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Mineral Components, Bioactive Constituents and Antifungal Activity of Whole parts of Tailor Ant (*Oecophylla Longinoda)*

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Abstract

The study was conducted to investigate the mineral components, bioactive chemical constituents and antifungal activity of whole parts of Oecophylla longinoda (Tailor ant). The Tailor ants were collected from their natural habitat in a nest and extracted with methanol using maceration method for forty-eight hours. The ant crude extract was concentrated using a rotary evaporator (Model RE, 200, USA) and mineral elements and bioactive chemical constituents were performed using standard methods. Candida albican, Penicillium notatum and Epidermophyton floccosum were selected for antifungal activity using agar well diffusion method while column chromatography was adopted for isolation and characterization done by infra-red (I.R) and ultra violet (UV) analysis. Potassium was detected as the highest mineral element (67.80 mg/mL) while crude protein from the whole ant was 65.03%. The result indicated that saponins, terpenes, flavonoids, sterol and alkaloid were present among others in the ant extract. The antifungal analysis revealed significant activities with zones of inhibition at a concentration of 80 mg/mL: 15 mm (E. floccosum), 10 mm (P. notatum) and 17 mm (C. albican). All the extract concentration showed a dose dependent activity when compared with the standard control antibiotics (Ketoconazole). The functional groups observed at 1461.10cm^{-1} indicated (C-H) bend of alkyl groups, 1707.10cm^{-1} (C=O) stretch of ketone or amide and 2120.90cm^{-1} ($C \equiv N$) stretch of nitrile. The band at 1707.10 cm^{-1} of C=O stretch suggests that the methanol ant isolate is rich in ketone groupswhile the UV maximum wavelength at 269 nm indicated unsaturated group chromophore. The study revealed that Oecophylla longinoda (Tailor ant) extract contains essential macro elements and bioactive constituents with high antifungal activity.

1.0. Introduction

Tailor ants are eusocial predatory insects belonging to the ant genus *Oecophylla* of the family *Formicidae* [1]. These ants are widely distributed in Asia, Australia and Africa [2]. Average minimum temperature and mean annual rainfall are two main factors that affect their distribution in forest environment. In fact, low temperatures below 17 °C have been found to inhibit the metamorphosis of these arboreal ants especially the larval development [2]. The treatment of various diseases by natural products is more of the application by flora species than fauna. For example, medicinal plants have been used as antimalarial [3], [4] 5], [6]; anticancer [7]; antimicrobial [8]; antiasthmatic [9]; blood lipid regulator [10]; analgesic [11]; anxiolytic [12], [13] and antisickling [14]. While fauna species like the ants have scanty reported scientific studies for the treatment of

diseases. However, most *Oecophylla* species have been reported for its antimicrobial activity [15], [16] and pesticidal activity among others. In most African countries, Tailor ant (*Oecophylla longinoda*) is one of the dominant ant species in forest canopies. Their colonies are exceptionally aggressive and territorial, tolerating almost no other insect ant species in the trees they occupy. They also exclude one another, in aggressive interactions so severe as to create narrow, unoccupied corridors that are in effect "no-ant's land" [17] and [18]. The Chinese since 304 A.D have recognized tha Oecophylla sppcies serve as the earliest biological control [19] and it has been reported that O. *smaragdina* and *O. longinoda* can control more than 50 different pests in multiple crops and forest trees [20] and [16].

Based on their pest control ability, the Tailor ants have also been reported to successfully reduce damages caused by four most important cashew insect pests; the tea mosquito bug, *Helopeltis* spp, the mango tip borer, *Penicillaria jocosatrix*, the fruit spotting bug, *Amblypelta* species and the leaf roller, *Anigraea* species. According to Peng in [20]. Edible insects have been reported to be good sources of Vitamins B12, folate and C compared with plant and animal sources [21] while the whole extract of *Oecophylla longinoda* grown in Africa exhibit anti-microbial activities [15] and [16]. Insects are potential sources of protein [21] and recently, the scientific study of the whole ant body revealed the presence of Mg, Ca, Zn, Fe and Mn in higher proportions than Na, K and P for Australian green ant (*Oecophylla smaragdina*). Thus, this study is aimed at determining the mineral components, bioactive constituents and antifungal activity of whole body of Tailor ant (*Oecophylla longinoda)*

2. Materials and Methods

2.1 Sample Collection and Extraction

Oecophylla longinoda (Tailor ant) were collected from their nest in Mango tree (*Mangifera indica*) in Ogheghe community at Ikpoba Okha local government area of Edo State and identified in the Department of Animal and Environmental Biology, University of Benin, Nigeria. The nests of the ants were immersed in a plastic bucket containing 4 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. Eighty two grammes (82 g) of the crushed ants were extracted by maceration for fourty-eight hours using 400 mL methanol solvent. The filtrate was collected using a whatman's number 4 filter paper and concentrated using a rotary evaporator (model RE 200, USA) at 50^0C

2.2 Bioactive chemical constituents screening

The bioactive chemical screening of the methanol extract of *O. longinoda* were performed using standard procedures prescribed by Sofowora [22]; Trease and Evans [23] and Rajasudha and Manikandan [24].

2.2.1. Test for glycosides (Modified Borntrager's Test)

1 mL of ant extract was treated were dissolved in 1 mL of ferric chloride solution and immersed in boiling water for 5 minutes. Both mixtures were cooled and extracted with 50 mL equal volumes of benzene. The benzene layer was separated and treated with 5 mL ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

2.2.2. Test for saponins (Froth Test)

1 mL of ant extract was diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2.2.3. Test for flavonoid (Lead acetate Test)
2 mL of ant extract was treated with few drops of lead acetate solution respectively. Formation of yellow colour precipitate indicates the presence of flavonoids**.**

2.2.4. Test for phenolic compounds (Ferric Chloride Test)
1 mL of ant extract was treated with 3-4 drops of ferric chloride solution. Formation of a bluish black colour indicates the presence of phenols

2.2.5. Test for tannins (Gelatin Test)
To 2 mL of ant extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

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

2.2.7. Test for Sterols. (Libermann Burchard's test)
0.5 g of ant extract was treated with 2mL chloroform and filtered. The filtrates were respectively treated with 2 mL of acetic anhydride, boiled and cooled. 2 mL Conc. Sulphuric acid was added to both solution. Formation of a brown ring at the junction indicates the presence of sterols.

2.2.8. 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 the test tubes to form a layer. A reddish brown colouration of the inter-phase is required for the presence of terpenoids.

2.2.9. Test for Triterpenes (Salkowski's Test)
5 mL of ant extract was mixed in 2 mL of chloroform and filtered. The filtrates were treated with 3mL of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpenes.

2.2.10. Test of Diterpenes (Copper acetate Test)
5 mL of ant extract was dissolved in 5 mL of distilled water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes

2.2.11. Test for alkaloids (Hager's Test: Hager's reagent (Saturated picric acid solution)
2 mL of Picric acid was added to the ant extract. A yellowish precipitate test is a positive test.

2.3 Antifungal activity
2.3.1. Fungi
The fungi employed in this study were procured from the University of Benin Teaching Hospital, Benin City which includes clinical isolates of *Candida albican, Penicillium notatum* and *Epidermophyton floccosum*
2.3.2. Media
Nutrient broth and potatoe dextrose, all 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.

2.3.3. Agar well diffusion assay

The antifungal activity of the methanol extract of *O. longinoda* was determined by using agar well diffusion technique. Nutrient agar plates were seeded with 0.1 ml of an overnight culture of each microbe (10^6 CFU/mL). The 24 h broth culture of each fungus was 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 essential oil extract added into each well. The fungi and plates respectively were incubated at 37°C for 24 h after which diameter of zones of inhibition was measured [25].

2.3.4. 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 methanol ant extract was determined using increasing volumes of 10, 20, 40 and 80 mg/mL concentrations and a drop of the fungi suspension that had been previously diluted to 10^6 CFU/ml were aseptically incorporated into molten potatoe dextrose 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. Ketoconazole was used as the positive control for the fungi.

2.4 Isolation of compound

Identification of pure compound from the crude methanol ant extract was done by combination of thin layer chromatography (TLC) and column chromatography (CC).

2.4.1. Column Chromatography (CC)

The crude extract was examined on TLC plate (Silica Gel 60 F254 Merck) to monitor the separation of extracts and find the best solvent systems for column chromatography. The solvent used were n-hexane, chloroform, ethyl acetate, methanol, n-hexane-chloroform, n-hexane –ethyl acetate, hexane-methanol, chloroform-methanol and ethyl acetate-methanol. A glass column of small size measuring 30 cm length by 1.5cm diameter (300 mm x 15 mm) was used for chromatography, and the adsorbent used was silica gel 60 (Merck 70-230 Mesh).The slurry of the silica gel was prepared by dissolving silica gel (100 g) in hexane solvent. While the column was prepared by pouring the slurry mixture into the glass column and allowed to settle down according to the procedure of [26]. The packed column was left over night before 12.4 g of the ant extract was introduced onto the top of the packed column and covered with a fitted whatman's No. 4 filter paper. The column was then eluted with selected suitable solvent systems with increasing polarity [27]. Collections of the compounds by the eluants were done by opening the opening the column's valve and about 10-20 mL fraction of the solvent coming out from the column was collected in test tubes [28]. The procedure was repeated by using different solvent systems, based on increasing polarity. Samples from the column fractions were examined by TLC plates in few suitable solvent systems to obtain the retention factor (Rf) of any components that appeared as spots. Fractions with similar Rf values were combined [28]. Fractions which contain more than one components were further isolated and purified by using smaller glass column of sizes 150 mm/15 mm with suitable solvent systems. Fraction with single component (one spot) that appeared in TLC plate was treated as possible pure bioactive metabolite. The combined fractions which contain the same single component were then allowed to air-dried to obtain a pure bioactive constituent.

2.5 Infra-red (I.R) analysis

The functional groups of the isolated compound was detected by using Fourier Transform Infra-Red spectrometry (FTIR). The pure compound was introduced directly into FTIR. Scan range employed was from 350 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1}, based on the method described by

Shalini and Sampathkumar [29]. Characteristic of the chemical bond was read by spectrum produced through transmittance of wavelength of the light. The chemical bond in a molecule was detected by interpreting the infra-red transmittance spectrum while identification of functional group in the compound was based on the Table of characteristic IR absorptions published in Organic Chemistry [30]

2.6 Ultra-Violet (U.V) analysis

The UV analysis for the isolated compound was done in a Philips 6405 UV/VIS scanning spectrophotometer

2.7 Mineral element determination

For the elemental analysis of the crude ant extract, one gram of the sample was digested with concentrated HNO_3 and $HClO_4$. The sample was then filtered and made up to 50 mL with distilled water [31]. The metal concentrations were determined using an Atomic Absorption Spectrophotometer (Buck Scientific model 210) and a Flame Spectrophotometer (Sherwood, model 410). The mineral elements investigated were Iron (Fe), Zinc (Zn), Calcium (Ca), Magnesium (Mg), Potassium (K), Nickel (Ni), Sodium (Na), Cadmium (Cd), Cupper (Cu), Chromium (Cr) and Lead (Pb).Crude protein content was determined by Kjeldahl method in accordance with the method recommended by Association of Official Analytica Chemist [31]

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	Flavonoids	+
4	Phenolics	+
5	Tannins	-
6	Eugenols	+
7	Sterols	+
8	Terpenoids	+
9	Triterpenes	-
10	Diterpenes	-
11	Alkaloids	+

Key: - = absent , + = present

In Table 1, glycosides, sterols, phenolics, flavonoids, alkaloids and terpenoids were indicated in the methanol ant extract while triterpenes, diterpenes and tannins were absent. These phytochemicals are useful bioactive agents that have physiological effect in man [3]. The presence of essential oils, terpenenoids and hydrocatrbons have been reported as bioactive chemical constituents in *O. longinoda* [32]. While glycosides, saponins and phenolics detection agreed with the results of Iyekowa et al. [16].

3.2 **Antifungal activity of methanol ant extract**

Table 2: Antifungal zone of inhibition of methanol ant extract of *O .longinoda*

Microorganisms	**Minimum inhibitory concentration (MIC) (mg/ml)**				
	10 mg/ml	**20 mg/ml**	**40 mg/ml**	**80 mg/ml**	**Ketoconazole 0.01mg/mL**
	Zone of inhibition *(mm)				
E. floccosum	08	08	10	15	30
P. notatum	05	05	07	10	26
C. albican	05	10	14	17	29

(-) - No activity, < 10 mm – Non significant activity; 10-19 mm – Significant activity
> - 20 mm – high activity
(National committee for clinical laboratory standard [33]
*Average of three observations adjusted to the nearest whole number

The antifungal activity of the methanol extract of Tailor ant (Table 2) indicated that the diameter of zone of inhibition increased with increase in concentration of the methanol ant extract. The growth of *C. albican* was inhibited more (17 mm) as the concentration increased to 80 mg/mL while the least inhibited (10 mm) fungus was *P.notatum* at 80 mg/mL when compared with the positive control, Ketoconazole . This antifungal result thus, shows a dose dependent activity. However, Oladumoye [15] had reported antimicrobial activity for green weaver ant species but not Tailor ant as reported in this study.

3.3 Purification of Compound of methanol ant extract

The 12.4 g of the ant crude extract was introduced into the column using slurry pack method with 100% hexane. The sample was then eluted with suitable solvent ration as shown in Table 2.

Table 3: Solvent system used for column chromatography (200 mL each solvent)

Solvents	Volume to volume
Hexane	1
Chloroform	1
Ethyl acetae	1
Methanol	1
Hexane-chloroform	1:2
Hexane-ethyl acetate	1:2
Hexane-methanol	1:2
Chloroform-methanol	1:2
Ethyl acetate-methanol	1:2

All the fractions with similar Rf values were pooled together and labeled A, B, C and D

Table 4: Combined fractions of the ant crude methanol extraction from the column chromatography

Fractions code	Fraction weight (mg)	Fraction colour
A	116	Dark brown
B	96	Light brown
C	98	Dark yellow
D	120	Light yellow

The pure compound was obtained from the Fraction D with 120.0 mg of light yellow colour of the ant extract with solvent system ethyl acetate-methanol (1:2) and the TLC analysis of the fraction was carried out to further purify the compound in the following solvent ratio shown in Table 5.

Table 5: TLC and Rf value of fraction D of different solvent ration system under white light

Solvent system (v/v)	Number of spots	Rf value	Stained TLC colour
Hexane –ethyl acetate	2	0.45 and 0.32	Dark brown
Ethylacetate-methanol	2	0.68 and 0.40	Light brown

The spot with Rf value of 0.68 was targeted and recovered as the pure compound obtained from the ant extract.

3.4 Infra- red analysis

The I.R spectrum of the ant methanol fraction is shown in Figure 1 and the wave numbers of functional groups are given in Table 6

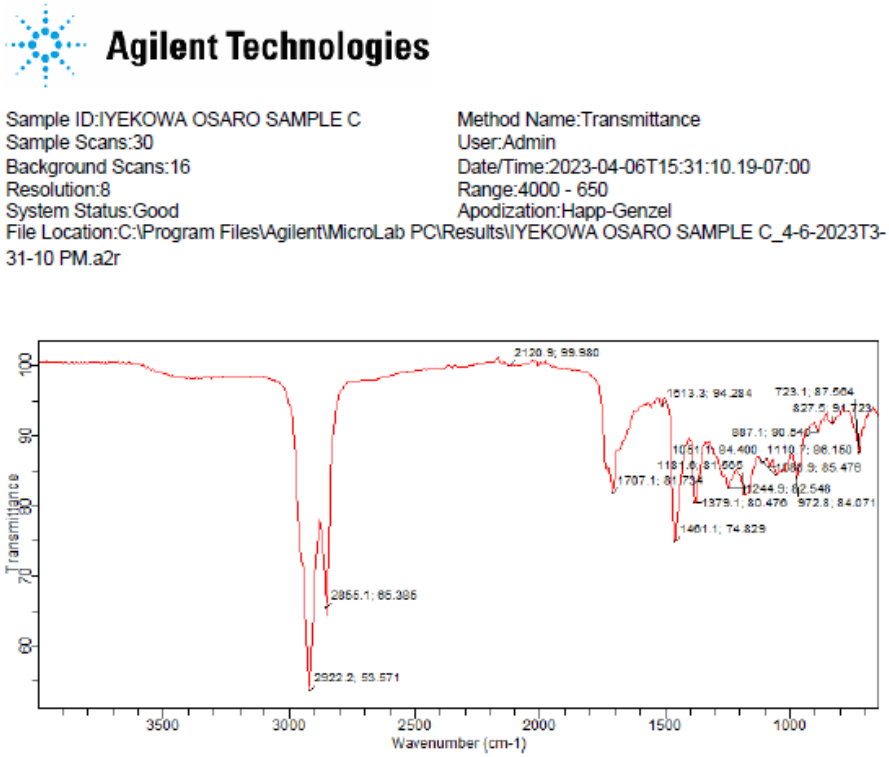

Figure 1: Infra- red spectrum of isolated compound of Tailor ant

Table 6: I.R Absorption bands of functional groups detected in isolated compound of Tailor ant

S/N	Peak(cm^{-1})	Appearance	Band	Functional group
1	1379.10	Sharp	C-H bend	Methyl group, CH_3
2.	1461.10	Long, sharp	C-H bend	Alkyl groups, CH_3, CH_2, CH
3.	1707.10	Sharp	C=O stretch	Ketone, amide
4.	2120.90	Short	(C ≡ N) stretch	Nitrile
5.	2855.10	Sharp	C-H stretch	Alkyl groups, CH_3, CH_2, CH
5.	2922.20	Long, sharp	C-H stretch	Alkyl groups, CH_3, CH_2, CH

Range: 4000 cm^{-1} to 650 cm^{-1}

From the FT-IR analysis, the functional groups observed at 1379.10cm^{-1} indicated (C-H) stretch of methyl; 1461.10cm^{-1} (C-H) bend of alkyl groups, 1707.10cm^{-1} (C=O) stretch of ketone or amide; 2120.90cm^{-1} (C ≡ N) stretch of nitrile; 2855.10cm^{-1} (C-H) stretch of alkyl groups and 2922.20cm^{-1} (C-H) bend of alkyl groups. The band at 1707.10 cm^{-1} of C=O stretch suggests that the methanol

ant isolate is rich in ketonic groups. This could be a functional group in the alarm pheromones (aromatic compounds) released by the insects for communication.

3.5 U.V Analysis

The U.V spectrum of isolated compound of Tailor ant is shown in Figure 2 and the maximum absorption wavelength given in Table 7.

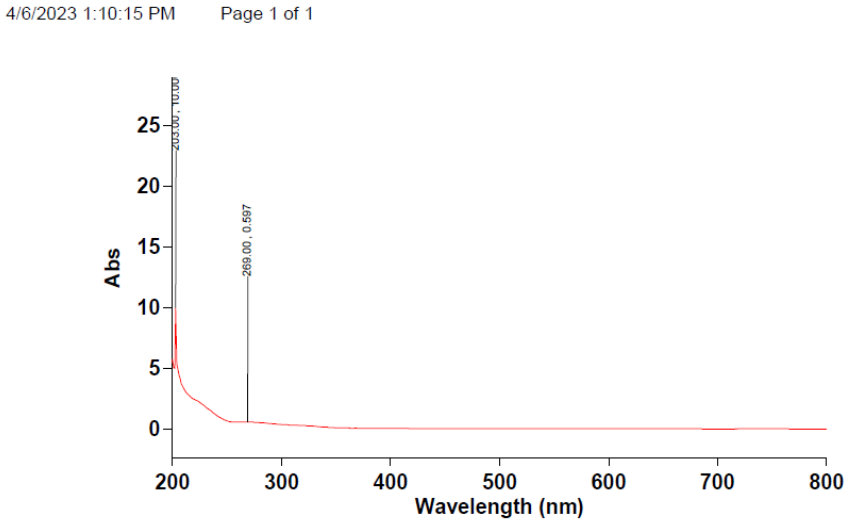

Figure 2: Ultra violet spectrum of isolated compound of Tailor ant

Table 7: Maximum absorption wavelength of the isolated compound of Tailor ant

Maximum wavelength (nm)	Emax	Absorbance	Peak	Chromophore
203.00	21,000	10.000	Sharp	$(-C=C-)_2$
269.00	35,000	0.597	Short	$(-C=C-)_3$

Range: 600 nm - 200 nm

The UV spectrum gave UVmax at 269 nm (Emax 35,000) with a sharp peak indicating the chromophore $(-C=C-)_3$ suggestive of unsaturated groups possibly in aromatic compounds (K-band, Emax> 10,000).

3.6 Mineral element composition

Table 8: Mineral elements detected in methanol ant extract of *O. longinoda*

Element	Content
Pb (mg/mL)	0.01
Na (mg/mL)	1.80
Mn (mg/mL)	0.40
Cu (mg/mL)	0.02
Fe (mg/mL)	0.30
K (mg/mL)	67.80
Ca (mg/mL)	64.50
CP (%)	65.03

CP = Crude protein

The mineral elements were detected in varying concentrations (Table 8) with potassium being the highest (67.80 mg/mL) while Pb was the least detect in the methanol ant extract, Meanwhile the value of crude protein from the whole ant was 65.03%, thus indicating that ant are very rich in protein. The role of K in human diet cannot be overstressed. It maintains normal fluid level within the cells while Na maintains fluids outside the cell. Potassium also help to support muscular contraction and normal blood pressure in humans, thus lowering the risk of heart diseases and stroke. Other functions of potassium include transmission of nerve signals, muscle contractions, fluid balance and various chemical reactions [34]. Among the macro elements detected Ca and K gave higher values and this corroborates the findings of Shamugan in [35] whose results indicated Mg and Ca in high proportion for Australian green ants (*Oecophylla smaragdina*). The high value of crude protein (65.03 %) also indicated that the ants may be a rich source of good diet.

4. Conclusion

The research findings have indicated that the methanol extract of Tailor ant contain essential minerals and chemical constituents which are beneficial to man when utilized in medicines while high zone of inhibition was observed against *Candidas albican.*

Conflict of Interest

The authors declare no conflict of interest in this work.

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