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In vitro anti-helminthic, antimicrobial, antioxidant, activity and GC-MS of fractions of the stem bark of *Memecylon accedens*

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Abstract
The three fractions, F_1, F_2, and F_3 obtained from TLC-guided bulking were tested for antimicrobial activity by broth microdilution method using the following microbes: *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella typhi,* and *Candida albicans*. F_1 demonstrated the highest activity in the antimicrobial analysis, followed by F_3, and F_2 had the least activity. Anthelmintic analysis of extract using *Pheretima posthuman* showed promising activity. The fractions also exhibited activity with F_1 having the highest activity. DPPH radical scavenging was performed for the fractions to test for antioxidant activities. The IC_{50} values recorded for extract, F_1, F_2, and F_3 DPPH assay were 54.46±0.104 µg/ml, 42.53±0.238 µg/ml, 115.44±0.496 µg/ml, and 45.23±0.058 µg/ml respectively. F_1 showed the greatest antioxidant activity. The fractions were also subjected to FT-IR and GC-MS analysis. This was done to obtain information to confirm the phytochemicals that were present in the extract and each of the fractions. The findings of this study provide insights into the possible use of *Memecylon accedens* as a potential agent for the discovery and development of drugs for the treatment of Helminth-related diseases, reactive oxidants, and microbial infections.

Keywords: *Memecylon accedens,* anti-helminthic, phytochemicals, broth microdilution

Introduction
Natural compounds have significantly contributed to drug discovery and the advancement of pharmacotherapy [1]. Since ancient times, numerous plants have been known for their medicinal use and have been used to treat various diseases [3]. Recently, there has been a great use on medicinal plants for disease treatment and health maintenance, even in developed nations [2]. Research suggests that approximately 40% of the world depends on traditional medicine or plant-based remedies for their healthcare needs [6]. For instance, extracts from plants like neem and basil are well known for their antibacterial properties, and multiple bioactive compounds have been identified from these sources [10]. Likewise, several species within the *Memecylon* genus have been widely used in traditional medicine, particularly in regions such as India and Madagascar. Studies conducted by Puttaswamy & Achur have shown that extracts from *Memecylon umbellatum* exhibit strong antibacterial and antioxidant properties, along with moderate antifungal activity [13]. Similarly, methanolic extracts of *Memecylon terminale Dalz* have demonstrated significant biological effects, including analgesic and antioxidant activities [13]. These therapeutic benefits are largely attributed to the presence of phytochemicals. Despite the recognized medicinal value of plants, one major limitation of traditional herbal medicine is the practice of using entire plant parts for treatment [12]. This method may not always be effective, as only certain components may contain the active compounds necessary for treating specific conditions, while other parts may be inactive or even toxic. Therefore, it is crucial to identify the specific bioactive fractions responsible for therapeutic effects. This study aims to investigate the phytochemical composition and pharmacological potential of the stem of *Memecylon accedens*, contributing to a deeper understanding of its medicinal properties.

Materials and Methods
The materials and methods used are described in Buah, P., *et al*. 2024. [15].

Results and discussion

Fractionation by column chromatography

Column chromatography for the separation of the crude extract into fractions for bioactivity analysis was performed. After the column was set up, the mobile phase was added to the column in small portions and allowed to percolate through the packed bed under gravity. The separation of the components takes place by selective retardation of the components of the mixture as a result of their interaction with the stationary phase. The mobile phase was allowed to flow through the column until the components in the mixture were successively collected in fractions (Heftmann *et al*, 2012). Sixty (60) fractions were collected since they were collected in small quantities. The collected fractions were bulked according to their TLC profiles to obtain three different fractions, labeled F_1, F_2, and F_3. [7].

Phytochemical analysis of fractions

Table 1: Summary of phytochemical analysis of fractions

Secondary metabolite	F_1	F_2	F_3
Phenolics	+	+	-
Alkaloids	+	-	-
Saponins	-	-	+
Steroids	+	+	+
Tannins	+	-	+
Terpenoid	+	+	+
Flavonoids	+	-	+
Polyphenols	+	+	-

Key: + Detected; - undetected

The fractions were analyzed for the presence of phytochemicals that were initially present in the extract. F_1 had the greatest number of phytoconstituents (phenolics, alkaloids, steroids, tannins, terpenoids, flavonoids, polyphenols), followed by F_3 showing the presence of saponins, steroids, tannins, terpenoids, flavonoids, and F_2 having the least number with only phenols, steroids terpenoids, and polyphenols out of the eight that were analyzed. All the fractions are likely to have biological activities due to the presence of these important phytochemicals.

FT-IR analysis of fractions

FT-IR analysis on the fractions revealed functional groups which further supported the results of the phytochemical analysis. For instance, the presence of phenol may reveal an OH group that is highly deshielded. The functional groups that were detected by the FT-IR in the various fractions have been reported. F_1 with the greater number of phytochemicals present displayed a number of functional groups of which some may be present in the secondary metabolites. F_2 and F_3 too showed the presence of several functional groups.

F_1

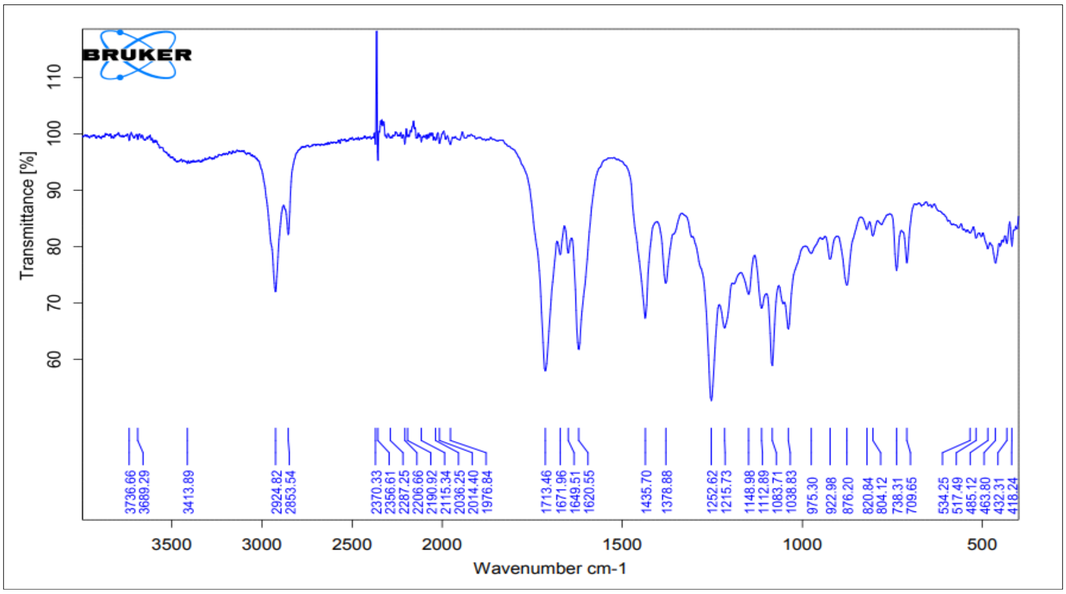

Fig 1: FT-IR spectrum of fraction F_1

F_2

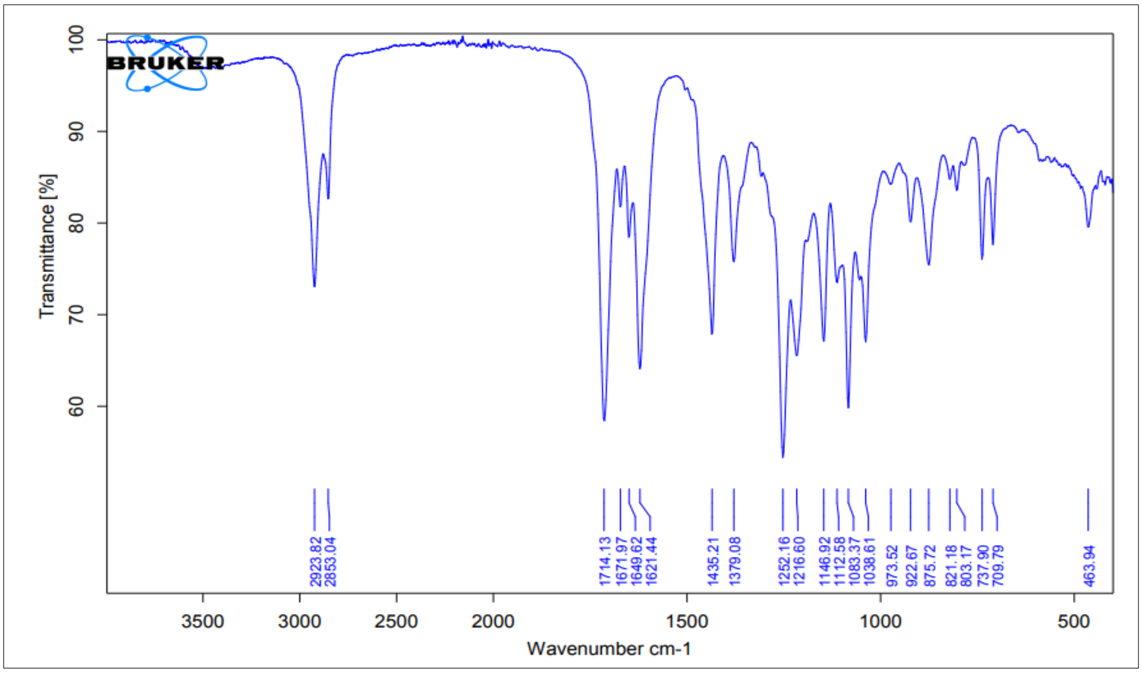

Fig 2: FT-IR spectrum of fraction F_2

F_3

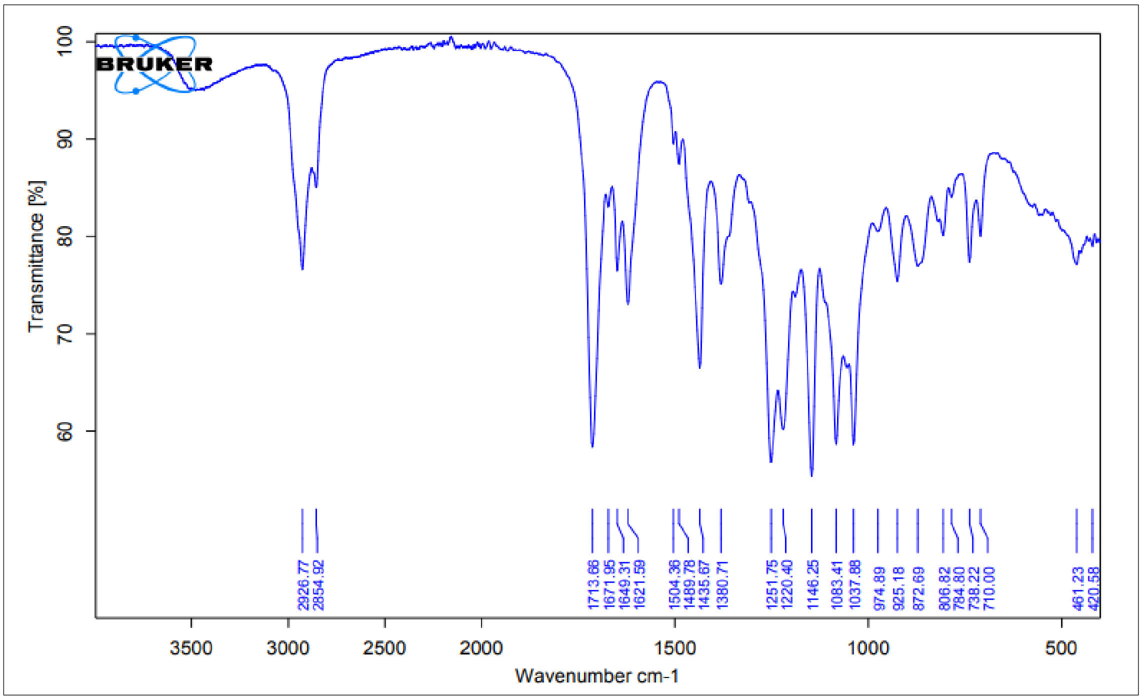

Fig 3: FT-IR spectrum of fraction F_3

GC-MS Analysis of the fractions

The fractions were further analyzed with gas chromatography-mass spectrometry (GC-MS) which combines the advantages of gas chromatography and mass spectrometry to identify various compounds in a test sample. The results from the GC-MS displayed several compounds whose functionality corresponded to the phytoconstituents and FT-IR analysis of the fractions. This further confirmed the presence of these secondary metabolites. The identification of the compounds in the GC-MS analysis was done by comparing the fragmentation patterns of the compounds with those of authentic standards from the NIST library presented in the appendix.

F$_1$

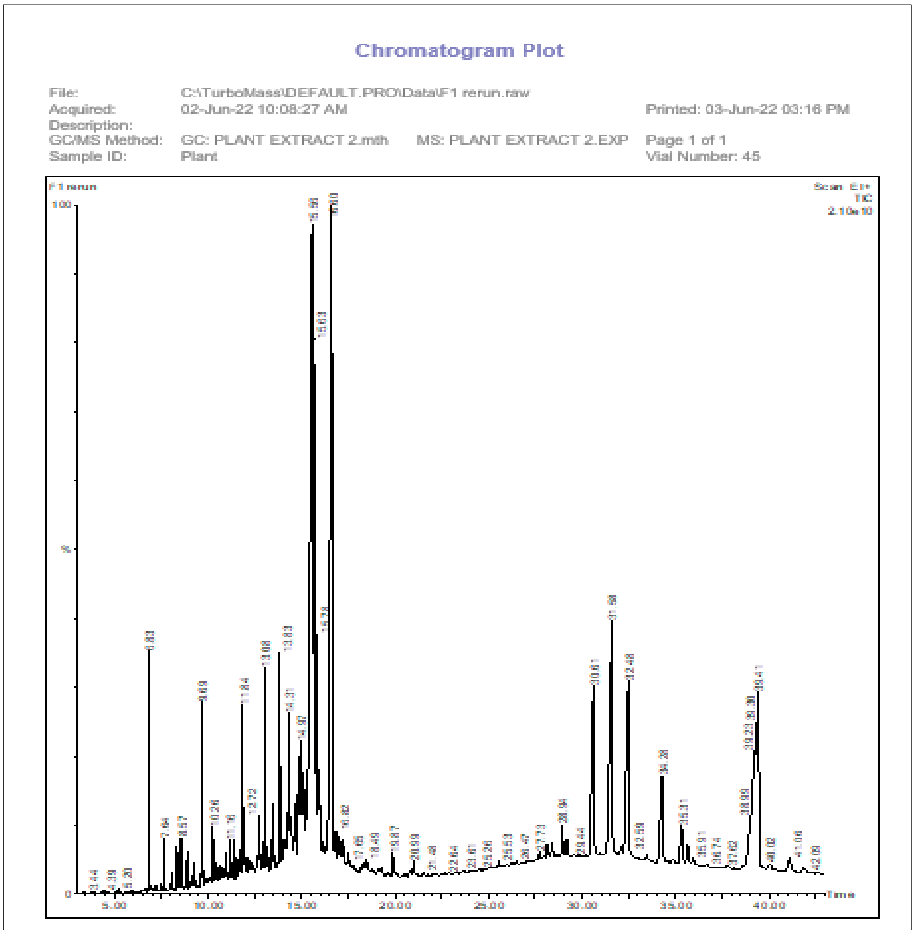

Fig 4: GC-MS Chromatogram of F$_1$

Table 2: Compounds identified in fraction F$_1$ by GC-MS and their retention time

Retention time/ min	Similarity index/%	Compound
6.830	99.79	Eugenol
11.836	98.47	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
12.716	95.02	7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2à(2E,6E),3, 2,6-Nonadienoic acid
13.083	97.38	9-(3,3-dimethyloxiranyl)-3,7-dimethyl-, methyl ester, (E,E)- 2,6-Nonadienoic acid
13.835	96.37	cis-Z-à-Bisabolene epoxide
14.311	95.31	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)]undecane
14.440	89.35	1-methyl-3-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-propenyl ester Acetic acid
14.678	97.73	3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)- 3-Buten-2-ol
14.861	97.53	1-Heptatriacotanol
14.971	79.83	[4-(1,1-dimethylethyl)phenoxy]-, methyl ester Acetic acid
15.118	79.61	cyclobutyl ester-3-Methyl-2-butenoic acid
15.210	93.34	3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl-2H-Indeno[1,2-b]furan-2-one
15.631	92.41	Methoprene
15.723	93.52	7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2à(2E,6E),3,2,6-Nonadienoic acid,
15.778	98.65	3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide
15.925	88.51	2-(5-(1-hydroxyethyl)-5-methyl-tetrahydrofuran-2-yl)propan-1-ol
16.603	93.10	4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-3H-Naphtho[2,3-b]furan-2-one
19.867	92.82	12,13-epoxy-, 3-acetate, (3à)- Trichothec-9-ene-3,15-diol
27.733	89.78	acetate, (3á,22E)- Ergosta-5,22-dien-3-ol
28.154	82.47	acetate Retinol
28.411	91.56	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane
28.943	84.75	(3á)-Cholesta-4,6-dien-3-ol
29.200	92.81	Desoximetasone
31.583	91.14	1,4-Dimethoxy-6,7,8,9-tetrahydro-5-benzocycloheptenone
32.482	93.49	1,4-bis(3,4-dimethoxyphenyl)tetrahydro-, [1R-(1à,3aà,4á,6aà)]- 1H,3H-Furo[3,4-c]furan
34.278	99.51	9,12-dim -15-oxapentacyclo[12.6.0.0(1,6).0(2,18).0(8,13)]icosa-8(13),9,11-triene-5-carboxylic acid,
35.305	99.51	1,2-Bis[1-(2-hydroxyethyl)-3,6-diazahomoadamantantydene-9]hydrazine
35.635	97.38	Stigmasta-3,5-dien-7-one
39.412	94.53	Yangambin
41.099	88.73	methyl ester 5,8,11,14-Eicosatetraynoic acid

F_2

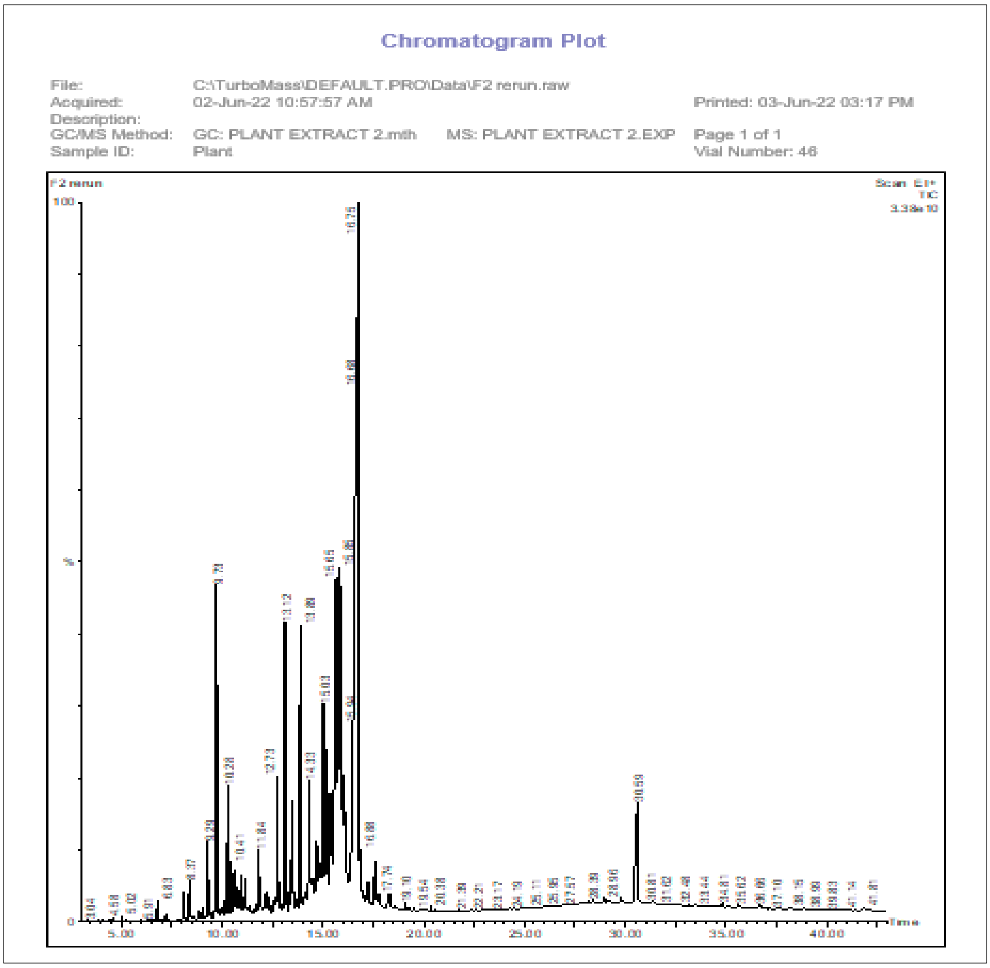

Fig 5: GC-MS Chromatogram of F_2

Table 3: Compounds identified in the fraction F_2 by GC-MS and their retention time

Retention time/ min	Similarity index/%	Compound
8.371	99.66	4-acetyl-, methyl ester Benzoic acid
9.287	98.93	l, 4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4á)]- Cyclohexanemethanol
10.406	99.21	tau.-Cadinol
11.836	99.45	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one
13.890	96.17	cis-Z-à-Bisabolene epoxide
14.330	99.12	4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-, [4ar-(4. 2(1H)-Naphthalenone,
14.696	98.41	5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol
15.026	97.92	Methyl 3-bromo-1-adamantaneacetate
15.155	81.92	pent-2-en-4-ynyl ester3-Methyl-2-butenoic acid
15.338	82.61	2,6-dimethylnon-1-en-3-yn-5-yl ester3-Methyl-2-butenoic acid
15.705	91.12	Methoprene
15.851	85.94	8,9-dehydro-9-formyl- Cycloisolongifolene
15.943	92.39	3,4,4'-Triaminodiphenylsulfone
16.053	87.90	8-acetoxy-2,6-dimethyl-, methyl ester2,6-Octadienoic acid
16.787	93.22	4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-3H-Naphtho[2,3-b]furan-2-one
17.575	88.73	1,4-dimethyl ester5,8-Dimethyl-1,4,6,7-tetrahydronaphthalene-1,4-dicarboxylic acid
18.308	88.72	11-methoxy-3,7,11-trimethyl-, methyl ester, (E,E)- 2,4-Dodecadienoic acid
25.954	98.38	3-[(6-deoxy-3,4-O-methylenehexopyranos-2-ulos-1- Card-20(22)-enolide yl)oxy]-5,11,14-trihydroxy-1.
26.229	99.03	3,5-dichloro-6-nitro-, (3á,5à,6á)- Cholestane
28.173	92.53	10,12,14-Nonacosatriynoic acid
28.393	96.42	3-Methoxymethoxy-3,7,16,20-tetramethyl-heneicosa-1,7,11,15,19-pentaene
28.961	90.64	7-Dehydrodiosgenin
29.145	85.95	7-Butyl-8-((2-(3,4-dimethoxyphenyl)ethyl)amino)-3-methyl-3,7-dihydro-1H-purine-2,6-dione
29.786	94.60	3-(2,2,5,7,8-Pentamethyl-chroman-6-sulfonyl)-6-phenyl-[1,3]oxazinane
30.593	100	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane
31.345	86.62	Gitoxigenin
34.150	97.54	Stigmasterol
35.617	93.32	Stigmasta-3,5-dien-7-one

F_3

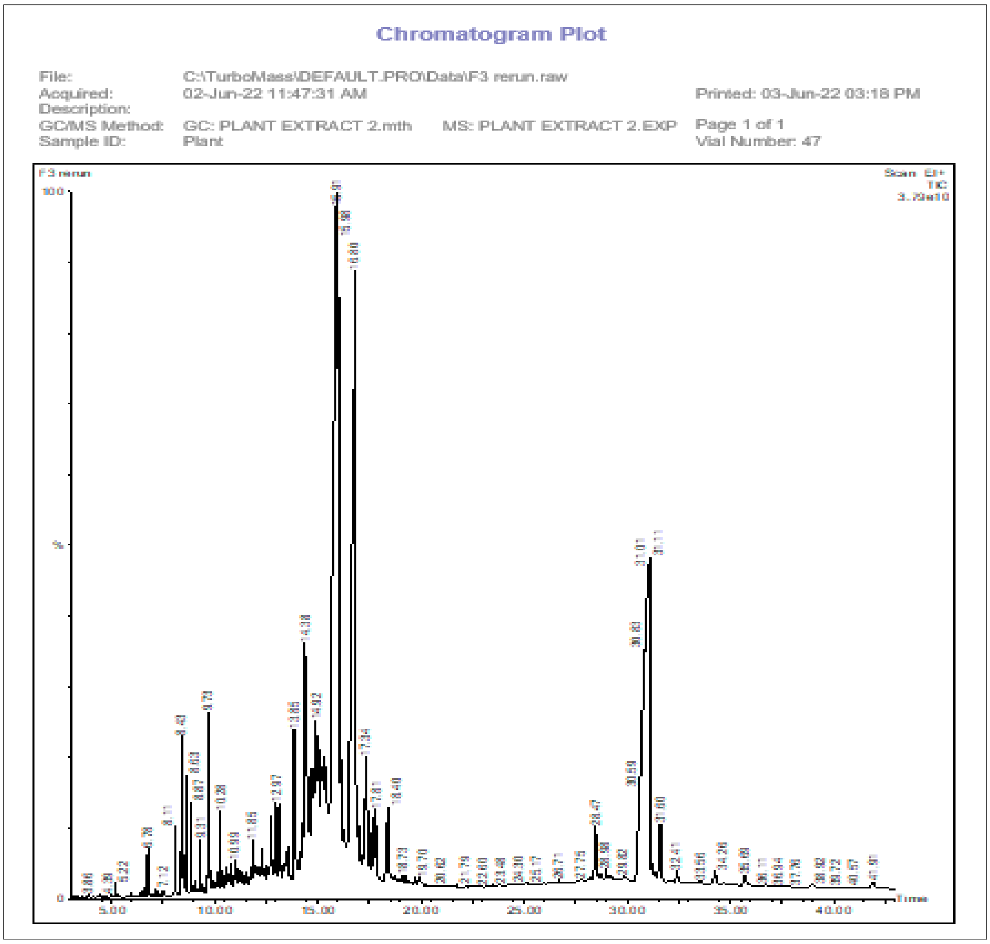

Fig 6: GC-MS Chromatogram of F_3

Table 4: Compounds identified in the fraction F_3 by GC-MS and their retention time

Retention time/ min	Similarity index/%	Compound
6.775	90.64	3-methoxy Pentane
8.114	84.70	2,6,6-trimethyl-2,4-Cycloheptadien-1-one
8.426	99.78	4-acetyl-, methyl ester Benzoic acid
8.627	99.83	1-(2-methyl-3-cyclohexenecarbonyl)-, methyl ester2-Cyclohexene-1-carboxylic acid
8.866	82.80	à-hydroxy-à-methyl-, methyl ester Benzeneacetic acid
10.993	98.14	4-(2-Acetyl-5,5-dimethylcyclopent-2-enylidene)butan-2-one
11.854	99.59	Corymbolone
12.734	98.17	7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2à(2E,6E),3,2,6-Nonadienoic acid,
12.973	94.85	3à,4à,9á,11-Diepoxymuurolan-10-ol
13.120	91.41	11-methoxy-3,7,11-trimethyl-, methyl ester, (E,E)- 2,4-Dodecadienoic acid
13.596	98.50	3-(6,6-Dimethyl-5-oxohept-2-enyl)-cycloheptanone
13.853	99.22	3,7,11,15-tetramethyl-, methyl ester, (E,E,E) 2,6,10,14-Hexadecatetraenoic acid
14.238	93.40	iso-propyl 5,8,11,14,17-eicosapentaenoate
14.385	89.19	Globulol
14.715	97.76	3-Buten-2-ol, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
14.916	98.47	3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester Formic acid
15.026	97.55	Methyl 3-bromo-1-adamantaneacetate
15.173	87.41	Binapacryl
15.338	94.32	3à,4à,9á,11-Diepoxymuurolan-10-ol
15.980	96.17	7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2à(2E,6E),3,2,6-Nonadienoic acid
16.163	82.21	2-Mercapto-4-methoxypyridine-1-oxide
16.805	93.00	4-hydroxy-4a,5-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydro-3H-Naphtho[2,3-b]furan-2-one
16.933	73.21	2,6-dimethyl- 3,7-Octadiene-2,6-diol
17.630	95.02	1,4-dimethyl ester -5,8-Dimethyl-1,4,6,7-tetrahydronaphthalene-1,4-dicarboxylic acid
17.813	88.68	Isopropyl 5,11-dihydroxy-3,7,11-trimethyl-2-dodecenoate
28.466	84.74	1,10-Epoxy-4-methoxy-7-methyl-8-(3,4-methylenedioxyphenyl)-spiro(5,5)undeca-1,4-dien-3-one
31.106	99.88	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane
31.602	90.34	1,4-Dimethoxy-6,7,8,9-tetrahydro-5-benzocycloheptenone

Antioxidant activities of fractions

DPPH radical scavenging activity

Table 5: DPPH radical scavenging activity of the fractions of the methanol extracts of Memecylon accedens and ascorbic acid expressed as IC_{50}

Sample	IC_{50} (µg/ml) ± SEM
Standard (Ascorbic acid)	40.87±0.178[a]
F_1	42.53±0.238[b]
F_2	115.44±0.496[d]
F_3	45.23±0.058[c]

The three fractions using ascorbic acid as the standard were analyzed for their antioxidant activity. The dose-dependent IC_{50} recorded for F_1, F_2, and F_3 was 42.53±0.238 µg/ml, 115.44±0.496 µg/ml, and 40.87±0.178 µg/ml respectively. Antioxidant activity is inversely proportional to the IC_{50} value, hence the lower the IC_{50} of a test solution the higher the antioxidant activity of the solution. Considering the IC_{50} values recorded, ascorbic acid (standard) showed only a slightly higher antioxidant activity than F_1. This depicts the strong antioxidant activity of F_1. This was followed by F_3 and then F_2. The antioxidant activity of F_2 was very low, and this is consistent with the lower number of phytoconstituents in the fraction, especially the absence of flavonoids and tannins that are known to exhibit antioxidant activities. The greater number of phytoconstituents in F_1, and the presence of flavonoids and tannins, as well as synergism, may be responsible for the high anti-oxidant activity of fraction F_1. Among the three fractions, F_1 could be explored for use as an active antioxidant agent. The activities of the fractions were also observed to be significantly different (F_1; $P<0.0001$, F_2; $P<0.0083$, F_3; $P<0.0014$) from the standard.

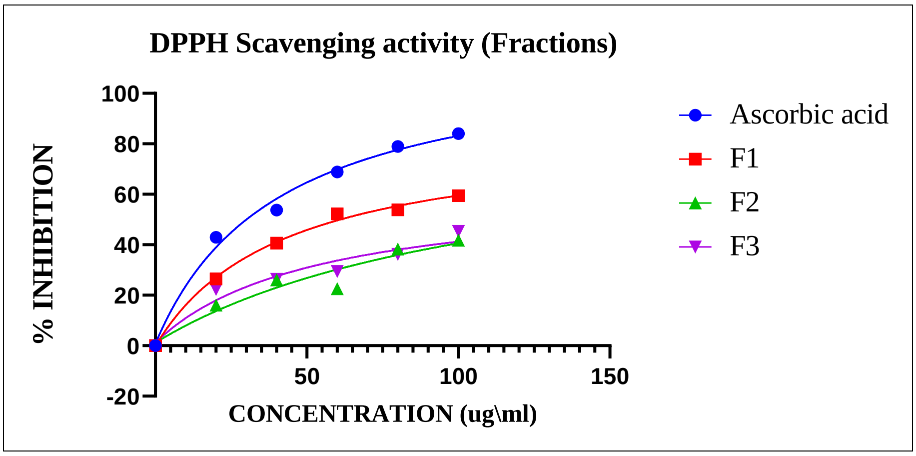

Fig 7: DPPH scavenging activity F_1, F_2, F_3, and ascorbic acid (standard)

Anthelmintic activity of the fractions

Table 6: Paralysis time of worms when fractions were administered

(Concentration mg/mL)	Paralysis time/mins			
	F_1	F_2	F_3	Mebendazole
20	10.00±0.808	28.00±0.943	12.03±0.448	25.49±0.812
10	16.07±0.945	57.11±0.776	24.00±0.471	41.81±0.296
5	27.14±0.485	72.02±1.224	42.08±1.416	59.25±1.580
2.5	56.43±1.007	114.51±2.239	67.48±1.209	84.02±0.270
1.25	115.11±1.417	203.43±1.651	126.74±0.769	157.47±0.427

Table 7: Death time of worms when fractions were administered

(Concentration mg/mL)	Death time/mins			
	F_1	F_2	F_3	Mebendazole
20	22.23±1.262	67.48±1.300	40.14±1.525	57.24±1.378
10	42.09±1.676	105.30±1.372	62.09±1.204	80.01±0.682
5	66.47±1.829	155.25±1.305	88.10±1.967	126.10±0.478
2.5	123.00±1.247	285.03±2.381	160.40±1.236	257.71±1.659
1.25	226.77±1.838	367.22±2.061	268.33±1.515	329.14±1.440

F_1 and F_3 displayed remarkable anthelmintic activity. F_1 and F_3 recorded shorter paralysis and death times than the standard, mebendazole. F_1, however, was more active than F_3. This is also consistent with the greater number of secondary metabolites that were detected in F_1 and F_3. Terpenoids, tannins, and saponins are known to possess anthelmintic activity [14], and they may be responsible for the anthelminthic activity of F_1 and F_3. Fraction F_2, however, took a very long time to cause paralysis and death, perhaps due to the absence of tannins and saponins. Also, F_1 exhibited better anthelmintic activity than the extract. This could be due to fewer impurities and synergism in the fraction F_1 as compared to the extract. F_1 could be further purified to isolate an anthelmintic compound. The activities of the fractions were also observed to be significantly different (F_1; $P<0.0002$, F_2; $P<0.0001$, F_3; $P<0.0001$) from the standard.

Antimicrobial activities of fractions

Table 8: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Fractions

Test organisms	F_1 (mg/ml)		F_2 (mg/ml)		F_3 (mg/ml)		Ciprofloxacin	Fluconazole
	MIC	MBC	MIC	MBC	MIC	MBC	MIC (µg/ml)	MIC (µg/ml)
Escherichia coli	25	50	37.5	>50	50	>50	7.6×10^{-1}	NA
Streptococcus pyogenes	31.25	50	37.5	>50	25	25	9.7×10^{-2}	NA
Staphylococcus aureus	31.25	50	25	>50	25	25	9.8×10^{-2}	NA
Salmonella typhi	18.75	50	12.5	>50	25	25	7.9×10^{-1}	NA
Candida. albicans	25	50	37.5	>50	18.75	25	NA	1.25×10^{1}

NA: Not Applicable

All three fractions showed antimicrobial activity against the various organisms that were used in the test. F_1 and F_3 further showed bactericidal and fungicidal properties within the prepared concentration. F_2, however, did not record MBC at concentrations equal to or lesser than 50 mg/ml, meaning it could not kill the bacteria at those concentrations. It however inhibited *Staphylococcus aureus* and *Salmonella typhi* at a pretty lower concentration of 25 mg/ml and 12.5 mg/ml respectively. The standard drugs ciprofloxacin and fluconazole, however, inhibited the microbes at a relatively lower concentration. The standard was more active than the fractions. The fractions could, however, be further investigated for a single antimicrobial compound. This could be very potent at a lower concentration. Some parts of the fractions might have acted antagonistically in rendering effect.

Evaluation of the bactericidal and bacteriostatic capacity of extract and fractions

Table 9: Evaluation of the bactericidal and bacteriostatic capacity of extract and fractions

Test organisms	MBC/MIC			
	Extract	F_1	F_2	F_3
Escherichia coli	1.2	2	>4	>4
Streptococcus pyogenes	1.2	1.6	>4	1
Staphylococcus aureus	1.2	1.6	>4	1
Salmonella typhi	1.2	2.67	>4	1
Candida. albicans	1.2	2	>4	1.33

Key: MBC/MIC ≤ 2 Bactericidal; MBC/MIC ≥ 4 Bacteriostatic

According to [4] the ratio of MBC/MIC ≤ 2 is considered bactericidal whilst MBC/MIC ≥ 4 implies a bacteriostatic effect. The extract synergistically showed a pronounced bactericidal effect. F_1 showed a bactericidal effect on almost all the test organisms except Salmonella typhi. F_3 showed neither MIC nor MBC effect on *E. Coli* at the tested concentrations. It however showed a good bactericidal effect on the rest of the test organisms. F_2 neither showed bactericidal nor bacteriostatic effect at the concentrations at which the experiment was carried out.

Conclusion

TLC-guided bulking revealed three distinct fractions, F_1, F_2, and F_3 that were further investigated for pharmacological activities. F_1 has the highest activity, followed by F_3 and F_2 recording the lowest biological activity. FT-IR and GC-MS analyses of the fractions were also consistent with their phytoconstituents. The ethnopharmacological profile of the methanolic stem fractions of *Memecylon accedens* showed the medicinal nature of the plant and hence it could be explored for new pharmacological agents to combat infection through drug discovery and development. Hence the traditional use of the plant has been justified.

Data Availability

All the data has been included in the manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

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