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Bioactive Chemical Constituents Screening and Inhibitory Activities of Methanol Extract of *Oecophylla Longinoda* (Tailor Ant) Against Some Pathogens

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Abstract

Traditional medicine practice in Benin City, Nigeria involves the use of many natural products originating from medicinal plants, certain animals like ants, snail, mineral ores, mushroom and a host of other fauna and flora species. In this study, the bioactive chemical constituents and antimicrobial potency of Oecophylla longinoda (Tailor ant) extract were investigated. The Tailor ants were collected from their natural habitat in a nest and extracted with methanol in soxhlet extractor apparatus. The ant crude extract was concentrated using a rotary evaporator (Model RE, 200, USA) and bioactive chemical constituents screening was performed using standard methods. The antimicrobial activity was conducted according to agar well diffusion method while alkaloid fraction was obtained from the ant extract using separation by extraction and characterized by infra-red (I.R) analysis. The result indicated that saponins, terpenes, and alkaloid were present among others in the extract. The antimicrobial analysis revealed zones of inhibition at a concentration of 100 mg/mL: 20 mm (Pseudomonas aeruginosa), 27 mm (Staphylococcus aureus), 20 mm (Escherichia coli) while Aspergillus niger and Penicillium notatum both had 27 mm. For the antifungal activity, inhibition against Mucormycetes increased as the concentration decreased but at the highest concentration of 100 mg/mL, there was no activity. All the extract concentration showed a dose dependent activity when compared with the standard control antibiotics (Ciprofloxacin). However, high zones of inhibition were observed with low concentration of the ant extract. The study revealed that Oecophylla longinoda (Tailor ant) extract contains bioactive constituents with high antimicrobial activity.

1. Introduction

Oecophylla longinoda (Tailor ant), sub-family *Formicanae* is an ant taxon frequently found in forested wild vegetation and orchards of mango (*Mangnifera indica*), custard apple (*Annona muricata*), citrus species, cashew (*Anacardium occidentale*), guava (*Psidium guajava*), cocoa (*Theobroma cacao*) and oil palm: *Elaeis guineensis* [1]. The ant extract in water formed the main ingredient used by traditional herbal practitioners in Benin metropolis for the treatment of rheumatoid arthritis, inflammation and muscular pain. The traditional healer applies the water extract of Tailor ant as poultice on the joints or parts of the body inflamed after being cut slightly with razor blade [2]. The believe that insect has healing power has been widespread since ancient

times [1]. For example, the red forest ant, *Formica rufa* has a long tradition of use in remedies in Sweden and neighbouring countries and has been available as oil and acid in the pharmacies at least the seventeenth century [3]. Reports revealed that ant spirit has been used to flavor alcohol [3] for the treatment of rheumatism and back pain [4]. In southern Nigeria ethno medicine, the root bark of walnut tree is mixed with palm kernel oil and some ant extract for the treatment of rheumatoid arthritis, inflammation and bone fracture [2]. *Oecophylla* ants have proven to be highly effective biological control agents being capable of controlling over 50 species of insect pests in at least 12 different tropical crops [5]. More so, the use of the ant for replacement of conventional synthetic insecticide has been established in Australian cashew and mango plantations [6].

Plate 1: Tailor ant

Plate 2: Tailor ant in their nest

Extracts of *Oecophylla longinoda* are a source of traditional Chinese and Indian medicines against arthritis and some other abnormal health conditions [7]. From the work of Oladunmoye [8], the high population of *O. longinoda* inhibited the infection of cocoa pod by bacteria like *Proteus penneri*, *Serratia* and *Citrobacter freundii* and fungi like *Aspergillus aculeatinus, Penicillium chrysogenum*, *Chloridium chlamydosporis* and *Phytophthora palmivora*. Meanwhile the Australian Formicine ants are also found to exhibit bactericidal properties [9]. *Oecophylla longinoda* generally have been found also to contain important vitmins like Vitamin B12, folate and Vitamin C [9]. Chemical constituents like alkaloids, triterpenoids, steroids have also been detected in Tailor ant [10]. The accumulation of different antibiotic resistance mechanism within the same strain has led to the appearance of the so called superbugs or multi drug resistant bacteria and this has led to the problem of resistance to antibiotics. Attention is now being shifted towards biologically active components isolated from plant and animal species for possible sources of new drugs or precursors. The study is aimed at the bioactive chemical constituents screening and antimicrobial activity of the methanol extract of *Oecophylla longinoda* in selected pathogens.

2. Materials and Methods

2.1 Sample Collection and Treatment

Oecophylla longinoda (Tailor ant) were collected from their nest in Almond tree (*Terminalia catappa*) and identified in the Department of Animal and Environmental Biology, University of Benin, Nigeria. The nest of the Tailor ants was immersed in a plastic bucket containing 3 Litres of distilled water to immobilize the ant while the leaves of the nest were carefully sorted out. The ants were then filtered using a sieve and crushed in mortar with a pestle.

2.2 Extraction

Seventy six grammes (76 g) of the crushed ants were packed in a thimble and exhaustively extracted in a soxhlet extractor using500 mL of methanol solvent for eight-hourly period. The crude extract was concentrated using a rotary evaporator (model RE 200, USA) at 50^0C.

2.3 Bioactive chemical constituents screening of methanol extract of Tailor ant

Screening was done to identify the presence of chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, and eugenols by using standard procedures by Sofowora [11] and Trease and Evans [12].

2.4. Test for glycosides

1 mL of the ant extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of conc. H_2SO_4. A brown ring is required for the presence of glycoside.

2.5. Test for saponins

0.5 g of ant extract was shaken with water in a test-tube and observed for frothing. Saponin rein Weiss (supplied by Merck) was used as a standard.

2.6.Test for flavonoid

2 mL of the ant extract was boiled with distilled water and filtered. 5 mL of 20% NaOH and few drops of dilute HCl were added to the solution. Formation of a colourless solution is indicative of a positive test.

2.7. Test for phenolic compounds

1 mL of the ant extract was added to 5 mL of 90% ethanol. In addition, 1 drop of 10% $FeCl_3$ was added. A pale yellow colouration of indicative of positive test.

2.8. Test for tannins

To 2 mL of the ant extract, 10 mL of distilled water was added and boiled for 5 minutes and then filtered into halves. To about 2 drops of the filtrate, ferric ($FeCl_3$) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.

2.9. Test for Eugenols

2 mL of the ant extract was mixed with 5% KOH solution. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate. A pale yellow precipitate is indicative of a positive test.

2.10. Test for steroids

2 mL of acetic anhydride was added to 0.5 g ant extract in 2 mL of dilute H_2SO_4. Acolour change from violet to blue or green is required for the presence of steroids.

2.11. **Test for terpenoids (Salkowski test)**

5 mL of ant extract was mixed in 2 mL of chloroform and 3mls of conc. H_2SO_4 was carefully added down the side of the inner wall of test tube to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

2.12. Test for alkaloids

2 mL of Picric acid was added to the ant extract. A yellowish precipitate test is a positive test.

2.13. Microorganisms

The microorganisms employed in this study were procured from the University of Benin Teaching Hospital, Benin City which includes clinical isolates of *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Mucormycetes, Aspergillus niger* and *Penicillum notatum.*

2.14. Media

Nutrient broth and nutrient agar, both products of Himedia Laboratories Mumbai (India) were used in this study. The composition of the medium was Beef extract -3.0 g, peptone - 5.0 g, sodium chloride -8.0 g, agar-15.0 g.

2.15. Agar well diffusion assay

The antimicrobial activity of the extracts was determined by using agar well diffusion technique. Nutrient agar plates were seeded with 0.1 mL of an overnight culture of each bacterial (106 CFU/mL). The 24 h broth culture of each bacterium were used to seed molten nutrient agar at 45°C, allowed to set and a well was made by sterile standard cork borer (6.0 mm in diameter and 200 µl (0.2 ml) of various concentration of methanol ant extract added into each well. Then bacterial plates incubated at 37°C for 24 h after which diameter of zones of inhibition were measured [13].

2.16. Determination of minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MIC values of the ant extract were determined using two fold micro-dilution to prepare concentrations of 100, 50, 25 and 12.5 mg/ml of each extract and a drop of the microbial suspension that had been previously diluted to 106 CFU/ mL were aseptically incorporated into molten nutrient agar and allowed to set. The plates were incubated to at 37°C for 24 hours. The lowest concentration preventing visible growth for each of the test organisms was recorded as the MIC. Ciprofloxacin was used as the positive control.

2.17 Isolation of alkaloid fraction

Twenty three grams (23g), of dried methanol extract of Tailor ant was dissolved in 50 mL of diethyl ether and treated with 20ml, three times, each of 2M HCl. The aqueous layer (lower layer) which contains the organic bases was separated and treated with 60 mL of sodium carbonate (Na_2CO_3) to release the soluble bases as insoluble precipitate. The precipitate which should contain basic fraction was then re-extracted with ether and dried for IR analysis.

3. Results and Discussion

3.1 Bioactive chemical constituents

The bioactive chemical constituents present in the methanol extract of the ants are shown in Table 1.

Table 1: Bioactive chemical constituents in methanol extract of *Oecophylla longinoda* (Tailor ant)

S/N	Bioactive chemical constituents	Methanol extract (Tailor ant)
1	Glycoside	+
2	Saponin	+
3	Phenolics	+
4	Tannins	-
5	Eugenol	+
6	Steroid	-
7	Terpenoids	+

8	Alkaloids	+
9	Flavonoids	+

Key: - = absent , + = present

In Table 1, glycosides, flavonoids, phenolics, alkaloids and terpenes were indicated in the ant extract while steroid and tannins were absent. These phytochemicals are useful bioactive agents that have physiological effect in man [11]. The presence of essential oils, terpenes and hydrocatrbons have been reported as bioactive chemical constituents in *O. longinoda* [14].

Alkaloids were also detected in the ant extract of *O. longinoda* from the study of Mylonakis [15]. The study confirmed that insects from the Hymenoptera family (ant family) are known to contain antimicrobial peptides rich in proline (an alkaloid of the pyrolidine class) which are active against a variety of gram positive and gram negative bacteria species.

Table 2: Antimicrobial activity of Methanol extract of Tailor ant

Microorganisms	**Minimum inhibitory concentration (MIC) (mg/ml)**				
	100 mg/mL	**50 mg/mL**	**25 mg/mL**	**12.5 mg/mL**	**Ciprofloxacin 0.01 mg/mL**
	Zone of inhibition *(mm)				
P. aeruginosa	20	25	27	-	28
S. aureus	27	20	22	26	28
E. coli	20	20	25	-	28
Mucormycetes	-	21	30	45	28
A. niger	27	25	22	21	28
P. notatum	27	25	19	15	28

(-) - No activity, < 10 mm – Non significant activity; 10-19mm – Significant activity
> - 20 mm – high activity
(National committee for clinical laboratory standard [16]
*Average of three observations adjusted to the nearest whole number.

The antimicrobial activity of aqueous extract of Tailor ant (Table 2) indicated high inhibitory activity against *P. aeruginosa* (27 mm) as the ant concentration decreased to 25 mg/mL. However, for *S. aureus* there was an irregular pattern of inhibition. An upward trend was observed for the result of *E.coli* from 20 mm (100 mg/mL) to 25 mm (25 mg/mL) as the concentration decreased. For the antifungi activity, inhibition against *Mucormycetes* increased as the concentration decreased but for both *A. niger* and *P. notatum*, there was a dose dependent activity. Decrease of zones of inhibition were directly proportional to a decrease in the concentration of the ant extract. From the work of Shanmugan in [17], similar trend of dose dependent activity was recorded. In all, the ant extract showed significant antimicrobial activity against all the pathogens when compared to standard antibiotic (ciprofloxacin). The acceptable standard zones of inhibition for sensitive organism for the antibiotic ciprofloxacin is greater than 21 mm [16]. Based on the antimicrobial activity of the methanol extract, these findings may support the traditional use of the ant decoction as poultice for the treatment of skin diseases like necrotic lesions caused by *P. aeruginosa* [2]. The diameters of zone of inhibition of the ant methanol extracts for the microorganism selected were lower than the control (ciprofloxacin) at all the concentrations except *Mucormycetes* which indicated high inhibition of 45 mm over 28 mm (ciprofloxacin) at 12.5 mg/mL. In this study, the inhibition of *E. coli* corroborates the findings of Oladunmoye *et al.* in [8]. They reported that extracts of green weaver ant (Tailor ant) samples from Nigeria might exhibit antimicrobial activity against several species of fungi and bacteria especially *E. coli*. Oladunmoye *et al.* [18] also stated in their

results that, the bacterial isolates indicated increase in susceptibility as the concentration of the *O. longinoda* extract increased.

3.3 Infra- red analysis

The I.R spectrum of the alkaloid fraction is shown in Figure 1 and the wave numbers of functional groups are given in Table 3.

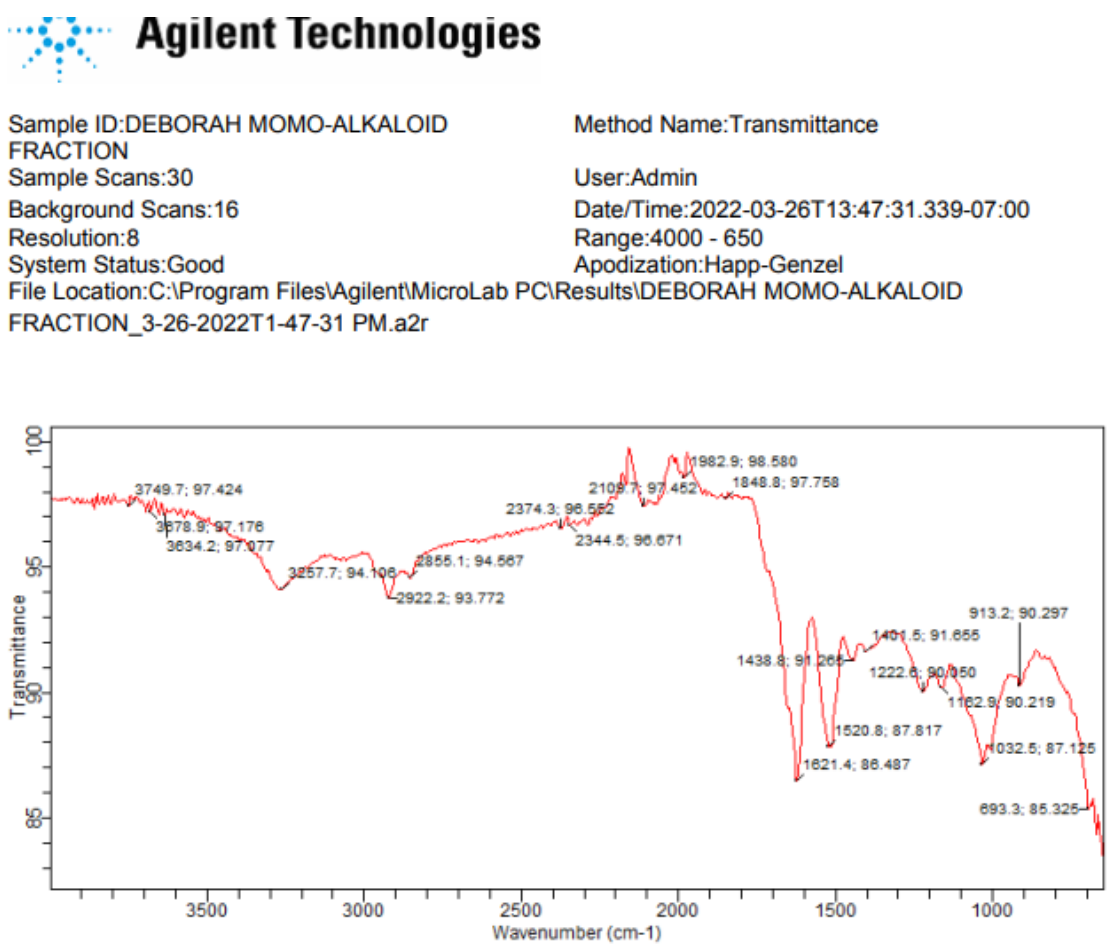

Figure 1: Infra- red spectrum of alkaloid fraction of Tailor ant

Table 3: I.R Absorption bands of functional groups detected in alkaloid fraction of Tailor ant

S/N	Peak(cm^{-1})	Appearance	Band	Functional group
1.	1032.50	Short	C-O stretch	Alcohol
2.	1438.90	Strong, short	C-H bend	Alkyl groups
3.	1621.40	Strong	N-H stretch	Amine, amide
4.	2109.70	Short	(C ≡ N) stretch	Nitrile
5.	3634.20	Broad	N-H stretch	Amine (RNH_2)

From the FT-IR analysis, the functional groups observed at 1032.50cm^{-1} indicated (C-O) stretch of alcohol; 1438.90cm^{-1} (C-H) bend of alkyl groups, 1621.40cm^{-1} (N-H) stretch of amine; 2109.70cm^{-1} (C ≡ N) stretch of nitrile and 3634.20cm^{-1} (N-H) stretch of amine. The band at 1621.40cm^{-1} of N-H stretch suggests that the methanol ant isolate is rich in alkaloid. This supports the detection of alkaloid in the bioactive chemical screening and the work of Mylonakis *et al.* [17].

4. Conclusion

The research findings have indicated that the methanol extract of Tailor ant contain bioactive components like terpenes, saponins and alkaloids which have physiological effect in humans while high inhibitory zone of activities were observed against the selected germs. Therefore, this work has corroborated the use of Tailor ant as poultice in traditional medicine for the treatment of microbial infection from bacteria and fungi. Chemical characterization of the isolated constituents and other pharmacological studies are suggested for further study.

Conflict of Interest

The authors declare no conflict of interest in this work.

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