International Journal of Bioassays 7.5 (2018) pp. 5630-5637

Research Article

Open Access
CODEN: IJBNHY
ISSN: 2278-778X
International Journal of Bioassays

Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea L* (Rubiaceae) collected from North Central Nigeria

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Received: 8/16/2018; **Revised:** 8/30/2018; **Accepted:** 9/07/2018
Available online: 12th September 2018

Abstract: Essential oils have found vast applications in many fields including aromatherapy, flavor and fragrance industries. *Ixora coccinea* Linn. is a reputable medicinal plant with long history of use in Nigeria. This study aimed to investigate the chemical constituents and antimicrobial activity of the leaf Essential Oil (EO) of *Ixora coccinea* grown in Nigeria. EO was obtained by hydrodistillation with yield of 0.16% (w/w). Chemical constituents of EO were determined using Gas Chromatography coupled to Mass Spectrometry (GC-MS). The GC-MS analysis identified 43 compounds, representing 94.67% of the oil constituents. The analysis revealed eight classes of compounds including hydrocarbons, alcohols, carboxylic acids, esters, aldehydes, ketones, sesquiterpenoids and triterpenoids. Hydrocarbons accounted for 33.77% with decane (11.12%) as the highest; alcohols comprised 28.86% of the oil with the highest being linalool (10.54%). Esters made up 14.15% of the oil. Carboxylic acid (10.91%) was dominated by malonic acid (10.26%); sesquiterpenoids made up 6.84% of the oil dominated by 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (3.07%). Aldehydes made up 3.36% of EO dominated by heptadecanal (2.30%). Ketones accounted for 1.38% of the oil. The inhibitory effect of EO was evaluated against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, *Candida albicans* and *Mycobacterium* tuberculosis (BCG) using broth microdilution method. The essential oil showed significant antimicrobial effects against the test organisms, with Minimum Inhibitory Concentration (MIC) ranging from 50 to 200 µg/mL. This result showed that EO could serve as adjunct therapy in the treatment of community acquired infections.

Keywords: *Ixora coccinea*, Leaf, Essential oil, Antimicrobial

Introduction

Some medical and aromatic plants produce fragrances and essence that are of immense benefit to man. Humans have exploited several plants for these allures and essence termed "*essential oil*". Essential oils are volatile constituents extracted from various parts of aromatic plants using mechanical expression or hydrodistillation. Essential oils are made up of a large array of chemical constituents that consist essentially of terpenoids and non-terpenoids found in various plant parts [1]. Herbal therapy has gained popularity among physicians and patients [2] as several medicinal and aromatic herbs are loaded with metabolites that have demonstrated several therapeutic effects [3].

Ixora, a genus of flowering plants in the Rubiaceae family consists of tropical evergreen trees that are native to the tropical regions of Asia [4] comprising about 500 different species with its centre of diversity in Tropical Asia [5]. The word "*Ixora*" was coined from a Portuguese version of Iswari, which is the name of the Goddess "*Parvati*" to which Ixora coccinea flowers are offered, while "*coccinea*" is a Latin word that means scarlet coloured [6]. Ixora also grows in subtropical climates in the United States, such as Florida and is distributed widely in Nigeria [7,8]. The common names of *Ixora coccinea* are Rangan, West Indian Jasmine, Kheme Chann tanea, Jarum-jarum, Techi, Pan, Santan, Jungle flame [7] and Jungle of Geranium or vetchi in Ayurveda [4]. The plant *Ixora coccinea* (synonyms included *Ixora grandiflora* Bot and Ixora bandhuca Roxbg) is cultivated as an ornamental plant. It is a multi-branched, glabrous ever green shrub commonly 1-2 m in height, but capable of reaching up to 3.6 m in height; bearing numerous bright scarlet coloured flowers which are in dense, senssile corymbiform cymes. Leaves are coriaceous, from 2 cm to 15 cm in length, oblong, sessile or sub-sessile and obtuse [9]. The plant is distributed preferably in acidic soils and thrives in moist, well drained acidic

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DOI: http://dx.doi.org/10.21746/ijbio.2018.7.5.1

soil with tolerance for shade. Its pharmacological profile include traditional uses as anti-mitotic, hepatoprotective, antimicrobial, chemoprotective, anti-oxidant, anti-nociceptive and anti-inflammatory activities [10]. The roots of *I. coccinea* is used as antiseptic, astringent, stomachic, sedative and also in the management of dysentery, diarrhea and gonorrhea; anorexia, hiccups, sores, cough, fever and chronic ulcers [11,12]. In Indo–china, root decoction is useful in clarifying the urine and also as poultice; fresh stems and leaves are used for eczema, sprains, boils and contusions [6]. Flowers are used in managing leucorrhoea, dysmenorrhea, haemoptysis, dysentery and catarrhal bronchitis [6,13,14]. The leaves have shown antimicrobial [15-17], antinociceptive and anti-inflammatory properties [15,18]. The plant also has antioxidant properties, anthelmintic activities, antileishmanial activity, anti-asthmatic activity, anti-diarrhoeal activity, hypoglycaemic and hypolipidaemic activity, hepatoprotective activity, wound healing activity, cytotoxic and antitumour activity, cardioprotective activity, anti-ulcer activity, neuroprotective activity and anxiolytic activity [15,17-35].

The leaves of *Ixora coccinea* yielded kaemferol, flavonoids, quercetin, anthrocyanidins, ferulic acids and other phenolic acids [26]. The flower extract contain flavonoids, triterpenoids and tannins. The flowers are used topically to treat scabies, sores, chronic ulcers and some type of dermatitis [16] and also traditionally to enhance sexuality and the re-kindling of passion [7]. Phytochemical investigation of *I. coccinea* revealed important phytochemicals such as ursolic acid, lupeol, oleanolic acid, rutin, sitosterol, lecocyanadin, anthocyanins, proanthocyanidins, quercetin, and kaempferol glycosides [7] many of which have antimicrobial activities [17].

Phytochemical investigation of the root resulted in the isolation of six phytoconstituents namely β-amyrin, 9,12-octadecadienoic acid, kaempferitrin, kaempferol-7-O-glucoside and quercetin [6,36]. The root of *I. coccinea* also contained palmitic acid, stearic acid, oleic acid, linoleic acid and mannitol [18,37].

Fifty-four components had been identified in the essential oil of *I. coccinea* flower, representing 99.97% of the total components detected [7]. The oil is composed mainly of triterpenes 62.60%, monoterpenes 31.73%, sesquiterpenes 3.35% and an ester 2.29%. The major triterpenes are ursolic acid (27.34%), oleanolic (20.16%) and lupeol (15.10%). Geranyl acetate (8.74%) is the major monoterpenes, followed by linalyl acetate (6.79%), neryl acetate (6.49%), terpineol acetate (4.91%), and borneol acetate (4.77%); ethyl cinnamate (2.29%) an ester while the sesquiterpenes are cyperene (2.72%) and α–copaene (0.63%) [38]. A new triterpene, ixorene with dammarane skeleton has been isolated from the leaves of *I. coccinea*, along with β-sitosterol, lupeol and D-mannitol [7]. Furthermore, the air-dried flowers of *I. coccinea* afforded cycloartenol esters, lupeol fatty ester, lupeol, ursolic acid, oleanolic acid and sitosterol [39].

A new natural terpenoid, ixoroid, was isolated from the flower of *Ixora coccinea* along with the known constituents stigmast-5-en-3-O-β-D-glucoside, 5-O-caffeoylquinic acid and D-mannitol [40]. In addition, ixorapeptide I and ixorapeptide II [41], as well as compounds like biochin A, myricetin, quercetin, rutin, diadzein and formononetin from the methanolic flower extract had been reported [42]. The leaves of *Ixora coccinea* had been reported to contain Ixora tannin A-2 (a trimeric A-type proanthocyanidin), procyanidin A2, cinnamtannin B-1 [43,44].

The increasing prevalence of multi-drug drugs resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to clinically used antibiotics has led to the quest for new antimicrobial agent from plant sources to overcome this challenge. *Ixora coccinea* L is a reputable medicinal plant in North Central Nigeria, where it is used for the treatment of infectious diseases, among other ailments. There is no reported work on the chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* from North Central Nigeria. The aimed of this study was to investigate the chemical composition and antimicrobial activity of the leaf essential oil of *Ixora coccinea* collected from North Central Nigeria.

Materials and Methods

Plant materials and volatile oil Collection

The plant *Ixora coccinea* was collected in July at the NIPRD Garden, Abuja, Nigeria. The plant was identified by a taxonomist at the herbarium of the National Institute of Pharmaceutical Research Development, Abuja, Nigeria, where a voucher specimen (NIPRD/H/5229) was deposited.

Fresh leaves of *Ixora coccinea* (500 g) were chopped into small pieces and the material was then hydrodistilled for 4 h using Clavenger type apparatus. Light-yellow coloured oil in yield of 0.16% (w/w) was obtained. The volatile oil obtained was dried over anhydrous sodium sulphate and stored at 4°C in sealed vials until analysis.

Gas Chromatography–Mass Spectrometry (GC-MS) analyses

The essential oil was analyzed by GC-MS using

Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector [MSD, operated in the EI mode (electron energy=70 eV), scan range of 45-400 amu, and scan rate of 3.99 scans/sec], and Shimadzu GC-MS solution data system. The Gas chromatography column was Optima-5 ms fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for Gas chromatography oven temperature was 60-180°C at a rate of 10°C/min, then held at 180°C for 2 min, followed by 18-280°C at a rate of 15°C/min, then again held at 280°C for 4 min. The injection port temperature was 250°C while detector temperature was 280°C. Helium was used as a carrier gas, at a flow rate 1.61 mL/min. Diluted sample (1/100 in hexane, v/v) of 1.0 µL was injected using autosampler and in the split mode with ratio of 10:90. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentages of each component are reported as raw percentages based on the total ion current without standardization. The essential oil constituents of *Ixora coccinea* leaf is as detailed in Table 1.

Table 1: Percentage of compounds in the volatile oil of *Ixora coccinea* leaf.

SN	Name of compound	Classification	RT (min)	% Composition
1	Cyclohexanepropanol	Alcohol	3.286	4.67
2	2-methylpropyl cyclohexane	Cyclic hydrocarbon	3.495	1.20
3	Malonic acid, 2-heptyl tetradecyl ester	Carboxylic acid	3.722	10.26
4	Mesitylene	Aromatic hydrocarbon	4.116	6.13
5	Decane	Saturated hydrocarbon	4.192	11.12
6	1,3,3-trimethylnonyl benzene	Aromatic hydrocarbon	4.493	3.16
7	Linalool	Alcohol	5.495	10.54
8	Methyl salicylate	Ester	6.827	3.40
9	Geraniol	Terpene alcohol	7.617	2.42
10	Ionone	Ketone	8.045	0.02
11	Citronellyl acetate	Ester	8.931	0.52
12	β-Damascenone	Ketone	9.411	0.41
13	Tetradecane	Saturated hydrocarbon	9.603	1.41
14	5,9-Undecadien-2-one,6,10-dimethyl-	Ketone	10.218	0.95
15	1-Dodecanol	Alcohol	10.503	1.67
16	β-Selinene	Aromatic hydrocarbon	10.837	2.30
17	α-Selinene	Sesquiterpenoid	10.945	1.60
18	(-)-Spathulenol	Sesquiterpenoid alcohol	11.320	0.63
19	Nerolidol	Sesquiterpene	11.631	3.07
20	cis-3-Hexenyl benzoate	Ester	11.709	1.89
21	Supraene	Triterpenoid	11.807	0.94
22	Hexadecane	Saturated hydrocarbon	12.055	1.42
23	Tetradecanal	Aldehyde	12.113	0.12
24	1-Hexadecanol	Alcohol	13.004	1.50
25	Heptadecanal	Aldehyde	13.554	2.30
26	Hexadecane	Saturated hydrocarbon	14.937	0.79
27	Isopropyl myristate	Ester	15.233	0.59
28	6,10,14-Trimethyl-2-pentadecanone	Sesquiterpenoid	15.488	2.17
29	Benzyl salicylate	Ester	15.801	1.11
30	1-Hexadecanol	Alcohol	15.976	0.29
31	cis,cis,cis-7,10,13-Hexadecatrienal	Aldehyde	16.041	0.94
32	Hexadecanoic acid, methyl ester	Ester	16.312	0.41
33	n-Hexadecanoic acid	Carboxylic acid	16.715	0.65
34	n-Nonadecanol	Alcohol	17.070	4.84
35	2-Methylhexacosane	Acyclic hydrocarbon	17.134	0.84
36	Isopropyl palmitate	Ester	17.324	1.12
37	Geranylgeranylacetate	Ester	17.479	0.13
38	Methyl-6-octadecenoate	Ester	17.908	0.68
39	Phytol	Alcohol	18.036	0.31
40	Nonadecene	Unsaturated hydrocarbon	18.666	1.46
41	Tetracosane	Saturated hydrocarbon	19.324	0.95
42	1,54-Dibromotetrapentaconntane	Hydrocarbon	21.748	2.78
43	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl]-cyclohexanol	Alcohol	22.751	1.99
	Total			94.67%

RT=Retention time.

Microbial strains

The following microorganisms were used in the evaluation of the antibacterial activity of the essential oil: Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 13883), *Escherichia coli* (ATCC 10798), and fungi *Candida albicans* (ATCC 2876).

Antimicrobial activity

The Minimum Inhibitory Concentration (MIC) values of the essential oil of *Ixora coccinea* leaf were determined in triplicate by the broth microdilution method in 96-well microplates. The oil sample was dissolved in Dimethyl Sulfoxide (DMSO) followed by addition of sterile Mueller-Hinton broth for bacteria and Saboraud-Dextrose broth for *Candida albicans*, to achieve concentration of 200 µg/mL. The final DMSO concentration was 10% (v/v) and this solution was used as a negative control. The inoculum was adjusted for each organism to yield a cell concentration of 2×10^{-7} colony forming units (cfu) per mL. Ciprofloxacin (Fidson, Lagos Nigeria) was used as a positive control for bacteria and Fluconazole (Pfizer, UK) was used as the standard drug for fungi at stock concentration of 50 µg/mL. Controls of sterility for the Mueller-Hinton nutrient broth, control culture (inoculum), ciprofloxacin, fluconazole, essential oil and DMSO were performed. The microplates were closed and incubated aerobically at 37°C for 24 h. The MIC values were determined as the lowest concentration of essential oil capable of inhibiting the growth of the microorganisms. All assays were carried out in triplicate. Results are shown in Table 2.

Results and Discussion

Plants contain a vast array of secondary metabolites with diverse pharmacological activities [45,46]. Among these secondary metabolites are essential oils. The application of essential oils in aromatherapy for treating different ailments has been reported [47-49]. The antimicrobial activities of essential oils from plants had been reported [50 - 53] and *Ixora coccinea* leaf extracts had been reported to have antimicrobial properties [54]. This study focused on evaluating the chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* grown in Nigeria.

The essential oil isolated from the leaves have characteristic odour and a light yellow colour. Further analysis using GC-MS resulted in the identification of 43 compounds as shown in (Table 1) representing 94.67% of the total essential oil constituents. Essential oil formation is affected by seasonal variation, climatic conditions such as temperature, sunlight, frequency and magnitude of precipitation and time of harvesting. The leaf volatile oil composed of several classes of chemicals such as alcohols (also consisting monoterpene and sesquiterpene alcohols) hydrocarbons; carboxylic acids, esters, aldehydes, ketones, sesquiterpenoids and triterpenoids. The leaf oil was highest in hydrocarbons (cyclic, aromatic, saturated and unsaturated hydrocarbons), amounting to 33.77% of the total constituents such as decane (11.12%), mesitylene (6.13%), cyclohexane (1.20%), benzene (3.16%), tetradecane (1.41%), naphthalene (2.30%); hexadecane (2.21%), 2-methylhexacosane (0.84%), nonadecane (1.46%), tetracosane (0.95%) and tetrapentacontane,1,54-dibromo (2.99%)). It has been reported that steam-distillation and hydro-distillation methods yield oils rich in terpene hydrocarbons, while in contrast, the super-critical extracted oils contained a higher percentage of oxygenated compounds [55–58] which implies that the qualitative and quantitative chemical composition of the essential oil differs according to the technique of extraction applied.

The second largest total constituent was alcohol (28.86%) comprising of linalool (10.54%), cyclohexanepropanol (4.67%), geraniol (0.74%), geraniol, a terpene alcohol (2.42%), dodecanol (1.67%), sputhulenol (0.63%), hexadecanol (1.79%), n-nonadecanol (4.84%), phytol (0.31%) and 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl]-cyclohexanol (1.99%). The leaf oil had esters of fatty acid and carboxylic acid

Table 2: Inhibitory effects on the growth of bacteria (MIC values µg/mL) of the volatile oil from the leaf of *Ixora coccinea.*

S/N	Micro-organisms	Minimum inhibitory concentrations (MIC values µg/mL)	
		Essential oil	Standard drug
1*	*Pseudomonas aeruginosa* (ATCC 27853)	100	0.05
2*	*Klebsiella pneumonia* (ATCC 13883)	50	0.39
3*	*Escherichia coli* (ATCC 10798)	100	0.39
4*	*Staphylococcus aureus* (ATCC 25923)	200	0.10
5**	*Candida albicans* (ATCC No. 2876)	100	6.25
7***	*Mycobacterium bovis* BCG (ATCC 27290)	200	0.04

**Bacterial strain; **fungal strain; ***mycobacterium strain. Ciprofloxacin (standard drug for bacterial strains); fluconazole (standard drug for fungal strain); isoniazid (standard drug for mycobacterium strain)*

which made up 14.15% of the total components of the volatile oil such as methylsalicylate (3.40%), citronellyl acetate (0.52%), 3-hexenyl benzoate (1.89%), isopropl myristate (0.59%), benzyl salicylate (1.11%), methyl hexadecanoate (0.41%), isopropyl palmitate (1.12%); geranylgeranylacetate (0.13%), methyl-6-octadecenoate (0.68%). Aldehydes made up 3.36% of the total oil constituents and included tetradecanal (0.125%), heptadecanal (2.30%) and 7,10,13-hexadecatrienal (0.94%). The percentage content of sesquiterpenoids was 6.84% of the total volatile oil components and comprised of α-selinene (1.60%); 3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (3.07%) and 6,10,14-trimethyl-2-pentadecanone (2.17%). Ketones accounted for 1.38% of the leaf volatile oil constituents and comprised ionone (0.02%); 6,10-dimethyl-5,9-undecadien-2-one (0.95%) and β-damascenone. Triterpenoids made up 0.94% of the total essential oil constituents and comprised of supraene (0.94%); carboxylic acids were malonic acid (10.26%) and hexadecanoic acid (0.65%).

Sesquiterpenes are known to delete bad information from the cell's memory and is useful as fixative in perfume industry while monoterpenes inhibits the accumulation of toxin in the body [59]. Methylsalicylate is used as fragrance in beverages and food products [60,61] and also used as an antimicrobial agent [61]. The antibacterial activity of some monoterpenes, diterpenoids, sesquiterpenes, triterpenoids and their derivatives had been reported [62]. Diterpenoids and sesquiterpenes isolated from different plants inhibited the growth of *Mycobacterium tuberculosis* [63, 64] and exhibited bactericidal activity against Gram-positive bacteria. The mechanism of action of terpenoids is not fully understood, but speculated to involve membrane disruption by the lipophilic compounds. Sesquiterpenoids have been reported to have antimicrobial properties [65]; and alcohols such as phytol have antibacterial activity against *Pseudomonas aeruginosa* [66]; geraniol, occurring in the essential oils of several aromatic plants is most important molecules in the flavour and fragrance industries because of its pleasant odour, geraniol is also known to have antimicrobial activities [67]. The preserving quality of benzoic acid is due to its ability to delay the multiplication of several groups of microorganisms making its action bacteriostatic [68]. Most of the antimicrobial activities of essential oils were attributable to the oxygenated terpenoids, while some hydrocarbons also exhibited antimicrobial effects [69–71]. Interactions between the different components may lead to additive, antagonistic or synergistic effects.

The susceptibility of some micro-organisms were evaluated against the essential oil obtained from the leaves of *Ixora coccinea* (Table 2). The MIC values were determined as the lowest concentration of the essential oil capable of inhibiting the microbial growth. *Klebsiella pneumonia* had Minimum Inhibitory Concentration (MIC) of 50 µg/mL); *Pseudomonas aeruginosa* with MIC of 100 µg/mL; *Escherichia coli* with MIC of 100 µg/mL; *Staphylococcus aureus* with MIC of 200 µg/mL; *Candida albicans* with MIC of 100 µg/mL and *Mycobacterium tuberculosis* (BCG) with MIC of 200 µg/mL. The test pathogens are contaminants in food, man as well as animals and air [72]. The essential oil of *Ixora coccinea* was more active against *Klebsiella pneumonia* with MIC of 50 µg/mL. The antibacterial activity exhibited by *Ixora coccinea* leaf essential oil supports its use in traditional medicine for treatment of infectious diseases.

Conclusion

Essential oils are natural plant products containing complex mixture of components and thus have multiple antimicrobial properties with the interactions between these components resulting in antagonism, additive or synergistic effects as observed in the leaf essential oil of *Ixora coccinea*. The antibacterial activity exhibited by the essential oil suggested that it can be exploited in herbal medicine for the management of respiratory and gastrointestinal diseases.

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Cite this article as:

Okhale SE, Ugbabe GE, Oladosu PO, Ibra-him JA, Egharevba HO, et al. Chemical constituents and antimicrobial activity of the leaf essential oil of Ixora coccinea L (Rubiaceae) collected from North Central Nigeria. *International Journal of Bioassays 7.5 (2018) pp. 5630-5637*. DOI: http://dx.doi.org/10.21746/ijbio.2018.7.1.1