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Essential oil constituents and study of Antioxdiant, Antidiabetic Activities of *Ixora coccinea* L. extract from South West, Nigeria

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Abstract
Ixora coccinea L.is a woody, evergreen and flowering shrub that is traditionally used in treatment of hypertension, menstrual irregularities, sprains and other varieties of ailments. This study investigated chemical constituents, antioxidant and antidiabetic activities of the methanol extract and essential oil from *Ixora coccinea.* The phytochemical analysis and in-vitro study was achieved by using standard procedures. The essential oil composition was analyzed using gas chromatography-mass spectrometry (GC-MS). The methanol extract revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, tannins and phenolics. The quantitative analysis showed higher content of saponins (0.32 mg/g), phenolic compounds (0.30 mg/g) and alkaloids (0.14mg/g). The antidiabetic activities were concentration dependent, with lowest inhibitory activity at 20µg/mL and highest antioxidant potential at 100µg/mL. However, methanol extract exhibited lower antioxidant activities compare to the standard. Also, the extracts displayed inhibitory activity that was dose dependent against the action of α-glucosidase (IC_{50}: 59.31µg/mL) and α-amylase (IC_{50}:61.68µg/mL). A total of 62 constituents, 95.2% of *Ixora coccinea* essential oil constituents were identified. The oil wasdominatedbyselina-1, 3, 7 (11)-trien-8-one (27.6%), selina-1, 3, 7 (11)-trien-8-one epoxide (12.7%), spathulenol (9.2%), (*E*)-β-caryophyllene (7.6%), germacrene B (4.5%) and β-selinene (4.7%) as major constitutents. The results indicated that *Ixora coccinea* contain phytochemicals with promising antidiabetics and antioxidant activities, which can serve as future lead drugs in combating diabetics and oxidative stress in related diseases.

Keywords *Ixora coccinea*, antidiabetic, antioxidant, phytochemicals, essential oil

1. Introduction
The *Ixora coccinea* L., a woody flowering shrub of the Rubiaceae, grows in the tropical areas of Asia and Africa [1-2]. The genus consists of evergreen trees and shrubs with a wide variety of about 500 species [3]. Leaves of *I. coccinea* are used in folk medicine with reported antioxidant, antimicrobial, chemoprotective, antinociceptive, anti-inflammatory, antidiarrheal and antimitotic activities [4-9]. Medicinally, a decoction of the roots part of *I. coccinea*is taken as a remedy for nausea,anorexia, and chronic ulcers. Fresh leaves and stems of the plant are used for treatment of skin diseases, diarrhea, feversores, dysentery, gonorrhea,and sprains [10-11]. Phytochemical screening of the *I. coccinea* plant revealed the presence of secondary metabolites such as steroids, terpenoids, flavonoids, fatty acids, and reducing sugars [12-15]. A recent study has revealed that flowers of *I. coccinea* contain the tirucallaneixoroid, stigmast-5-en-3-*O*-β-D-glucoside, 5-*O*-caffeoylquinic acid, and D-mannitol [16]. This present report is aimed at the characterization of the essential oil composition, as well as the antioxidant, and antidiabetic potential of *I. coccinea* methanol extract for future exploitation in pharmaceutical applications.

2. Materials and Methods
2.1 Plant Materials and Identification
The fresh leaves part of *I. coccinea* were collected in the month of December, 2021 from farmland, Ile-Igbon Village, Lagelu, Oyo State, Nigeria at (7°28′59″ N, 4°4′59″ E). Botanical identification was done by Mr. S. A. Odewo at the Herbarium, Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State, Nigeria. The leaves of *I. coccinea* were dried in the laboratory over a period of seven days and then pulverized using electric blender and stored in polyethylene bags until ready for use.

2.2 Isolation of Essential Oil

A sample (300 g) of *I. coccinea* leaves was subjected to hydrodistillation thrice in an all-glass Clevenger-type apparatus according to British Pharmacopoeia [17] for 4 h until no additional oil was observed to be distilled. The essential oil was dried over anhydrous sodium sulfate to eliminate traces of water, and stored in a sealed vial under refrigeration (4 °C) prior to analysis.

2.3Gas Chromatographic: Mass Spectral Analysis

The leaf essential oil from *I. coccinea* was subjected to gas chromatographic-mass spectral (GC-MS) analysis as previously reported [18]: Shimadzu GCMS-QP2010 Ultra, electron impact (EI) mode with electron energy = 70 eV, scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and Shimadzu GC-MS solution software v. 4.45 (Shimadzu Scientific Instruments, Columbia, MD, USA); ZB-5ms fused silica capillary GC column (Phenomenex, Torrance, CA, USA); (5% phenyl)-polymethylsiloxane stationary phase, 0.25 µm film thickness; helium carrier gas, column head pressure = 552 kPa, flow rate = 1.37 mL/min; injector temperature = 260 °C, ion source temperature = 260 °C; GC oven temperature program: initial temperature = 50 °C, temperature increased 2 °C/min to 260 °C. A 5% *w*/*v* solution in CH_2Cl_2 was prepared, 0.1 µL was injected using a split ratio of 30:1. Identification of the individual components of the essential oil was prepared by injection of pure samples and determined by comparison of the retention indices, determined using a series of *n*-alkanes, in addition to comparison of the mass spectral fragmentation patterns with those found in the MS databases [19-23] using the LabSolutions GCMS solution software version 4.45 (Shimadzu Scientific Instruments, Columbia, MD, USA) and with matching factors > 80%. Quantification was done by external standard method. Calibration curves of representative compounds from each class were drawn and used for quantification.

2.4 Methanolic Extract for Phytochemical Screening

100g of the pulverized plant of *I. coccinea* was weighed and macerated in sufficient amount of 70% methanol for 72 hours. The infusion obtained was filtered using muslin cloth, the filtrate was evaporated using rotary evaporator giving a dark down syrup and dried at 40 ℃ in the oven to get a solid concentrate. The concentrate was stored in an air tight sample bottle and stored in a refrigerator until required for analysis.

2.5 Phytochemical Screening

Phytochemical screening of the plant methanol extracts and other powdered specimen were carried out by a standard procedure that was reported earlier by [24-26] with little modification.

2.5.1 Screening of Alkaloids: 5 mg sample of the extract dissolved in 3 ml of acidified ethanol warmed slightly and filtered. Drops of Mayer's reagent and 1 ml of Dragendroff's reagent were added the filtrate and turbidity was observed.

2.5.2 Screening of Flavonoids: 5 ml of dilute ammonia solution were added to aqueous filtrate of the plant extract followed by addition of concentrated H_2S0_4.

2.5.3 Screening of Cardiac Glycoside (Keller Killani test): 5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was followed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.5.4 Screening of Saponins: 2g of the sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observe the formation of emulsion.

2.5.5. Screening of Tannins*:* 0.5g of the sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

2.5.6 Screening of Phenols: Ferric Chloride Test; 5ml extracts were added with few drop of ferric chloride solution. Formation of bluish black color indicate the presence of phenol.

Determination of total contents of: tannins, saponin, alkaloids, flavonoids, cardiac glycosides and phenols respectively were also carried using standard methods with little modification [27-30].

2.6 Determination of Alkaloids content

5g of the sample, 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. It was filtered and the filtrate was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the filtrate until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

2.7 Determination of Flavonoids content

1 ml of sample solution (100µg/ ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol.

2.8 Determination of Cardiac Glycoside content

1 ml of test extract solution was transferred into 10ml volumetric flasks, Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5%w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2^0C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

2.9 Determination of Saponins content

20g of the sample, 100 mL of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90 °C. The concentrate was transferred into a 250 mL separation funnel and 20 mL of diethyl ether was added and shaked vigorously. The aqueous layer was

recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath for evaporation and were further dried in the oven to a constant weight; the saponin content was calculated.

2.10 Determination of Tannins content

500 mg sample of the concentrate dissolve in 50 ml of distilled water and shake for one hour. A 5 ml aliquot of the filtrate was mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm within 10 minutes.

2.11 Determination of Phenolic content

0.5g sample of extract dissolve in 50 ml of water. Take 0.5 ml add 0.1 ml of Folin- Ciocalteu reagent (0.5 N) mix and incubate at room temperature for 15 minutes. After this, add 2.5 ml sodium carbonate solution (7.5% w/v) and further incubated for 30 minutes at room temperature.

The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

2.12 Antioxidants Assay

2.12.1 2, 2-Diphenyl-1-Picrylhydryzyl (Dpph) Radical Scavenging Activity Assay

0.1 mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 3ml of isolated compound solution in water at different concentrations (10-80 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm, lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard. The difference in absorbance between test and control was calculated and expressed as percentage scavenging of DPPH radical [31].

DPPH Scavenging effect (%) = $[(A_0 – A_1)/A_0]$ X 100

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample or extract.

2.12.2 Nitric Oxide Scavenging Activity Assay

Nitric oxide was generated from sodium nitroprusside and measured by Griess reagent. Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4) saline was mixed with different concentration (10-80 µg/ml) of isolated compound dissolved in water and incubated at 25 °C for 150 minutes. At interval, sample (1.5 ml) Griess reagent. The absorbance was read at 550 nm and ascorbic acid was used as standard [32].

Nitric oxide (%) = $[(A_0 – A_1)/A_0]$ X 100

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample or extract.

2.13 Antidiabetics Assay

2.13.1 Alpha Amylase Inhibitory Assay

α-amylase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification [33]. In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100mM, pH=6.8), 10µl α-amylase (2µ/ml), and 20 µl of varying concentrations of extract and fractions (20, 40, 60, 80 and 100 µg/ml) was pre-incubated at 37 ˚C for 20 minutes. Then, the 20 µl of 1% soluble starch in 100mM phosphate (buffer pH 6.8) was added as a substrate and incubated further at 37 ˚C for 30 min, 100 µl of the dinitrisalicyclic acid (DNS) colour reagent was added, incubated in boiling water bath for 10 minutes and then cooled to room temperature. After cooling, the reaction mixture was diluted with distilled water. The absorbance of the resulting mixture was measured at 540 nm. Acarbose at several concentrations (20 µg/ml-100 µg/ml) was used as standard. The standard was set up in parallel as control and each experiment was carried out in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula.

(% inhibition) = $[(A_0 – A_1)/A_0]$ X 100

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample or extract.

2.13.2 Alpha Glucosidase Inhibitory Assay

Assessment of the α-glucosidase inhibitory activity was slightly modified from the method established in the literature. Various concentrations of extract (5 µL) were added to phosphate buffer (140 µL, pH 6.8), followed by α-glucosidase (10 µL, 0.3 µ/mL). After incubation at 37 °C for 10 minutes, 2 mM p-nitrophenylpyranoside (PNPG, 45 µL) was added. Then, 0.1 M Na_2CO_3 (50 µL) was added, and, after 30 minutes of reaction at 37 °C, the absorbance was recorded at 410 nm. Background absorbance was determined using a non-enzyme control microplate containing the phosphate buffer (140 µL/well) and was subtracted from the absorbance of samples and controls. Acarbose was used as a standard reference [34].

(% inhibition) = $[(A_0 – A_1)/A_0]$ X 100

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample

2.14 Statistical Analysis

The results of triplicate experiments were expressed in terms of ± standard error of mean (SEM) and the IC_{50} values were calculated using Microsoft Excel software.

3. Results and Discussion

3.1 Chemical composition: A light-yellow essential oil was obtained in 0.55% yield. A total of 62 compounds were identified in the essential oil (Table 1). The essential oil was predominantly 46.75% oxygenated sesquiterpene with selina-1, 3, 7 (11)-trien-8-one (27.6%) and selina-1, 3, 7 (11)-trien-8-one epoxide (12.7%) as the dominant components. Sesquiterpene hydrocarbons (34.8%) also made up a large percentage of the composition with (*E*)-caryophyllene (7.6%),β-selinene(4.7%), and germacrene B (4.5%). A Previous report{35] on chemical constituents of *I. coccinea* revealed hydrocarbons with decane (11.2%), alcohols (28.86%) with linalool (10.54%), carboxylic acids (10.96%) dominanted by malonic acid (10.26%) and sesquiterpenoids made up 6.84% of the oil dominated by 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (3.07%). However, present study showed oxygenated sesquiterpene with selina-1, 3, 7 (11)-trien-8-one (27.6%) and selina-1,3,7(11)-trien-8-one epoxide (12.7%) as the dominant components. In contrast, sesquiterpene hydrocarbons (34.8%) reported in this study

were made up of (*E*)-caryophyllene (7.6%), β-selinene(4.7%), and germacrene B (4.5%) as major constituents different from those previously reported. Similarly, triterpenes (62.60%) and monoterpenes (31.73%); with geranyl acetate (8.74%) as the major monoterpenes, followed by linalyl acetate (6.79%), neryl acetate (6.49%), terpineol acetate (4.91%), and borneol acetate (4.77%) had been previously reported [36-37]. but Monoterpene hydrocarbons and oxygenated monoterpenoids occurred in trace amount < 0.01- 0.5% respectively in this present study. Monoterpene hydrocarbons only occurred in trace amount < 0.01% while oxygenated monoterpenoids occur in 0.5%.

Table 1: Chemical composition of the leaf essential oil of *Ixora coccinea*

RI_{calc}	RI_{db}	Compound	%
984	984	6-Methylhept-5-en-2-one	Tr
989	989	2-Pentylfuran	Tr
1024	1024	*p*-Cymene	Tr
1028	1030	Limonene	Tr
1035	1034	(*Z*)-β-Ocimene	Tr
1045	1045	(*E*)-β-Ocimene	Tr
1069	1069	*cis*-Linalool oxide (furanoid)	Tr
1084	1086	Terpinolene	Tr
1085	1086	*trans*-Linalool oxide (furanoid)	Tr
1099	1101	Linalool	0.4
1103	1104	Hotrienol	Tr
1105	1104	Nonanal	Tr
1191	1190	Methyl salicylate	0.1
1194	1195	α-Terpineol	0.1
1333	1335	δ-Elemene	0.1
1345	1346	α-Cubebene	0.1
1367	1370	α-Ylangene	0.1
1373	1375	α-Copaene	0.2
1380	1383	*cis*-β-Elemene	0.1
1381	1382	β-Bourbonene	0.1
1388	1390	*trans*-β-Elemene	2.3
1391	1394	Sative ne	0.4
1404	1406	α-Gurjunene	0.3
1418	1417	(*E*)-β-Caryophyllene	7.6
1420	1421	(*E*)-α-Ionone	0.2
1428	1427	γ-Elemene	2.2
1436	1438	Aromadendrene	0.6
1443	1446	Myltayl-4(12)-ene	0.1
1446	1447	Geranyl acetone	0.1
1453	1454	α-Humulene	0.6
1457	1458	*allo*-Aromadendrene	0.8
1467	1473	4,5-di-*epi*-Aristolochene	0.2
1471	1475	Selina-4,11-diene	0.7
1473	1478	γ-Muurolene	0.4
1474	1476	γ-Gurjunene	0.1
1477	1479	α-Amorphene	0.3
1487	1487	β-Selinene	4.7
1489	1491	Viridiflorene	0.4
1490	1490	γ-Amorphene	0.2
1494	1497	α-Selinene	4.0
1496	1497	α-Muurolene	0.2
1503	1505	α-Bulnesene	0.2
1511	1512	γ-Cadinene	0.3
1516	1514	δ-Amorphene	1.3
1534	1540	Selina-4(15),7(11)-diene	1.1
1535	1531	(*Z*)-Nerolidol	0.2
1539	1542	Selina-3,7(11)-diene	1.0
1557	1557	Germacrene B	4.5
1577	1578	Spathulenol	9.2
1580	1587	Caryophyllene oxide	3.8
1583	1582	*epi*-Globulol	0.3
1596	1596	*trans*-β-Elemenone	0.3
1607	1613	Humulene epoxide II	0.2
1629	1633	Selina-1, 3, 7(11)-trien-8-one	27.6
1632	1629	*iso*-Spathulenol	1.3
1653	1655	α-Cadinol	0.2
1656	1658	Selin-11-en-4α-ol	0.8
1690	1694	Germacrone	2.2
1695	1696	Juniper camphor	0.1

1709	1709	Nootkatol	0.2
1741	1750	Selina-1,3,7 (11)-trien-8-one epoxide	12.7
1840	1841	Phytone	0.3
		Monoterpene hydrocarbons	Traces
		Oxygenated monoterpenoids	0.5
		Sesquiterpene hydrocarbons	34.8
		Oxygenated sesquiterpenoids	59.3
		Benzenoid aromatics	0.1
		Others	0.6
		Total identified	95.2

RT = Retention time (min). RI_{calc}= Calculated retention index values. RI_{db}= Retention index values from the databases. t = trace (< 0.05%).

3.2 Qualitative Phytochemical Analysis

The results of the phytochemical screening of the methanol extract from *I. coccinea* leaves indicated that alkaloids, flavonoids, cardiac glycoside, saponins, tannins and phenol were present (Table 2).

Table 2: Phytochemicals of extract from *Ixora Coccinea*

Plant Extract	Alkaloids	Flavonoids	Cardiac glycosides	Saponin	Tannins	phenol
I. coccinea	++	++	++	++	++	++

++: Present; -: Absent;

3.3 Quantitative Phytochemical Analysis

Quantitative assessment of phytochemical constituents is summarized in Table 3. Saponin > phenolics > alkaloids > cardiac glycosides > tannins > flavonoids.

Table 3: Quantification of phytochemicals of extract from *Ixora Coccinea.*

Plant Extract (mg/g)	Alkaloids	Flavonoids	Cardiac glycosides	Saponin	Tannins	Phenol
I. coccinea	28 ±0.01	14 ±0.03	23 ±0.02	32 ±0.04	22 ±0.01	30 ±0.01

The values are presented as ±SEM for triplicate determination.

3.4 Antioxidant Assay

Figure 1 and 2: displayed the in-vitro antioxidant potential of methanol extracts from *I. coccinea* leaves. The percentage inhibition graph for DPPH radical scavenging activity and Nitric oxide radical scavenging activity were concentration dependent with lowest inhibition at 25 µg/mL and highest inhibition at 100 µg/mL. The standard (ascorbic acid) showed a better activity compared to the methanol extract at all tested concentrations.

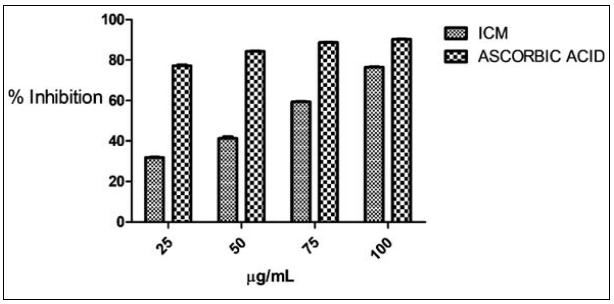

Fig 1: 2, 2-diphenyl-1-picylhydrazyl (DPPH) radical scavenging effect of *I. coccinea* methanol leaf extract (ICM). The values are expressed as mean value ± Standard error of mean of triplicate (SEM).

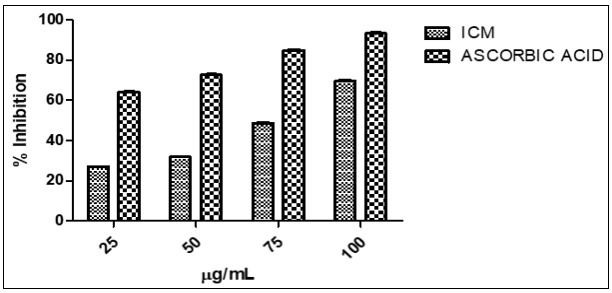

Fig 2: Nitric Oxide (NO) radical scavenging effect of *I. coccinea* methanol leaf extract. Values are expressedas a mean ± SEM.

Table 4: IC_{50} of *I. coccinea* methanol leaf extract for antioxidant activities each value of IC_{50} were obtained by linear regression equation and expressed as means ± SEM

Assays	I. *Coccinea* IC_{50}, µg/mL	Ascorbic acid IC_{50}, µg/mL
DPPH	71.88 ± 0.01	7.77 ±0.02
Nitric Oxide	58.83 ± 0.04	10.52 ± 0.04

The IC_{50} of DPPH and NO radical scavenging potential of methanol extract of *I. coccinea* are displayed in table 4. The methanol extract for DPPH exhibited higher IC_{50} value (71.88 µg/mL) compared to that of the standard (ascorbic acid) which the value is (7.77 µg/mL). The results of NO revealed that the IC_{50} of the ascorbic acid to be lower than that of methanol extract.

3.5 Antidiabetic Potential: Figures 3 and 4 showed the percentage inhibition of α-amylase and glucosidase by the methanol extract of *I. coccinea*. The graph clearly shows a dose-dependent trend with the lowest inhibition at 20 µg/mL and highest at 100 µg/mL. The standard (acarbose) displayed higher inhibitory potency at all tested concentrations.

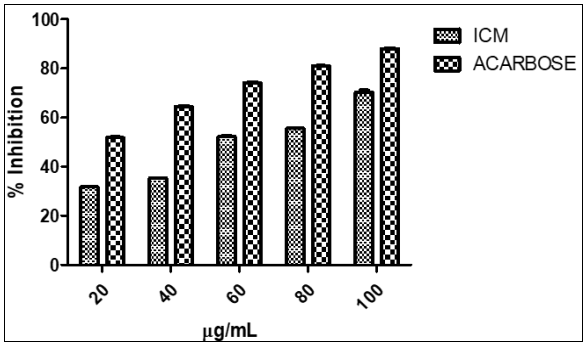

Fig 3: α-Amylase inhibitory effect of *I.coccinea* methanol leaf extract (ICM). Values are expressed as means ± SEM of triplicate determinations.

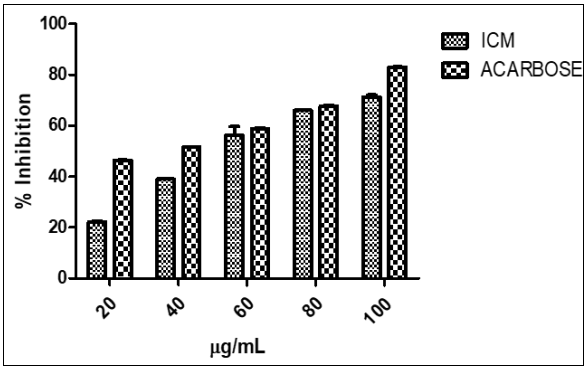

Fig 4: α-Glucosidase inhibitory effect of *I.coccinea* methanol leaf extract (ICM). Values are expressed as means ± SEM of triplicate determinations

Table 5: The median inhibitory concentrations (IC_{50}) of *I. coccinea* methanol leaf extract (ICM) against α-amylase and α-glucosidase.

Assays	*I. Coccinea* IC_{50}, µg/mL	Ascorbic acid, IC_{50}, µg/mL
α-Amylase	61.68 ±0.04	10.72 ±0.04
α-glucosidase	59.31 ±0.03	34.02 ±0.03

The methanol leaf extract displayed higher IC_{50} for α-amylase compared to the standard (acarbose).

It has been widely observed and accepted that the medicinal value of plants lies in the phytochemical constituents. Phytochemical screening of the methanol leaf extract of *I. coccinea* revealed the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and phenolics, while the quantification determination is in order of saponins> phenolics> alkaloids> cardiac glycosides> tannins> flavonoids. Saponin compounds have been observed to show antiprotozoal, molluscicidal activities. Also it exhibited antioxidants, to impair the digestion of protein and uptake of vitamins and minerals in the gut, to cause hypoglycemia and to act as antifungal and antiviral agnts [38-39]. Phenolic compounds and flavonoids are very important in displaying antioxidant activity because of their ability to scavenging of free radicals of hydroxyl groups [40-44]. It is suggested that polyphenolic compounds with different biological and enthnomedicinal activities of *I. coccinea* might make a potential contribution to the antioxidant and antidiabetic activity of this methanolic extract of *I. coccinea*.

4. Conclusion

The present study revealed that *I. coccinea* contain bioactive constituents with promising antioxidant and antidiabetic activities. It also showed that the methanol leaf extract of *I. coccinea* contains phytochemical that could serve as antioxidative agents and inhibitors of α-glucosidase and α-amylase, in glucose utilization and metabolism and serve as future lead drugs in combating diabetics and oxidative stress related diseases. However further studies would be carried out to investigate the mechanism by which the essential oil of *I. coccinea* could normalize the blood glucose levels and its potential efficacy on mammal.

Ethical Approval

Ethical Approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Data Availability Statement

Data from this investigation are available in the manuscript.

References

1. Sunitha D, Hemalatha K, Bhagavanraju M. Phytochemical and Pharmacological Profile Of *Ixora*: A Review, International Journal of Pharmaceutical Sciences and Research. 2015;6(2):567-584.
2. Neelamegam R. Allelopathic effect of *Ixora coccinea* Linn on seed germination and early seedling growth of paddy (*Oryza sativa* L.). J Phytol. 2011;3(6):51-55.
3. Varier VPS. Indian Medicinal Plants, a compendium of 500 species, University press Pvt. Ltd, Hyderabad. 2010, 239.
4. Saha MR, Alam MA, Akter R, Jahangir R. *In vitro* free radical scavenging activity of *Ixora coccinea* L. Bangladesh Journal of Pharmacology. 2008;3:90–96.
5. Annapurna J, Amarnath PVS, Kumar DA, Rmakrishna SV, Raghavan KV. Antimicrobial activity of *Ixora coccinea* L. leaves. Fitoterapia. 2003;74:291-293.
6. Latha PG, Panikkar, KR. Chemoprotective effect of *Ixora coccinea* L. flowers on cisplatin induced toxicity in mice. Phytotherapy Research 2001;15:364-366.
7. Ratnasooriya WD, Deraniyagala SA, Bathige SDNK, Goonasekara CL, Jayakody JRAC. Antinociceptive action of aqueous extract of the leaves of *Ixora coccinea* L. Acta Biologica Hungarica. 2005;56:21-34.
8. Ratnasooriya WD, Deraniyagala SA, Galhena G, Liyanage SSP, Bathige SDNK, Jayakody IRAC. Anti-inflammatory activity of the aqueous leaf extract of *Ixora coccinea*, Pharmaceutical Biology**.** 2005;43:147–152.
9. Yasmeen MB, Prabhu B, Agashikar NV. Evaluation of the antidiarrheal activity of *Ixora coccinea* L. in rats. Journal of Clinical and Diagnostic Research. 2010;4:3298–3303.
10. Glossary of Indian Medicinal plants with active principles. National Institute of Science communication and Information Resources New Delhi. 1992;1:374.
11. Manjeshwar S, Baliga I, Poruthukaran JK. *Ixora coccinea* Linn: A Review of its Traditional uses, phytochemistry and pharmacology, Chinese Journal of Integrative Medicine. 2012;17(10):1-9.
12. Ragasa CY, Tiu F, Rideout JA. New cycloartenol ester from *Ixora coccinea* L. Natural Product Research. 2004;18:319–323
13. Lee CL, Liao YC, Hwang TL, Wu CC, Chang FR, Wu YC. Ixora peptide I and ixora peptide II, bioactive peptides isolated from *Ixora coccinea* Biorganic& Medicinal Chemistry Letters. 2010;20:7354–7357.
14. Yadava RN. Analysis of the fixed oil from the roots of *Ixora coccinea* Linn. Asian Journal of Chemistry. 1989;1:307–308.
15. Kartha ARS, Menon KN. The isolation and constitution of an acid from the root bark of *Ixora coccinea* L. Proceedings of Mathematical Sciences, 1943, 11–15.
16. Muhammad AV, Ambreen IK, Salman K, Shaheen F, Iftikhar AT. Ixoroid: A New Triterpenoid from the Flowers of *Ixora coccinea*, Natural Product Communications. 2012;7(7):831-834.
17. British Pharmacopoeia HM, Starionery OCE: London, UK 1993, 1.

18. Owolabi MS, Ogundajo AL, Dosoky NS, Setzer WN. Chemical composition and antimicrobial potential of essential oils of leaf and stem bark of *Haematostaphis barteri* Hook. f. (Anacardiaceae). J Essent Oil Bear Plants. 2020;23(3):583-593. doi: 10. 1080/0972060X. 2020. 1787868
19. Adams RP. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry, 4th ed. Allured Publishing 2007;
20. Mondello L FFNSC 3. Shimadzu Scientific Instruments 2016;
21. National Institute of Standards and Technology NIST17 2017;
22. Satyal P. Development of GC-MS Database of Essential Oil Components by the Analysis of Natural Essential Oils and Synthetic Compounds and Discovery of Biologically Active Novel Chemotypes in Essential Oils [Ph.D Thesis]. University of Alabama in Huntsville 2015;
23. Lawson SK, Satyal P, Setzer WN. The wood essential oil of Sassafras albidum. Am. J Essent. Oils Nat Product. 2022;10(1):1-5.
24. Harborne JB. Phytochemical Methods. Chapman and Hall Ltd., London, 1973, 49-188
25. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2nd Edition, Sunshine House, Ibadan, Nigeria, 1993, 134-156.
26. Trease GE, Evans WO. Trease and Evans Pharmacognosy 16th Edition. New York: Sauder Elsevier Limited, A. 2009, 365-650.
27. Szydłowska A, Czerniak C, Dianoczki K, Recseg-Karlovits G, Szłyk E. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. Talanta. 2008;76:899-905.
28. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocols. 2007;2:875-877.
29. Agbor GA, Vinson JA, Donnelly PE. Folin-Ciocalteau reagent for polyphenolic assay. International Journal of Food Science, Nutrition and Dietetics. 2014;3:147-156.
30. Bohm BA, Koupai-Abyazani MR. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium reticulatum* and *V. calycinum* (Ericaceae). Pacific Science. 1994;48:458-463.
31. Sanchez-Moreno LJA & Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. J Sci. Technol. Int. 1998;8:121-137.
32. Mercocci L, Marguire JJ, Droy-Lefaiz MT, Parker L. The nitric oxide scavenging properties of Ginkgo biloba extract EGB 761. Biochem. Biophys. Res. Comm. 1994;201:748-755.
33. Reshma MV, Namitha LK, Sundaresan A, Kiran CR. Total phenol content, antioxidant activities and α-glucosidase inhibition of sesame cake extracts J. Food Biochem. 2012;22:1-9.
34. Shinde J, Taldone T, Barletta M, Kunaparaju N, Kumar VS, Placido J. Alpha-glucosidase inhibitory activity of *Syzygium cumini* Linn.Skeels seed kernelin vitro and in Goto-Kakizaki. GK.rats Carbohydr. Res. 2008;343:1278-128.
35. Okhale SE, Ugbabe GE, Oladosu PO, Ibrahim JA, Egharevba HO, Kunle OF. Chemical constituents and antimicrobial activity of the leaf essential oil of Ixoracoccinea L (Rubiaceae) collected from North Central Nigeria. International Journal of Bioassays. 2018;7(5):5630-5637
36. Sunitha D, Hemalatha K, Bhagavanraju M. Phytochemical and Pharmacological Profile of *Ixora:* a review. Int J Pharm Sci Res. 2015;6(2):567-584.
37. Gloria UO, Gibson UN. Chemical composition of Essential oil of *Ixora coccinea* flower from Port Harcourt, Nigeria. Int J Acad Res. 2011;2:381.
38. Veermuthu D, Muniappan A, Savarimuthu I. Antimicrobial activity of some ethnomedicinal plant used by Paliyar tribe from Tamil Nadu, India. Biomedical Central Complementary and Alternate Medicine. 2006;6(35):95-97.
39. Traore F, Faure R, Ollivier E, Gasquet M, Azas N, Debrauwer L. Structure and antiprotozoal activity of triterpenoid saponins from *Glinusoppositi folius*. Planta Medication. 2000;66:368–371.
40. Rajeshwar Y, Senthil GP, Gupta M, Mazumder UK. Studies on in-vitro antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. European Bulletin Drug Residue. 2005;13(1):31-39.
41. Batubara I, Darusman LK, Mitsunaga T, Rahminiwati M, Djauhari E. Potency of Inndonesian medicinal plants as tyrosinase inhibitor and anti oxidant agent J Biol. Sci. 2010;10:138-144.
42. Mohomed AEH, El-Saye MA, Hegay ME, Helay SE, Esmail AM, Mohamed NS. Chemical constituents and biological activities of *Artemisia herba-alba.* Record of Natural Products. 2010;4:1-25
43. Rahimi R, Nikfat S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed. Pharmacother. 2005;59:365-373.
44. Ogundajo AL, Kazeem MI, Owoyele OA, Ogunmoye AO, Ogunwande IA. Inhibition of α-amylase and α-glucosidase by *Acanthus montanus* Leaf extracts. British Journal of Pharmceutical Research. 2016;9:1–8.