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## Phytochemical analysis and antimicrobial activity of *Aframomum chrysanthum* seed extracts

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### Abstract

Three extracts of *Aframomum chrysanthum* seeds were investigated for antimicrobial activity using disc diffusion method. Varied concentration-dependent antimicrobial activities were exhibited by n-hexane and ethylacetate extracts against the investigated pathogenic microorganisms especially *Staphylococcus aureus*. Susceptibility testing conducted on all the extracts showed that n-hexane extract had moderate antimicrobial activity at 200 mg/ml against *Staphylococcus aureus*, *Candida albicans* and *Trichophyton rubrum*. All the isolates were observed to be resistant to the methanol extract. n-Hexane extract displayed potent antimicrobial activity against *Trichophyton rubrum* when compared to the standard antifungal drug (fungisol) used in the investigation. Phytochemical analysis carried out on the n-hexane extract of *Aframomum chrysanthum* seed, revealed the presence of eighty-one (81) compounds made up of phytosterols, alkaloids, anthraquinone, phospholipids and fatty acids. The presence of these phytochemicals is an indication that the plant could be used as antimicrobial agent.

**Keywords:** *Aframomum chrysanthum*, antimicrobial activity, seed, microorganism, phytochemical analysis

### Introduction

The appearance of drugs resistant microorganisms in medical and healthcare environment requires continuous investigation and identification of new effective natural products with potential of solving the problem. In recent years, there has been a renewed focus on herbs and natural product to derive medicines, both for public health and other uses. One of the medicinal plants that has served several traditional therapeutic purposes is *Aframomum chrysanthum* J. M. Lock.

*Aframomum chrysanthum* J. M. Lock belongs to the genus *Aframomum*. *Aframomum* has over seventy species including *chrysanthum*. *Aframomum chrysanthum* is a member of the family Zingiberaceae which is also known as 'the ginger family' [1]. *Aframomum* is the common name of *A. chrysanthum*. In Nigeria, it is commonly called 'Banga spice'. *Aframomum chrysanthum* is a perennial plant that is commonly found in tropical African forests and savannah, where they could be found as dense clumps. Wildlife animals such as elephants and gorillas feed on the leaves and shoots of the plant. The plant is essentially used as spice in drinks and foods. Extracts from *A. chrysanthum* seeds (Figure 1) have been found to be useful antidiarrhea supplements [2]. The reported presence of phenols in the seed of *A. chrysanthum* suggests that the plant could be an antimicrobial agent because phenolic compounds have been widely used as disinfectants and standards for comparison of bactericides [3]. Very few works on *A. chrysanthum* have been reported unlike *A. melegueta* which is a more popular member of the *Aframomum* class.

This study evaluated the antimicrobial activities of n-hexane, ethylacetate and methanol extracts of *Aframomum chrysanthum* seeds against selected pathogenic microorganisms and the phytochemicals of n-hexane extract of the seed.



**Fig 1:** *A. chrysanthum* seeds

## Materials and Methods

### Solvents

Solvents used for extraction (n-hexane, ethylacetate and methanol) were redistilled before use. Analytical grade (Sigma-Aldrich) of dimethyl sulphoxide (DMSO) was used as dissolving and diluting solvent for antimicrobial analysis.

### Preparation of extracts

Dry seeds of *A. chrysanthum* were bought from Mile One market in Port Harcourt, Rivers State, Nigeria. Mechanical grinding machine was used to pulverize the seeds. Maceration method of extraction was used to prevent denaturation of thermo-labile phytochemicals in the plant [4]. n-Hexane (950 ml) was added to the pulverized seeds (797.8 g) and the resulting mixture was stirred repeatedly and then allowed to stay for 48 hours. The dark-coloured mixture was filtered to obtain n-hexane extract which was further concentrated using a rotatory evaporator at 36 – 40 °C. This process was repeated 4 times. The combined concentrated n-hexane extract was kept at room temperature to dry and a weight of 21.7 g (2.72% yield) was obtained. The colour of the oily extract was dark brown. The process described above was repeated on the residue using ethylacetate and methanol sequentially. The extracts obtained were 54.88 g (6.88% yield) and 27.76 g (3.48% yield) solids.

### Susceptibility testing

Pathogenic microorganisms used for the susceptibility testing included two Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*), two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Shigella dysenteriae*), a yeast (*Candida albicans*), and a fungus (*Trichophyton rubrum*). The identity of the microorganisms was confirmed through macroscopic and microscopic examinations (catalase, oxidase, motility, citrate utilization, salt tolerance, starch hydrolysis, indole production, Methyl Red-Vogues Proskauer (MRVP) and blood haemolysis). All the microorganisms used were isolated and identified at the Department of Microbiology, Rivers State University, Nigeria.

Susceptibility of the microorganisms to *A. chrysanthum* seed extracts and selected standard antimicrobial drugs (as positive control) was determined using the disc diffusion method [5, 6]. The disc diffusion method was adopted in the research work due to its ease of application and the dependability of its results. Broth cultures of the bacteria were prepared by inoculating a colony of each microorganism, separately, into 10 ml sterile nutrient broth tubes. Broth cultures of the yeast and fungus were prepared by inoculating a colony of the yeast

and colonial material of the fungus into separate potato dextrose broth (PDB) tubes. Inoculated PDB and nutrient broth tubes were incubated at 37 °C for about 24 hours. After incubation, the broth cultures of the bacteria were swab plated, separately, onto sterile nutrient agar plates. This process was also done for *C. albicans* and *T. rubrum*. The Sabouraud dextrose agar plates used corresponded to the number of the investigated extracts, the number of microorganisms involved in the testing and the number of positive controls used.

Filter paper discs impregnated separately with the test extracts (200 mg/ml) and standard antimicrobial drugs respectively, were placed on the swab inoculated plates with the aid of sterile forceps. The plates were incubated at 37 °C for 24 - 48 hours, after which the zones of inhibition around the discs were measured and recorded. This was done by using a meter rule to measure their diameters in millimeter (mm).

The extracts of *A. chrysanthum* seed having zone of inhibition against the microorganisms were noted. Different concentrations of the bioactive extracts were prepared and used for further susceptibility testing against the susceptible microorganisms. Various concentrations of each of the extract (200, 100, 50, 25, 12.5 and 6.25 mg/ml) were prepared by dissolving 1 g of each extract in 5 ml of DMSO to give a standard solution of 200 mg/ml which was serially diluted to appropriate concentrations of 100, 50, 25, 12.5 and 6.25 mg/ml.

Bioactive extracts selected for the second round of testing were n-hexane and ethylacetate extracts. Pathogenic microorganisms used for the second round of testing were *S. aureus*, *C. albicans* and *T. rubrum*. Susceptibility testing was done in triplicates for each investigated extract concentration, and zone of inhibition readings taken. The recorded zone of inhibition is the mean of three readings plus the standard deviation. The minimum inhibitory concentration (MIC) which is the lowest concentration of the extracts that inhibited the growth of the microbe was also noted.

### Phytochemical analysis

#### Phospholipids analysis

The modified method employed in n-hexane extract phospholipids content determination was taken from Raheja *et al.* [7]. A stream of nitrogen gas was passed through the test tube containing 10 mg of n-hexane extract to completely remove any leftover solvent in order to ensure complete dryness of the extract for the phospholipids analysis. Then 0.40 ml of chloroform and 0.10 ml of chromogenic substance was added to a test tube and heated to 100 °C in a water bath for 80 secs. n-Hexane (5 ml) was added to the content after it was allowed to cool at room temperature. The content was gently shaken repeatedly and left for a while for the aqueous and solvent layers to separate. Gas chromatography and pulse flame photometric detector was employed in the chromatographic analysis of the recovered n-hexane extract which was concentrated to 1 ml.

#### Phytosterols analysis

n-Hexane extract (0.5 g) was dried by passing nitrogen stream through it. The extract was put into a screw-capped test tube and saponified at 95 °C for 30 mins using 3 ml of 10% KOH in ethanol to which 0.2 ml of benzene (to make sure miscibility occurred) and 3 ml of deionized water was added.

n-Hexane (2 ml) was utilized in removing non-saponifiable materials. n-Hexane (2 ml each) was used to carry out three extractions for 1 hour, 30 mins and 40 mins respectively to achieve complete extraction of the sterols. The combined n-hexane extracts were concentrated to 2 ml and poured into Agilent vial which was used for gas chromatographic analysis [8].

#### Fatty acid methyl esters analysis

About 50 mg of n-hexane extract was esterified at 95 °C for 5 mins with 3.4 ml of 0.5 M KOH in dry methanol. HCL (0.7 M) was used to neutralize the mixture and 3 ml of 14% boron trifluoride in methanol was added. The mixture was heated at 90 °C for 5 mins to reach complete methylation. n-Hexane was used to extract the fatty acid methyl esters three times from the mixture. The extract was concentrated to 1 ml for gas chromatography analysis and 1 µl was introduced into the injection port of a gas chromatograph [8].

#### Alkaloids analysis

n-Hexane extract was refrigerated at >4 °C before the analysis. The extract was added to 10 ml of 70% methanol in a borosilicate beaker and extraction done on the mixture at room temperature for 20 mins in an ultrasonic bath. The mixture was later centrifuged at 10000 rpm for 10 mins twice and the supernatant collected and filtered with a 0.22 µm membrane filter. Standards of different concentrations were made ready for introduction into a high-performance liquid chromatography (HPLC) system for calibration and correlation coefficient establishment; after which the sample was fed into the HPLC system following similar procedure used for the standard mixtures [8].

#### Anthraquinones analysis

Analysis of anthraquinones of the n-hexane extract of *A. chrysanthum* seed was performed using gas chromatography - mass spectrometer (GC-MS) HP 5890 model powered with HP Chem Station Rev. A 09.01 (1206) Software. The carrier gas used was nitrogen with inlet temperature of 250 °C and column dimension of 30 m × 0.25 mm × 0.25 µm. n-Hexane extract of *A. chrysanthum* seed (0.01 g) was put in a test tube. A stream of nitrogen gas was passed through the sample to dry it completely before analysis. Chloroform (0.40 ml) and 0.10 ml of chromogenic solution was added to the sample in the test tube and it was heated to 100 °C in water bath for 2 mins. The sample was then allowed to cool to room temperature and 6 ml of n-hexane was added and stirred for 5 mins. The n-hexane layer was collected after cooling and concentrated to 0.2 ml for gas chromatography analysis using pulse flame photometric detector [8].

## Results and Discussion

The n-hexane extract showed antimicrobial activities against Gram-positive bacterium - *S. aureus*, yeast - *C. albicans*, and fungus - *T. rubrum* (Table 1). Zones of inhibition of the extract against *S. aureus*, *C. albicans*, and *T. rubrum* were 17.33±1.70, 10.67±0.47 and 10.67±1.63 mm, respectively. Zero inhibition was observed for the extract against Gram-positive bacterium - *B. cereus*, Gram-negative bacteria - *P. aeruginosa* and *S. dysenteriae*. Ethylacetate extract was active against *S. aureus* only. The zone of inhibition of the extract against *S. aureus* was 11.33±0.47 mm. It can be observed that the methanol extract was not active against any of the microorganisms. Thus, all the microbes were resistant to the methanol extract (Table 1).

A comparison of the susceptibility of the pathogenic microorganisms to the investigated extracts and the standard drugs revealed that the microorganisms were more resistant to the extracts than the standard drug used; except for n-hexane extract which had slightly higher inhibition than the standard drug on *T. rubrum*.

*S. aureus* has many strains. Some strains produce toxins that can cause staphylococcus food poisoning, toxic shock syndrome or scalded skin syndrome. Many strains of *S. aureus* are resistant to antibiotics [9]. Table 2 shows the susceptibility of *S. aureus* to n-hexane and ethylacetate extracts when different concentrations were used. n-Hexane extract at 200 to 6.25 mg/ml had average zone of inhibition range of 19.00±0.25 to 8.00±0.47 mm. Ethylacetate extract at 200, 100 and 50 mg/ml had zones of inhibition of 12.00±1.41, 11.00±0.82 and 9.00±0.47 mm respectively, while the organism was observed to be resistant to the extract at 25, 12.5 and 6.25 mg/ml. Minimum inhibitory concentrations were 6.25 and 50 mg/ml for n-hexane and ethylacetate extracts, respectively. From Table 3, it can be observed that the n-hexane extract at 200 mg/ml had 10.67±0.47mm average zone of inhibition while the extract at 100, 50, 25, 12.5 and 6.25 mg/ml had average zones of inhibition of less than 10 mm against *C. albicans*.

Antifungal activities of various concentrations of n-hexane extract to the pathogenic fungi, *T. rubrum*, were quite marginal (Table 4). Zone of inhibition observed for the extract at 200 mg/ml was 10.67±0.47 mm while the extract at lower concentrations of 100, 50, 25, 12.5 and 6.25 mg/ml had inhibition zones of about 8 mm. *T. rubrum* was found to be resistant to ethylacetate extract at all concentrations. Based on the result of the antimicrobial activity of *A. chrysanthum* seed extracts against *S. aureus*, *C. albicans* and *T. rubrum*, it can be deduced that the seeds of *A. chrysanthum* have potential as antimicrobial agent, especially with the emergence of multi-drug-resistant microorganisms.

**Table 1:** Susceptibility of pathogenic microorganisms to *A. chrysanthum* seed extracts at 200 mg/ml

| Microorganisms        | Zone of inhabitation (mm) |                       |                  |                |      |
|-----------------------|---------------------------|-----------------------|------------------|----------------|------|
|                       | n-Hexane extract          | Ethyl acetate extract | Methanol extract | Standard drugs | DMSO |
| <i>B. cereus</i>      | NI                        | NI                    | NI               | 24±0 (Rf)      | NI   |
| <i>S. aureus</i>      | 17.33±1.70                | 11.33±0.47            | NI               | 22±0 (Sm)      | NI   |
| <i>P. aeruginosa</i>  | NI                        | NI                    | NI               | 30±0 (Cp)      | NI   |
| <i>S. dysenteriae</i> | NI                        | NI                    | NI               | 221±0 (Cp)     | NI   |
| <i>C. albicans</i>    | 10.67±0.47                | NI                    | NI               | 18±0 (Ny)      | NI   |
| <i>T. rubrum</i>      | 10.67±1.63                | NI                    | NI               | 10±0 (Fg)      | NI   |

**Key:** NI- No Inhibition, Rf – Rifampicin (10 µg/ml), Sm – Streptomycin (30 µg/ml), Cp – Ciprofloxacin (10 µg/ml), Ny – Nystatin (100 µg/ml), Fg – Fungisol (200 mg/ml), DMSO - Negative control

**Table 2:** Susceptibility of *S. aureus* to *A. chrysanthum* seed extracts

| Concentration (mg/ml) | Zone of inhibition (mm) |                      |
|-----------------------|-------------------------|----------------------|
|                       | n-Hexane extract        | Ethylacetate extract |
| 200                   | 19.00±0.82              | 12.0±1.41            |
| 100                   | 12.33±0.47              | 11.0±0.82            |

|      |            |          |
|------|------------|----------|
| 50   | 10.00±0.00 | 9.0±0.47 |
| 25   | 9.00±0.47  | NI       |
| 12.5 | 9.00±0.47  | NI       |
| 6.25 | 8.00±0.47  | NI       |
| NC   | NI         | NI       |

**Key:** NC - Negative Control (DMSO); NI - No Inhibition

**Table 3:** Susceptibility of *C. albicans* to *A. chrysanthum* seed extracts

| Concentration (mg/ml) | Zone of inhibition (mm) |                      |
|-----------------------|-------------------------|----------------------|
|                       | n-Hexane extract        | Ethylacetate extract |
| 200                   | 10.67±0.47              | NI                   |
| 100                   | 9.67±0.47               | NI                   |
| 50                    | 8.33±0.47               | NI                   |
| 25                    | 8.33±0.47               | NI                   |
| 12.5                  | 8.00±0.00               | NI                   |
| 6.25                  | 8.00±0.00               | NI                   |
| NC                    | NI                      | NI                   |

**Key:** NC - Negative Control (DMSO), NI - No Inhibition

**Table 4:** Susceptibility of *T. rubrum* to extracts of *A. chrysanthum* seed extracts

| Concentration (mg/ml) | Zone of inhibition (mm) |                      |
|-----------------------|-------------------------|----------------------|
|                       | n-Hexane extract        | Ethylacetate extract |
| 200                   | 10.67±0.47              | NI                   |
| 100                   | 8.67±0.47               | NI                   |
| 50                    | 8.33±0.47               | NI                   |
| 25                    | 8.00±0.00               | NI                   |
| 12.5                  | 8.00±0.00               | NI                   |
| 6.25                  | 8.00±0.00               | NI                   |
| NC                    | NI                      | NI                   |

**Key:** NC - Negative Control (DMSO), NI - No Inhibition

Table 5 shows 6 phospholipids found in the n-hexane extract.

**Table 5:** Phospholipids of n-hexane extract of *A. chrysanthum* seeds

| S/N | Compound                 | Retention time (min) | Concentration (mg/100 g) |
|-----|--------------------------|----------------------|--------------------------|
| 1   | Phosphatidylethanolamine | 14.175               | 89.98375                 |
| 2   | Phosphatidylcholine      | 15.405               | 186.98584                |
| 3   | Phosphatidylserine       | 16.568               | 26.90626                 |
| 4   | Lysophosphatidylcholine  | 17.665               | 4.76927                  |
| 5   | Phosphatidylinositol     | 18.709               | 182.81293                |
| 6   | Phosphatidic acid        | 19.665               | 5.18250                  |

**Table 6:** Phytosterols of n-Hexane extract of *A. chrysanthum* seeds

| S/N | Compound     | Retention time (min) | Concentration (mg/100 g) |
|-----|--------------|----------------------|--------------------------|
| 1   | Cholesterol  | 19.502               | 6.70148e-1               |
| 2   | Cholestanol  | 20.586               | 9.12747e-4               |
| 3   | Ergosterol   | 21.418               | 6.62632e-4               |
| 4   | Campesterol  | 22.394               | 12.93549                 |
| 5   | Stigmasterol | 23.239               | 14.33356                 |
| 6   | Savenasterol | 24.040               | 10.98555                 |
| 7   | β-Sitosterol | 25.067               | 175.90816                |

**Table 7:** Fatty acid and methyl esters of n-Hexane extract of *A. chrysanthum* seeds

| S/N | Compound                      | Retention time (min) | Concentration (mg/100 g) |
|-----|-------------------------------|----------------------|--------------------------|
| 1   | Caprylic acid methyl ester    | 8.921                | 0.000000                 |
| 2   | Capric acid methyl ester      | 11.064               | 0.000000                 |
| 3   | Lauric acid methyl ester      | 12.830               | 0.000000                 |
| 4   | Myristic acid methyl ester    | 14.440               | 2.17149e-7               |
| 5   | Palmitic acid methyl ester    | 16.044               | 2.07287e-5               |
| 6   | Palmitoleic acid methyl ester | 16.677               | 3.71098e-7               |
| 7   | Margaric acid methyl ester    | 17.367               | 3.82330e-8               |
| 8   | Stearic acid methyl ester     | 18.059               | 2.55130e-6               |
| 9   | Oleic acid methyl ester       | 18.939               | 1.44534e-5               |
| 10  | Linoleic acid methyl ester    | 19.526               | 2.44802e-5               |
| 11  | Linolenic acid methyl ester   | 20.795               | 2.85790e-6               |

Phosphatidylcholine had the highest concentration of 186.98584 mg/100 g while the lowest was lysophosphatidylcholine (4.76927 mg/100 g). The total concentration of phospholipids in the extract was 496.641 mg/100 g. There were 7 identified sterols in the n-hexane extract (Table 6). Amongst which β-Sitosterol (175.90816 mg/100 g) was predominant while ergosterol (6.62632e-4 mg/100 g) was the least in terms of concentration. Phytosterols are lipophilic, naturally occurring compounds. Over 40 natural plant sterols have been identified so far. The major phytosterols reported from plants are β-sitosterol, campesterol and stigmasterol and the most predominant phytosterol is β-sitosterol that exhibit hyperlipoproteinemic activity [10].

Fatty acid methyl esters analysis revealed the presence of 16 compounds. Linoleic acid methyl ester (2.44802e-5 mg/100 g) was found to be more than the others. Table 8 shows 42 alkaloids that were identified during the analysis of n-hexane extract. The most abundant of all the identified alkaloids is crinine-3-α-ol (6.28911e-5 mg/100 g) while echitamine (8.10436e-7 mg/100 g) was the least. Ten compounds were identified during anthraquinones analysis of the n-hexane extract (Table 9). An anti-cancer and antimicrobial compound, 1,4,5-trihydroxyanthraquinone (6.79067e-2 mg/100 g) was found to be more predominant in the extract while damnacanthol (6.81859e-6 mg/100 g) was the least.

|    |                               |        |            |
|----|-------------------------------|--------|------------|
| 12 | Arachidic acid methyl ester   | 21.913 | 6.18252e-7 |
| 13 | Arachidonic acid methyl ester | 23.000 | 9.36377e-7 |
| 14 | Behenic acid methyl ester     | 23.972 | 3.40725e-7 |
| 15 | Erucic acid methyl ester      | 24.893 | 5.85874e-7 |
| 16 | Lignoceric acid methyl ester  | 25.487 | 1.07202e-6 |

**Table 8:** Alkaloids of n-Hexane extract of *A. chrysanthum* seeds

| S/N | Compound                              | Retention time (min) | Concentration (mg/100 g) |
|-----|---------------------------------------|----------------------|--------------------------|
| 1   | Choline                               | 6.935                | 1.21199e-6               |
| 2   | Trigonelline                          | 7.650                | 1.41509e-6               |
| 3   | Theobromine                           | 7.793                | 1.08103e-6               |
| 4   | Theophylline                          | 7.964                | 1.01708e-6               |
| 5   | Caffein                               | 8.424                | 8.48535e-6               |
| 6   | Angustifoline                         | 8.578                | 9.25266e-6               |
| 7   | Sparteine                             | 9.079                | 7.47724e-6               |
| 8   | Ellipticine                           | 9.687                | 8.41732e-7               |
| 9   | Lupanine                              | 11.026               | 2.16140e-6               |
| 10  | 13- $\alpha$ -hydrorhombi-foline      | 11.302               | 1.09066e-5               |
| 11  | 9-Octadecenamamide                    | 12.905               | 7.43917e-6               |
| 12  | Dihydro-oxo-dimethoxy heemanthamine   | 14.060               | 1.37955e-5               |
| 13  | Augustamine                           | 14.871               | 3.70105e-5               |
| 14  | Oxoassanine                           | 14.927               | 3.67568e-5               |
| 15  | Crinane-3- $\alpha$ -ol               | 15.387               | 6.28911e-5               |
| 16  | Cinchonidine                          | 16.382               | 7.86549e-6               |
| 17  | Cinchonine                            | 16.446               | 2.20033e-5               |
| 18  | Buphanidrine                          | 16.543               | 1.97500e-5               |
| 19  | Indicine-N-oxide                      | 17.764               | 2.42824e-5               |
| 20  | Powelline                             | 18.677               | 1.71418e-5               |
| 21  | Undulatine                            | 18.774               | 1.60851e-5               |
| 22  | Ambelline                             | 19.728               | 2.82880e-6               |
| 23  | 6-Hydroxybuphanidrine                 | 20.604               | 3.84541e-6               |
| 24  | Acronycine                            | 21.255               | 4.69730e-6               |
| 25  | Monocrotaline                         | 21.305               | 1.87316e-5               |
| 26  | 6-Hydroxypowelline                    | 21.802               | 2.39868e-5               |
| 27  | Nitidine                              | 22.375               | 5.55729e-6               |
| 28  | Crinamidine                           | 24.022               | 2.80386e-5               |
| 29  | 1 $\beta$ , 2 $\beta$ -Epoxyambelline | 24.702               | 6.73534e-6               |
| 30  | 6-Hydrxyundulatine                    | 24.782               | 8.14428e-6               |
| 31  | Epoxy-3, 7-dimethoxycrinane-11-one    | 25.545               | 8.52265e-7               |
| 32  | Akuamidine                            | 26.896               | 2.51358e-5               |
| 33  | Echitamidine                          | 26.957               | 2.89943e-6               |
| 34  | Voacangine                            | 27.014               | 6.13058e-6               |
| 35  | Mitraphylin                           | 27.622               | 3.54735e-5               |
| 36  | Camptothecin                          | 28.218               | 6.21643e-6               |
| 37  | Echitamine                            | 28.565               | 8.10436e-7               |
| 38  | Colchicine                            | 28.997               | 2.74078e-6               |
| 39  | Emetine                               | 29.640               | 1.99280e-6               |
| 40  | Tetrandrine                           | 29.689               | 4.24824e-6               |
| 41  | Thalicarpin                           | 30.071               | 2.97411e-6               |
| 42  | Paclitaxel                            | 32.221               | 8.98459e-7               |

**Table 9:** Anthraquinones of n-Hexane extract of *Aframomum chrysanthum* seeds

| S/N | Compound                      | Retention time (min) | Concentration (mg/100 g) |
|-----|-------------------------------|----------------------|--------------------------|
| 1   | 2,6-Dimethoxybenzenoquinone   | 16.041               | 2.95736e-3               |
| 2   | 6-Methoxyquinoline-1-oxide    | 18.056               | 3.15454e-3               |
| 3   | Ziganein                      | 18.936               | 5.69439e-2               |
| 4   | 1,4,5-Trihydroxyanthraquinone | 19.106               | 6.79067e-2               |
| 5   | Emodin                        | 19.523               | 5.70011e-2               |
| 6   | Soranjidiol                   | 19.794               | 6.40859e-5               |
| 7   | Damnacanthal                  | 20.792               | 1.42501e-5               |
| 8   | Damnacanthol                  | 22.241               | 6.81859e-6               |
| 9   | Rhein methyl ester            | 22.699               | 4.49268e-3               |
| 10  | Citroreosin                   | 23.969               | 6.96810e-3               |

The study revealed that the seed extracts of *A. chrysanthum* inhibited the growth of some of the investigated microbes. From the obtained result (Table 1-4) of susceptibility studies, n-hexane extract showed the highest antimicrobial activity while the ethylacetate extract exhibited marginal antimicrobial property. The methanol extract showed no antimicrobial activity against the investigated microbes and may not have therapeutic and prophylactic effects against any disease caused by the tested microorganisms. In all the antimicrobial analysis carried out, *A. chrysanthum* seed extracts showed zero antimicrobial effect against *B. cereus*, *P. aeruginosa* and *S. dysenteriae*. The inability of some of the extracts used to diffuse through the nutrient agar medium could be the reason for the observed poor activity [5]. The

impairment in drug diffusion is a major drawback in the investigation of plant extracts when agar diffusion method is used [5, 11, 12, 13]. n-Hexane extract displayed good inhibitory property as an antimicrobial agent because it exhibited slightly greater effect against *T. rubrum* compared to fungisol (standard drug) used in the investigation (Table 4). It was also observed that for all the susceptibility testing carried out, the antimicrobial activities depended largely on concentration of the extract used; thus, higher concentrations resulted to wider zones of inhibition. This relationship between plant extract concentration and zone of inhibition has also been reported by other researchers [1, 14, 15]. From the results obtained, Gram-positive bacteria were more susceptible to the active extracts than the Gram-negative ones. Gram-negative bacteria have a largely impermeable cell wall which makes them more resistant to antibiotics when compared to Gram-positive bacteria [16].

The antimicrobial activities of *A. chrysanthum* seed extracts could be attributed to the phytochemicals contained in them. *A. chrysanthum* seed contains phytochemicals such as phospholipids, phytosterols, fatty acids, alkaloids and anthraquinones (Tables 5-9). These secondary metabolites have been proven to be responsible for the antimicrobial abilities of a lot of medicinal plants [11, 17, 18]. Results from the phytochemical analysis of the n-hexane extract (which was the most bioactive of all the investigated extracts) showed that *A. chrysanthum* seeds contain compounds which are most likely responsible for the antimicrobial qualities observed in the susceptibility tests. Studies have shown that phospholipids have antimicrobial activity against pathogenic microorganisms [19].  $\beta$ -sitosterol, stigmasterol and campesterol (which are all phytosterols) have been shown to have strong antimicrobial properties against a variety of bacterial and fungal strains [20]. The antifungal and bactericidal properties of fatty acids are well known [21]. Alkaloids are secondary metabolites of plants which have been known to have strong antimicrobial properties. They have other great uses in pharmacology such as anti-hypertensive, antimalaria and analgesics [22]. Alkaloids have been known to inhibit bacteria growth with similar effects as ampicillin, a standard antibiotic [23]. Anthraquinones and their derivatives have shown potential applications as antibacterial, antiviral and antioxidant agents. Healthcare industries and medicine have utilized both natural and synthetic anthraquinones in treatment of diverse diseases and ailments caused by pathogenic microorganisms [24].

## Conclusion

It can be concluded from this study that the n-hexane and ethylacetate extracts of the seed of *Aframomum chrysanthum* showed antimicrobial activity against some of the tested microorganisms. This probably justifies the use of the plant as an antimicrobial agent. The seed of *Aframomum chrysanthum* can be of great application in medicine due to the antimicrobial properties of its chemical components. Health care delivery systems will find the seed of this plant essential in treatment of diseases and ailments caused by some bacteria and fungi.

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