

**Chemical constituents, antibacterial, antifungal and antioxidant activities from the leaf extracts of *Drynaria laurentii***

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**ABSTRACT**

The chemical constituents of weighed, air-dried leaf samples of a native Nigerian plant, *Drynaria laurentii*, were examined. The bioactivity of n-hexane, ethyl acetate and methanol extracts of the plants were tested against ten (10) strains of bacteria and fungi, and their antioxidant activities and antimicrobial properties were studied. Ethyl acetate extract of *Drynaria laurentii* leaves exhibited 2,2-diphenyl-1-hydrazine (DPPH) radical scavenging property with Inhibition Concentration at 50% (IC<sub>50</sub>) of 298.912 µg/mL while hexane and methanol extracts of the plant showed no significant antioxidant activity. From the antimicrobial study, the methanol extract of the plant inhibited the growth of all the test organisms at all concentrations while the n-hexane extract specifically inhibited the growth of the test bacteria at 200-25 mg/mL and have low inhibitory effects on all the test fungi. Meanwhile, the ethyl acetate extract inhibited the growth of all the test bacteria from a range of 200–12.5 mg/mL and all the test fungi at the range 200-25 mg/mL. However, some bacteria (6.25 mg/mL) and fungi (12.5-6.25 mg/mL) displayed resistance to the ethyl acetate extract of the plant. The Gas Chromatography-Mass Spectrometry (GC-MS) characterisation of n-hexane leaf extract of *D. laurentii* afforded nineteen (19) compounds with heptadecanal constituting 15.64% as the most abundant constituent of the extract. Ethyl acetate and methanol extracts of the plant afforded eighteen (18) and seventeen (17) compounds with γ-sitosterol (14.34%) and clionasterol (15.32%) as the abundant constituents in both extracts respectively.

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**KEY WORDS:** *Drynaria laurentii*, Antioxidant activity, Antimicrobial properties, γ-sitosterol, urs-12-ene, GC-MS.

## INTRODUCTION

*Drynaria* belongs to the family of Polypodiaceae and its species are characterized by an epiphytic fern with stout, rhizomatous tree trunks and small to large heterophyllous leaves (Singh et al., 2008; Brahmachari, 2010). The plants' species are widely distributed in south eastern Asia and Oceania. Furthermore, two species of *Drynaria* occur in central Africa, with one in Madagascar and the other adjacent islands (Singh et al., 2008). The plant serves as ornamental plants and found in the foothills to submontane elevations across the region of Guinea to Southern Nigeria and with a widespread also elsewhere in tropical Africa (Burkill, 1985). *Drynaria* is used in Chinese medicine as one of the most efficient and important herbs used to treat damaged bones and ligaments. Moreover, *Drynaria* is literally translated "mender of shattered bones" by Chinese practitioners. *Drynaria* is also used to treat kidney disorders, act as kidney tonic, treats liver diseases, diarrhea, toothache, bleeding gums and tinnitus (Lin et al., 2002; Li *et al.*, 1997). Alopecia, a condition connected with hair loss has been successfully treated with *Drynaria* as it stimulates hair growth and strength. Researchers also have revealed the use of *D. laurentii* as sex and heart-tonic (Lin et al., 2002).

This research focuses on the chemical constituents, antimicrobial and antioxidant properties of the plant leaf extracts, as there are very few research reports on the antimicrobial and antioxidant properties of the plant leaves to the best of our knowledge.

## MATERIALS AND METHODS

The aerial parts of the *Drynaria laurentii* were sourced from Ondo and Oyo states, Nigeria. They were identified by the plant taxonomist, Mr. Bolu Ajayi of the Department of Plant Biology, University of Ilorin where

voucher specimen (UIH/007/1238) was deposited in the herbarium. The leaf parts of *D. laurentii* were air-dried and crushed into smaller pieces using mortar and pestle. The plant samples were weighed and extracted using serial exhaustive extraction method by moving from a non-polar solvent (n-hexane) to a medium polar solvent (ethyl acetate) and then to a polar solvent (methanol).

**Phytochemical screening:** Preliminary phytochemical examination of the crude extracts was carried out using the modified methods described by Pranshant *et al.*, 2011.

### Antimicrobial assays

**Test Microorganisms:** Cultures of six human pathogenic bacteria made up of four Gram-negative and two Gram-positive were used for the antibacterial assay. The Gram-negative bacteria are *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while *Bacillus subtilis* and *Staphylococcus aureus* are the Gram-positive bacteria used. The four fungi utilized for the antifungal assay are *Candida albicans*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium notatum*. All the microorganisms used are clinical strains from the Department of Medical Microbiology (University College Hospital, Ibadan) and were screened in the Laboratory of Pharmaceutical Microbiology department, University of Ibadan, Ibadan, Nigeria.

**Media:** Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar (Oxoid Ltd) were used in this study. N-hexane, ethyl acetate and methanol were also used in solubilizing the extracts and act as negative controls in the assays.

**Antimicrobial agents used:** Gentamicin (10 µg/mL) and tioconazole (0.7 mg/mL) were employed as standard reference drugs in these studies.

#### Determination of Antimicrobial activity

##### Agar diffusion-Ditch method (for bacteria):

Overnight, culture of each organism was prepared by taking two wire-loop of the organism from the stock, and each inoculated into 5 mL of sterile nutrient broth and incubated for 24 hrs at 37°C. Then 0.1 mL of each organism was taken from the overnight culture and put into a 9.9 mL of sterile distilled water to obtain 10<sup>-2</sup>M inoculum concentration of the test organism. 0.2 mL was taken from the diluted test organism (10<sup>-2</sup>) into the prepared sterile nutrient agar cooled to about 45°C, then poured into sterile petri dishes and allowed to solidify for about 60 min. A sterile cork borer of 8 mm diameter was used to make 8 wells on the media according to the number of the diluted extracts for the experiment. The graded concentrations (6.25 – 200 mg/mL) of the extracts were put into each well and separated from the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 24 hr at 37°C.

**Agar diffusion-Surface method (fungi):** A sterile sabouraud dextrose agar was prepared accordingly and poured into the sterile plates in triplicates and was properly solidified. 0.2 mL of the 10<sup>-2</sup> inoculum concentration of the test organism was spread on the surface of the agar. Eight wells were bored by using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were separately put into each well with the controls. All the plates were left on

the bench for 2 hrs to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25°C for 72 hrs (Akinpelu and Onakoya. 2006).

#### Antioxidant activity

**Antioxidant Activity:** Since DPPH is widely used to test the ability of compounds to trap free radical or hydrogen donors, and to evaluate antioxidant activity, the ability of the plant samples to scavenge DPPH free radicals was assessed by the standard method adopted with suitable modifications (Sies, 1997). The stock solutions of extracts were prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 µg/mL. The absorbance was measured in triplicate at varying concentrations and the mean absorbance was determined. Parallel to examination of the antioxidant activity of plant extracts, the value for the standard compound (Ascorbic acid) was obtained (Table 3.16) and compared to the values of the antioxidant activity, the percentage inhibitions of the serial concentrations of the methanol DPPH extracts and that of the standard which was determined at different concentrations using the expression below.

$$\% \text{ inhibition} = \left( \frac{A \text{ of control} - A \text{ of sample}}{A \text{ of control}} \right) \times 100$$

The IC<sub>50</sub> values (Inhibition Concentration at 50%) were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

**GC-MS analysis of the extracts:** GC-MS analysis of the *D. laurentii* extracts was performed with Agilent

19091GC plus automatic sampler system coupled with a quadruple Mass Spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenylmethylsilox, (length; 30m x 250µm; film thickness 0.25µm). Samples were injected at a temperature of about 250 °C with a split ratio of 10:1, the flow rate of helium being 1mL/min.

## RESULTS AND DISCUSSION

### 1. Phytochemical Screening

The preliminary phytochemical screening of the crude extracts of *D. laurentii* revealed the presence of bioactive compounds such as phenolics, tannins, flavonoids, fats and oils, terpenoids, alkaloids, steroids, glycosides and carbohydrate as shown in Table 1. The presence of these bioactive compounds is an indication that these plants may possess some pharmacological activities.

**Table 1 : Phytochemical Screening of hexane, ethyl acetate and methanol extracts of the leaves of *D. laurentii***

Chemical constituents	Hexane extract	Ethyl acetate extract	Methanol extract
Alkaloids	+	-	+
Glycoside	+	+	+
Carbohydrate	-	-	+
Flavonoids	+	+	+
Tannins	+	-	+
Saponins	-	-	+
Terpenoids	+	+	-
Steroids	+	-	+
Anthraquinone	-	-	+
Fat & Oils	+	+	-
Phenols	-	+	+
Protein	-	-	-

**KEYS:** + = Present; - = Absent

### 2. Antimicrobial activity

The three crude extracts *D. laurentii* gave a clear zone of inhibition against the growth of the test bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*). N-hexane extract inhibited the growth of the test bacteria at moderate to high concentration (see Table 2). It inhibited *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*,

*Pseudomonas aeruginosa* and *Klebsiella pneumoniae* fairly at concentration range of 200-25 mg/mL and have low inhibitory effects on all test fungi. The ethyl acetate extract of *D. laurentii* leaves inhibited the growth of all test bacteria from a range of 200–12.5 mg/mL and all the test fungi at the range 200-25 mg/mL. The ethyl acetate extract inhibited all the test organisms at concentration range of 200-25 mg/mL. However, bacteria like *Bacillus subtilis*, *Pseudomonas*

*aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* show resistance to the ethyl acetate extract at concentration of 6.25 mg/mL. The fungi *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer* are resistant to the extract at concentration range of 12.5-6.25 mg/mL. Methanol

extract of the aerial parts of *D. laurentii* inhibited the growth of all the test organisms at all concentrations. These inhibitory properties give credence to the fact that leaf parts of *D. laurentii* exhibit antibacterial and antifungal activities and hence can be used for the treatment of various illnesses caused by the strains of bacteria and fungi used in this research.

**Table 2: Antimicrobial activity of n-hexane, ethyl acetate, and methanol extracts of *D. laurentii* leaves**

Microorganism	Mean zone of Inhibition (mm)																							
	n-hexane extract				Ethyl acetate extract				Methanol extract															
<i>S. aureus</i>	21	18	14	12	-	-	-	38	26	20	18	14	12	10	-	40	27	24	20	18	14	11	-	40
<i>E. coli</i>	19	15	12	10	-	-	-	36	24	20	18	16	14	10	-	38	25	22	19	16	13	10	-	40
<i>B. subtilis</i>	20	18	14	10	-	-	-	40	22	18	15	12	10	-	-	40	27	24	20	17	14	10	-	40
<i>P. aeruginosa</i>	16	14	12	10	-	-	-	38	20	18	14	14	10	-	-	38	25	21	18	14	12	10	-	40
<i>K. pneumoniae</i>	17	14	12	10	-	-	-	38	20	18	14	12	10	-	-	38	25	21	18	15	13	10	-	38
<i>S. typhi</i>	15	14	10	-	-	-	-	36	18	16	14	12	10	-	-	38	24	20	17	14	12	10	-	38
<i>C. albicans</i>	15	12	10	-	-	-	-	26	18	14	12	10	-	-	-	28	21	18	16	14	12	10	-	28
<i>A. Niger</i>	15	12	10	-	-	-	-	26	18	16	12	10	-	-	-	28	20	18	16	14	12	10	-	28
<i>P. notatum</i>	15	12	10	-	-	-	-	28	16	14	12	10	-	-	-	28	21	18	16	14	12	10	-	26
<i>R. stolonifera</i>	15	14	10	-	-	-	-	26	17	14	12	10	-	-	-	28	20	18	16	14	12	10	-	28
Conc of extracts (mg/mL)	200	100	50	25	12.5	6.25	-ve	+ve	200	100	50	25	12.5	6.25	-ve	+ve	200	100	50	25	12.5	6.25	-ve	+ve

Key: +ve = Gentamicin 10 µg/mL (bacteria), Tioconazole 70% (fungi); -ve = Solvent of dilution

### 3. Antioxidant activities

The ability of the plants' extracts (n-hexane, ethyl acetate and methanol) to scavenge DPPH radicals and

reducing their effects was analyzed. The results of this analysis are as shown in the tables and figures below:

**Table 3: Absorbance (A1-3) and percentage inhibition of Ascorbic Acid (%I of A) Standard for DPPH Antioxidant activity of the leaf extract of *D. laurentii*. The absorbance of control is 1.265**

Conc. ( $\mu\text{g/mL}$ )	A1	A2	A3	AV $\pm$ SD	%I of A
1000	0.138	0.138	0.140	0.139 $\pm$ 0.0012	89.02
500	0.150	0.150	0.150	0.15 $\pm$ 0.000	88.14
250	0.161	0.162	0.160	0.161 $\pm$ 0.001	87.26
125	0.180	0.180	0.180	0.180 $\pm$ 0.000	85.79
62.5	0.193	0.195	0.194	0.194 $\pm$ 0.001	84.26
31.25	0.245	0.245	0.245	0.245 $\pm$ 0.000	80.67
15.62	0.311	0.311	0.311	0.311 $\pm$ 0.000	75.44
7.81	0.453	0.452	0.454	0.453 $\pm$ 0.001	64.18
3.9	0.782	0.781	0.78	0.781 $\pm$ 0.001	38.26
1.95	0.991	0.991	0.991	0.991 $\pm$ 0.000	21.66

AV= Average Value of Absorbance; SD = Standard Deviation

**Table 4: DPPH Antioxidant activity and %inhibition of hexane extract of *D. laurentii*. 0.432 is the absorbance of control.**

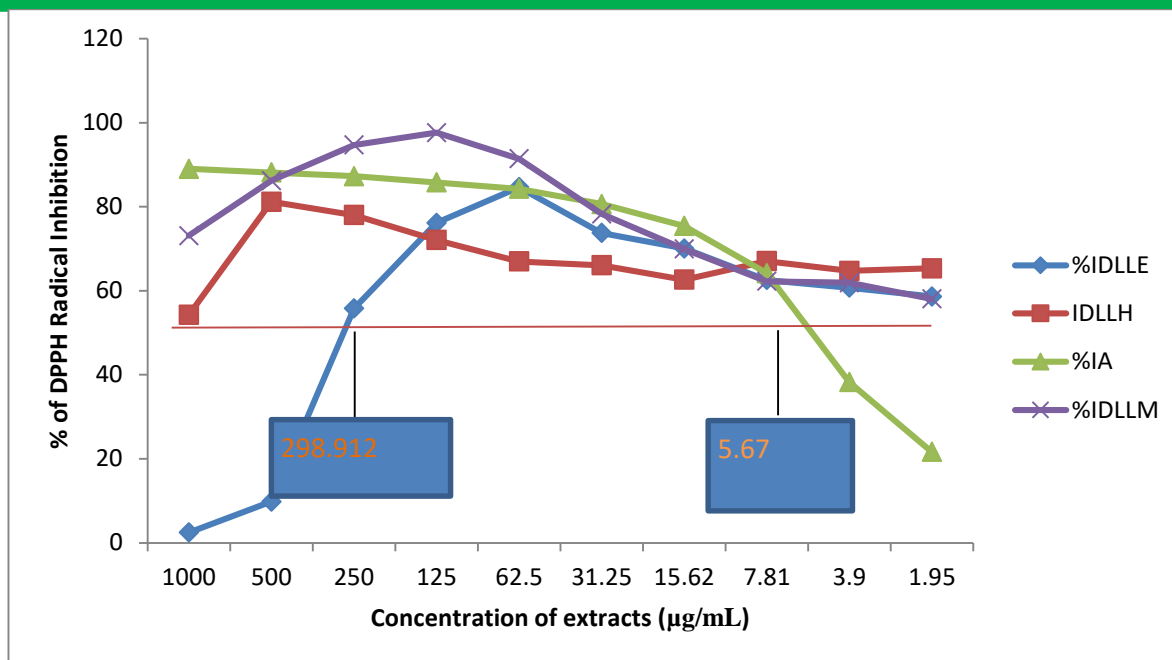
Conc. ( $\mu\text{g/mL}$ )	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.181	0.203	0.209	0.198 $\pm$ 0.0147	54.24
500	0.080	0.082	0.082	0.081 $\pm$ 0.0012	81.17
250	0.095	0.095	0.095	0.095 $\pm$ 0.0000	78.01
125	0.116	0.123	0.123	0.121 $\pm$ 0.00040	72.07
62.5	0.140	0.144	0.144	0.143 $\pm$ 0.0023	66.96
31.25	0.147	0.147	0.146	0.147 $\pm$ 0.0006	66.05
15.62	0.16	0.158	0.166	0.161 $\pm$ 0.0042	62.65
7.8	0.137	0.143	0.147	0.142 $\pm$ 0.0050	67.05
3.9	0.151	0.152	0.154	0.152 $\pm$ 0.0015	64.74
1.95	0.150	0.150	0.149	0.149 $\pm$ 0.0006	65.35

**Table 5: DPPH Antioxidant activity and %inhibition of ethyl acetate extract of *D. laurentii*. 0.432 is the absorbance of control.**

Conc.( $\mu\text{g/mL}$ )	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.422	0.421	0.421	0.421 $\pm$ 0.0006	2.46
500	0.389	0.390	0.390	0.389 $\pm$ 0.0006	9.79
250	0.190	0.194	0.189	0.191 $\pm$ 0.0026	55.78
125	0.102	0.103	0.104	0.103 $\pm$ 0.0010	76.15
62.5	0.065	0.067	0.067	0.066 $\pm$ 0.0012	84.64
31.25	0.116	0.112	0.112	0.113 $\pm$ 0.0023	73.76
15.62	0.129	0.129	0.130	0.129 $\pm$ 0.0006	70.06
7.8	0.162	0.162	0.161	0.161 $\pm$ 0.0006	62.57
3.9	0.170	0.169	0.170	0.169 $\pm$ 0.0006	60.72
1.95	0.179	0.178	0.179	0.179 $\pm$ 0.0006	58.64

**Table 6: DPPH antioxidant activity and % inhibition of methanol extract of *D. laurentii*. 0.432 is the absorbance of control.**

Conc.( $\mu\text{g/mL}$ )	Absorbance	Absorbance	Absorbance	Mean absorbance	% Inhibition
1000	0.138	0.139	0.140	0.138 $\pm$ 0.0012	89.03
500	0.15	0.15	0.15	0.15 $\pm$ 0.0000	88.14
250	0.162	0.162	0.160	0.161 $\pm$ 0.0001	87.27
125	0.180	0.180	0.180	0.18 $\pm$ 0.0000	85.77
62.5	0.195	0.195	0.194	0.194 $\pm$ 0.0010	84.66
31.25	0.245	0.245	0.245	0.245 $\pm$ 0.0000	80.63
15.62	0.311	0.311	0.311	0.311 $\pm$ 0.0000	75.41
7.8	0.453	0.452	0.454	0.453 $\pm$ 0.0001	64.18
3.9	0.782	0.781	0.780	0.781 $\pm$ 0.0001	38.26
1.95	0.991	0.991	0.991	0.991 $\pm$ 0.0000	21.66



KEYS: % I= Percentage inhibition;; IDLLH – N-hexane extract inhibition; IDLLE – Ethyl acetate extract inhibition; IDLLM – Methanol extract inhibition; IA= Ascorbic acid (reference) inhibition

**Figure 1: Antioxidant activity of leaf extracts of *D. laurentii***

The antioxidant activity of *D. laurentii* showed that only the ethyl acetate extract possessed significant DPPH free radical scavenging property when compared to the control Ascorbic acid. GC-MS analyses of hexane extract of *D. laurentii* leaves revealed hexadecanoic acid, octadecamethyl-cyclonilosiloxane (phytol), 2-dodecenoic acid, heptadecanal and 2-methyl hexacosane as the major compounds with their corresponding percentage of abundance of 4.73 %,

15.29 %, 6.57 %, 15.64 % and 14.21 %. Further, hexadecanoic acid (13.71 %), phytol (12.60 %),  $\gamma$ -sitosterol (14.34%),  $\alpha$ -amyrin (7.90%) and  $\beta$ -amyrin (7.55%) were the principal compounds revealed in ethyl acetate extract of the plant, while oleyl amide (15.16%), clionasterol (15.32%) and urs-12-ene (14.52%) were the principal constituents present in methanol extract of the plant (Table 7 – 9).

#### 4. GC-MS Results



**Table 7: GC-MS Analysis of N-hexane leaf extract of *Drynaria laurentii***

S/N	Compound name	Peak Area (%)	Molecular Weight (g/mol)	Retention time
1	N-Hexadecanoic acid	4.73	256	16.708
2	3,4-dimethylcyclohexanol	1.51	128	18.299
3	1,9-Nonadiol,dimethyl Sulfonate	3.15	316	18.554
4	1,1,1,5,7,7-Heptamethyl-3,3-bis(trimethylsiloxy) tetrasiloxane	3.34	758	31234
5	18-methyl-nonadiene-1,2dio,trimethylsilylether	3.16	458	22.373
6	Bisoflex 81	8.42	390	22.058
7	Octadecamethyl-cyclonosiloxane	15.29	1022	23.434
8	Sulforous acid,octadecyl-2-propylester	10.08	376	24.719
9	2-Dodecenoic acid	6.57	198	25.484
10	9,10-Dibromopentacosane	6.72	510	25.636
11	Heptadecanal	15.64	254	25.967
12	2-Methylhexacosane	14.21	380	26.658
13	Z,Z-4,16-Octadecadien hexacosane	2.38	308	24.102
14	Phthalic acid, 2-chloropropyl ethylester	0.91	270	24.102
15	1-Decyl iodide	0.54	268	11.457
16	2,2-Dimethyl-3-heptanone	0.66	142	14.699
17	Allyl glycol	0.72	102	15.256
18	1-Iodononane	0.75	254	17.133
19	4-Methoxy-2-butyne-1-ol	0.67	100	19.148

**Table 8: GC-MS Analysis of ethyl acetate leaf extract of *Drynaria laurentii***

S/N	Compound Name	Peak Area %	Molecular Weight (g/mol)	Retention time
1	2-Bornanone	8.43	152	5.315
2	Menthol	2.25	156	5.724
3	Hexahydrofarnesyl Acetone	1.15	268	15.257
4	n-Hexadecanoic acid	13.71	256	16.764
5	Ethylpalmitate	1.33	284	17.039
6	Phytol	12.60	296	17.039
7	(2,2,6-Trimethyl-bicyclo [4.1.0]hept-1-yl)-methanol	3.75	168	18.565
8	Phytol acetate	5.11	338	19.306
9	9-Octadecenamide	488	281	20.525
10	Bisoflex- 81	1.90	562	22.057
11	Gama-Sitosterol	14.34	414	22.720
12	Beta-Amyrin	7.90	426	23.367
13	Alpha-Amyrin	7.55	426	24.258
14	Tetratetracontane	4.33	618	24.724
15	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)	1.71	426	25.007
16	Globulol	2.56	222	25.601
17	Hexatriacontane	4.36	506	26.660
18	3,7-Dimethylundecane	0.58	184	14.701

**Table 9: GC-MS Analysis of methanol leaf extract of *Drynaria laurentii***

S/N	Compound Name	Peak Area %	Molecular Weight (g/mol)	Retention time
1	Diethyl Phthalate	1.10	222	11.220
2	3-Phenoxybenzaldehyde	1.02	198	13.001
3	Methyl hexadecanoate	1.57	270	16.255
4	n-Hexadecanoic acid	4.75	256	16.708
5	Phytol	2.67	296	18.295
6	Olealdehyde	1.35	266	18.840
7	Stearic amide	1.14	283	18.88
8	Oleyl amide	15.16	281	20.530
9	9-t-Butyltricyclo [4.2.1.12,5] decane-9,10-diol	5.83	224	22.049
10	Octadecamethyl- cyclononasiloxane	2.76	666	22368
11	Betulinol	1.94	442	22.680
12	Clionasterol	15.32	414	23.070
13	1H-Cycloprop[e]azulene decahydro-1,1,7-trimethyl-4-methylene	11.79	204	23.161
14	Octadecamethyl- Cyclononasiloxane	6.75	666	23.47
15	Urs-12-ene	14.55	410	23.996
16	Octadecamethyl- Cyclononasiloxane	5.96	666	24.440
17	2-methyloctacosane	1.29	408	26.637

## CONCLUSION

The leaf parts of *Drynaria laurentii* have been investigated in this research and preliminary phytochemical screening of the crude extracts shows the presence of bioactive compounds -that are of medicinal uses- such as tannins, glycosides, flavonoids, saponin, phenolics, terpenoids, steroids and anthraquinone. Antimicrobial activity of crude extracts from *Drynaria laurentii* against all the test bacteria and fungi was found to be interesting at moderate to high concentration which justifies the ethnomedicinal uses of the plant for treating some diseases attributed to bacteria and fungi. *D. laurentii* extracts inhibited the growth of the organisms at some characteristic concentrations. The GC-MS reveals various peaks of bioactive compounds

of which the activities of the plants' extracts against bacteria and fungi, as well as their activities against free radicals, may be attributed. The most prominent compound with probable synergistic effect with all other compounds present in smaller quantities in the extracts proffers an explanation to the activity of the whole plant against the test organisms (bacteria and fungi) or free radical (DPPH).

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