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Chemical Composition, Antibacterial, and Antioxidant Activity of Methanol extracts from Aerial part of *Indigofera nummularifolia* and *Zornia* glochidiata

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ABSTRACT

The aim of the study is to investigate the chemical composition, antibacterial and antioxidant properties of methanol extracts of *Indigofera nummularifolia* aerial parts and *Zornia glochidiata* leaves. The chemical constituents of weighed, air-dried, and pulverized leaf samples of *Indigofera nummularifolia* and *Zornia glochidiata* were examined using Gas Chromatography-Mass Spectroscopy (GC-MS). The antimicrobial activities of methanol extract of *Indigofera nummularifolia* and *Zornia glochidiata* were tested against six (6) strains of bacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterococcus faecalis*, and *Escherichia coli* using agar diffusion pour-plate method. The antioxidant activities of the methanol extracts *Indigofera nummularifolia* and *Zornia glochidiata* were carried out using the DPPH method. The methanol extract of *I. nummulariifolia* showed weak activity against *E. coli* (5.5 mm). On the other hand, the methanol extract of *Z. glochidiata* showed significant activity against *S. aureus* (10.50 mm). The methanol extract of *Z. glochidiata* exhibits significant antioxidant activity by scavenging DPPH radicals in a dose-dependent manner. The methanol extract of *Z. glochidiata* exhibited antioxidant activity with IC_{50} value at 31.15 µg/mL, comparable with that of the standard control, ascorbic acid (IC_{50} value of 52.10 µg/mL). The GC-MS analyses of the extracts of the two plants revealed various bioactive compounds including fatty acids, carboxylic acids, carboxylic acids esters, terpenes, alcohols, sugars, and other bioactive metabolites. The chemical profiling of the two plants (*Indigofera nummularifolia* and *Zornia glochidiata*) employed in this study exhibits broad spectrum of bioactive metabolites with promising antibacterial and antioxidant activities.

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For years some plants have been used traditionally for the treatment of sicknesses and diseases (Edewor *et al*., 2014). These medicinal plants have been shown to possess chemical components that exhibit therapeutic properties. There is a growing interest in the study of medicinal plants that possess antioxidant property as well as antimicrobial. These natural antioxidants have been shown to be effective in the inhibition of oxidation of food, reduction of age-related diseases (Burda and Oleszek, 2001) and have the ability to increase the antioxidant capacity of the plasma thereby reducing the risk of contracting cancer and heart related diseases (Zou *et al*., 2004). Globally, these plants are also used medicinally and often are good sources of many potent and powerful drugs to treat microbial infections (Terhide *et al*., 2025). Many medicinal plants have sparked substantial interest based on ethno-pharmacological usage, particularly in the treatment of various illnesses such as chronic inflammatory diseases (Moro *et al*., 2012). In the search for new analgesics and anti-inflammatory medications, study into medicinal plants with putative folkloric use as analgesics and anti-inflammatory agents should be seen as a fruitful and reasonable research technique.

Indigofera nummulariifolia, a medicinal plant that grows in tropical and subtropical climates, is used to treat a variety of liver problems and viral infections (Okafor 2025). Many other species of the Indigofera genus have been proven to have a vast array of pharmacological activities; *I. pulchra* is known to have anti-venom properties (Abubakar *et al*., 2006), *I. heterantha* is used as herbal medicine as well as folk medicine to treat gastrointestinal disorder and abdominal pain, *I. tinctoria* has shown antioxidant, free radical scavenging activity and anti-dyslipidemic activity, and *I. oblongifolia* proven to possess antimicrobial, hepatoprotective and strong lipoxygenase inhibitory activity (Mohammad *et al*., 2011).

On the other hand, *Zornia glochidiata* is an annual (rarely perennial) herb that is found throughout tropical Africa, from Senegal and Eritrea to Cape Province, South-West Africa, and Madagascar. The stems are 10 to 70 cm long, erect or decumbent, slightly woody at the base, and rarely survive the dry season. It has oblong to ovate-lanceolate upper leaflets that are rounded at the base, while the lower leaflets are wider. Flowers are small and inconspicuous, usually yellow but often white or orange in color and with or without reddish-purple veins (Verdcourt, 2000). *Z. glochidiata* grows in grassland,

roadsides, open woods, waste sites, and riverbanks at altitudes ranging from 0 to 1800 m; mostly on sandy soil, in rocky regions, or on sand dunes, but occasionally in the semi-evergreen forest (Verdcourt, 2000). Root decoctions are used to treat paralysis, epilepsy, convulsions, and spasms, as well as yellow fever and malaria. Various formulations containing *Zornia glochidiata* are also used to treat the common cold, oedema, bronchitis, cor pulmonale, and even gastric cancer (Poschner, 2013). A study has shown that spasmolytic and antibacterial effects of *Zornia glochidiata* support its usage for the treatment of gastrointestinal diseases (Rojas et al., 1999). An n-hexane extract of the plant demonstrated promising anticancer actions by inducing apoptosis in DLA-cells and mice *in vitro* and *in vivo*, with no acute toxicity, which justifies its historic use for the treatment of stomach cancer (Arunkumar *et al*., 2012).

Hence, there are little research conducted on chemical compositions of these two plants. Therefore, this research focuses on the chemical compositions, antibacterial and antioxidant properties of the leaf extracts of the plants as there are very few research reports on the antibacterial and antioxidant properties of the plants' leaves to the best of our knowledge.

MATERIAL AND METHODS

Instruments, Reagents and Chemicals

Gas Chromatography-Mass Spectrometry (Shimadzu, GC-MS QP2010), Gas Chromatograph Trace GC ultra in mode split) equipped with a flame ionization detector (FID), Mass Spectrometer (Agilent Technologies, USA) equipped with a DB-5 capillary column (30m x 0.32 mm ID; ϕ1 µm film thickness), (DPPH) 2,2-diphenyl1-picryl-hydrazyl, were obtained from Sigma Aldrich Chemical Company (St. Louis). Media for bacteria were from Biorad (France). All the other chemicals were of analytical grade and were also obtained from Sigma Aldrich Chemical Company.

Plant Collection and Sample Preparation

The aerial parts of *Indigofera nummulariifolia* and the leaves of *Zornia glochidiata* were collected from various locations in Ilorin, Kwara State, Nigeria. They were identified by the plant taxonomist, Mr. Bolu Ajayi of the Department of Plant Biology, University of Ilorin, where voucher specimens (Herbarium Numbers: UILH/001/1538/2022 and UILH/002/1539/2022 respectively) of the plants were deposited at the herbarium.

Preparation of Plant Extracts

The leaf parts of *I. nummulariifolia* and *Z. glochidiata* were separately air-dried, squeezed, and pulverized into powdery form to increase the surface area of the plant and enhance the efficiency of extraction. The obtained powdery samples of *I. nummulariifolia* were weighed (500 g) and extracted using successive extraction method known as maceration with 500 g in 200 cm^3 of the solvents in increasing order of polarity, (n-hexane, chloroform, ethyl acetate and methanol) respectively (Haruna, 2018). Non polar extracts of *Z. glochidiata* have been reported in previous research (Arunkumar *et al*., 2012), except methanol fraction. Hence, Methanol extract of the plant was reported in this work

Antibacterial Activity

The antibacterial activity was determined using the agar diffusion pour-plate method (Satish and Mahesh, 2008). The organisms used in this study were *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Enterococcus faecalis, and Escherichia coli*. They were clinically isolated and obtained from University of Ilorin Teaching Hospital. The strains were maintained and eventually sub-cultured on nutrient agar. Agar well diffusion method was used to evaluate the antimicrobial activities of the extract as described by Asian National Committee for Clinical Laboratory recommendation, 2000. Culture of 0.1 ml of various organisms in nutrient broth was seeded in molten nutrient agar that had poured and allowed to set in to sterile Petri dish. Wells were made into the set agar using sterile cork borer (6 mm to 8 mm in diameter) and the wells were filled with the extracts solution of 0.1ml (at different concentration for each extract) left to pre-diffuse for 30 min. Dimethyl sulphoxide was used as negative control and Gentamycin (antibacterial) as positive control. The bacteria plates were incubated at 37^{o}C for 24 hour The degree of inhibition was determined by the size of the zone of inhibition measured in mm and were taken as evidence of antimicrobial activity of each of the extracts.

Antioxidant Activity

Since DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical is widely used to test the ability of compounds to trap free radical or donate hydrogen for the evaluation of antioxidant activity, the ability of the plant extracts on DPPH free radicals was assessed by the standard method adopted with modifications (Johnson and Ayoola, 2015). The stock solutions of extracts were prepared in methanol to achieve a concentration of 1 mg/*mL*. Serial dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/*mL*. The absorbance was measured in triplicate at varying concentrations and the mean absorbance was determined. The mixtures were kept in darkness and allowed to stand for 15 min at room temperature, and the absorbance was taken at 517 nm, methanol was used to zero the spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{AO - A1}{AO} \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample. The IC_{50} values (Inhibition Concentration at 50%) were estimated from the % inhibition versus concentration graph.

GC-MS Analysis of the Extracts

Methanol extracts of the two plants were subjected to GC-MS analysis. The injector temperature was set at 250°C. The column oven temperature was held at 40°C for 2 min, and then it was increased to 300°C at a heating rate of 5°C min^{-1}. The carrier gas used was helium (purity 99.99%) at a flow rate of 1.0 ml min^{-1}. The samples were injected in the splitless mode and the splitter was opened after 10 min (delay time). The sample volume in the direct injection mode was 1µl. In the transfer line, temperature is 300°C. The GC-MS was connected with a database of NIST6 main-Mass in order to identify and predict the structure of compounds present.

RESULTS AND DISCUSSION

Antibacterial Activity

The antibacterial activity was measured as zone of inhibition, which is the diameter in (mm) of the clear zone of inhibition of microbial growth that surrounds the well. The methanol extract of *I. nummulariifolia* showed significant activity against *E. coli* while the methanol extract of *Z. glochidiata* was only active against *P. aeruginosa* and *S. aureus* bacteria (Table 1).

Table 1: Results of the Antibacterial Activities of the Methanol Extract of *Indigofera nummulariifolia* and *Z. glochidiata* Leaves

Test organisms	*I. nummulariifolia*			*Z. glochidiata*		
	Methanol extract	Control (mm)	Gentamycin (mm)	Methanol extract	Control (mm)	Gentamycin (mm)
Acinetobacter baunmanni	_	_	10.00	_	_	10.00
Entericoccus Faecalis	_	_	_	_	7.50	21.00
Escherichia coli	5.5	7.5	21.00	_	_	10.50
Klebsiella pneumonia	_	_	4.50	_	_	4.50
Pseudomonas aeruginosa	_	_	11.00	3.50	_	11.00
Staphylococcus aureus	_	_	19.00	10.50	_	19.00

Antioxidant Activity

The ability of the plants' extracts to scavenge DPPH radicals and reduce their effects was analyzed. The results of this analysis are as shown in Tables 2 and Figures 1.

Table 2: Results of the Antioxidant Activity of the Methanol Extract of *Indigofera nummulariifolia* and *Zornia glochidiata* Leaves

Conc. (µg/mL)	*I. nummulariifolia*		*Z. glochidiata*	
	% Inhibition	%I A	% Inhibition	%I A
15.625	10.53	21.66	17.086	15.03
31.25	50.25	38.26	18.345	13.46
62.50	26.28	64.18	19.065	72.66
125	52.98	96.2	28.597	77.34
250	82.50	65.3	6.475	65.3
500	99.98	68.2	7.194	68.2
1000	79.67	71.4	18.885	71.4

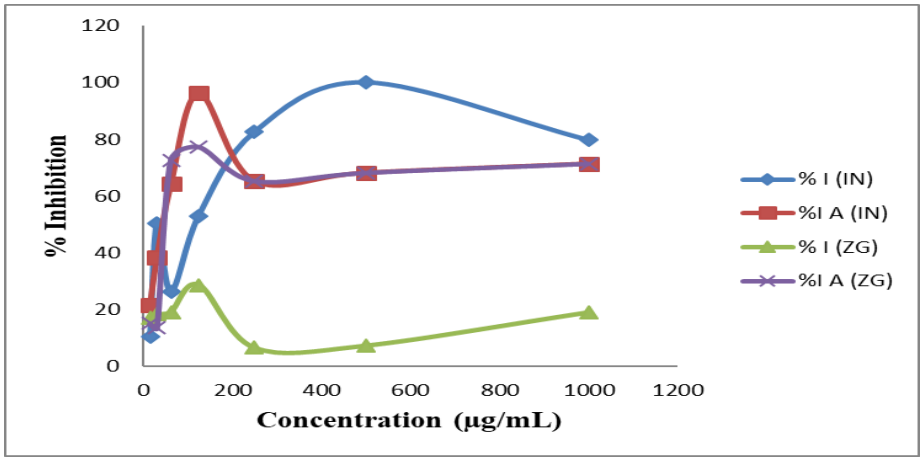


Figure 1: Graph Showing the Antioxidant Activities of Methanol Extracts of *Indigofera nummulariifolia* (IN) *and Zornia glochidiata* (ZG) leaves. (% Inhibition)

Tables 1-2 and Figure 1 showed that the methanol extracts of *Indigofera nummulariifolia* leaves and the methanol extract of *Zornia glochidiata* exhibit significant antioxidant activities by scavenging DPPH radicals in a dose-dependent manner. Methanol extract exhibited antioxidant activity with IC_{50} value at 31.15 µg/mL, comparable with that of the standard control, ascorbic acid (IC_{50} value of 52.10 µg/mL). Based on this result, it is found that the sample contains a pronounced antioxidant property.

GC-MS Results

The major constituents of methanol extract of *Zornia glochidiata* (Table 3) are 4-O-methylmannose (11.88 %), hexadecanoic acid (11.22 %) and 11-octadecenoic acid (5.10 %), and the minor constituents being 1-butanol-3-methylformate (2.17%), 1-ethynyl-3,5-dimethyladamantane (2.34 %), 9,12-octadecadienoic acid (3.92 %), cis-9-hexadecenal (3.20 %), 9-octadeceamide (Z) (2.29 %), heptadecafluorononanoic acid (3.34 %), and fumaric acid (3.56 %). In the same vein, the principal constituents from the methanol extract of *Indigofera nummulariifolia* (Table 4) are 4-((1E)-3-hydroxyl-1-propenyl)-2-methoxy (7.37 %), 6-methyl-cyclodec-5-enol (4.34 %), 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene (7.08 %), 1,7-dioxa-10-thia-4,13-diazacyclo-pentadecane-5,9,12-trione (4.19 %), methyl 4-O-methyl-d-arabinopyranoside (4.42 %), 4-O-

methylmannose (5.21 %), and n-hexadecanoic acid (6.34 %) while the minor constituents are endo-1,5,6,7-Tetramethylbicyclo[3.2.0]hept-6-en-3-ol (2.62 %), benzene methanol (2.15 %), 9,12-octadecadienoic acid (3.42 %) and E-10,13,13,trimethyl-11-tetradecen-1-ol acetate (2.23 %).

Table 3: GC-MS Analysis of Methanol Extract of *Indigofera nummularifolia* Leaves

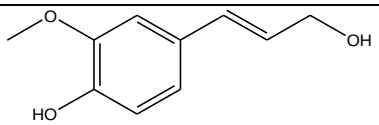
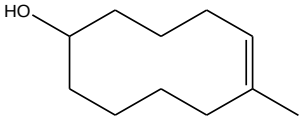
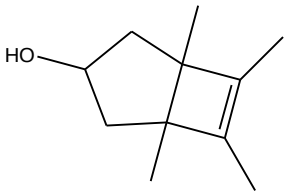
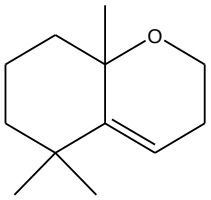
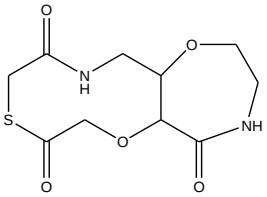
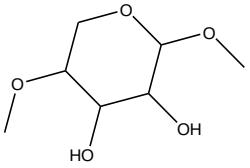
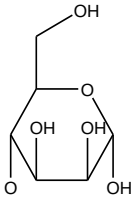
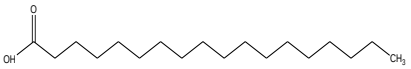
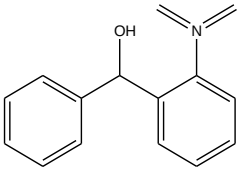
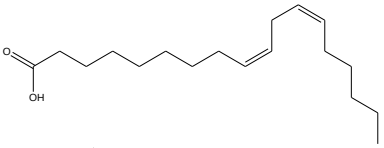
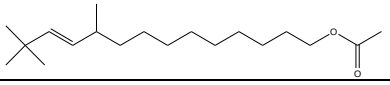
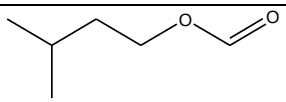
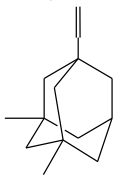
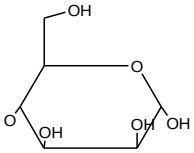
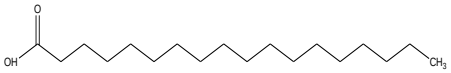
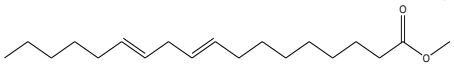
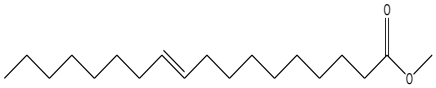
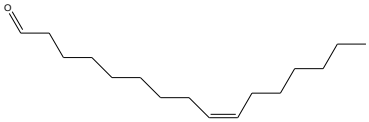
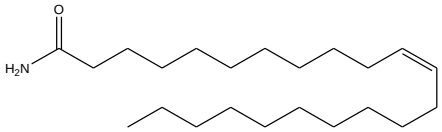
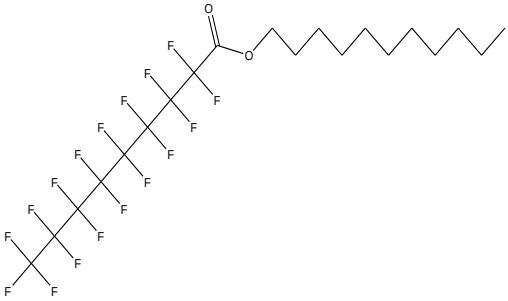
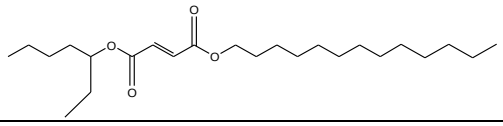
S/N	RT	% AB	Compound name	Formula	M.W	Structure
1	12.533	7.37	4-((1E)-3-hydroxyl-1-propenyl)-2-methoxy	$C_{10}H_{12}O_3$	180	
2	12.681	4.34	6-methyl-cyclodec-5-enol	$C_{11}H_{20}O$	168	
3	12.861	2.62	endo-1,5,6,7-Tetramethylbicyclo[3.2.0]hept-6-en-3-ol	$C_{11}H_{18}O$	160	
4	12.942	7.08	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	$C_{12}H_{20}O$	180	
5	13.100	4.19	1,7-dioxa-10-thia-4,13-diazacyclo-pentadecane-5,9,12-trione	$C_{10}H_{16}N_2O_5S$	276	
6	13.208	4.42	Methyl 4-O-methyl-d-arabinopyranoside	$C_7H_{14}O_5$	178	
7	13.275	5.21	4-O-Methylmannose	$C_7H_{14}O_6$	194	
8	14.313	6.34	n-hexadecanoic acid	$C_{17}H_{34}O_2$	270	
9	14.368	2.15	Benzene methanol	$C_{13}H_{11}NO_3$	229	
10	15.461	3.42	9,12-octadecadienoic acid	$C_{19}H_{34}O_2$	294	
11	16.299	2.23	E-10,13,13,Trimethyl-11-tetradecen-1-ol acetate	$C_{19}H_{36}O_2$	296	

Table 4: GCMS Analysis of the Methanol extract of *Zornia glochidiata* Leaves

S/N	RT	% AB	Compound name	Formula	M.W	Structure
2	6.716	2.17	1-Butanol-3-methylformate	$C_{18}H_{36}O$	268	
15	11.866	2.34	1-ethynyl-3,5-dimethyladamantane	$C_{16}H_{22}O_4$	278	
17	12.312	11.88	4-O-methylmannose	$C_{16}H_{32}O_2$	256	
24	13.991	11.22	Hexadecanoic acid	$C_{20}H_{40}O$	296	
31	15.136	3.92	9,12-Octadecadienoic acid	$C_{18}H_{36}O_2$	284	
32	15.190	5.10	11-Octadecenoic acid	$C_{19}H_{36}O_2$	296	
35	15.448	3.20	Cis-9-hexadecenal	$C_{28}H_{44}N_2O_7$	520	
40	16.986	2.29	9-octadeceamide (Z)	$C_{20}H_{23}F_{17}O_2$	618	
58	21.609	3.34	Heptadecafluorononanoic acid	$C_{20}H_{23}F_{17}O_2$	618	
59	21.781	3.56	Fumaric acid			

CONCLUSION

The methanol extracts from the leaves of *Zornia glochidiata* and *Indigofera nummulariifolia* have been investigated in this research. Though *Indigofera Nummularifolia* methanol extract displayed weak antibacterial activity against the organisms used, the methanol extract of *Z. glochidiata* showed moderate activity against *Staphylococcus aureus* with zone of inhibition of 10.50 mm and significant antioxidant activity with IC_{50} of 31.15 µg/mL compared with standard ascorbic acid (IC_{50} 52.10 µg/mL) revealing the efficacy of this plant either as antioxidant agent or as useful agent in the management of oxidative disorders. The GC-MS reveals bioactive compounds of which the activities of the plants' extract against free radicals might be attributed.

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