

PHYTOCHEMICAL PROFILE, NEMATICIDAL AND PHYTOTOXIC POTENTIAL OF
LEAVE EXTRACT OF ACACIA AURICULIFORMIS (EAR LEAF)¹Jemirin O. D., ²Ibikunle G. J., ³Edewor T. I., ¹Ahmed A. A.¹Department of Industrial Chemistry University of Ilorin, Ilorin, Nigeria.²Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.³Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.***Corresponding Author: Ibikunle G. J.**

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ABSTRACT

Natural products, particularly standardized plant extracts, provide a valuable reservoir of structurally diverse secondary metabolites with pharmaceutical and agricultural potential. *Acacia auriculiformis* (earleaf acacia) is a multipurpose plant with recognized medicinal, nutritional, ecological, and agricultural importance. This study therefore evaluated the phytochemical profile, phytotoxic, and nematicidal activities, and bioactive compounds of the ethanolic leaf extract of *A. auriculiformis*. Fresh leaves of *A. auriculiformis* were collected, washed, air-dried, pulverised, and extracted by static maceration in ethanol at room temperature for seven days. The extract was filtered, concentrated, and its percentage yield determined gravimetrically. Qualitative and quantitative phytochemical analyses were performed using established procedures. Phytotoxicity was assessed using a seed-germination bioassay, while nematicidal activity was evaluated against juveniles of *Radopholus similis*. Gas chromatography–mass spectrometry (GC–MS) was employed to characterize detectable compounds. The ethanolic leaf extract yielded 5.86%, and contained alkaloids, flavonoids, saponins, cardiac glycosides, and phenolic compounds qualitatively. Quantitative analysis revealed alkaloids (28.60 mg/mL), flavonoids (20.40 mg/mL), saponins (10.40 mg/mL), and cardiac glycosides (6.20 mg/mL). The phytotoxicity assay demonstrated concentration-dependent inhibition of seedling growth, with shoot and root lengths decreasing from 12.10 and 11.00 cm at 0.25% extract concentration to 6.99 and 6.33 cm, respectively, at 2.0%, compared with 13.92 and 15.34 cm in the control. The extract also demonstrated pronounced, concentration-dependent nematicidal activity against *R. similis*, with mortality increasing from 89% at 0.1% to 99% at 0.5% after 24 h, compared with 1% in the distilled-water control. GC–MS analysis identified seventeen bioactive compounds, with decanoic acid ethyl ester and hexadecanoic acid ethyl ester occurring prominently, alongside octanoic acid ethyl ester, *cis*-9-hexadecenal, and decane, 1,1'-oxybis-. This study showed that *A. auriculiformis* leaves contain diverse phytochemicals associated with measurable phytotoxic, and nematicidal activities.

KEYWORDS: *Acacia auriculiformis*, *Nematodes*, *Radopholus similis*, *Nematicida*, *phytotoxic*, *phytochemical*, *ear leaf*.**INTRODUCTION**

Acacia auriculiformis is indigenous to the tropical region of Northern Australia, Papua New Guinea, Eastern Indonesia and sub-Saharan Africa. The species occurs in open forest, woody savannah and coastal plain where it thrives under warm temperature and seasonal rainfall patterns. Because of its exceptional adaptability, the

species has been widely introduced into tropical and subtropical region around the world through afforestation and agro forestry program (Hague *et al.*, 2019). In Nigeria, *A. auriculiformis* is commonly found in forest plantation, research institutes, university campus, highway, parks and urban landscape. It performs very

well within the Guinea and Sudan Savannah ecological zone (Buba, 2015).

A.auriculiformis is recognized as one of the most economically valuable multipurpose tree species cultivated throughout the tropical and subtropical region of the World. The species has gain considerable attention because of it rapid growth, adaptability to marginal environments, high biomass yield and wide range of commercial application. (Tonouewa *et al.*, 2023). One of the principal economic values of *A.auricuformis* lies in it timber. The wood possesses high density, moderate hardness and resistance to biological deterioration, making it useful for construction.

MATERIALS AND METHODS

Collection and extraction: The leaves of *A. auricuformis* were collected from University of Ilorin, Ilorin, Nigeria. The plant had earlier been identified in the Department of Plant Biology of the University. The leaves were air dried on the laboratory bench for seven days at the ambient temperature (35⁰C- 38⁰C). Thereafter, it was pulverized into powdered and sieved into fine particles, which was extracted with ethanol by soaking for a week. The extract solution was concentrated using Rotary evaporator to obtain 3.2g dry mass of the extract.

Phytochemical screening of the extract: The extract was assessed for the presence of alkaloids, tannins, glycosides, saponins, sterols and flavonoids, using qualitative and quantitative methods.

Test for Alkaloids: One cm³ of 1% HC1 was added to 3 cm³ of each of the solvent extracts in a test tube, heated for 20 min, cooled and filtered and the filtrate was used for the following tests:

- (i). Two drops of Mayers reagent were added to 1 cm³ of the extract in a test tube. A creamy white precipitate was observed in the extracts, indicating the presence of alkaloids in the extract.
- (ii). Two drop of Wagners reagent were added to 1 cm³ of extract in a test tube. A reddish brown precipitate was observed in all the extracts, indicating the presence of alkaloids in the extract.

Test for Tannins: Two drops of Ferric chloride were added to 1 cm³ of extract in a test tube. A dirty green precipitate was observed in the ethanol extract, indicating the presence of tannins.

Test for Saponins: Exactly 3cm³ of extract was shaken vigorously in the test for 2 min. No frothing was observed in the extract. This indicated the absence of saponins.

Test for Flavonoids: (i). One cm³ of 10 % NaOH was added to 3 cm³ of each extract. A yellow precipitate was observed in all the extract. This indicated the presence of flavonoid in the extracts, (ii).Two gram of magnesium

and three drops of concentrated HC1 were added to 3 cm³ of extract. Red colouration was developed in the extract, indicating the presence of flavonoid in the extract.

Test for Sterols: Five drops of concentrated H₂SO₄ were added to 1 cm³ extract. A brownish-red colour was observed, indicating the presence of sterols in the tested extract.

Test of Glycosides: Exactly 10 cm³ of 50% concentrated H₂SO₄ was added to 1 cm³ of the extract in the test tube. The mixture was heated on water bath for 15 min. 10 cm³ of Fehling solutions (5 cm³ each of Fehling solutions A and B) was added and the mixture was boiled. A brick red precipitate was observed in the extract. This indicated the presence of glycosides in the extract (Ajayi, *et al.*, 2026).

Phytotoxic assay of test solutions: In order to study phytotoxic activity of the extract solution, different concentrations of the test solution were prepared and applied to check germination of both root and shoot of the germinating seeds. Seed of maize (*Zea may* and were purchased from Ipata market, Ilorin. The assay seed were sorted for uniformity of size and damaged seeds were discarded, these were washed tap water and surface sterilized using NaCL (10%v/v for 10min, followed washing with sterile distilled water. Bioassays were carried out using Petri dishes (90mm diameter containing cotton wool as support. Test solution (5ml was added to the cotton wool in the petri dishes and dried completely in vacuum at 40⁰C. Five selected seeds were placed on the cotton wool and incubated for 7days at 25⁰C in dark using prepared solution of 0.25%, 0.5%, 1% and 2% respectively. The treated maize seed were allow to germinate on moist cotton wool. Addition of 10ml of distilled water was done daily for seven days. After seven days of incubation, the length of roots and shoot of germinated seeds were measured in centimeter cm. Treated experimental sets were compared with the control (ibikunle, *et al.*, 2024).

Extraction of *Radopholus similis* Juveniles: Infected yam tubers showing characteristic symptoms of nematode infestation were selected from the stock culture. The tubers were carefully washed to remove adhering soil and other extraneous materials without damaging the tissues excessively. A clean sieve was placed over a plastic Petri tray or suitable collecting container, and a paper serviette was carefully positioned on the sieve as a supporting medium.

The affected yam tubers were cut into small pieces using a sterile knife and evenly distributed on the paper serviette placed on the sieve. The sieve containing the infected yam tissue was then positioned in such a way that its lower portion was suspended above the bottom of the collecting tray. Approximately 300 mL of clean water was carefully added to the plastic tray, ensuring

that the water level reached the material on the sieve without completely submerging the yam tissue.

The entire extraction assembly was left undisturbed at room temperature for 24 h. During this period, the active nematodes migrated from the infected yam tissue through the paper serviette and sieve into the surrounding water. After 24 h, the sieve and its contents were gently removed to avoid disturbing the nematode suspension. The water collected in the plastic tray was examined microscopically for the presence of *R. similis* juveniles. The extraction procedure was based on the tray method described by Ibikunle, *et al.*, (2024). The nematode suspension was carefully collected and examined under a compound or stereomicroscope. Active juveniles displaying characteristic movement were selected for the Nematicidal assay. Where necessary, the suspension was diluted with distilled water to obtain a relatively uniform nematode concentration for the experimental treatments.

In vitro Nematicidal Assay: A measured volume of the nematode suspension containing a predetermined number of active *R. similis* juveniles was transferred into appropriately labelled multi-well assay plates containing the different concentrations of the ethanolic leaf extract. Each treatment concentration was tested in replicate. The negative control consisted of nematode suspension exposed to distilled water or the corresponding solvent without plant extract, while the positive control consisted of nematodes exposed to the selected reference nematicide, where applicable. The experimental preparations were maintained at room temperature under controlled laboratory conditions. Nematode mortality

was assessed at a predetermined exposure period of 72 h. At each observation period, the nematodes were examined microscopically for movement. Juveniles that remained completely immobile after gentle mechanical stimulation were considered apparently dead. To distinguish temporary paralysis from mortality, apparently immobile nematodes were, where applicable, transferred to clean distilled water and observed for recovery. Juveniles that failed to resume movement were recorded as dead. The number of living and dead juveniles was recorded for each concentration and exposure period.

Determination of Percentage Mortality

The percentage mortality of *R. similis* juveniles was calculated using:

$$\% \text{ mortality} = \frac{\text{Number of dead juveniles}}{\text{Total number of juveniles}} \times 100$$

Where necessary, control mortality was accounted for using Abbott's correction. A concentration-dependent increase in mortality and/or reduction in survival over time was interpreted as evidence of nematicidal activity.

GC–MS Conditions: GC–MS analysis was performed using a Shimadzu 2010 gas chromatography system with a split mode mass selective detector, in a fused silica column with helium as carrier gas at a flow rate of 0.80 mL/min. The GC oven temperature was 60 °C with a split ratio of 10:1, and the injector temperature was 250 °C.

RESULTS

Table1: Qualitative phytochemical screening of *A. auriculiformis* leaves extract.

Phytochemicals	Leave Extract % Ethanol
Alkaloids	+++
Flavonoids	+++
Phenols	++
Saponins	++
Tanins	++
Cardiac glycosides	++
Steroids	+
Anthocyanins	+
Terpenoids	+

Key: +++ Presence in abundant; ++ Moderately present; + Sparingly present

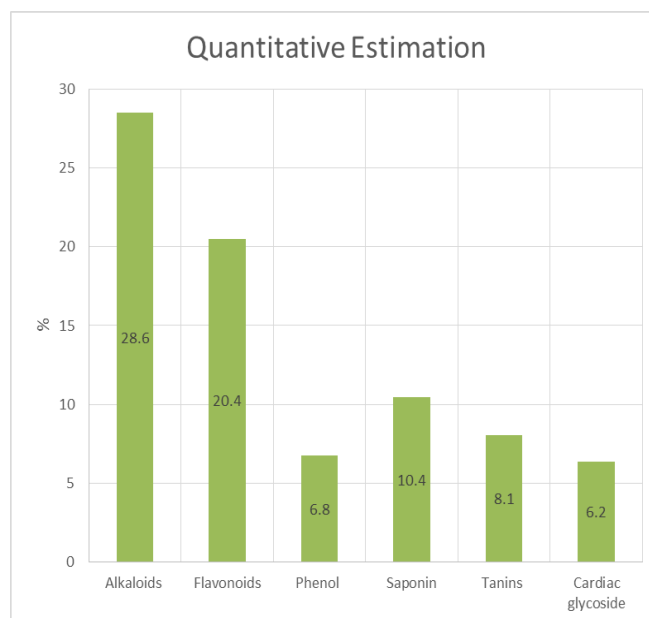


Figure 1: Quantitative phytochemical screening of *A. auriculiformis* extract.

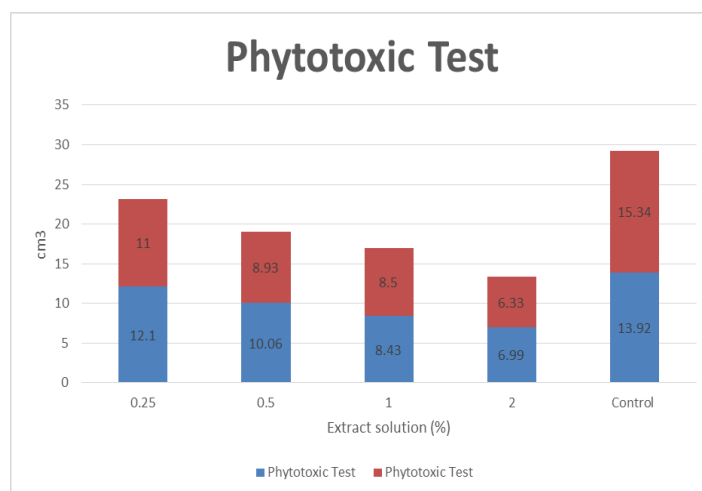


Figure 2: Quantitative Estimation of Phytotoxicity *Acacia auriculiformis* leave extract.

Table 2: Effect of Ethanolic extract of *A. auriculiformis* on activity and Survival of *R. similis* Juveniles.

Treatment concentration	Active %	Quiescent %	Dead %
0.1% extract	2a	9b	89a
0.2% extract	1a	9b	90a
0.3% extract	1a	8b	91a
0.4% extract	0a	6b	94a
0.5%l extract	0a	1a	99a
Distilled water control	96b	3b	1b

Values carrying different superscript letters differ significantly according to the statistical procedure used

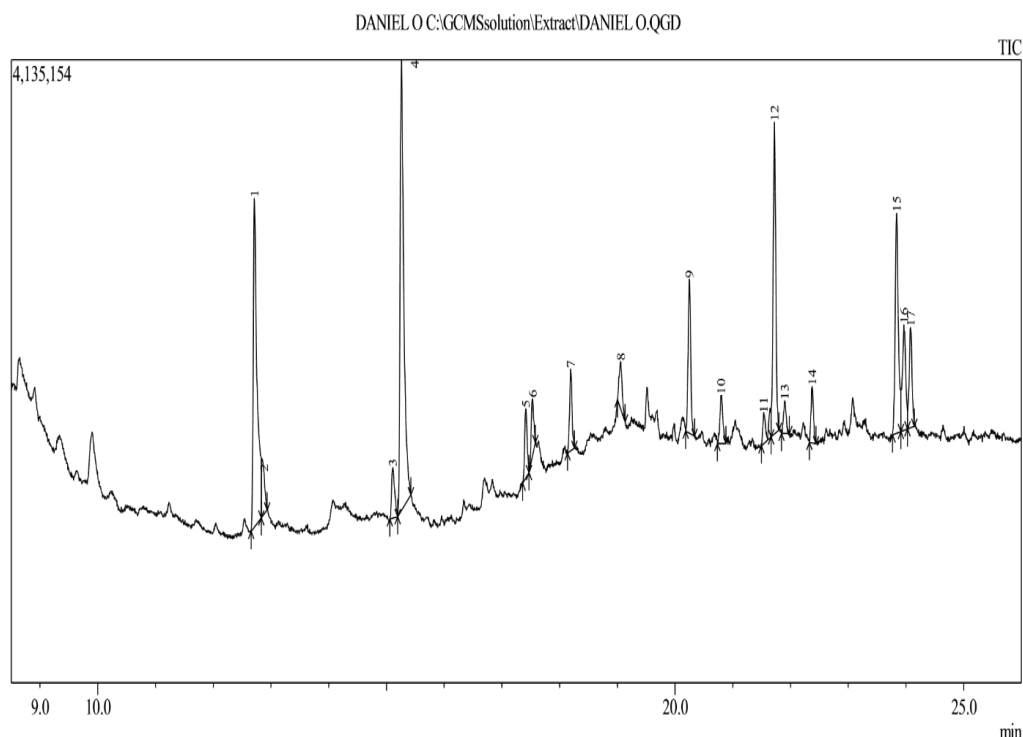


Figure 3: GC-MS Spectrum of the Ethanolic Extract of the leaves of *A. Auriculiformis*.

Table 3: GC-MS Result Analysis of the Ethanolic Extract of *A. auriculiformis*.

Peak#	Name	R.Time	Height	Molecular weight	Molecular formula
1	Octanoic acid, ethyl ester	12.712	2182701	172	C ₁₀ H ₂₀ O ₂
2	endo-Borneol	12.880	275562	154	C ₁₀ H ₁₈ O
3	1-Tetradecene	15.109	337294	196	C ₁₄ H ₂₈
4	Decanoic acid, ethyl ester	15.260	2989386	200	C ₁₂ H ₂₄ O ₂
5	1-Pentadecene	17.413	462141	210	C ₁₅ H ₃₀
6	Decanoic acid, ethyl ester	17.530	384740	200	C ₁₂ H ₂₄ O ₂
7	Octane, 1,1'-oxybis-	18.191	542922	242	C ₁₆ H ₃₄ O
8	Squalene	19.058	317544	410	C ₃₀ H ₅₀
9	Decane, 1,1'-oxybis-	20.247	1023694	298	C ₂₀ H ₄₂ O
10	1,2-Benzenedicarboxylic acid, bis(2-methylpro	20.796	320390	278	C ₁₆ H ₂₂ O ₄
11	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien	21.530	198315	276	C ₁₇ H ₂₄ O ₃
12	Hexadecanoic acid, ethyl ester	21.720	2070774	284	C ₁₈ H ₃₆ O ₂
13	Dibutyl phthalate	21.898	217454	278	C ₁₆ H ₂₂ O ₄
14	Decane, 1,1'-oxybis-	22.374	376752	298	C ₂₀ H ₄₂ O
15	cis-9-Hexadecenal	23.835	1462027	238	C ₁₆ H ₃₀ O
16	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	23.965	702839	278	C ₁₈ H ₃₀ O ₂
17	Octadecanoic acid, ethyl ester	24.078	665583	312	C ₂₀ H ₄₀ O ₂

Statistical Analysis

Experimental data were entered into SPSS version 20 for statistical analysis. Results were expressed as mean $\pm$ standard deviation (SD).

For comparisons involving more than two treatment groups, one-way analysis of variance (ANOVA) was used where the assumptions of the test were reasonably satisfied. Where a significant overall difference was observed, an appropriate post-hoc multiple-comparison test, such as Tukey's test, was applied to identify differences between specific groups. For nematocidal

studies involving multiple exposure times and concentrations, two-way ANOVA was employed to evaluate the effects of concentration, exposure time, and their interaction. Statistical significance was set at: < 0.05 . The statistical procedure selected for each dataset was based on the experimental design and distributional characteristics of the data.

DISCUSSION

Table 1 shows the result of phytochemical analysis and it shows that alkaloids, flavonoids are abundantly present while phenols, saponin, tannins and cardiac glycoside

were moderately available in the extract. Steroids and terpenoids were present sparingly.

Figure 2, the extract show inhibitory activity on the root and shoot growth of maize plant.

The lowest concentration of the plant extract has the higher value in shoot 12.10 cm and root with 11.10 cm, while the higher concentration has at least value of 6.99cm for shoot and 6.33cm for root. It was discovered that the value decrease as the concentration of the extract increase. This result showed that shoot is more sensitive to the phytotoxic effect of the extract compared with the control having 15.34 cm for shoot and 13.92 cm for roots. The results of the research show that flavonoid, steroids, glycosides, Tannins and alkaloids that were present in one leaf extract of *A. auriculiformis* *in vitro*. In conclusion, the result of this study showed that the leaves extract of *Acacia auriculiformis* plant contained these substances (flavonoid, alkaloid, steroids, glycoside, phenols) which were implicated for the phytotoxic potential of the plant, which is in agreement with literature. Therefore, extract from the leaves of *A. auriculiformis* could be exploited for use in the control and management of weeds (allelopathically), as a plant based natural herbicides alternative to synthetic herbicides.

GC-MS Characterization of *A. auriculiformis*:

Analysis of the ethanolic crude extracts by GC-MS of *A. auriculiformis* was carried out. The compounds were determined by carefully analyzing the GC-MS spectra and chromatogram of the crude extracts, where the mass spectral data obtained were compared with the mass spectral library to identify the compounds present in the sample and establish the one with higher potency. From the chromatogram, it is clear that the ethanolic crude extract contains several different compounds. The chromatograms provide peaks at different retention times for the ethanolic crude extract. An extensive library search using the NIST11 mass spectral library was done to identify the compounds in the library.

Many bioactive compounds were identified in the ethanolic crude extract of *A. auriculiformis* and are shown in Table 3 with the chromatograms in Figure 3. The components are listed in order of their elution. The GC-MS analysis of the ethanolic leaf extract revealed the presence of seventeen (17) compounds corresponding to 17 peaks, of which two compounds were reported at more than one retention time. Decanoic acid ethyl ester was assigned to peaks 4 and 6, while decane, 1,1'-oxybis- was assigned to peaks 9 and 14. Their % height and % area were added up to give a single compound, thus reducing the 17 compounds to 16 compounds, while the GC-MS analysis of the ethanolic extract of the leaf of *A. auriculiformis* revealed the presence of several detectable chemical constituents. The most prominent identified compounds are decanoic acid ethyl ester at approximately 20.57%, octanoic acid ethyl ester at

15.02%, hexadecanoic acid ethyl ester at 14.25%, cis-9-hexadecenal at 10.06%, and decane, 1,1'-oxybis- at approximately 7.05%. The detection of fatty-acid ethyl esters is notable. Decanoic acid ethyl ester, octanoic acid ethyl ester, hexadecanoic acid ethyl ester, and octadecanoic acid ethyl ester constituted prominent chromatographic signals in the supplied data. These compounds may contribute to the biological characteristics of the extract. Squalene was also detected. Squalene is a triterpene hydrocarbon associated with biological and structural functions in organisms. Its detection contributes to the overall chemical diversity of the extract.

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