Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2024; 13(2): 799-805
Received: 18-02-2024
Accepted: 24-03-2024

Akwasi Acheampong
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Emmanuel Quartey
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Maud Adoley Acquaye
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Abdulai A Naazo
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Kennedy Ameyaw Baah
[1] Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[2] Department of Science Education, Wesley College of Education, Kumasi, Ghana

Lydia Tima Amankwaa
Department of Pharmacy Technician, Sunyani Technical University, Sunyani, Ghana

Patrick Buah
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Silas Ofori Frimpong
[1] Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[4] Department of Chemistry, University of Maryland, College Park, U.S.A

Corresponding Author:
Akwasi Acheampong
Department of Chemistry, Faculty of Physical and Computational Sciences, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

In vitro anthelminthic and anti-inflammatory activities, and GC-MS analysis of methanol and acetone extracts of *Mareya micrantha* leaves

Akwasi Acheampong, Emmanuel Quartey, Maud Adoley Acquaye, Abdulai A Naazo, Kennedy Ameyaw Baah, Lydia Tima Amankwaa, Patrick Buah and Silas Ofori Frimpong

DOI: https://doi.org/10.22271/phyto.2024.v13.i2f.14926

Abstract
Mareya micrantha, a medicinal plant, is used to treat pains, wounds, worm infestations and gastrointestinal disorders. The aim of this research was to investigate the anthelmintic and anti-inflammatory properties of the methanol and acetone extracts of *Mareya micrantha* leaves. Phytochemical screening was performed using standard methods with GC-MS used for the identification of the phytochemicals. Egg albumen denaturation was used for the determination of the anti-inflammatory (*in vitro*) activities of the extracts. Anthelmintic activity (*in vitro*) of the extracts was investigated against *Millsonia ghanensis*. Phytochemical investigation revealed the presence of phenols, terpenoids, polyphenols, flavonoids, tannins, steroids, saponins, and glycosides. Twenty phytochemicals, most of which have known bioactivities, were identified for each extract with five being common to them, and they are n-hexadecanoic acid, Tributyl acetyl citrate, hexadecanoic acid methyl ester, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, and 2, 2, 4-trimethyl-3-(3, 8, 12, 16-tetramethyl-heptadeca-3, 7, 11, 15-tetraethyl)-cyclohexanol. The extracts had anti-inflammatory activity. The anthelmintic activity of the extracts was significantly higher than mebendazole-treated helminths. The outcome of this study points to the fact that *Mareya micrantha* could be exploited as a source of potential drug candidates against helminthic and microbial infection as well as inflammation and oxidative stress.

Keywords: *Mareya micrantha*, anthelmintic, antimicrobial, anti-inflammatory, antioxidant

1. Introduction
Plants with medicinal value have been in use since ancient times. Natural products have been used in traditional medicine to treat and cure diseases. In most developing countries including Ghana, traditional medicine, specifically herbal medicine is a key element in health delivery [1-3]. The World Health Organization (WHO) has estimated that 70 to 95% of populations in developing nations depend on plants for their healthcare needs [4]. The WHO in its report concerning complementary and traditional medicine (2019) indicated that some national policies have been developed (98 Member States), some national laws or regulations had been launched (109 Member States), and implementation of the laws or regulations on herbal medicine had been done by 2018 (124 Member States) [5]. This indicates how member states of the World Health Organization have integrated traditional or herbal medicines into their healthcare delivery system.
M. micrantha belongs to the family, *Euphorbiaceae*, a member of the angiosperms and it is among the largest family with 300 genera and 8000 species. It is an important source of medicines [6]. Most members are shrubs or trees. *M. micrantha* is commonly called in twi as "odubrafo" in Ghana [7] and "Oyia and Ochibouo-m'pi" in Cote d'lvoire [8]. It is innate to Central and Western in the tropics [9].
The *M. micrantha* leaves have uses such as treatment of waist pains, have antibacterial, antifungal, antiplasmodial activities, and act as an abortifacient [2, 3, 7, 10]. It is used to fight dermatoid infections, leprosy, leishmaniasis, and have cardio-depressive, laxative, hypotensive and oxytocic properties, and act as oxytocic agent [3, 8].
M. micrantha aqueous extract has been found to increase the amplitude of the contractions of isolated rabbit duodenum, showed laxative, and myorelaxant activities [10]. The extracts of the leaves (methanol and cold aqueous) exhibited antibacterial activity against various bacteria including *Bacillus subtilis*, *Clostridium sporogenes*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Agrobacterium tumefaciens*, and *Escherichia coli* [11].

Reported on three new nor-cucurbitacins that had been isolated from the leaves, and the antioxidant activity that was exhibited by the isolated compounds [12].

Even though some scientific research has been conducted to confirm the plant's ethnobotanical use, there appears to be scanty information about the plant's anthelmintic and anti-inflammatory activities. This study aims to examine the methanol and acetone leaves extracts of *M. micrantha* for their anthelmintic and anti-inflammatory activities using *in vitro* assays and analyse the phytochemicals using GC-MS.

2. Materials and Methods

2.1. Collection of sample and authentication

Fresh leaves of *M. micrantha* were collected from Obuasi in Ghana (latitude: 6°19′10′ N and longitude: 1°64′97′ W) in November 2020. They were taxonomically identified and authenticated (KNUST/HMI/2014/L083) at the Herbal Medicine Department, KNUST, Ghana by Mr. Osafo Asare Clifford.

Chemicals and Reagents

Sigma Aldrich (Irvine, U.K.) is where the chemicals were procured but Kinapharma Ghana Limited generously donated the standard drugs. Organic solvents (analytical grade) were procured from BDH Lab. Suppl. (England).

2.3 Extraction of Phytoconstituents

Prior to extraction, running water and subsequently distilled water were employed to wash the leaves of *M. micrantha* thoroughly. This helped to remove debris and heavy metals. The leaves were dried for 21 days in the shade, pulverized, and kept in a desiccator until needed for analysis.

A mass of 300 g of the crude sample of *M. micrantha* was weighed separately into the reflux apparatus set up using separately acetone and methanol as solvents. Soxhlet extraction was carried out by placing the crude sample in a thimble which was then inserted inside the Soxhlet apparatus. This was exposed to two different solvents of different polarities (Methanol and acetone). The extraction process was carried out for 10 hours for acetone and 24 hours for methanol until the solvent appeared colourless in the upper extraction chamber [13]. After the effective extraction, extracts were concentrated at 50 ºC, and dried under nitrogen gas to completely remove any residual solvent. The yield of extract (%) was determined. Glass containers were used to store the crude extracts and kept in a freezer at 4 ºC until further analyses.

2.4 Phytochemical Screening of Extracts

Phytochemical screening of the pulverized sample and crude extracts (acetone and methanol) was carried out qualitatively using the standard method of Trease and Evans (2009) to establish the presence of secondary metabolites [14].

2.5. Collection of Worm and Authentication

The collection of the earthworms, *Millsonia ghanensis*, was done at a water-logged site at KNUST (longitude 1º30 W-1º35 W, and latitude 6º35 N- 6º40 N). The worms were transferred into glass bottles with some quantity of the soil from which they were taken. Mr. Lawrence Yeboah of KNUST Zoology Unit. authenticated the worm type.

2.5.1. *In-vitro* anthelminthic activity

The anthelminthic activity of the two extracts (Acetone and methanol) on *Millsonia ghanensis* (Earthworms) was performed with a method by Ajaiyeoba *et al*. [15] as reported in our earlier work [13]. With water and DMSO as solvents, 500 µg/mL stock solutions were prepared for each extract. Serial dilution was employed to prepare four solutions of concentrations 31, 63, 125, and 250 µg/mL. A 500 µg/mL solution of Mebendazole and distilled water (sterile) were respectively used as the reference standard and negative control. It was ensured that solutions were freshly prepared before commencement of the experiments. Into separate Petri dishes was poured 50 mL of each of the test solutions and four worms that were of comparable sizes were released into each of the Petri dish. The worms were monitored for the time of paralysis and death. When there is no movement seen after vigorously shaking the worms, the worms are to be considered paralyzed, and the time is recorded as the time of paralysis. When worms did not move upon being shaken vigorously or being submerged in warm water (50 ºC), followed by a body colour fading, the time was recorded as time of death since the worms were considered to be dead. Triplicate measurements were taken, and the results were reported as a mean ± SEM (standard error of the mean) (N = 3).

2.6. Anti-Inflammatory Assay with Denaturation of Egg Albumin

Inhibition of protein thermal denaturation have been found to be an activity caused by Anti-inflammatory agents, hence egg albumin denaturation was employed to assess anti-inflammatory activities in this study. The method utilized by Kumari *et al.* [16] was employed for the anti-inflammatory assay with slight modification as reported by Akoto and co-workers [13]. Concentrations of 5000 µg/mL of both methanol and acetone extracts solutions utilizing a solvent of sterile distilled water for the methanol extract and DMSO for the acetone extract were prepared. Diluted solutions were prepared utilizing the appropriate solvents to yield concentrations of 1000, 2000, 3000, and 4000 µg/mL. Aspirin, acetylsalicylic acid, a standard anti-inflammatory drug was utilized as a positive control, whereas DMSO and water were utilized as negative control. A total volume of 5mL of reaction mixtures containing 2.8 mL of phosphate-buffered saline (pH 6.4), 0.2 mL of egg albumin, and 2 mL of extract solutions (various concentrations) was prepared. An aliquot of aspirin (2 mL) was utilized as the standard reference drug, and the negative control was either doubled distilled water or DMSO solution (2 mL). Incubation of the mixtures was then done in an incubator (BOD) for 15 minutes at a temperature of 37 °C. Denaturation was then induced by heating the mixtures in a water bath at 70 °C for 5 minutes. With a UV-Vis spectrophotometer the solutions' absorbances were measured at 660 nm [13, 16]. Triplicate measurements were obtained by independently repeating the procedure. Protein denaturation inhibition (%) was calculated as follows:

$$\% \text{ inhibition} = \frac{A_0 - A}{A_0} \; x \; 100$$

Where and A = absorbance of test solution, and A_0 = absorbance of negative control

2.7 Analysis of Extracts with GC-MS

Analysis of the methanol and acetone extracts was done with a Gas Chromatograph (GC Clarus 580 - PerkinElmer) having a Mass Spectrometer (Clarus SQ 8S - PerkinElmer) as detector (GC-MS) to identify the phytoconstituents present. A

fused capillary column (5% diphenyl / 95% dimethylpolysiloxane - ZB-5HTMS) with dimensions (30 x 0.25 um ID x 0.25 um DF) was utilized. The temperature of oven was scheduled from 100 °C (isothermal for 2 min) to 200 °C, increasing at 10 °C /min, and then to 280 °C with 5 °C/min increase and holding for 15 min. A 1 uL helium gas (99.9999%) acting as a carrier gas and flowing at a rate of 1 ml/min was utilized. The temperature of the ion source and the injector were maintained at 220 °C and 250 °C respectively. The mass detector operated in scan mode from 45 to 500 Da, at 70 eV, and 1s scan interval. The total run time was 40 min with 0 - 3 min solvent delay.

2.8 Data analysis: Analysis of all experimental data and graphs were evaluated statistically by ANOVA (one-way analysis of variance) and "Bonferroni's Multiple Comparison Test" using Microsoft Excel 2019 and GraphPad Prism 8.0 for Windows (Graph Pad Software, San Diego, CA, USA).

3. Results and Discussions

3.1. Plant Material Extraction

The percent yields were respectively 14.74 and 10.50 for methanol and acetone extracts. The differences in yield could be as a result of the differences in the two solvents' polarity. Methanol is more polar than acetone, and the nature of the solvent determines the extent of extraction. The more polar solvent can dissolve the important polar therapeutic drug constituents thereby obtaining a better yield during extraction [17].

3.2. Phytochemical Screening

The phytochemical screening results is presented in Table 1. Only two, carotenoids and alkaloids, out of the thirteen phytoconstituents screened for in the crude sample and extracts were found to be absent.

Table 1: Summary of phytochemical constituents of crude sample, methanol and acetone extracts of *M. micrantha*

Phytochemical	Crude sample	Methanol extract	Acetone extract
Flavonoids	+	+	+
Alkaloids	-	-	-
Phenols	+	+	+
Saponin	+	+	-
Polyphenols	+	+	+
Terpenoids	+	+	-
Steroids	+	+	+
Phytosterol	+	+	+
Tannins	+	+	+
Carotenoids	-	-	-
Anthraquinones	+	+	-
Glycosides	+	+	-
Quinones	+	+	+

Key: (-) = absence of phytochemical; (+) = presence of phytochemical

Eleven and seven phytochemicals were respectively shown to be present in the methanol acetone extracts. This may be due to the higher polarity of methanol enabling it to extract saponin, terpenoids, anthraquinones, and glycosides. The lower polarity of acetone might have prevented it from extracting these phytochemicals, which are highly polar in nature with polyphenolic OH and many alcohol functional groups.

Medicinal or pharmacological properties of plants depend on the phytochemical composition or bioactive compounds in the extracts [18]. Tannins, flavonoids, and phenols present in both extracts have been found to be the primary free radical scavenging agents. Tannins are used in healing wounds, treating diarrhoea, and slowing down transudates such as those associated with atopic dermatitis skin lesions, all indicating antimicrobial property [18]. Saponins, present in the methanol extract, have the properties of being antitumor, and have induced cytotoxicity effect. They lower human cancer risk and prevent cancer cells from growing [18].

Flavonoids possess medicinal properties like being antimicrobial, anti-inflammatory, and antioxidant [6]. Glycosides have anti-inflammatory activity, used in cardiac congestive heart failure treatment, and protect against lethal endotoxemia [18]. Terpenoids are known to have anti-malarial, anti-inflammatory, anti-viral, cholesterol synthesis inhibition, anti-bacterial, and wound and scar healing properties [19]. The identified phytoconstituents in *M. micrantha* leaves could be responsible for their pharmacological activities and ethnobotanical uses such as helminthic, inflammation, oxidative-stress, and bacterial infections.

3.3 Anthelmintic Activity

The methanol and acetone extracts produced significant anthelmintic activity on adult earthworms (*Millsonia ghanensis*) in a dose-dependent manner. Adult earthworms (*Millsonia ghanensis*) were used as the test organisms because of their resemblance (anatomical and physiological) to the intestinal roundworms in humans, and also because of their availability [20]. The results of the anthelminthic activity of the extracts and the standard drug (Mebendazole) have been presented in Table 2.

Table 2: The Anthelmintic activity of mebendazole, acetone, and methanol extracts of *M. micrantha* leaves

Conc (mg/ml)	Paralysis Time / mins			Death time / mins		
	Methanol	Acetone	Mebendazole	Methanol	Acetone	Mebendazole
	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM
0.5	241.25 ±0.25^{a}	207.50 ±0.25^{b}	237.88 ±0.88^{c}	519.00 ±6.50^{a}	487.38±2.56^{b}	994.00 ±9.81^{c}
0.25	747.88 ±0.38^{a}	338.13 ±0.13^{b}	708.38 ±0.13^{c}	843.38±2.76^{a}	731.00±3.25^{b}	1096.56 ±8.31^{c}
0.125	771.88 ±0.13^{a}	628.75 ±0.25^{b}	743.38 ±0.38^{c}	886.25±1.50^{a}	1011.75 ±2.05^{b}	1157.88 ±1.41^{c}

0.063	794.13 ±0.38 [a]	755.50 ±0.50 [b]	796.25 ±0.75 [c]	994.38±6.13 [a]	1181.13±1.13 [b]	1394.44 ±1.69 [c]
0.031	825.75 ±0.75 [a]	865.00 ±0.75 [b]	861.13 ±0.13 [c]	1286.38±6.13[a]	1277.13 ±5.13[a]	1502.19 ±1.69[b]

Paralysis time or death time with the different alphabets at a particular concentration are statistically different at $p<0.05$.

Paralysis time and death time were used to assess the anthelminthic potency of the extracts and Mebendazole. This is because some anthelmintics, such as piperazine citrate, work by paralyzing worms to enable their evacuation in human and animal faeces. Others cause spastic paralysis including morantel, Levamisole, and pyrantel. Paralysis is also caused by Dichlorvos and haloxon anthelmintics by acting as organophosphorus cholinesterase antagonists [21]. Paralysis time generally decreased with the increasing concentration of the extracts and Mebendazole. The acetone extract showed the greatest potential of paralyzing the worms at all concentrations except at 0.031 mg/mL, where methanol extract showed a higher potency. Also, the extracts exhibited a greater ability to paralyze the worms than the standard drug at all concentrations except at 0.031 mg/mL where mebendazole exhibited a slightly higher potency than the acetone extract. Mebendazole, a benzimidazole drug, inhibits the formation of microtubules of cestodes, nematodes, and fluke, as it selectively binds to their beta-tubulins [21].

The death times of the worms in the acetone and methanol extracts were recorded at 487 mins and 519 mins respectively. The acetone extract exhibited a higher anthelminthic activity than the methanol extract at lower concentrations, whereas the methanol extracted exhibited a higher activity at higher concentrations. Both the paralysis and death times of methanol and acetone extracts were concentration-dependent. No activity (paralysis and death) was observed in the negative control, distilled water. The anthelmintic properties of acetone and methanol extracts at test concentration were statistically significant with P-value < 0.003, and the activity of the mebendazole-treated worms were significantly lower in comparison. Not only did the extracts have remarkable potency, but they also killed the worms at a considerably faster rate than mebendazole. The high activity of the acetone and methanol extracts against helminths could be linked to the tannins [[22], and perhaps other phytochemicals in the extracts that act in synergy with the tannins.

3.4. *In vitro* Anti-inflammatory Activity

The results of the anti-inflammatory efficacy of the methanol and acetone extracts are presented in Fig. 1. The percentage inhibition increased generally with increasing concentration.

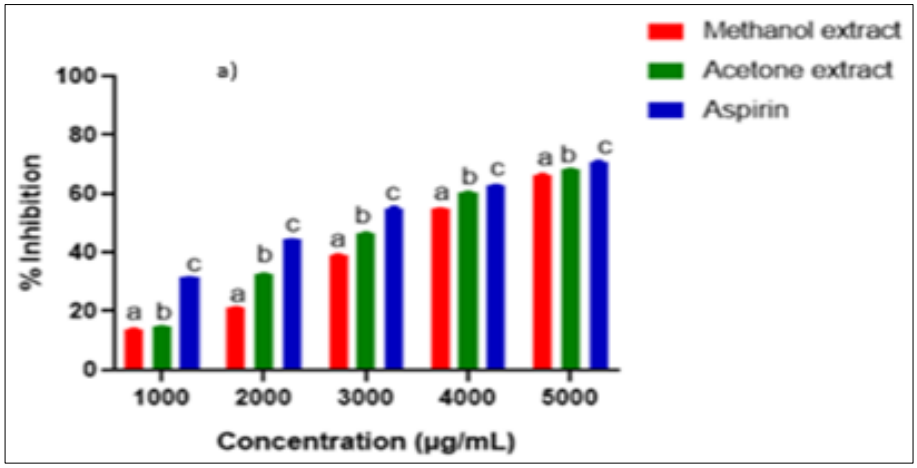

Fig 1: Percentage inhibition of aspirin and the extracts (methanol and acetone)

Each bar represents a mean of three replicate measurements. The bars with different alphabets on them at a particular concentration are statistically different at 5% probability level when Turkey's M.C.T. is used.

At all levels of concentration, the aspirin exhibited the highest inhibition, followed by acetone extract and then methanol extract. Even though the inhibitory activity was markedly different for the reference drug and the extracts at lower concentrations, this difference was drastically reduced at higher concentrations. The inhibition (percent) of methanol extract, acetone extract, and aspirin (the reference drug) at 5000 µg/mL concentration were 66.568±0.043, 68.299±0.113 and 70.994±0.074% respectively as shown in Fig. 1. Both extracts exhibited anti-inflammatory activity and the result was statistically significant with P-value < 0.005. The methanolic and acetone extracts of *M. micrantha* leaves effectively reduced albumin denaturation, according to the findings. Furthermore, the extracts had strong protein-protective capacities comparable to aspirin at higher concentrations, implying that *M. micrantha* extracts could be used as sources of novel anti-inflammatory drugs. The data obtained indicate that the acetone extract of *M. micrantha* leaves has greater activity and could be a viable source of anti-inflammatory compounds.

Protein denaturation occurs when there is loss of proteins structure and biological function of proteins because of heat, external stress, or other chemicals. Denaturation of protein is widely known and is induced by the inflammatory process, which is seen in asthma, diabetes, cancer, autoimmune and disorders like rheumatism, neuro-degenerative and rheumatoid arthritis [23]. Therefore, protein denaturation medicines could be of benefit to research on anti-inflammatory medication. The ability to block denaturation of protein caused by heat has been demonstrated by many anti-inflammatory medicines [24]. Protein denaturation inhibition is the principal mode of action of NSAIDs (Non-Steroidal Anti-inflammatory Drugs). The anti-inflammatory actions of *M. micrantha* extracts may be due to their capacity to suppress protein denaturation. The phytochemicals, glycosides, steroids, phenolics, flavonoids, alkaloids, and terpenoids present in the methanol and acetone extracts may be the cause of the extract's anti-inflammatory activities. These

phytochemicals have, in studies, been indicated to have potent anti-inflammatory activities. Flavonoids have been shown to inhibit inflammatory mediators such as adhesion molecules and C-reactive protein, and enzymes [23]. Extracts of *Croton penduliflorus* which contained flavonoids, glycosides, and triterpenoids possessed anti-inflammatory activity [26].

3.5 Identification of Phytochemicals using GC-MS

The National Institute of Standards and Technology (NIST) library containing fragmentation patterns of numerous compounds was utilized to identify (tentatively) the phytochemicals in the extracts by comparison. The GC-MS analysis led to the identification of various compounds in the methanol and acetone extracts, as presented in Table 3.

Table 3: Compounds identified in the methanol and acetone extracts of *M. micrantha*

Methanol extract			Acetone extract		
Rt	**Compound**	**Similarity index**	**Rt**	**Compound**	**Similarity index**
4.53	Dodecane	89.99	11.86	Tetradecanoic acid	78.00
7.76	1-methyl-1-n-octyloxy-1-silacyclobutane	88.45	12.38	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol*	97.00
8.32	1H-Pyrazole, 3-5-dimethyl-1-phenyl	92.97	12.65	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol*	86.33
8.99	α-D-Glucopyranose, 1, 6-anhydro	90.53	12.85	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol*	90.05
9.99	Methyl-6-O-[1-methylpropyl]-α-D-galactopyranoside	96.61	13.39	Hexadecanoic acid, methyl ester	97
10.36	Methoprene	93.34	13.99	Hexadecanoic acid	92.43
10.67	Cyclohexane, 1, 3, 5-trimethyl-2-octadecyl	94.79	15.55	Cyclopropanedecanoic acid, -2-octyl, methyl ester	88.69
10.84	2, 4, 6-Triisopropylbenzene sulfonamide	89.29	15.86	Heptadecanoic acid, 19-methyl-, methyl ester	72.23
12.36	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol*	95.36	17.73	Tributyl acetyl citrate*	92.26
12.63	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol*	91.69	19.62	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	92.35
12.84	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol	92.62	20.16	4H-cyclopropa[5', 6']benz[1', 2', 7]azuleno[5, 6b]oxiren-4-one, 8, 8a bis(acetoloxy)	95.86
13.35	Hexadecanoic acid, methyl ester	99.45	20.48	2, 3-diphenylcyclopropylmethyl phenyl sulfoxide trans-	87.25
13.95	n-hexadecanoic acid	97.39	20.65	17-pentatriacontene	91.61
15.55	9-octadecenoic acid (Z)-, methyl ester	99.45	20.76	1H-indene, 1-hexadecyl-2, 3-dihydro-	82.44
15.86	Methyl stearate	95.25	21.01	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	96.72
16.32	E, E, Z-1, 3, 12-nonadecatriene-5, 14-diol	93.00	21.78	Diisooctyl phthalate	72.96
16.54	Octadecanoic acid	75.52	23.80	Octadecane, 3-ethyl-5-(2-ethylbutyl-	93.41
17.73	Tributyl acetyl citrate*	96.04	24.44	Tricyclo[5.4.3.0] (1, 8)tetradecan-6-one, 4-ethenyl-3-hydroxy-2, 4, 7, 14-tetramethyl	71.74
25.16	7, 8-epoxylanostan-11-ol, 3-acetoxy-	96.45	24.57	1, 3-benzenedicarboxylic acid, bis(2-ethylhexyl)ester	85.17
25.56	2, 2, 4-trimethyl-3-(3, 8, 12, 16-tetramethyl, heptadeca-3, 7, 11, 15-tetraethyl)-cyclohexanol	93.15	25.56	2, 2, 4-trimethyl-3-(3, 8, 12, 16-tetramethyl-heptadeca-3, 7, 11, 15-tetraethyl)-cyclohexanol	

Rt=retention time; *Compounds that were identified in both the methanol and acetone extracts

As indicated in the phytochemical screening, the methanol extract contained glycosides which were absent in the acetone extract, and this has been corroborated by the presence of α-D-Glucopyranose, 1, 6-anhydro (Levoglucosan) and Methyl-6-O-[1-methylpropyl]-α-D-galactopyranoside in the methanol extract. The differences in the compounds identified in the two extracts explain why their pharmacological activities are not the same. The functional groups in the identified compounds corroborate the FTIR spectrum obtained for the two extracts. A few compounds were found in both extracts and they include Hexadecanoic acid, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid, methyl ester, Tributyl acetyl citrate and 2, 2, 4-trimethyl-3-(3, 8, 12, 16-tetramethyl-heptadeca-3, 7, 11, 15-tetraethyl)-cyclohexanol.

Most of the identified phytochemicals are present in other medicinal plants, and they are known to have varying pharmacological activities [27, 28]. They may act alone or in synergy to produce therapeutic effects. Tetradecanoic acid is known to have uses as Antioxidant, Lubricant, Hypercholesterolemic, Cancer-preventive, and Cosmetic [28]. Hexadecanoic acid is known to possess antimicrobial, Pesticidal, Antifibrinolytic, Hemolytic, Nematicidal, Antialopecic activities [28]. Hexadecanoic acid, methyl ester is known to possess anti-inflammatory and cytotoxicity activities [29]. The phytoconstituent, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol has Cancer-preventive properties [28]. The ester, 9-octadecenoic acid, methyl ester, and tetradecanoic acid (myristic acid) possess strong antioxidant and antimicrobial activities [27]. The phytoconstituent, E, E, Z-1, 3, 12-nonadecatriene-5, 14-diol occurs in many medicinal plant extracts, and in the extract of a marine red algae (*Halymenia durvillei*) and it has been indicated as a potential inhibitor for SARS-COV-2 due to its pharmacophore features [30]. The steroid triterpenoid, 7, 8-epoxylanostan-11-ol, 3-acetoxy-, possesses antimicrobial activity [31]. Diisooctyl phthalate, a fatty acid ester, possesses antimicrobial activity [32].

The glycosides, α-D-Glucopyranose, 1, 6-anhydro (Levoglucosan) and Methyl-6-O-[1-methylpropyl]-α-D-galactopyranoside, possess anti-inflammatory activities [18]. The identified compounds in the extracts and others acting in synergy with them are likely the source of the pharmacological activities.

4. Conclusion

The acetone and methanol extracts possess anti-inflammatory and anthelmintic potency but that of the acetone extract is higher than the methanol extract. The methanol and acetone extracts of *M. micrantha* contain bioactive phytoconstituents with promising anthelmintic and anti-inflammatory properties, thus validating their traditional medicinal uses. Ongoing research in our laboratory focuses on further fractionation, isolation, and assessment of the biological activities of the isolated compounds from *M. micrantha* leaves, as well as characterization, and structure elucidation of isolates.

Data Availability
All data on the findings of this study have been included in the article.

Conflicts of Interest
The authors have no competing professional, financial, or personal interests to declare. Regarding the publication of this paper, the authors have no conflicts of interest.

Acknowledgments
The authors express gratitude to the Departments of Pharmacognosy, Chemistry, Pharmaceutical Microbiology, and the Central Laboratory of KNUST for making their facilities available for this study. Mr. Francis Amankwaah of Pharmaceutical Microbiology, KNUST, is appreciated for his technical support.

Funding Statement
No specific funding was received for this research but funding was done by the authors as part of research required by their tertiary institutions.

References
1. Boadu AA, Asase A. Documentation of Herbal Medicines Used for the Treatment and Management of Human Diseases by Some Communities in Southern Ghana. Evid Based Complement Alternat Med. 2017;2017:1-12.
2. Komlaga G, Cojean S, Dickson RA, Beniddir MA, Suyyagh-Albouz Mensah MLK, Agyare C, *et al*. Antiplasmodial activity of selected medicinal plants used to treat malaria in Ghana. Parasitol Res. 2016;115:3185-95.
3. Doumbia I, Ouattara K, Amadou CF, Yapi HF, Djaman AJ, N'guessan JD. Assessment of serum ionic profiles in rabbits treated with aqueous extract of *Mareya micrantha* (Euphorbiaceae). Pharm Innov J. 2017;6(3):226-31.
4. World Health Organization. The World medicines situation 2011 - Traditional Medicines: Global situation, Issues and Challenges. Geneva, Switzerland: World Health Organization; c2011.
5. World Health Organization. World Health Organization Global Report on traditional and complementary medicine 2019. Geneva, Switzerland: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO.
6. Mwine JT, van Damme P. Why do euphorbiaceae tick as medicinal plants? A review of euphorbiaceae family and its medicinal features. J Med Plants Res. 2011;5(5):652-62.
7. Appiah KS, Oppong CP, Mordani HK, Omari RA, Koabitey S, Amoatey CA, *et al*. Medicinal Plants Used in the Ejisu-Juaben Municipality, Southern Ghana: An Ethnobotanical Study. Medicines. 2018;6(1):2-27. DOI:10.3390/medicines6010001.
8. Dosso M, Soro TY, Libra MA, Traore F, N'guessan JD. Anticholinesterase Properties of *Mareya micrantha* (Benth) Mull. Arg. (Euphorbiaceae) and its Fractions on the Cholinesterase Model of Isolated Rabbit Duodenum. Int J Appl Biol Pharm Technol. 2020;11(2):121-30.
9. Barberá P, Velayos M, Aedo C. Annotated checklist and identification keys of the Acalyphoideae (Euphorbiaceae) of Equatorial Guinea (Annobón, Bioko and Río Muni). Phytotaxa. 2013;140(1):1-25. doi: 10.11646/phytotaxa.140.1.1.
10. Koffi KL, Bony FN, Okpekon AT, Bitty MLA, Guessan KJN, Séon-Méniel B. Compounds with myorelaxant activity from the leaves of *Mareya micrantha* (Benth.) Müll. Arg. (Euphorbiaceae). J Pharmacogn Phytochem. 2020;9(4):48-59.
11. Macfoy CA, Cline EI. *In vitro* antibacterial activities of three plants used in traditional medicine in Sierra Leone. J Ethnopharmacol. 1990;28(3):323-7.
12. Douhoré GYT, Attioua KB, Soro Y, Kabran FA, Kablan LCA, Vedrenne M, Vaca-Garcia C. Nor-cucurbitacins from the leaves of *Mareya micrantha* (Benth.) Müll. Arg. (Euphorbiaceae). Fitoterapia. 2020;143:104538.
13. Akoto CO, Acheampong A, Duah Boakye Y, Kokloku BK, Kwarteng G, Kwadzo BK. *In vitro* anthelmintic, anti-inflammatory, antioxidant activities and FTIR analysis of Sclerocarya birrea root. J Pharmacogn Phytochem. 2020;9(2):1389-401.
14. Trease GE, Evans WC. Pharmacognosy. 16th ed. New York, USA: W.B. Sanders Company Limited; c2009. p. 1-604.
15. Ajaiyeoba BO, Onocha PA, Olarenwaju OT. *In vitro* Anthelminthic properties of *Buchholzia coriacea* and *Gynandropsis gynandra* Extracts. Pharm Biol. 2001;39(3):217-20.
16. Kumari CS, Yasmin N, Hussain MR, Babuselvam M. *In vitro* anti-inflammatory and anti-artheritic property of *Rhizophora mucronata* leaves. Int J Pharma Sci Res. 2015;6(3):482-5.
17. Acheampong A, Amankwaa LT, Afriyie IO, Baah KA. Antioxidant and Antimicrobial Activity of the Methanol and Petroleum Ether Extracts of the Stem of *Microdesmis puberula*. Pharm Chem J. 2018;5(1):38-48.
18. Sood A, Kaur P, Gupta R. Phytochemical screening and antimicrobial assay of various seeds extract of cucurbitaceae family. Int J Appl Biol Pharm Technol. 2012;3(3):401-9.
19. Karande AM, Kamble HV, Kumbhar VH, Kane SR, Magdum CS. Preliminary phytochemical screening of *Glochidion ellipticum*. Eur J Exp Biol. 2016;6(4):41-5.
20. Danquah CA, Koffuor GA, Annan K, Ketor EC. The anthelmintic activity of *Vernonia amygdalina* (Asteraceae) and *Alstonia boonei* de wild (Apocynaceae). J Med Biomed Sci. 2012;1(1):21-7.
21. Martin RJ. Modes of action of anthelmintic drugs. Vet J. 1997;154(1):11-34. DOI:10.1016/s1090-0233(05)80005-x.
22. Akoto CO, Acheampong A, Boakye YD, Naazo AA, Adomah DH. Anti-inflammatory, antioxidant, and anthelmintic activities of *Ocimum basilicum* (Sweet Basil) fruits. J Chem. 2020. Article ID:2153534. doi: 10.1155/2020/2153534.
23. Baah KA, Acheampong A, Amponsah IK, Jibira Y, Owusu MO, Tima LA. Evaluation of the Anti-inflammatory, Antimicrobial, and Antioxidant activities of the stem bark extract of *Croton penduliflorus* Hutch (Euphorbiaceae). J Pharmacogn Phytochem. 2017;6(6):1705-10.
24. Swamy MK, Sinniah UR, Akhtar M. *In vitro* pharmacological activities and GC-MS analysis of different solvent extracts of *Lantana camara* leaves collected from tropical region of Malaysia. Evid Based Complement Altern Med. 2015;2015:506413. http://dx.doi.org/10.1155/2015/50641
25. Ponnamma SU, Manjunath K. GC-MS analysis of phytocomponents in the methanolic extract of *Justicia*

wynaadensis (Nees) T. anders. Int J Pharma Bio Sci. 2012;3(3):570-6.
26. Othman AR, Abdullah N, Ahmad S, Ismail IS, Zakaria MP. Elucidation of *in-vitro* anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. BMC Complement Altern Med. 2015;15:1-10.
27. Tassakka ACMA, Sumule O, Massi MN, Manggau M, Iskandar IW, Alam JF, *et al*. Potential bioactive compounds as SARS-CoV-2 inhibitors from extracts of the marine red alga *Halymenia durvillei* (Rhodophyta)-A computational study. Arab J Chem. 2021;14(11):103393.
28. Zubair MF, Atolani O, Ibrahim SO, Adebisi OO, Hamid AA, Sowunmi RA. Chemical constituents and antimicrobial properties of *Phyllanthus amarus* (Schum & Thonn). Bayero J Pure Appl Sci. 2017;10(1):238-46.
29. Shanab SMM, Shalaby EA, Lightfoot DA, El-Shemy HA. Allelopathic effects of water hyacinth [*Eichhornia crassipes*]. PLoS One. 2010;5(10):e13200.