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Evaluation of the antimicrobial and larvicidal activities of n-hexane root extract of *Ixoria coccinea* L. (Rubiaceae)

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Abstract

The antimicrobial as well as the larvicidal activities of n-hexane root extract of *Ixoria coccinea* against *Aedes aegypti* was evaluated in this work. The extraction was done using maceration method. Agar well diffusion method was used to assess the antimicrobial activity of the extract against different clinical isolates of gram positive and gram-negative bacteria. The larvicidal activity was evaluated according to World Health Organization (WHO) guideline for larvicidal assay as follow: a stock solution of 10mg/ml was prepared for each extract from which five different concentrations (1.0, 2.0, 3.0, 4.0 and 5mg/ml in 100ml) were made and used for the test. A control was also prepared for each extract. A total of 20 larvae were used for each of the concentration and mortality was recorded at 24, 48 and 72 hours. The result of the antimicrobial assay showed that at 100mg/ml concentration, the extract gave an inhibition zone diameter of 17±1.41mm for *Staphylococcus aureus*, 8±0.82mm for *Bacillus spp,* 2±0.00mm for Stapylococcus epidermidis, 4±0.58mm for *Pseudomonas aeruginosa* and 6±1.29mm for *Escherichia coli.* The extract was most active against *Staphylococcus aureus* with an MIC of 50mg/ml. The extract is not active against all the fungi species used in the test. Examination of the result for larvicidal assay against *Aedes aegypti* larvae showed that the LC50 after 24, 48 and 72 hours are 1.782, 1.376 and 0.686mg/ml respectively. At concentration of 3mg/ml there was 100% mortality after 72 hours. The results showed that the n-hexane root could serve as a source of both antibiotic and mosquito larvicides.

Keywords: Larvicidal; Antimicrobial; *Lxoria coccinea*; N-hexane Extract

1. Introduction

Plants have long been recognised as important natural product sources for preserving both human and animal health. There have been reports suggesting a wide variety of chemical compounds found in plants have significant therapeutic and preventative properties [1]. Approximately 80% of people in developing nations utilise traditional medicines made of substances derived from medicinal plants [2]. Despite the availability of several drug development methods, plants remain the main source of natural medicines. Because of antibiotic resistance, there is renewed interest in plants that possess antibacterial characteristics. This resistance may be linked to the careless use of commercial medications or to not following prescription guidelines when using antibiotics, such as not finishing the entire course of therapy for infectious infections [3]. Furthermore, some antibiotics have unfavourable side effects such nausea, bone marrow suppression, thrombocytopenic purpura, and agranulocytosis, which might cause rare disorders to arise [4]. This has encouraged scientists to look for more innovative and different microbial compounds from therapeutic plants [3].

As a result of the ongoing use of artificial pesticides to control the *Aedes aegypti* vector, mosquito populations have become naturally resistant to various chemical groups.

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As one of the leading causes of illness and mortality around the world, the etiological agents of human viral infections spread by mosquito vectors are of significant public health importance. *Aedes aegypti* is considered a significant arbovirus carrier among mosquito vectors [5]. This vector is capable of transmitting the pathogens that cause diseases such as dengue, Zika, chikungunya, and yellow fever. Chemical insecticides like carbamates, pyrethroids, organochlorines, organophosphates, and synthetic larvicides are used extensively to control these mosquitoes. In addition to harming the ecosystem, the ongoing use of these artificial chemicals has led to the rise of resistant populations of pathogens and insects [6,7].

The mosquito, *Aedes aegypti*, is found throughout America and tropical countries, with the exception of Canada and Chile. Additionally, it is the vector for a number of human-transmitted illnesses, such as dengue, malaria, and, more recently, zika and chikungunya. In Ecuador, vector-borne illnesses rank second in terms of morbidity, with dengue and chikungunya leading the pack.

The flowering plant *Ixora coccinea*, often called jungle geranium, flame of the woods, jungle flame, or pendkuli, belongs to the Rubiaceae family. This ubiquitous flowering shrub is indigenous to Bangladesh, Sri Lanka, and Southern India. It is now one of the most widely used blooming shrubs in landscapes and gardens in South Florida [8]. Ayurveda uses this commercially significant medicinal herb. Every portion, including the flower, leaves, and root, is used to make different therapeutic remedies for conditions like diabetes and skin diseases.

The objective of this work is to evaluate the antimicrobial activity of the n-hexane root extract of *Ixora coccinea* using standard methods. The larvicidal activity of the same extract will also be evaluated against *Aedes Aegypti* larvae by standard guideline of larvicidal assay

2. Material and methods

Rotary evaporator (Labscience, England), a water bath (Techmel and Techmel, USA), an analytical weighing scale, glass funnels, beakers, crucibles, a measuring cylinder, a desiccator, acetone, water, and DMSO solvent etc. are a few of the materials utilized in this work.

2.1. Plant collection

Ixora coccinea Linn. (Rubiaceae) roots were collected from Rivers state, Nigeria on the month of July. it was identified by Dr. Sulieman Mikailu of Department of Pharmacognosy and Phytotherapy, University of Port Harcourt and deposited at the same Department Herbarium, with the herbarium voucher number UPHRO615.

2.2. Plant Preparation

The collected plant roots were cut into smaller pieces, subjected to thorough washing, and air-dried under a shade until well dried. Following the drying period, the roots were finely pulverized and securely stored in an airtight container for subsequent utilization.

2.3. Extraction of Plant Materials

The plant material extraction process was done using the maceration method. This was done with the measurement of 200g of the sample, which was subsequently placed into a maceration jar. Sufficient volume of analytical-grade N-Hexane solvent was added to the sample within the jar and covered. Thorough mixing was achieved through shaking. The resulting mixture was left to macerate for a 24-hour duration, with intermittent shaking to facilitate enhanced extraction. Subsequently, the mixture was subjected to filtration. This entire procedure was repeated using the same marc to ensure exhaustive extraction. The resulting extract was concentrated using a rotary evaporator and finally dried in a water bath at a temperature 45 °C.

2.4. Phytochemical Screening

The n-hexane extract of the pulverized plant roots was subjected to phytochemical evaluation in accordance with the method by Harborne [9].

2.5. Antimicrobial Assay

2.5.1. Standardization of Culture

After being aseptically injected into regular saline, the microbes that were isolated were cultured for 24 hours at 37 °C. Next, the 0.5 MaC-Farland turbidity standard was used for comparison.

Antimicrobial Susceptibility Test (AST) of n-hexane extract of *Ixora coccinea* on clinical isolates Using Agar Diffusion Method

Cotton wool and antiseptic were used to clean the worktop. For each test organism, sterile Petri plates were marked in triplicate. The prepared Nutrient Agar pour in the universal bottle was filled with 0.1 ml of each microbe aseptically and well mixed. After that, the mixture was transferred to the appropriate Petri dish and left to harden on the workbench. A sterile cork borer with a 6 mm diameter was used to extract an agar disc from the agar layer once the agar had set on the petri dish. This allowed each agar plate to have a well. The N-hexane stock concentrations (100 mg/ml) of *Ixora coccinea* extract were marked on the wells. A sterile Pasteur's pipette was used to carefully transfer 0.1 ml of each of the prepared stock concentrations into the well. The well was then set on the workbench for 15 minutes to allow for optimal diffusion. For twenty-four hours, the plates (Petri dishes) were incubated at 37 °C. The same procedure was repeated using Sabouraud dextrose agar (SDA) which was inoculated with the fungi and incubated at ambient temperature for 2- 7 days. Using a metre rule, the diameter of the resultant Zones of inhibition was measured in millimetres (mm) via the plate bases and results recorded.

Determination of the Minimum Inhibitory Concentration (MIC)

Cotton wool and antiseptic were used to clean the worktop. For each test organism, sterile Petri plates were marked in triplicate. The agar pours were inoculated with 0.1ml of standardized bacteria culture and mixed well. The inoculated agar was poured into the labelled petri dishes and left to solidify on the workbench. After solidification of the agar, five (5) wells were bored using a sterile cork borer. These wells were labelled to accommodate five (5) concentrations of the extract, the concentrations were 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.125mg/ml. Using a sterile pasteur's pipette, 0.1ml of each concentration of the extract was introduced into the corresponding wells. The plates were left on the workbench 15 minutes for proper diffusion and it was the incubated upside down at 37^{o}C for 24 hours. After incubation the plates were observed for presence of zones of inhibition.

2.6. Larvicidal Bioassay

The *Aedes aegypti* egg for the assay was obtained from the National airborne viral research centre Enugu, Nigeria. It was hatch and the larvae grow in the department of Pharmacognosy and Phytotherapy of University of Port Harcourt. The Larvicidal bioassay was carried using the 3" and 4" instar larvae of *Aedes aegypti* and this was done according to World Health Organization [WHO, 2005] [10] standard method for larvicidal assay. A stock solution of 10mg/ml of the n-hexane root extract was prepared by dissolving 4.5 g of the n-hexane root extract in 450 ml of distilled water, sufficient amount of acetone was used to dissolve the n-hexane root extract before adding the 450 ml of distilled water and properly stirred using a glass rod, From the stock solution that was prepared, several concentrations was prepared in triplicates (1,2,3,4 and 5mg/ml). Each of the concentrations had a control containing 100ml of distilled water, 20 healthy larvae put in each of the plastic plate used to carry out the assay which was run simultaneously at the same conditions at room temperature. Mortality was checked for after 24,48 and 72hours. The dead larvae were confirmed by not respond to stimuli of being probed using a needle. results were carefully observed and recorded

2.7. Statistical Analysis

The statistical tools that were used in this study is the Ldp line software based on the standard method of probits by Finney [11], to calculate LC 50 values of each extract after the various time of observations which are 24, 48 and 72 hours.

3. Results

Table 1 Percentage yield from extraction of *Ixora coccinea* Linn

Weight of plant material (g)	Solvent	Weight of extract (g)	Percentage yield (%)
200	n-hexane	8.75	4.375

Table 2 Result of the Phytochemical screening of *Ixora coccinea* root extract

S/No	Phytochemical	Result
1	Anthraquinone	+
2	Alkaloids	-
3	Flavonoids	+
4	Saponin	+
5	Phenolic compounds	+
6	Carbohydrate	+
7	Triterpenoids	+

Key: (+): - positive, (-): - negative

Table 3 Result of the Antimicrobial Susceptibility Test (AST) of N-Hexane extract of *Ixora coccinea* root

Microorganism	Conc. mg/ml	n-hexane extract mean IZD (mm)	Positive control (gentamicin 2mg/ml) mean IZD (mm)	Negative control DMSO
Gram positive				
Staphylococcus aureus	50 100	8 ± 0.82 17±1.41	4 ± 1.41	Nil
Bacillus spp	50 100	Nil 3 ± 0.82	3 ± 0.00	Nil
Staphylococcus epidermidis	50 100	Nil 2 ± 0.00	6 ± 1.73	Nil
Gram negative				
Pseudomonas aeruginosa	50 100	Nil 4 ± 0.58	3 ± 0.00	Nil
Escherichia coli	50 100	Nil 6 ± 1.29	7 ± 0.82	Nil
Klebsiella spp	50 100	Nil Nil	9 ± 1.00	Nil
Proteus spp	50 100	Nil Nil	8 ± 2.16	Nil
Fungi			Positive control (Fluconazole 5mg/ml)	Negative control DMSO
Candida spp	50 100	Nil Nil	18 ±2.45	Nil
Aspergillus spp	50 100	Nil Nil	Nil	Nil
Penicillium spp	50 100	Nil Nil	Nil	Nil

Values for means are represented as mean of ± S.E.M (standard error of means). Key: IZD =Inhibition zone diameter

From the table 3 above the result of the antimicrobial susceptibility test showed that the extract is very active against *Staphylococcus aureus* moderately active against *Pseudomonas aeruginosa* and *Escherichia coli and* poorly active against *Bacillus spp* and *Staphylococcus Epidermidis*. All the fungi spps are resistant to the extract.

Table 4 Minimum Inhibitory Concentration (MIC) of N-Hexane extract of *Ixora coccinea* root

Organisms	**Concentration**					
	3.125	6.25	12.5	25	50	100
Staphylococcus aureus	+	+	+	+	-	-
Bacillus spp	+	+	+	+	+	-
Staphylococcus epidermidis	+	+	+	+	+	-
Pseudomonas aeruginosa	+	+	+	+	+	-
Escherichia coli	+	+	+	+	+	-

Key: (+) growth, (-) inhibition

From the result of MIC in table 4 above, 100mg/ml is the minimum inhibitory concentration for all the organisms with the exception of *Staphylococcus aureus* which mic of 50mg/ml.

Table 5 Result of larvicidal assay of n-hexane root extract of *Ixora coccinea* against *Aedes aegypti*

Con. Mg/ml	**24 hours**			**48 hours**			**72 hours**		
	% mortality	**LC50**	**Control**	**% mortality**	**LC50**	**Control**	**% mortality**	**LC50**	**Control**
1	18.3±19.3	1.782	0	26.7±20.1	1.376	0	70±4.1	0.686	0
2	61.7±20.9		0	81.7±13.1		0	91.7±8.5		0
3	81.7±8.5		0	86.7±2.4		0	100±0.0		0
4	81.7±4.7		0	90±8.2		0	98.3±2.4		0
5	90±0.0		0	96.7±4.7		0	100±0.0		0

Values for means are represented as mean of ± S.E.M (standard error of means)

Result of the larvicidal assay in the table 5 above showed that the extract gave strong larvicidal action against the *Aedes aegypti* larva with LC50 of 1.78mg/ml after 24 hours and 0.686mg/ml after 72 hours

4. Discussion

Examination of result of percentage yield showed the yield to be 4.375%. the low percentage yield may be due to the solvent of extraction

Phytochemical screening gave the presence of anthraquinone, flavonoids, saponin, phenolic compounds, carbohydrates and triterpenoids. There is absence of alkaloids. This is similar to result obtained by Okwubie and Ajogwu, (2021) [12] which recorded the presence of these compounds with additional presence of alkaloids and cyanogenetic glycosides, while Joshi et al., (2013) [13] reported the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins, resins, saponins, triterpenoids and steroids in the ethanolic root extract. This may be due to different solvents of extractions. One or two of these phytochemicals may be responsible for the larvicidal and the antimicrobial activities.

The result of the antimicrobial activities of the extract showed very good activity against *Staphylococcus aureus* with inhibition zone diameter of 8 ± 0.82 and 17±1.41mm for dose concentration of 50 and 100mg/ml respectively. This gave more activity than the positive control of gentamycin with inhibition zone diameter of 4 ± 1.41mm. Though there were activities against *Bacillus spp, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli* but they were at higher concentration of 100mg/ml as such the extracts has lower antibacterial activities against these organisms when compare with the standard, as shown on the table 3 above. Marimuthu et al., (2014) [14] showed that the methanolic extract of this plant gave good activities against the bacteria used in this study, though the activities tend to

be higher with the methanolic leaf, stem and flower extracts from that study than the hexane root extract used in this study. However, Marimuthu et al (2014) [14] showed that the methanolic extracts gave good activities against fungi while this study showed that the n-hexane root extract gave no activity against fungi. Also, Nagaraj Selvaraj et al., (2011) [15] showed that ethanol extract gave higher antibacterial activity when compare to lesser polar solvents. However, they reported very low antifungal activities with all the solvents used in their work which is closer to the result on the antifungal activity in this work.

Examination of the larvicidal activity showed that the n-hexane extract exhibited a very good larvicidal activity against *Aedes aegypti* larvae. At concentration of 1mg/ml there was 70±4.1 percentage mortality after 72 hours. The percentage mortality increased to 91.7±8.5 after 72 hour at concentration of 2mg/ml, at concentration of 3mg/ml the percentage mortality became 100 percentage after 72 hours. The larvicidal activities of this extract is dose and time dependent. This is collaborated by the work done by Okwubie and John, 2017 [16] and Nwabor et al, 2014 [17] which showed the activity is dose and concentration dependent.

Okwubie and Ajogwu, (2021) [12] had shown that acetone root extract of this plant has very high activity, while extracts with high polar solvent of methanol and water gave low and very low activity respectively. This showed that the larvicidal activity reside with extracts from non-polar solvents or mid polar solvents as the result on the use of non-polar solvent n-hexane in this work showed very good activity. Therefore, the best solvent of extraction for good larvicidal activity is non-polar solvents.

5. Conclusion

This work has shown that n-hexane root extract of *Ixora coccinea* has both antibacterial and larvicidal activity but has no antifungal activity. On the larvicidal activity, further work could be made to isolate and characterize the compound or agent responsible for larvicidal activity.

Compliance with ethical standards

Acknowledgment

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Disclosure of conflict of interest

We the authors do declare that there is no conflict of interest.

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