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CHARACTERIZATION AND ANTIMICROBIAL PROPERTIES OF *Prosopis africana, Pentaclethra macrophlla,* AND *Erythrophleum suaveolen* (FABACEAE) SEED EXTRACTS AND FRACTIONS ON SELECTED WOOD PATHOGENS

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ABSTRACT

The study investigated antimicrobial properties of extracts and fractions characterized from seeds of three tropical trees species (Prosopis africana, Pentaclethra macrophlla, and Erythrophleum suaveolen). Methanol extracts of P. africana, P. macrophylla, and of E. suaveolens seeds were prepared and characterized using X-ray diffraction (XRD), column chromatography, and nuclear magnetic resonance (NMR) spectrometry. Results obtained revealed that P. macrophylla fractions contained lupenone in PM35, while a combination of triterpenes and lupeol in PM57 and daucosterol present in PM72 from P. africana. In contrast, E. suaveolens seed fraction EM86 contain Sitosterol/stigmasterol, wax and fatty substances. The extracts were active against Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescens, and Klebsiella spp test bacteria at zone of inhibition (ZoI) ranged from 18 mm to 20 mm. E. suaveolens methanol seed extract demonstrated notable activity against six fungi viz: Aspergillus nigre, Coniophora puteana, Fusarium sp, Rhizopus spp, and Sclerotium rolfsii, with ZoI values ranging from 16 mm to 20 mm. The results demonstrated varying degrees of antibacterial and fungicidal activities among the extracts against test bacterial and fungal strains. Only P. mycrophylla extract inhibited and killed Aspergillus nigre and Serpula lacrymans at MIC of 2.5 and 5 mg/ml, and MFC of 10 mg/Ml respectively.

Keywords: Antimicrobial, Bacteria, Compound, characterize, Extract, fraction, fungi

INTRODUCTION

The increasing resistance of pathogens to conventional antibiotics has driven research into alternative antimicrobial agents from natural sources. The seeds of *Prosopis africana*, *Pentaclethra macrophylla*, and *Erythrophleum suaveolen* have shown promise due to their rich phytochemical profiles and traditional medicinal uses. This review synthesizes the current knowledge on the antimicrobial properties of extracts and fractions derived from these seeds.

Prosopis africana (Guill. & Perr.) Taub. Is in the family of leguminous (Fabaceae). It is

called different names in Nigeria as *Ihi* in Igbo, *Obo* or *Erun-obo* in Yoruba, *Oginni* or *Oginyi* in Edo language. It is a multipurpose tree native to Africa, has attracted attention for its potential as a natural antimicrobial agent (Ezike *et al.,* 2010). Studies by Yanda *et al.* (2022*)* indicate that extracts from its leaves seeds possess significant antibacterial properties, potentially offering a natural alternative to synthetic antibiotics. The bioactive compounds within *P. africana,* such as flavonoids, tannins, and saponins, are believed to contribute to its antimicrobial efficacy. Research has demonstrated its effectiveness against various bacterial strains, reinforcing its potential in combating antibiotic-resistant pathogens (John, 2024)

Pentaclethra macrophylla, Benth. commonly known as the African oil bean tree, is valued for its medicinal and nutritional properties. It is in the family Fabaceae. This species is called Congo acacia (English). In Nigeria, it is called *Ugba* or *Ogba* in Igbo, *Apara* or *Apawa* in Yoruba) and *Okpagha* or *Akpagha* in Edo language. Extracts from its seeds have shown important antibacterial and antioxidant activities. The seeds contain a variety of bioactive compounds, including phenolic acids and flavonoids, which contribute to their medicinal properties (Ajayi *et al.,* 2002). Available literatures have highlighted the antimicrobial properties of *P. marophylla,* but paid little attention to the chemical structure. Winrock International (1995) reported that despite limited chemical composition studies, existing research highlights the antimicrobial potential of *P. macrophylla*, particularly in inhibiting the growth of pathogenic bacteria.

Erythrophleum suaveolen, another significant African tree known for its potent bioactive compounds. It belongs to the family Fabaceae (Leguminosae). It is commonly known as Ordeal Tree, Red Water Tree, Sasswood, Sassy Bark (Akinpelu *et al.,* 2021; Ninh The Son, 2019). Extracts from its seeds exhibit strong antimicrobial activities, attributed to the presence of alkaloids, flavonoids, and other phytochemicals. Research indicates that *E. suaveolen* is effective against a broad spectrum of bacteria, making it a valuable candidate for developing natural antimicrobial agents. Its traditional use in African medicine underscores its potential and the need for further scientific exploration (Kasilo *et al.,* 2019).

Despite the antimicrobial potentials of *P. africana*, *P. macrophlla* and *E. suaveolens*, limited studies in this regard have so far been carried out against wood bacteria and fungi. Therefore, this study was aimed to identify column chromatography profiles of seeds from *P. africana*, *P. macrophylla* and of *E. suaveolens* and characterize the compounds identified using nuclear magnetic resonance (NMR) spectrometry and evaluate antimicrobial activities of *P. africana, P. macrophylla* and of *E. suaveolens* seeds extracts on selected wood bacteria and fungi.

MATERIALS AND METHODS

Plant materials collection and preparation

The pods of *P. africana*, *P. macrophlla* and *E. suaveolens* were collected from the ground under the trees. The pods were air-dried under the sun for a period of two weeks before they were broken to remove the seeds and air-dried for an additional one week. The seeds were prepared in powder form according to the methods described by Siddig (1991) and Ahmed (1995), and stored in polythene bags until this study was completed.

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Prosopis africana **seed**

Pentaclethra macrophlla **seed**

Erythrophleum suaveolens **seed**

Distillation of Solvents

Ethyl acetate N-hexane, and methanol solvents used for the extraction were bought from Showcrown Laboratry. Ltd., Ibadan. The solvents were distilled in chemistry laboratory at Joseph Sarwuan Tarka University, Makurdi (JOSTUM) to remove impurity. Distilled solvent was collected and stored in bottles before extraction.

Crude extraction of *P. africana, P. macrophylla* and *E. suaveolens* seeds

The extraction of dried seeds of *P. africana, P. macrophylla* and *E. suaveolens* was carried out in the Chemistry Laboratory at the (JOSTUM). Extraction of pulverized seeds was done sequentially by macerating 1000 g into 1000 mL (w/v) of n-hexane for 24 hours. The marc from the hexane extraction was sequentially macerated with ethyl acetate, again for 24 hours. Then, the mixtures were filtered using No. 1 Whiteman filter papers into well-labelled glass bottle. The filtrate was evaporated using electric fan to obtain dried extracts and the bottle re-weighed (w_2).

Column Chromatography (CCT)

Separation of Mixture of N-hexane and Ethyl acetate pod Extracts

Dried crude extracts of *P. africana, P. macrophlla,* and *E. suaveolens* seeds were subjected to column chromatography to produce fractions. Each dried extract was mixed with commensurate powdered silica gel and loaded onto a column. The column was prepared by inserting a ball of cotton wool, followed by a layer of silica gel, and then saturated with n-hexane. The solvent was allowed to drain for 10 minutes before the column was eluted with a mixture of increasing polarity, starting with hexane: ethyl acetate (95:5) and gradually increasing to 0:100. This process allowed for the separation and collection of different fractions based on their interactions with the silica gel and solvents. The column chromatography process involved introducing a mixture of N-hexane and ethyl acetate solvents in a progressive ratio at the beginning of the extraction, followed by methanol solvents in the final stage to elute the extracts in the stationary phase. The fractions collected from the column were stored in labeled and numbered vials with volumes ranging from 2.5 to 3 mL. These fractions were then evaporated to dryness using an electric fan and weighed using a sensitive weighing balance. The vials containing crystals were separated for further analysis via NMR and antimicrobial tests.

Antimicrobial screening of unprocessed s extracts

The antimicrobial activity of seed methanol extracts from *P. africana*, *P. macrophylla*, and

E. suaveolens was evaluated against selected wood bacteria (*Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus salvarius, Erwinia carotovora, Serratia marcescens, Pseudomonas contexa, Enterobacter* sp.,*Rhanella* sp., *Klebsiella* sp., *Pectobacterium carotovorum*) and fungi (*Aspergillus flavus, Aspergillus fumigatus, Aspergillus nigre, Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicola, Fusarium* sp.,*Rhizopus spp., Sclerotium rolfsii, Tricoderma* sp.and *Serpula lacrymans*). The extracts were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10mg/ml and tested using the diffusion method. Mueller Hinton agar and Sabouraud dextrose agar were used as growth media for bacteria and fungi respectively, and the extracts were introduced into wells cut in the media. The plates were incubated at 37°C for 24 hours for bacteria and 30°C for 7 days for fungi, after which the zone of inhibition was measured and recorded. Two antifungi agents (Sparfloxacin and Fulcin) were used as controls for fungi, while keteconazole and sparfloxacin were used as a control for bacteria.

Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration (MIC) of the crude extract was determined using the broth dilution method. Mueller Hinton broth and Sabouraud dextrose broth were sterilized and cooled, then inoculated with a standardized suspension of the test microbe. The crude extract was serially diluted in the broth to obtain concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.63 mg/ml. The test microbe was then inoculated into each concentration, and the tubes were incubated at 37°C for 24 hours for bacteria and 30°C for 1-7 days for fungi. The lowest concentration of the crude extract that showed no turbidity (growth) was recorded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were determined to assess whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar and Sabouraud dextrose agar were sterilized and cooled, then inoculated with the contents of the MIC in serial dilutions. The plates were incubated at 37°C for 24 hours for bacteria and 30°C for 1-7 days for fungi. The MBC/MFC was the lowest concentration of the crude extract that showed no colony growth, indicating that the microbes were killed.

RESULTS

Table 1 presents the chemical compositions of seed fractions from two distinct tree species, *P. africana* and *E. suaveolens,* employing NMR spectroscopy. The analysis involved fractionation of the seeds into discrete components, denoted by identifiers PM35, PM57, PM72 for *P. africana,* and EM86 for *E. suaveolens.* NMR analysis of these fractions revealed intriguing insights into their chemical constituents.

In the case of *P. africana,* the PM35 fraction exhibited characteristics associated with lupenone, while PM57 indicated a combination of triterpenes and lupeol. Moreover, the PM72 fraction suggested a mixture of triterpenes with a potential presence of daucosterol. Contrastingly, the seed fraction EM86 from *E. suaveolens* showcased constituents' indicative of Sitosterol/stigmasterol, wax and fatty substances wax and fatty substances.

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Table 1: Chemical compositions of seed fractions from two distinct tree species, *P. africana* and *E. suaveolens*

Seed of tree Species	Fraction	Nuclear Magnetic Resonance inference
	PM35	Lupenone
P. africana Seeds	PM57	Mixture of triterpenes plus lupeol
	PM72	Mixture of triterpenes plus possibly daucosterol
E. suaveolens Seeds	EM86	Wax, fatty substances, Sitosterol/stigmasterol,

^{1}H-NMR Characterisation of PM35 as Lupenone

1.75 - 1.98 (2H, m, H-l), 2.20-2.40 (3H, m, H-2, H-19), 0.69 (lH,d,H-5), 1.45(2H,m,H-6), l.40(lH,m,H-7), 1.17(1H, d, H-9), 1.35 (2H, m, H-11), 1.07, 1.68 (lH, each, H-12), 1.67 (lH, t, H-13), 1.02, 1.75 (lH, each, H-15), 1.38, 1.41 (lH, each, H-16), 1.35 (lH, t, H-8), 1.30, 1.93 (lH, each m, H-21), 1.20, 1.42 (1H each, m, H-22), 0.97 (3H, s, Me-23), 0.75 (3H, s, Me-24), 0.82 (3H, s, Me-25), 1.02 (3H, s, Me26), 0.94 (3H, s, Me-27), 0.78 (3H, s, Me-28), 4.63,4.50 (lH, each, br s, Me-29) and 1.76 (3H, s, Me-30) **(**Prashant and Krupadanam, 1993).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.69 (s, 1H), 4.57 (s, 1H), 1.95 – 1.87 (m, 2H), 1.68 (s, 3H), 1.07 (s, 5H), 1.03 (s, 3H), 0.96 (s, 2H), 0.93 (s, 2H), 0.80 (s, 3H). The spectral and structure of Lupenone are presented in Figures 1(a & b) respectively.

^{1}H-NMR Characterisation of PM57 as a Mixture of Triterpenes including Lupeol

The HNMR spectrum of ...the H-3 proton appeared as a triplet of a triplet of double doublets (tdd) at delta 3.21, H-29 gave two multiplets at delta 4.71 and delta 4.56 was found to be Lupeol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.59 (d, J = 3.3 Hz, 1H), 4.47 (q, J = 3.7, 3.0 Hz, 1H), 3.10 (ddd, J = 15.2, 7.7, 4.0 Hz, 1H), 1.10 (s, 2H), 0.94 (s, 1H), 0.88 (s, 2H), 0.85 (s, 1H), 0.80 (s, 1H), 0.67 (s, 2H), 0.59 (s, 2H). The spectraland structure of Triterpenesare shown if Figures 2 (a & b) respectively.

^{1}H-NMR Characterisation of EM86 as Sitosterol/stigmasterol

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (d, J = 5.5 Hz, 10H), 5.16 (dd, J = 15.7, 8.5 Hz, 2H), 5.02 (dd, J = 15.2, 8.3 Hz, 2H), 3.52 (t, J = 7.8 Hz, 1H), 1.02 (d, J = 7.7 Hz, 13H), 0.69 (d, J = 7.4 Hz, 10H). Figures 3 (a & b) and 4 (a &b) present the spectral and structures of Sitosterol and stigmasterol respectively. As indicated in Table 1, the spectral and structure of daucosterol characterised from *E. suaveolens* seeds (EM 86) are presented in Figures 5(a) and 5(b) respectively.

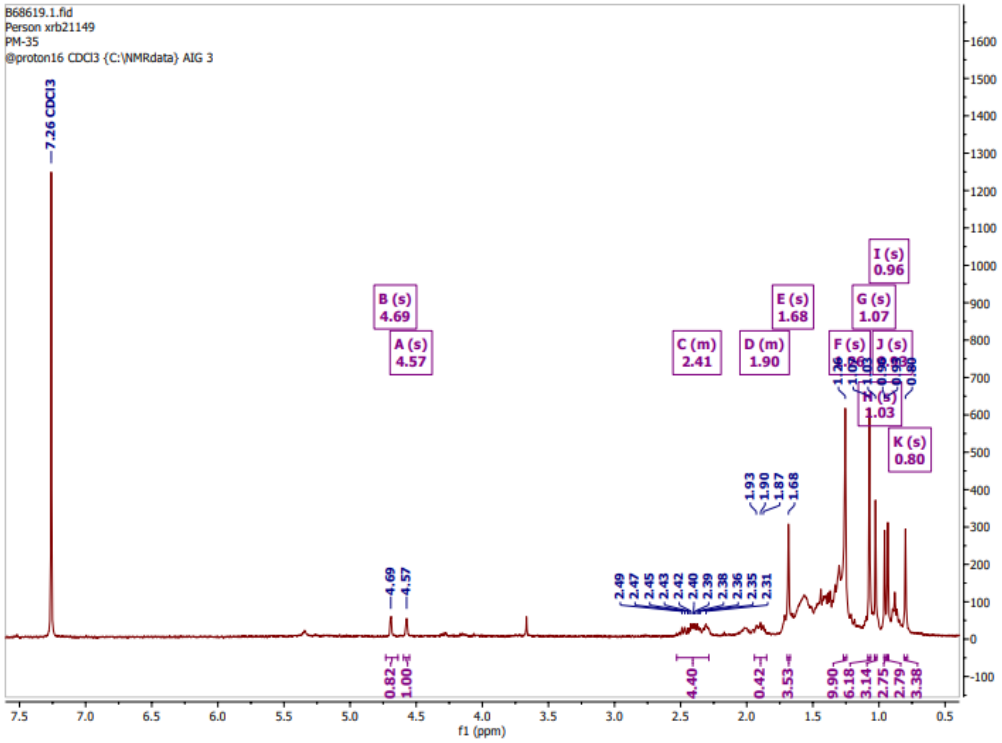

Figure 1 (a): Spectral of of Lupenone from PM35 fraction *(P. africana* Seed)

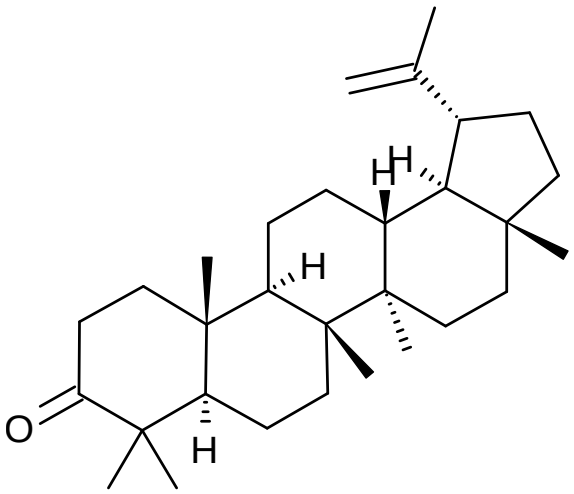

Figure 1 (b): Structure of Lupenone

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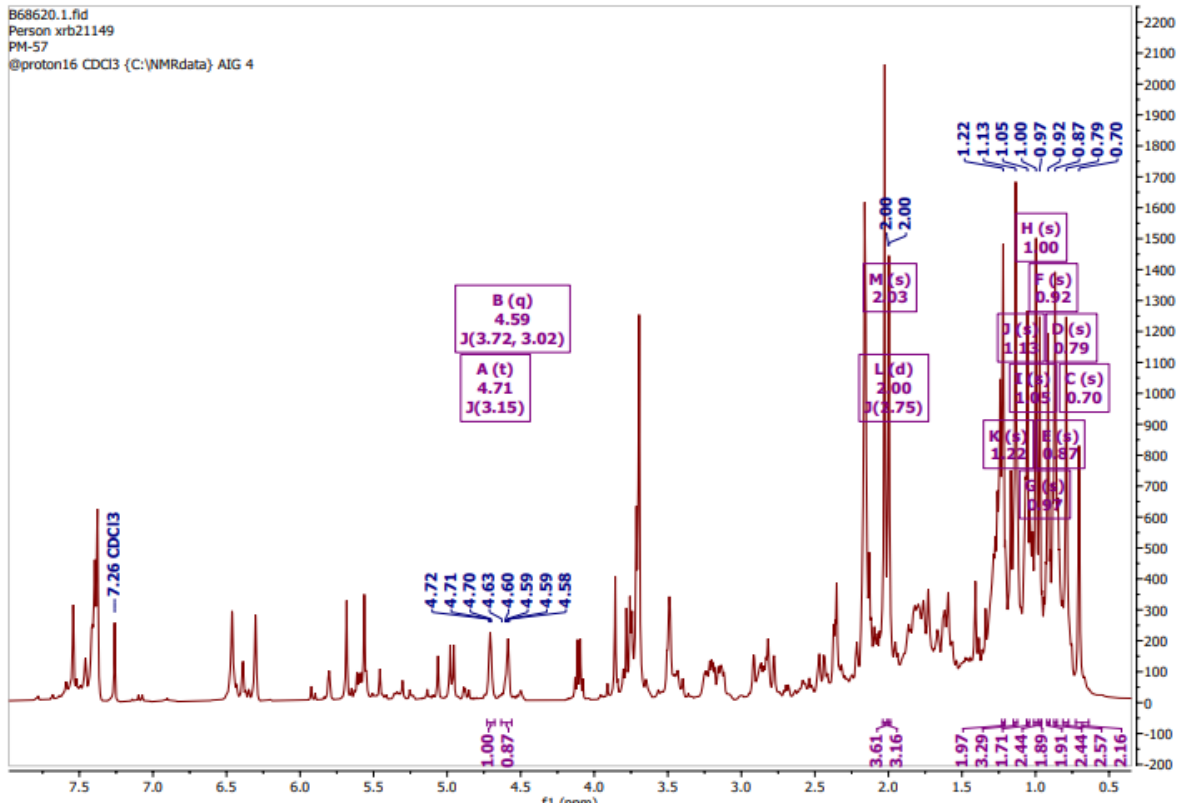

Figure 2 (a) Spectrum of Triterpenes from PM57 Fraction *(P. africana* Seed)

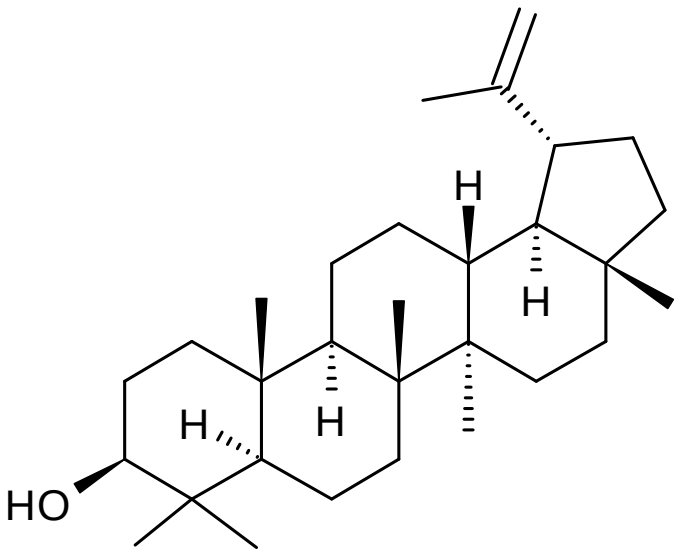

Figure 2 (b): Structure of Triterpenes

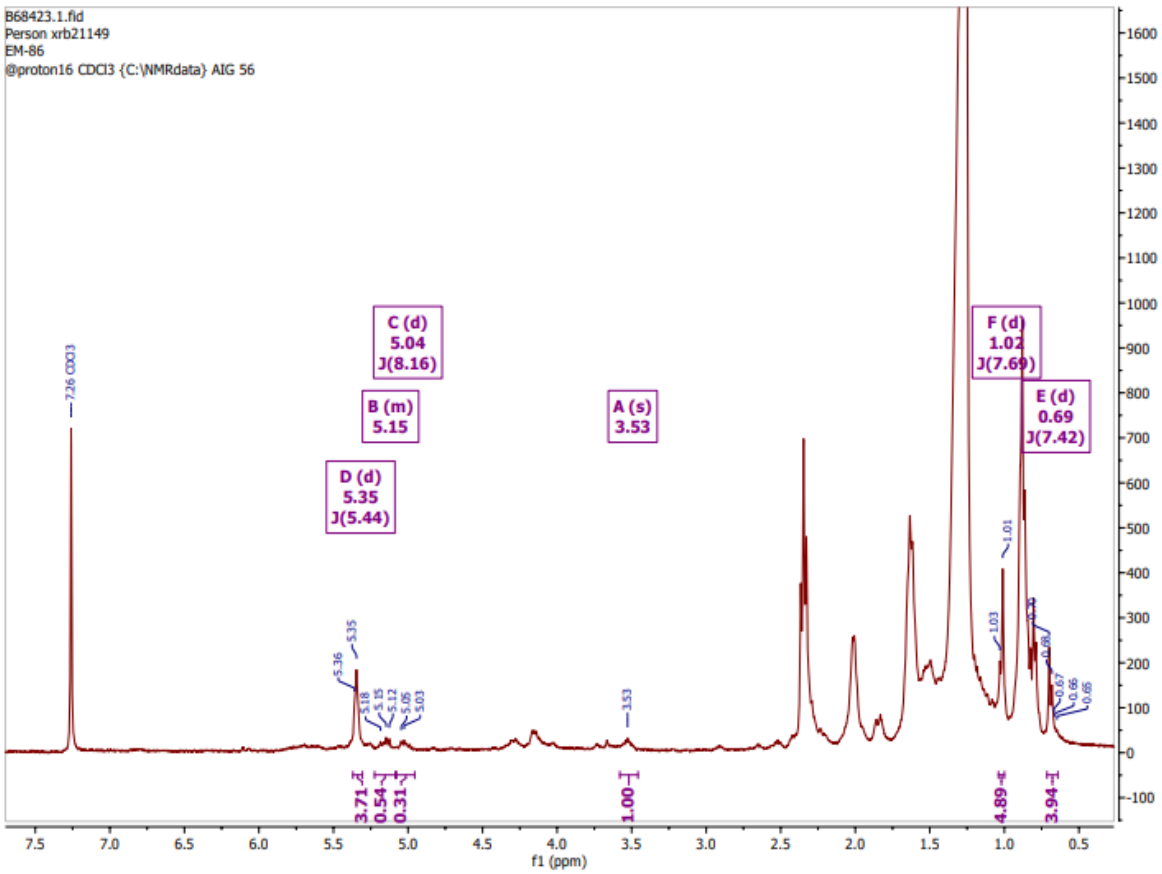

Figure 3 (a): Spectrum of Sitosterol from EM86 fraction (*E. suaveolens* Seeds)

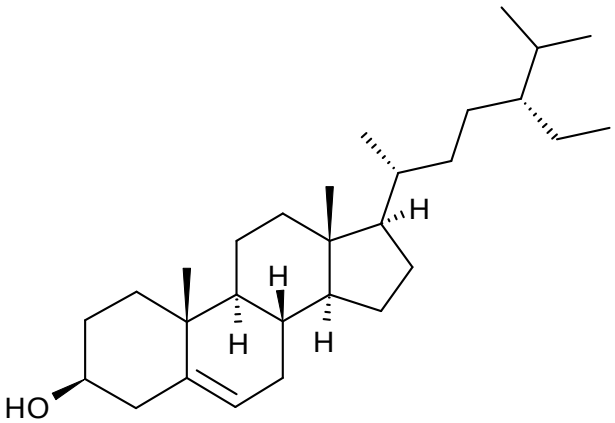

Figure 3 (b): Structure of Sitosterol

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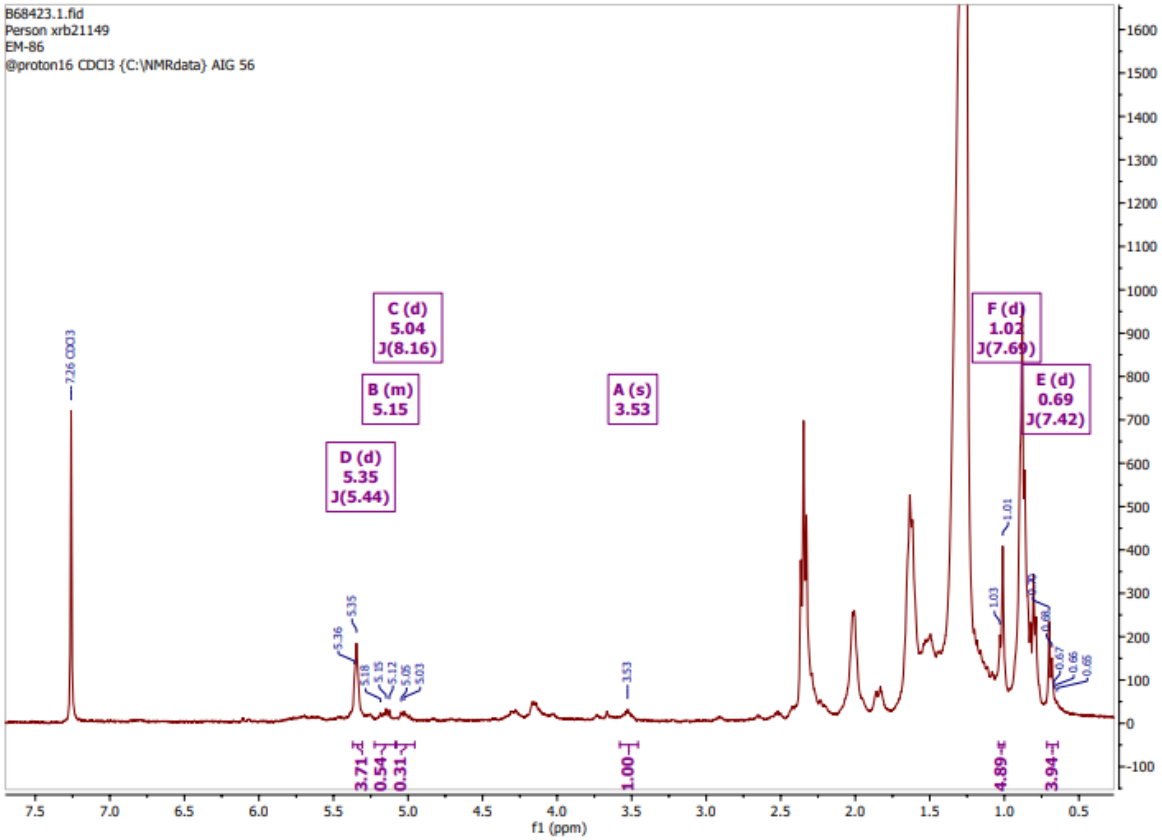

Figure 4 (a): Spectrum of stigmasterol from EM86 fraction (*E. suaveolens* Seeds)

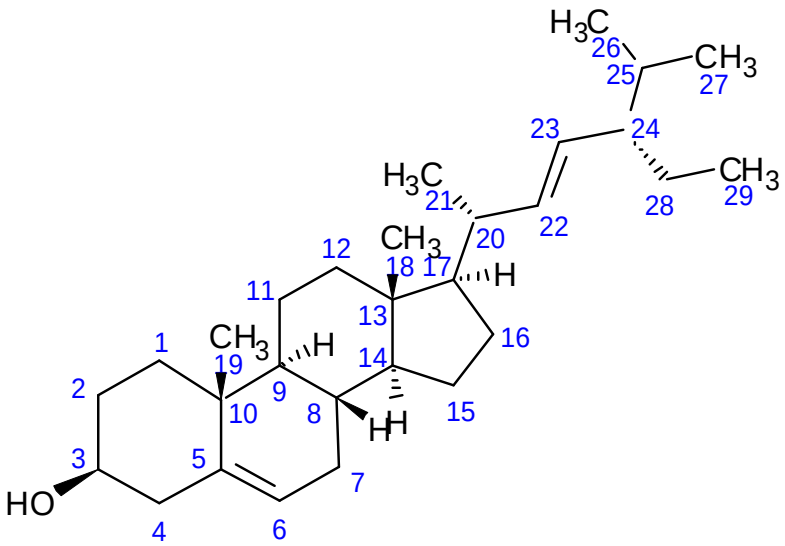

Figure 4 (b): Structure of stigmasterol

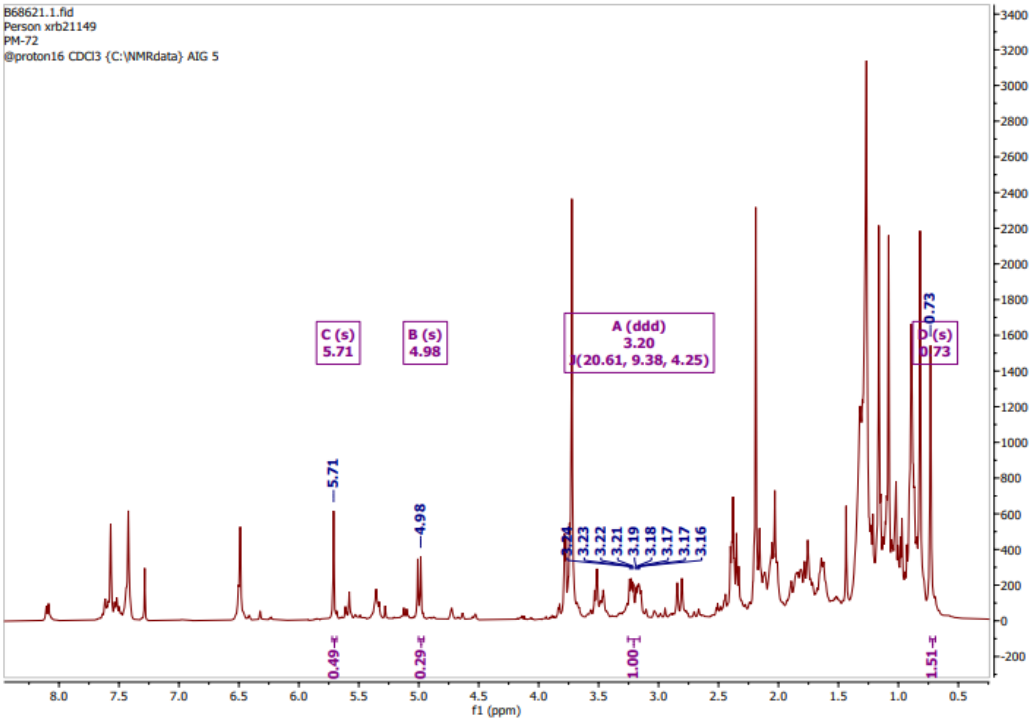

Figure 5 (a): Spectrum of Mixture of triterpenes plus possibly daucosterol

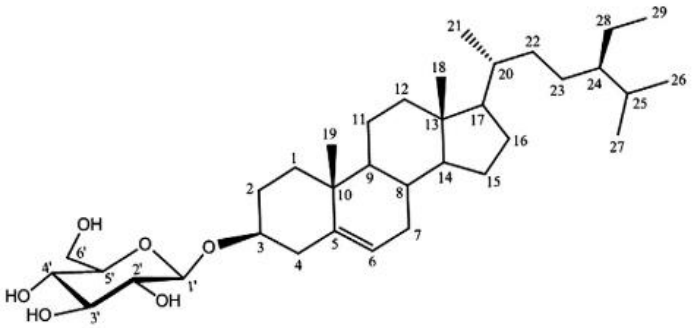

Figure 5 (b): Chemical Structure of daucosterol

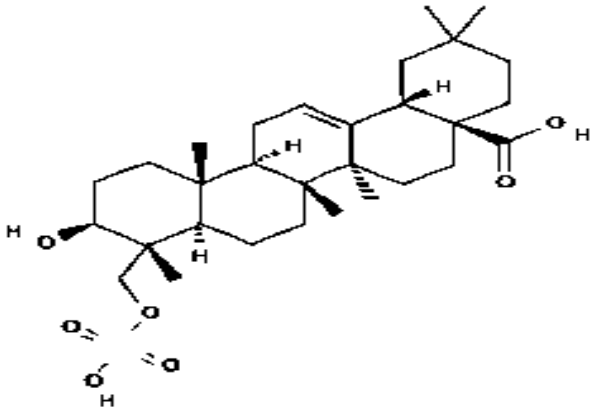

Figure 5 (c): Chemical Structure of triterpenes

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Table 2 shows results of the antibacterial activities of methanol crude extracts obtained from the seeds of three plant species, namely *P. africana, P. macrophylla,* and *E. suaveolens,* against a panel of test bacteria. The results demonstrated varying levels of antibacterial efficacy among the extracts against different bacterial strains. For *P. africana* and *P. macrophylla* methanol seed extracts with ZoI values ranging from 18 - 21 mm and 18 - 24 mm, respectively, were very active against most bacteria such as *Bacillus subtilis, Streptococcus salvarius, Erwinia carotovora, Enterobacter* sp, *Rhanella sp, Klebsiella* sp and *Pectobacterium carotovorum. E. suaveolens* methanol seed extract exhibited very active antibacterial activities against *Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescens, Pseudomonas contexa* and *Klebsiella* sp and range of ZoI of 18 – 20 mm. Interestingly, although *Streptococcus salvarius, Erwinia carotovora, Enterobacter* sp, *Rhanella* sp and *Pectobacterium carotovoru* were resistant against *E. suaveolens* methanol seed extract, they were inhibited by *P. africana* and *P. macrophylla* extracts at ZoI range of 18 – 24 mm.

Table 3 presents the fungicidal potential of methanol crude extracts derived from the seeds of *P. africana, P. macrophylla,* and *E. suaveolens,* against test fungi. The results revealed varying degrees of fungicidal efficacy among the extracts when tested against different fungal strains. The *P. africana* methanol seed extract displayed moderate efficacy against *Aspergillus fumigatus, Coniophora puteana, Fusarium sp, Rhizopus spp* and *Tricoderma sp* at ZoI of between 16 – 21 mm. However, it demonstrated resistance (ZoI = 0 mm) against *Aspergillus flavus, Aspergillus nigre, Fibroporia vaillantii, Fomitopsis pinicola, Sclerotium rolfsii* and *Serpula lacrymans.*

Inversely, the *P. macrophylla* methanol seed extract exhibited limited good fungicidal activity, showcasing sensitivity against four fungi which include *Aspergillus fumigatus, Coniophora puteana, Rhizopus spp* and *Sclerotium rolfsii* with ZoI values ranging between 16 mm and 22 mm. Yet, it demonstrated resistance against several fungi which include *Aspergillus flavus, Aspergillus nigre, Fibroporia vaillantii, Fomitopsis pinicola, Fusarium sp, Tricoderma sp* and *Serpula lacrymans.*

The *E. suaveolens* methanol seed extract demonstrated outstanding activity against certain fungi, displaying sensitivity against five fungi viz: *Aspergillus nigre, Coniophora puteana, Fusarium* sp, *Rhizopus* spp, *and Sclerotium rolfsii*, with ZoI values ranging from 16 mm to 20 mm. However, it exhibited reduced efficacy or resistance against other fungi. *Aspergillus flavus, Fibroporia vaillantii* and *Fomitopsis pinicola* were resistant to the three plant extracts. Ketoconazole (25 – 31 mm) and Fulcin (27 – 30 mm), demonstrated varying levels against the test isolates (Table 4).

Table 2: Antibacterial activities and Zone of Inhibition of unprocessed with pods methanol crude extracts against test bacteria

S/No.	Test bacteria	*P. africana* methanol seed & pod extract		*P. macrophylla* methanol seed & pod extract		*E. suaveolens* methanol seed & pod extract		Sparfloxacin (10µg/ml)		Ketoconazole (10µg/ml)	
		ABA	ZoI (mm)	ABA	ZoI (mm)	ABA	ZoI(mm)	ABA	ZoI (mm)	ABA	ZoI (mm)
1.	*Bacillus subtilis*	S	19	S	20	S	18	R	0	S	32
2.	*Pseudomonas aeruginosa*	R	0	R	0	S	20	R	0	S	30
3.	*Streptococcus salvarius*	S	20	S	18	R	0	S	27	S	29
4.	*Erwinia carotovora*	S	18	S	21	R	0	S	30	R	0
5.	*Serratia marcescens*	R	0	R	0	S	20	S	23	R	0
6.	*Pseudomonas contexa*	R	0	R	0	S	18	R	0	S	27
7.	*Enterobacter* sp	S	22	S	20	R	0	S	30	R	0
8.	*Rhanella* sp	S	20	S	23	R	0	S	28	R	0
9.	*Klebsiella* sp	S	21	S	18	S	20	R	0	S	27
10.	*Pectobacterium carotovorum*	S	20	S	24	R	0	S	31	R	0

Key: S =Sensitive R =Resistance; ABA = Antibacterial activities; ZoI = Zone of Inhibition; When zone of inhibition (ZOI) values are < 10 mm the antibiotics are said to be inactive, at 10-13 mm they are partially active, 14-19 mm they are active, and >19 the antibiotics are very active. (Guevara, 2005)

Table 3: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of seed with pod methanol crude extracts against the test bacteria

S/No.	Test bacteria	*Prosopis africana* methanol seed extract	*Pentaclethra macrophylla* methanol seed extract	*Erythrophleum suaveolens* methanol seed pod extract	*Prosopis africana* methanol seed extract	*Pentaclethra macrophylla* methanol seed extract	*Erythrophleum suaveolens* methanol seed pod extract
		MIC (mg/ml)			MBC (mg/ml)		
1.	*Bacillus subtilis*	5	2.5	2.5	10	10	10
2.	*Pseudomonas aeruginosa*	R	R	2.5	R	R	10
3.	*Streptococcus salvarius*	2.5	5	R	10	10	R
4.	*Erwinia carotovora*	5	2.5	R	10	10	R
5.	*Serratia marcescens*	R	R	2.5	R	R	10
6.	*Pseudomonas contexa*	R	R	5	R	R	10
7.	*Enterobacter* sp	2.5	2.5	R	10	10	R
8.	*Rhanella* sp	2.5	2.5	R	10	5	R
9.	*Klebsiella* sp	2.5	2.5	2.5	10	10	10
10.	*Pectobacterium carotovorum*	2.5	2.5	R	10	5	R

Table 4: Fungicidal activities and zone of inhibition of unprocessed s methanol crude extracts against test fungi

S/No.	Test fungi	*P. africana* methanol seed & pod extract		*P. macrophylla* methanol seed & pod extract		*E. suaveolens* methanol seed &pod extract		Keteconazole (10µg/ml		Fulcin (10µg/ml)	
		ABA	ZoI (mm)	ABA	ZoI (mm)	ABA	ZoI (mm)	ABA	ZoI (mm)	ABA	ZoI
1.	*Aspergillus flavus*	R	0	R	0	R	0	R	0	S	30
2.	*Aspergillus fumigatus*	S	16	S	18	S	16	R	0	S	25
3.	*Aspergillus nigre*	R	0	R	0	S	20	R	0	S	27
4.	*Coniophora puteana*	S	20	S	22	S	18	S	26	R	0
5.	*Fibroporia vaillantii*	R	0	R	0	R	0	R	0	S	27
6.	*Fomitopsis pinicola*	R	0	R	0	R	0	S	31	R	0
7.	*Fusarium* sp	S	19	R	0	S	20	R	0	S	32
8.	*Rhizopus* spp	S	21	S	20	S	18	S	28	S	30
9.	*Sclerotium rolfsii*	R	0	S	21	R	0	S	25	S	29
10.	*Tricoderma* sp	S	20	R	0	R	0	R	0	S	27
11. -	*Serpula lacrymans*	R	0	R	0	S	17	R	0	R	0

Key: R =>Resistance; S => Sensitive R => Resistance; AFA =fungicidal activities; ZoI = Zone of Inhibition; When zone of inhibition (ZOI) values are< 10 mm the antibiotics are said to be inactive, at 10-13 mm they are partially active, 14-19 mm they are active, and >19 the antibiotics are very active. (Guevara, 2005)

Table 5 shows the antifungal potential of methanol extracts derived from *P. africana, P. macrophylla,* and *E. suaveolens* against test fungi by assessing minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The extracts exhibited varying degrees of efficacy against different fungal strains. Notably, *Aspergillus fumigatus* was inhibited by *P. africana, P. macrophylla* and *E. suaveolens* methanol extracts at 5 mg/ml MIC and killed at 10 mg/ml MFC. *Coniophora puteana* was inhibited by *P. africana* and *E. suaveolens* at MIC of 5 mg/ml and *P. macrophylla* at 2.5 mg/ml, whereas it was completely killed at MFC of 10 mg/ml. Only *E. suaveolens* methanol seed extracts was able to inhibit and killed *Aspergillus nigre* and *Serpula lacrymans* at MIC of 2.5 and 5 mg/ml, and MFC of 10 mg/ml respectively. *Fusarium sp* and *Rhizopus* spp were inhibited by *P. africana* and *E. suaveolens* extracts at MIC between of 2.5 and killed at 5 mg/ml MFC. Similar to *E. suaveolens* extract, only *P. macrophylla* extract was able to inhibit and killed *Sclerotium rolfsii* at MIC and MFC of 2.5 and 10 mg/ml, respectively.

Plate 1 shows pictorial view of zone of inhibition of bacteria inoculated with *E. suaveolens* seeds methanol extract. *Bacillus subtilis, Pseudomonas aeruginosa, Serratia*

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marcescens and *Klebsiella* spp plates showed various levels of ZoI whereas other bacteria were resistant. Plate2 presents a visual representation of the ZoI of bacteria exposed to*P. africana* seed methanol extract. The figure shows that while *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Pseudomonas convexa* did not exhibit any ZoI, the remaining bacteria strains were susceptible and inhibited by the *P. africana* seed extract. In other words, the extract was effective in inhibiting the growth of most of the tested bacteria strains, with the exception of *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Pseudomonas convexa.*

Table 5: Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of unprocessed s methanol extracts against test fungi

S/No	Test fungi	*P. africana* methanol seed extract	*P. macrophylla* methanol seed extract	*E. suaveolens* methanol seed pod extract	*P. africana* methanol seed extract	*P. macrophylla* methanol seed extract	*E. suaveolens* methanol seed pod extract
		Minimum Inhibitory Concentration(MIC)			Minimum Fungicidal Concentration(MFC)		
		mg/ml	mg/ml	mg/ml	mg/ml	mg/ml	mg/ml
1.	*Aspergillus flavus*	R	R	R	R	R	R
2.	*Aspergillus fumigates*	5	5	5	10	10	10
3.	*Aspergillus nigre*	R	R	2.5	R	R	10
4.	*Coniophora puteana*	5	2.5	5	10	10	10
5.	*Fibroporia vaillantii*	R	R	R	R	R	R
6.	*Fomitopsis pinicola*	R	R	R	R	R	R
7.	*Fusarium* sp	2.5	R	2.5	10	R	10
8.	*Rhizopus* spp	2.5	2.5	5	10	10	10
9.	*Sclerotium rolfsii*	R	2.5	R	R	10	R
10.	*Trichoderma* sp	2.5	R	R	10	R	R
11.	*Serpula lacrymans*	R	R	5	R	R	10

Key: *R =Resistant*

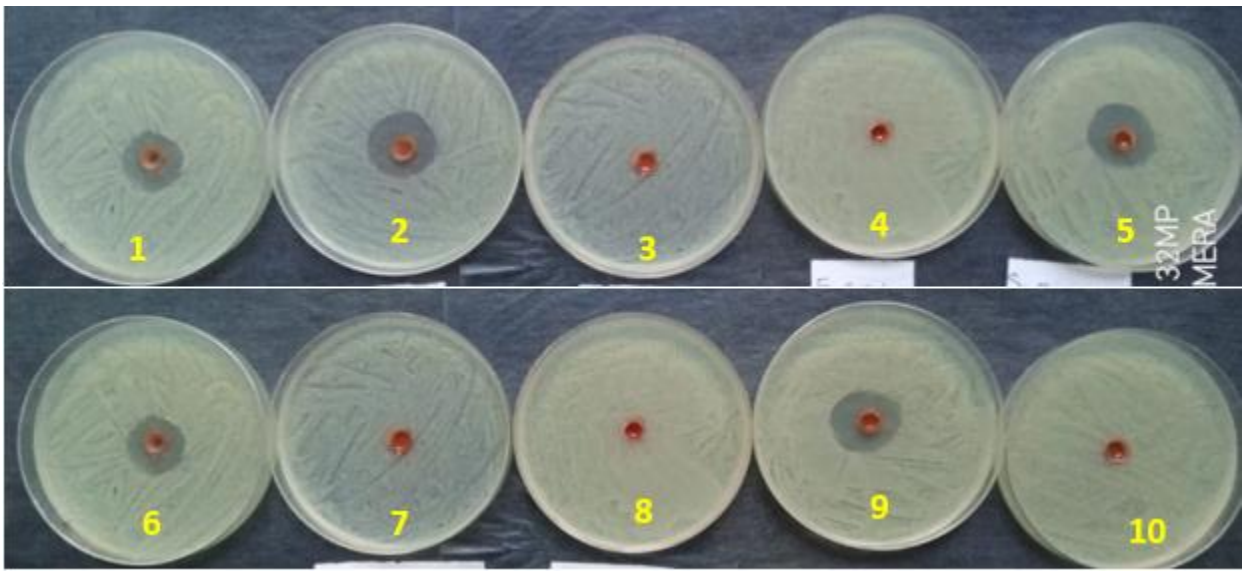

Plate 1: Zone of inhibition of unprocessed methanol extract of *E. suaveolens* seeds against the test bacteria

1. *Bacillus subtilis*, 2. *Pseudomonas aeruginosa*, 3. *Streptococcus salvarius*, 4. *Erwinia carotovora*, 5. *Serratia marcescens*, 6. *Pseudomonas convexa*, 7. *Enterobacter spp.*, 8. *Rahnella spp.*, 9. *Klebsiella spp*, 10. *Pectobacterium carotovorum*

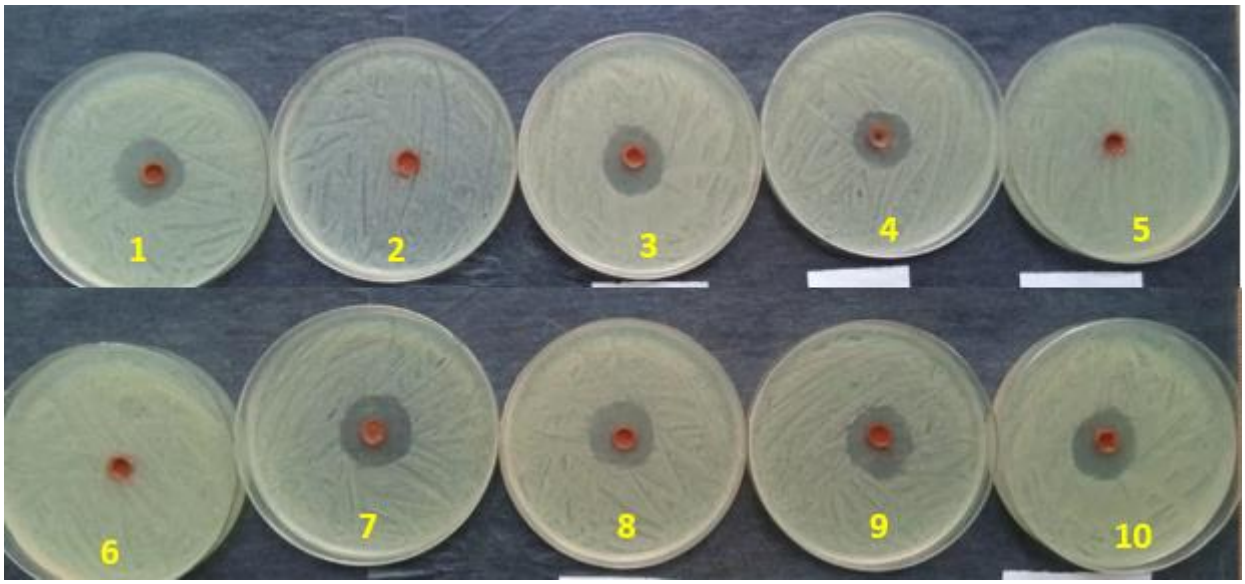

Plate 2: Zone of inhibition of unprocessed methanol extract of *P. africana* seeds against the test bacteria

1. *Bacillus subtilis,* 2. *Pseudomonas aeruginosa,* 3. *Streptococcus salvarius,* 4. *Erwinia carotovora,* 5. *Serratia marcescens,* 6. *Pseudomonas convexa,* 7. *Enterobacter spp.,* 8. *Rahnella spp.,* 9. *Klebsiella spp,*10. *Pectobacterium carotovorum*

Plate 3 shows the ZoI resulting from the introduction of methanol extract from *P. macrophylla* seeds to bacterial cultures. The result shows that *Pseudomonas aeruginosa*, *Serratiam arcescens,* and *Pseudomonas convexa,* exhibited resistance to the *P. macrophylla* extract. Conversely, the extract effectively controlled the growth of other bacterial.

Plates 4 and 5 illustrate a pictorial representation depicting the ZoI resulting from the application of methanol extracts from *E. suaveolens* and *P. africana* seeds against the test fungi. The culture plates showed that a majority of the fungi were effectively inhibited by both extracts. Plate 6 shows ZoI of fungi treated with extract of *P. macrophylla* methanol extract. *Aspergillus fumigatus, Coniophora puteana, Rhizopus* spp and *Sclerotium rolfsii* plates show various levels of ZoI whereas other fungal species were resistant. Plate 6 shows the ZoI observed in fungi treated with methanol extract of*P. macrophylla* seeds. The culture plates for *Aspergillus fumigatus, Coniophora puteana, Rhizopus* spp, *and Sclerotium rolfsii* exposed to methanol extract of *P. macrophylla* seeds varying levels of ZoI. In contrast, other fungal species displayed resistance to the extract.

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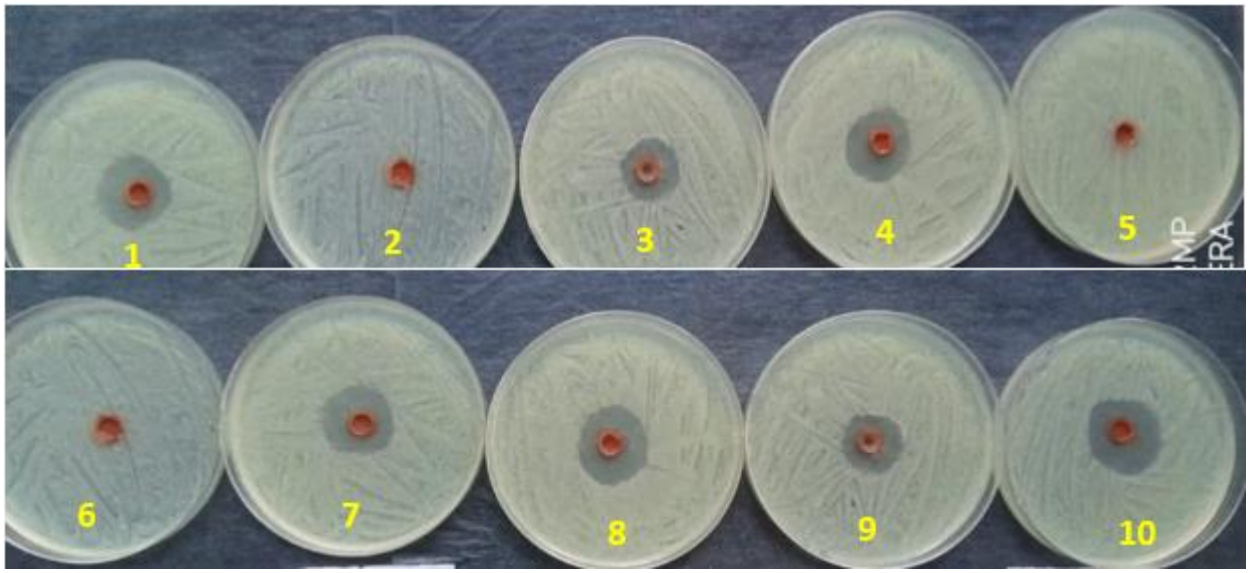

Plate 3: Zone of inhibition of *P. macrophylla* unprocessed seeds methanol extract on plates test bacteria

1.Bacillus subtilis, 2. *Pseudomonas aeruginosa,* 3. *Streptococcus salvarius,* 4. *Erwinia carotovora,* 5. *Serratiam arcescens,* 6. *Pseudomonas convexa,* 7. *Enterobacter spp.,* 8. *Rahnella spp.,* 9. *Klebsiella spp,* 10. *Pectobacterium carotovorum*

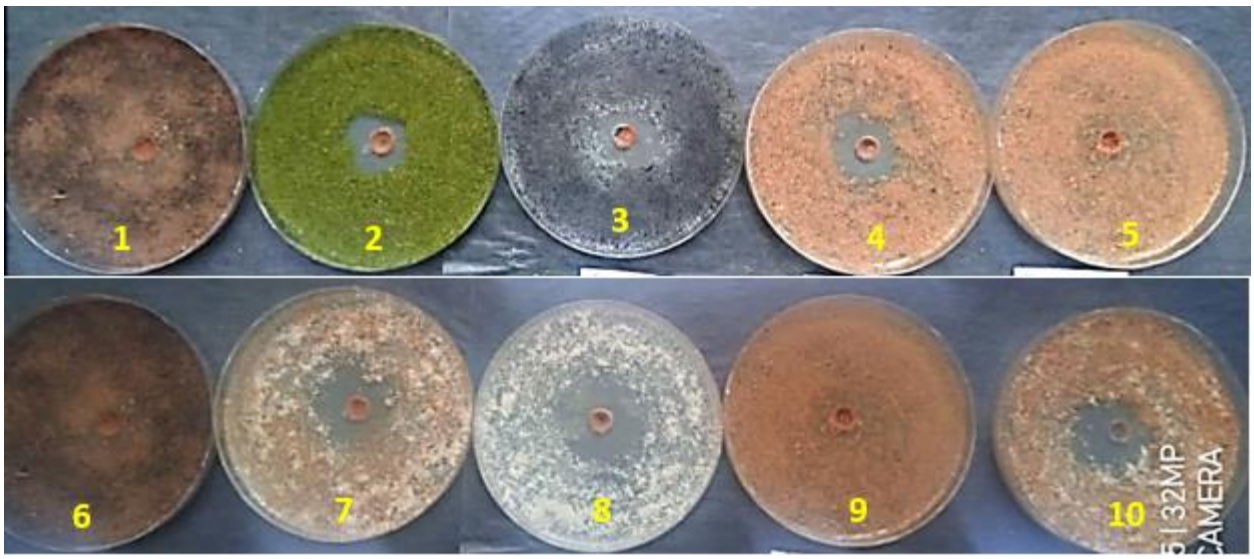

Plate 4: Zone of inhibition of unprocessed methanol extract of *E. suaveolens* seeds against the test bacteria

1. *Aspergillus flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;* 6. *Fomitopsis pinicola;* 7. *Fusarium sp;* 7. *Rhizopus spp;* 8. *Sclerotium rolfsii;* 9. *Tricoderma sp;* 10. *Serpula lacrymans*

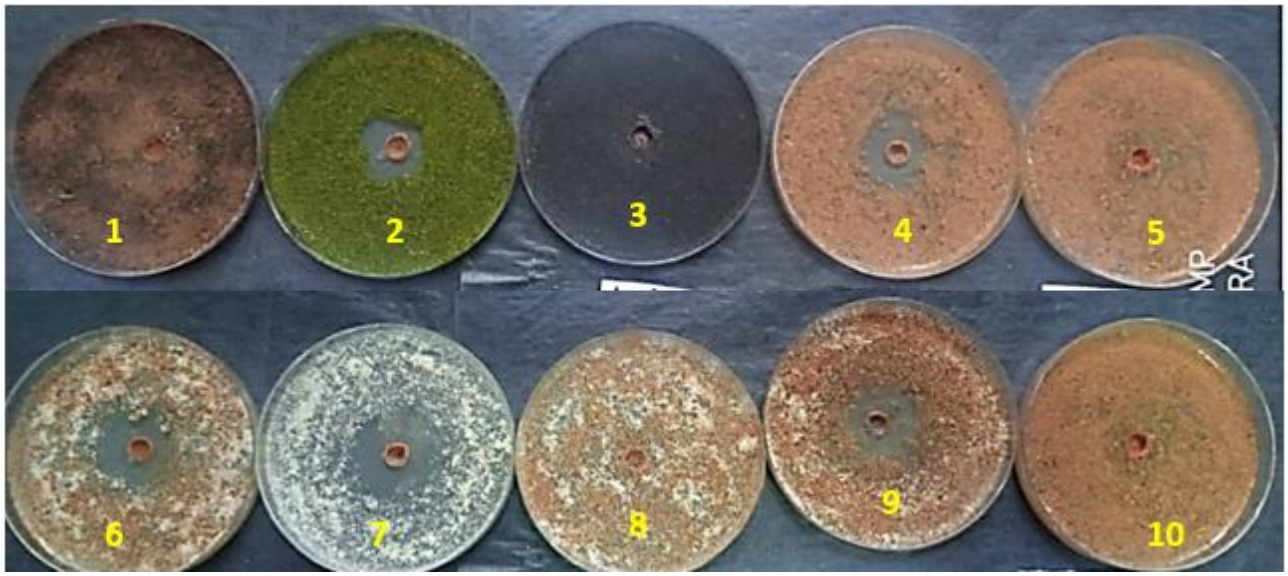

Plate 5: Zone of inhibition unprocessed methanol extract of *P. africana* seeds against the test fungi.

1. *Aspergillus flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;* 6. *Fomitopsis pinicola;* 7. *Fusarium* sp; 7. *Rhizopus* spp*;* 8. *Sclerotium rolfsii;* 9. *Tricoderma* sp; 10. *Serpula lacrymans*

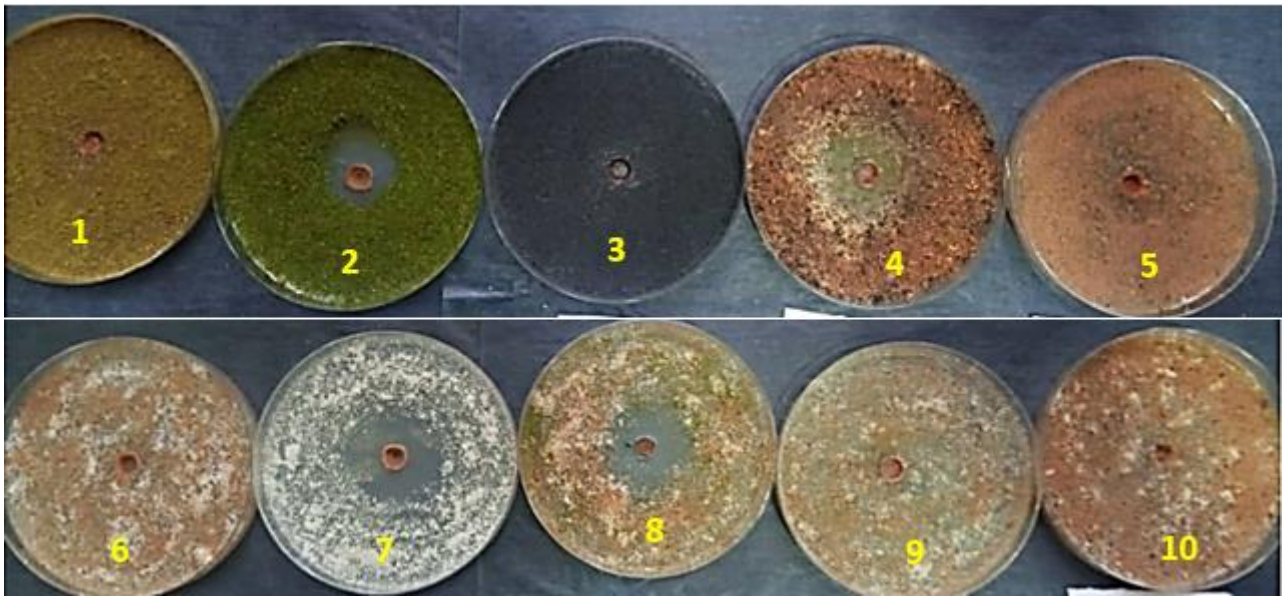

Plate 6: Zone of inhibition of unprocessed methanol extract of *Pentaclethra macrophylla* seeds against the test fungi.

1. *Aspergillus flavus;* 2. *Aspergillus fumigatus;* 3. *Aspergillus nigre;* 4. *Coniophora puteana;* 5. *Fibroporia vaillantii;*6. *Fomitopsis pinicola;* 7. *Fusarium* sp*;* 7. *Rhizopus* spp*;* 8. *Sclerotium rolfsii;* 9. *Tricoderma*sp*;* 10. *Serpula lacrymans*

DISCUSSION

Characterization of compounds identified from *P. africana* and *E. suaveolens* seeds

Findings from this study showed that PM35 fraction from *P. africana* exhibited lupenone features, while PM57 indicated triterpenes and lupeol. PM72 suggested a triterpene mixture with potential daucosterol presence. In contrast, EM86 from *E. suaveolens* showed constituents resembling wax and fatty substances, distinct from *P. africana*. Lupenone, a pentacyclic triterpene, has been

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found to possess significant antimicrobial properties.

Triterpenes, including lupenone and lupeol, have been found to exhibit significant antimicrobial properties. Research has demonstrated that these compounds possess antimicrobial activity against a variety of microorganisms. For instance, lupeol has been reported to exhibit antimicrobial activity in addition to its anti-inflammatory and anticancer properties (Gallo and Sarachine, 2009; Liu *et al.,* 2021). Similarly, lupenone has been shown to possess antimicrobial activity, among other beneficial health effects. Research has shown its antimicrobial activity against a variety of microorganisms. A study by Andrade *et al.* (2022) tested the antimicrobial activity of extracts and lupenone from *A. inundata* and found promising results. Lupenone also has been reported to exhibit beneficial health effects, including antimicrobial activity, in various studies (Xu *et al.,* 2018). Furthermore, a review article by Alfaro-Almaguer *et al.* (2022) highlighted the antimicrobial activity of lupenone, emphasizing its potential in this regard.

Research reported that daucosterol demonstrated antimicrobial activity against certain strains of bacteria (Sultana and Afolayan, 2007) and antifungal properties of wood extracts from various trees (Rodrigues *et al.,* 2010). Therefore, it is possible that daucosterol may exhibit antimicrobial properties on wood.

Effect of antibacterial activities and Zone of Inhibition methanol crude extracts against test bacteria

P. africana and *P. macrophylla* methanol seed extracts with ZoI ranging from 18 - 21 mm and 18 - 24 mm, respectively, were very active against most bacteria such as *Bacillus subtilis, Streptococcus salvarius, Erwinia carotovora, Enterobacter* sp, *Rhanella* sp, *Klebsiella* sp *and Pectobacterium carotovorum. E. suaveolens* methanol seed extracts exhibited very active antibacterial activities against *Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescens, Pseudomonas contexa* and *Klebsiella* spand the zone of inhibition was within the range of 18 to 20 mm. Interestingly, although *Streptococcus salvarius, Erwinia carotovora, Enterobacter* sp, *Rhanella* sp and *Pectobacterium carotovoru* were inhibited by *P. africana* and *P. macrophylla*seed extracts at ZoI range of 18 – 24 mm. This result from this study agrees with the findings of Seukep *et al.* (2015) who investigated the antibacterial activities of medicinal plants against various bacterial strains. The study suggested that plant extracts from *Fagara macrophylla, Canarium schweinfurthii* and *Myrianthus arboreus*, could be applied for the control some infections and especially those involving common multidrug-resistant organisms (MDROs) bacterial species. The activities of selected medicinal plants (*Pentaclethra macrophylla, Entada africana* and *Entada abyssinica)* against multi-drug resistant Gram-negative bacteria (*Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae* and *Providencia stuartii*) in Cameroon by Tchana *et al.* (2014). The study reported that the testes medicinal plant extracts could control of some bacterial infections.

Effect of fungicidal activities and zone of inhibition of test fungi

The fungicidal potential of methanol crude extracts derived from the seeds of *P. africana, P. macrophylla*, and *E. suaveolens* against a diverse array of test fungi. The findings reasonably agree with the findings by Kebede and Shibeshi (2022) who reported antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. The researchers reported that the ethyl acetate fraction of the crude methanol extract exhibited the best antimicrobial activity. Zanna *et al.* (2021) reported fungicidal potential of selected plant extracts against human pathogens which was demonstrate using methanol extracts of plant materials prepared by polarity-based solvent extraction. A study on the antifungal and antioxidant potential of methanolic extracts from *Acorus calamus L., Chlorella vulgaris*

Beijerinck, Lemna minuta Kunth, and *Scenedesmus dimorphus* (Turpin)carried out by Dinev *et al.* (2021) found that the methanol extracts of these plants showed antifungal activity against eight fungi (*F. oxysporum*, *A. flavus*, *A. niger*, *F. graminearum*, *A. ochraceus*, *Alt. alternata*, *A. carbonarius* and *P. chrysogenum)*. Also, a study on the phytochemical analysis, antioxidant, and antimicrobial screening of *Seriphidium oliverianum* plant extracts by Abbas *et al.* (2021) found that the methanol extract using conventional shaking process (CSP) showed the highest zone of inhibition (10.5 mm) against *Fusarium avenaceum* and *Fusarium brachygibbosum*. Similarly, a study on the antibacterial and antifungal activities and phytochemical profile of leaf extract from different extracts of *Ricinus communis* against selected pathogens by Suurbaar *et al.* (2017) reported that methanol extract showed the highest inhibitory activity against all the bacteria and fungi tested. Prempeh and Mensah-Attipoe, (2008) reported that aqueous extract of the root bark and root wood of *Zanthozylum zanthozyloides* showed bactericidal and fungicidal activity against selected clinical microorganisms, including *Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Aspergillus niger, Candida albicans,* and *Rhizopus oryzae.*

CONCLUSION

The study characterized Lupenone from PM35, mixture of triterpenes and lupeol from PM57, and mixture of triterpenes with daucosterol from PM72 fractions of *P. africana* seed. It was established that *P. africana, E. suaveolens* and *P. macrophylla* methanol seeds extracts were active against *Aspergillus fumigatus, Coniophora puteana* and *Rhizopus* sppat MIC of 5 mg/ml, and MFC of 10 mg/ml. The study also reported that *P. africana, E. suaveolens* and *P. macrophylla* methanol seeds extracts were active against *Bacillus subtilis* and *Klebsiella* spat MIC of between 2.5 mg/ml and 5 mg/ml, and MFC of 10 mg/ml.

REFERENCES

Abbas, A., Naqvi, S. A. R., Rasool, M. H., Noureen, A., Mubarik, M. S., and Tareen, R. B. (2021). Phytochemical Analysis, Antioxidant and Antimicrobial Screening of *Seriphidium oliverianum* Plant Extracts. *Dose-response: a publication of International Hormesis Society*, *19*(1), 15593258211004739.

Abdul, I. W., Mohamad, N. H., Mizaton, H. H., Mohsin, H. F., and Ponto, T. (2011). Stigmasterol from a *Pandanus* and *Myrmecodia* species. In *2011 International Symposium on Humanities, Science and Engineering Research* (pp. 1-3). IEEE.

Ahmed, E. E (1995). The effect of some Bruchid seed borers on the *Acacia nilotica*, *Prosopis chilensis a*nd their control M. Sc. Thesis, Faculty of agric. Khartoum university. 68-144.

Ajayi, I.A., Dawodu, F.A., Adebowale, K.O. and Oderinde, R.A. (2002). Chemical composition of *Pentaclethra macrophylla* seed and seed oil grown in Nigeria. *Rivista Italiana delle Sostanze Grasse,*74. 183-185.

Akinpelu, B. A., Dare C. A., Adebesin F. I., Iwalewa E. O. and Oyedapo O. O. (2012). Effect of stem - bark of *Erythrophleum suaveolens* (Guill. & Perri.) saponin on fresh water snail (*Lanistes lybicus*) tissues. *African Journal of Environmental Science and Technology,* 6(11), pp. 446-451,

Alfaro-Almaguer, J. A., Mejía-Manzano, L. A., and González-Valdez, J. (2022). State-of-the-Art and Opportunities for Bioactive Pentacyclic Triter). penes from Native Mexican Plants. *Plants (Basel, Switzerland)*, *11*(17), 2184. https://doi.org/10.3390/plants11172184

Andrade, E. M. J. Teixeira, J. S. Silva D. K. C. dos Santos, b, T. B., Korn, M. G. A., Brandão, H. N. Ferraz, C. G., Meira, C. S., Soares, M. B. P., Guimarães, E. T., Netaa, L. C. S. and Júnior A. F. S. (2022). Phytochemical Analysis, Multi-Element

Scientia Africana, Vol. 23 (No. 4), October, 2024. Pp 1-20
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Composition and Biological Activities of Extracts and Lupenone from *Albizia inundata* (Mart.) Barneby & J.W. Grimes. *Journal of the Brazilian Chemical Society*,31(9), 1825-1837.

Dinev, T.; Tzanova, M.; Velichkova, K.; Dermendzhieva, D. and Beev, G. (2021**).** Antifungal and Antioxidant Potential of Methanolic Extracts from *Acorus calamus* L., *Chlorella vulgaris* Beijerinck, *Lemna minuta* Kunth and *Scenedesmus dimorphus* (Turpin) Kützing. *Applied Sciences*, *11*, 4745.

Ezike, A. C., Akah, P. A., Okoli, C. O., Udegbunam, S., Okwume, N., Okeke, C., and Iloani, O. (2010). Medicinal Plants Used in Wound Care: A Study of Prosopis africana (Fabaceae) Stem Bark. *Indian Journal of Pharmaceutical Sciences*, *72*(3), 334–339.

Gallo M. B. C. and Sarachine M. J. (2009). Biological Activities of Lupeol. *International Journal of Biomedical and Pharmaceutical Sciences*, 3 (Special Issue 1), 46-66.

John A. O. (2024). *Prosopis africana* Extracts as Potential Natural Alternatives to Synthetic Antibiotics and a Key for Sustainable Broiler Production: A Review. *Bioequiv and Bioavailab International Journal*, 8(1): 000227.

Kasilo, O. M. J., Wambebe, C., Nikiema, J., and Nabyonga-Orem, J. (2019). Towards universal health coverage: advancing the development and use of traditional medicines in Africa. *BMJ Global Health*, *4* (Suppl 9), e001517. https://doi.org/10.1136/bmjgh-2019-001517

Kebede, B., and Shibeshi, W. (2022). In vitro antibacterial and antifungal activities of extracts and fractions of leaves of *Ricinus communis* Linn against selected pathogens. *Veterinary Medicine and Science*, *8*(4), 1802–1815.

Kwesi A. (2020). A Review of *Pentaclethra macrophylla* (African Oil Bean) Seed. International Digital Organization for Scientific Research. *IDOSR Journal of Experimental Sciences* 5(1) 1-7.

Liu, K., Zhang, X., Xie, L., Deng, M., Chen, H., Song, J., Long, J., Li, X., and Luo, J. (2021). Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. *Pharmacological Research*, *164*, 105373.

Ninh The, Son. (2019). Genus Erythrophleum: Botanical description, traditional use, phytochemistry and pharmacology. Phytochemistry Reviews. 10.1007/s11101-019-09640-0.

Prashant, A. and Krupadanam, G. D. (1993). Dehydro-6-hydroxyrotenoid and lupenone from Tephrosia villosa. *Phytochemistry*, *32*(2), 484-486.

Prempeh, A., and Mensah-Attipoe, J. (2008). Crude aqueous extract of the root bark of *Zanthoxylum xanthoxyloides* inhibits white blood cells migration in acute inflammation. *Ghana Medical Journal*, *42*(3), 117–119.

Rodrigues, A. M. S., Theodoro, P. N. E. T., Eparvier, V., Basset, C., Silva, M. R. R., Beauchêne, J., Espı´ndola L. S. and Stien, D. (2010). *Search for Antifungal Compounds from the Wood of Durable Tropical Trees. Journal of Natural Products, 73(10), 1706 - 1707.*

Scientific African, 13, e00864,https://doi.org/10.1016/j.sciaf.2021.e00864.

Seukep, J. A., Ngadjui, B., and Kuete, V. (2015). Antibacterial activities of *Fagara macrophylla*, *Canarium schweinfurthii*, *Myrianthus arboreus*, *Dischistocalyx grandifolius* and *Tragia benthamii* against multi-drug resistant Gram-negative bacteria. *SpringerPlus*, *4*, 567.

Siddig, S.A (1991). Evaluation of neem seed and leaf water extracts and powders foe the control of insect pests in the Sudan, Technical Bulletin. Agricultural Research Crop. Shambat Research Station, technical Bulletin No. 6. 39 pp.

Sultana, N., and Afolayan, A. J. (2007). A novel daucosterol derivative and

antibacterial activity of compounds from *Arctotis arctotoides. Natural Product Research*, 21(10), 889–896.

Suurbaar, J., Mosobil, R. and Donkor, A.M. (2017). Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of *Ricinus communis* against selected pathogens. BMC Research Notes, 10, 660

Tchana, M. E., Fankam, A. G., Mbaveng, A. T., Nkwengoua, E. T., Seukep, J. A., Tchouani, F. K., Nyassé, B., and Kuete, V. (2014). Activities of selected medicinal plants against multi-drug resistant Gram-negative bacteria in Cameroon. *African Health Sciences*, 14(1), 167–172.

Winrock International (1995). *Pentaclethra macrophylla:* a multipurpose tree from Africa with potential for agroforestry in the tropics. *https://winrock.org/ pentaclethra-macrophylla-a-multipurpose-tree-from-africa-with-potential-for-agroforestry-in-the-tropics/*

Xu, F., Huang, X., Wu, H., and Wang, X. (2018). *Beneficial health effects of lupenone triterpene: A review. Biomedicine and Pharmacotherapy, 103, 198–203.*

Yanda, L., Tatsimo, S. J. N., Tamokou, J. D., Matsuete-Takongmo, G., Meffo-Dongmo, S. C., Meli Lannang, A., and Sewald, N. (2022). Antibacterial and Antioxidant Activities of Isolated Compounds from *Prosopis africana* Leaves. *International Journal of Analytical chemistry*, 4205823. https://doi.org/10.1155/2022/4205823

Zanna, H., Tijani, Y., Shettima, A., Modu, B., Damasak, A., and Uzairu, S. (2021). Fungicidal potential of selected plant extracts against human pathogenic fungi. *Scientific African*. 13. e00864. 10.1016/j.sciaf.2021.e00864.