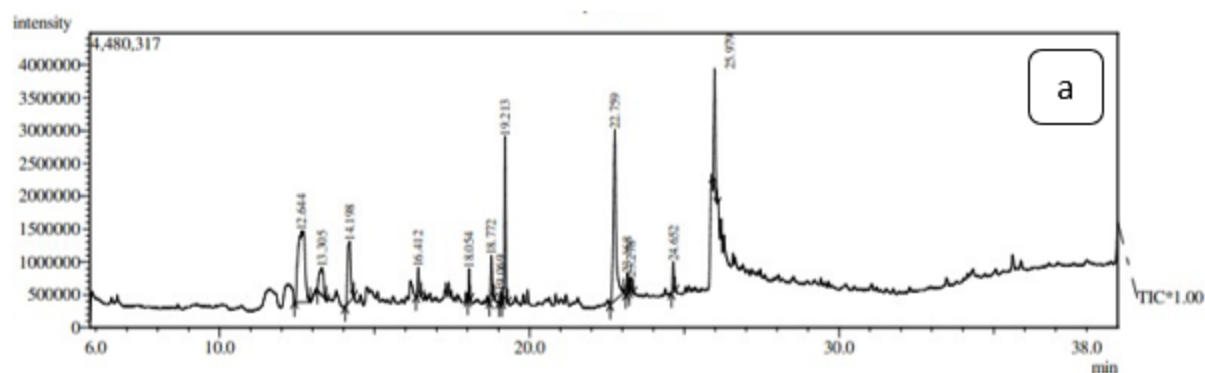


Figure 1

GC-MS/MS chromatogram of *I. coccinea*L. a) fruit pulp b) seeds c)leaves

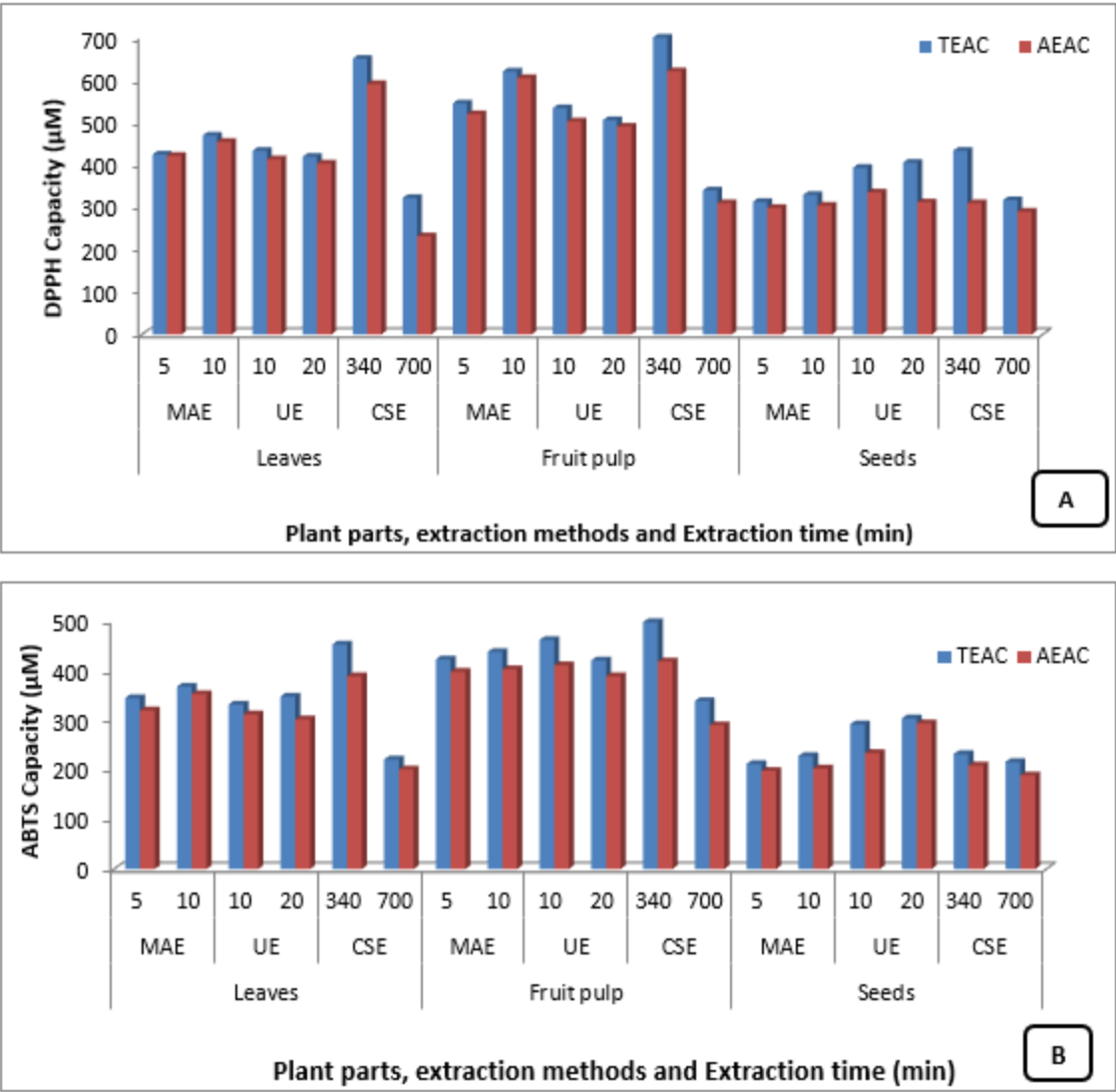


Figure 2

Antioxidant capacities of various parts of *I. coccinea*. A: DPPH, B: ABTS assay