



Chemical, Toxicity and Antibacterial Studies on Methanol Extracts of *Melanthera scandens*, *Ageratum conyzoides*, *Aspilia africana* and *Synedrella nodiflora*

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Abstract

Background and objective: Compositae species are applied as whole or part of the feed stock for animals and poultry. However, rabbits display varied preferences in their consumption of these plants, following the order of: *Melanthera scandens* (MS) > *Synedrella nodiflora* (SN) > *Aspilia africana* (AA) > *Ageratum conyzoides* (AC). This preference profile may be due to variation in chemical composition, flavor or toxicity of the plants. The rabbits in our farm feed on 100% MS or SN with excellent performance. This study, therefore, is set to: obtain the methanol extract of these plants, screen them for their antibacterial activity, determine their toxicity and investigate the chemical composition of their n-hexane fraction (volatile constituents).

Methods: Crushed leaves of MS, AC, AA and SN were extracted with methanol using soxhlet extraction technique to obtain the methanol extract of each plant. Phytochemical examination, antibacterial activity determination (using the agar-well diffusion technique) and toxicity studies (using experimental white albino mice) were carried out on the methanol extracts. Vacuum liquid chromatography (VLC) fractionation of each extract was carried out. The n-hexane fractions obtained from the VLC fractionation were subjected to gas chromatography-mass spectroscopy (GC-MS) analysis and the chemical constituents were identified.

Results: Phytochemical screening showed the presence of alkaloids, steroids and tannins in all the extracts. The extracts exhibited varying degrees of antibacterial activities against bacterial strains used for this study. AC showed activity against tested microbes, having zones of inhibition ranging from 10 mm and 22 mm, with the exception of *Bacillus stearothermophilus* and *Pseudomonas fluorescens*. MS inhibited the growth of the microbes, having zones of inhibition ranging from 12 mm and 20 mm, with the exception of *Bacillus anthracis*, *Bacillus stearothermophilus*, *Clostridium sporogens* and *Enterococcus faecalis*. AA did not show activity against four of the tested microbes: *Bacillus anthracis*, *Klebsiella pneumonia*, *Pseudomonas fluorescens* and *Enterococcus faecalis*. SN however, inhibited the growth of all the bacterial strains tested, with the exception of *Escherichia coli* only. On the other hand, when streptomycin was used as a positive control, the growth of all the bacterial strains was inhibited, having

zones of inhibition ranging from 15 mm and 22 mm. Toxicity study showed that the extracts were not toxic at the concentrations of 10 mg/kg through 1,000 mg/kg; however, at higher concentrations of 1,600 mg/kg through 2,500 mg/kg, the extracts became toxic. The LD₅₀ was determined to be 1,275 mg/kg for all the extracts. GC-MS analysis showed the presence of 4-tetradecene in all of the extracts, except AC.

Conclusions: The varied preferences observed in rabbit consumption of these plants definitely have nothing to do with toxicity. The presence of various fatty acids and unsaturated hydrocarbons in the volatile

Keywords: Phytochemical screening; Compositae (Asteraceae); Acute toxicity; *Melanthera scandens*; *Ageratum conyzoides*; *Aspilia africana*; *Synedrella nodiflora*.

Abbreviations: AC, *Ageratum conyzoides*; AA, *Aspilia Africana*; CNS, central nervous system; EtOAc, ethylacetate; GC-MS, gaschromatography-mass spectroscopy; LD, lethal dose; LIO, Locally Isolated Organism; MS, *Melanthera scandens*; MeOH, Methanol; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; NCIB, national collection of industrial bacterial; ND, not determined; SN, *Synedrella nodiflora*; VLC, vacuum liquid chromatography.

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components of the extracts, which influence the flavor, may be responsible for the wellness of rabbits and their relative preferences in consumption.

Introduction

Compositae (*Asteraceae*), with its approximately 1,620 genera and more than 23,600 species, is the largest family of flowering plants.¹ The majority of the Compositae species are herbaceous, yet an important component of the family is constituted by shrubs or even trees occurring primarily in the tropical regions of North and South America, Africa and Madagascar and on isolated islands in the Atlantic and Pacific oceans. The family is characterized by having a capitulum or head, an inferior, unilocular ovary with one ovule and with few exceptions fused anthers surrounding the style. The flowering sequence in the capitulum nearly always follows the direction from the outside to the centre.²

The biological and therapeutic application of the plants of Compositae have informed the systematically conducted chemical and pharmacological research of this family. The plants in this family are particularly rich in sesquiterpene lactones, polyacetylenes, steroids, terpenoids, alkaloids, saponins, and various heterocyclic compounds.³ Notable plants that belong to this family include: *Melanthera scandens* (MS), *Ageratum conyzoides* (AC), *Aspilia africana* (AA) and *Synedrella nodiflora* (SN). There are many reports on studies involving evaluation of the effects of supplement concentrate in diets of animals, such as rabbits⁴⁻⁶; moreover, these plants are among those commonly utilized locally as alternative non-conventional rabbit feed.

MS is a scandent annual or perennial herb that grows up to between 1 m and 4 m in height, with quadrangular and scabrid branches.⁷ It is distributed geographically in East, West and South Africa. It is known as 'ayaraedemerong' by the Ibibios of Akwa Ibom State of Nigeria. Its antioxidant, *in vitro* antiplasmodial and antidiabetic activities have been reported.⁸ Extract from its leaves have shown anti-inflammatory and analgesic effects, which may be mediated through the phytochemical constituents of the plant.⁹ Adams reported that caryophyllene (26.4%) and bicyclosesquiphellandrene (19.4%) were the major components of the oil from the leaves,¹⁰ whereas the stem oil was reported as rich in limonene (16.2%), phellandrene (18.6%) and bisabolol (7.8%). The leaves were predominated by the combination of pinene and bicyclosesquiphellandrene, with a high amount of oxygenated compounds in the stem oil.

AC is commonly called goat weed. It is native to tropical America. It is also found in all warm and subtropical areas of the world, such as West Africa and some parts of Asia and South America. The genus *Ageratum* consists of approximately 30 species, but only a few of those have been phytochemically investigated to date.¹¹ AC is beneficial in ethnomedicine all over the tropical areas. It is a common weed which spreads from its native range to all areas of the tropic. The flower is a hermaphrodite and is easily pollinated by insects. In Nigeria, leaves from the plant are used to relieve pain and heal wounds, and to treat skin diseases, neck pain, body infections and diarrhea.¹² The leaves are also applied to cuts and wounds, due to their antihemorrhagic and antiseptic properties. Aqueous extract of the leaves of AC was shown to prevent blood coagulation and to have analgesic activity; in addition, that water-soluble fraction of the leaves was demonstrated to have analgesic and anti-inflammatory activities in rats.¹² The toxicity study of this plant has not been well documented.¹²

AA is a shrub widely distributed across tropical Africa and oc-

curing in Savannah forests in Africa and Asia.¹³ In Nigeria, it is commonly known as 'yunriyun' by the Yorubas, 'orangila' by the Igbos and 'tozalin' by the Hausas.¹⁴ *A. africana* is used in traditional medicine for the treatment of several ailments in different parts of the world. For instance, it has been used in treating gonorrhoea, tuberculosis, cough, rheumatic pains, stomach upset, corneal opacity, wounds, and insect bites.¹⁴ Oral decoction of the leaves is used to relieve febrile head ache and quicken delivery in females, as well as to cure other ailments, including stomach ache.¹⁵⁻¹⁷ A decoction can also be used in treating pulmonary hemorrhages and hemostasis.¹⁸ The toxic effect of the methanol extract of AA on the estrous cycle and uterine tissues of Wistar rats has been studied¹⁹; Tamura *et al.*²⁰ has also reported on the inhibitory effect of steroidal saponin on the estrous cycle of Wistar rats.

SN is an erect herb, growing to a height of 60–120 cm. It is branched dichotomously, with 5–15 cm long and 2–9 cm wide ovate or elliptic leaves.²¹ It is usually found as a weed, growing along river and stream banks and also along road sides.²² *S. nodiflora* is used widely in Africa and Asia for various purposes. In Ghana, the whole plant is boiled and the aqueous extract is then administered for the treatment of epilepsy, and the leaves are used for threatened abortion, hiccup, laxative, and as a feed for livestock.^{22,23} Some indigenous tribes in Nigeria use the whole plant for the treatment of cardiac troubles and to stop wound bleeding.²⁴ In some parts of the world, for example Indonesia, the young foliage is eaten as a vegetable, while the leaf sap is used to treat stomach ache. The plant is also used as an embrocation for rheumatism.¹¹ In Malaysia, the leaf is used for poulticing sore legs and for the treatment of head ache. The sap is instilled into the ear for ear ache.¹¹

Methods

Apparatus

Soxhlet extractor, laboratory glass ware, weighing balance, condenser, chiller, rotary evaporator, retort stand with clamp, *etc.*

Solvents

Methanol (CH₃OH), ethyl acetate (EtOAc), n-hexane, acetone, distilled water. The solvents were distilled before use.

Plant material and extraction procedure

The leaves of MS, AC, AA and SN were collected from Ile-Ife, Osun State, Nigeria and authenticated at the Herbarium Section of Botany Department, Obafemi Awolowo University, Ile-Ife, Nigeria. The ground leaves of MS (800 g), AC (800 g), AA (2.2 kg) and SN (1.2 kg) were subjected to soxhlet extraction using CH₃OH as the extracting solvent and then concentrated using a rotary evaporator at 60 °C to obtain the crude CH₃OH extracts for each plant. About 20 g each of the crude extracts of MS, AC, AA and SN were separately pre-adsorbed on silica gel. For each, a vacuum liquid chromatography column was packed and loaded with the

pre-adsorbed sample, silica gel (70–230 mesh) as the stationary phase, mixtures of n-hexane in EtOAc, and EtOAc in CH₃OH in increasing polarity as the mobile phase. Fractions obtained with 100% n-hexane from each plant extract were analyzed using gas chromatography-mass spectroscopy (GC-MS).

The GC-MS of the n-hexane fractions was performed on an Agilent Technologies (Santa Clara, CA, USA) GC system 7890A with injector model 7683b, under the following analytical conditions: Agilent Technologies capillary column (30 m × 0.25 mm × 0.25 μm film thickness), injection volume of 1 μL at rate of 1.4963 mL/min at pressure of 3.2652 psi and average velocity of 45.21 m/s. Helium (99.999%) was used as the carrier gas, with a constant flow rate of 1.0 mL/min and ion-source temperature of 280 °C.

Phytochemical screening of crude extracts

The crude extracts of MS, AC, AA and SN were screened for the presence of different classes of phytochemicals. The phytochemical screening was carried out using standard procedures.^{25,26}

Biological activity

Animals

White albino mice (vom strain; weight range: 17–23 g) of both sexes were used for the experiment. They were obtained from the Department of Pharmacy Animal House, Obafemi Awolowo University, Ile-Ife. The animals were kept in cages and maintained on a standard commercial rat pelleted feed (Breedwell, Oyo State, Nigeria) and water was given *ad libitum*.

Acute toxicity

The acute toxicity of the CH₃OH extracts of MS, AC, AA and SN was determined using standard method.²⁷ The animals (mice) were acclimatized for 1 week, after which their weights were measured and recorded. Doses of 10 mg/kg, 100 mg/kg, 1,000 mg/kg, 1,600 mg/kg, 2,900 mg/kg and 5,000 mg/kg were prepared, and different volumes were injected intraperitoneally into three groups of 3 mice each. Behavioral changes, number of deaths and the effect of toxicity were observed for 72 h. The number of dead animals in each group within the period was recorded.

Antimicrobial activity

The antimicrobial activity of the CH₃OH extracts of MS, AC, AA and SN were determined using the agar-well diffusion method described by both Russell and Furr and Irobi *et al*.^{28,29} The microorganisms used were: *Bacillus anthracis* (Locally Isolated Organisms, LIO), *Bacillus cereus* [National Collection of Industrial Bacteria (NCIB), No. 6349], *Bacillus polymyxa* (LIO), *Bacillus stearothermophilus* (NCIB No. 8222), *Bacillus subtilis* (NCIB No. 3610), *Clostridium sporogens* (NCIB No. 532), *Corynebacterium pyogenes* (LIO), *Escherichia coli* (LIO), *Klebsiella pneumoniae* (NCIB No. 418), *Pseudomonas fluorescens* (NCIB No. 3756), *Proteus vulgaris* (NCIB No. 67), *Staphylococcus aureus* (NCIB No. 8588), and *Enterococcus faecalis* (LIO). These microorganisms were obtained from the culture collection of the Microbiology Department, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The antimicrobial tests were carried out in triplicate and

Table 1. Phytochemical screening of leaves of MS, AC, AA and SN

Phytochemicals	MS	AC	AA	SN
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Glycosides	+	+	–	–
Flavonoids	ND	ND	+	ND
Antraquinones	ND	+	ND	ND

+ = present; – = absent; ND = not determined. Abbreviations: AA, *Aspilia africana*; AC, *Ageratum conyzoides*; MS, *Melanthera scandens*; SN, *Synedrella nodiflora*.

the mean value was computed.

Results and discussion

Phytochemical screen

The results of the phytochemical analysis are presented in Table 1.

The various phytochemical compounds detected in the leaves of MS, AC, AA and SN are known to be biologically active and thus could be responsible for the use of the plants in traditional medicine for the treatment and prevention of various diseases. Tannins, for instance, have been implicated in hemostasis activity, wherein they stop bleeding from damaged or injured tissues by precipitating proteins to form plugs. The presence of tannins in a medicinal plant suggests the ability of the plant to play major roles as antimicrobial, antidiarrheal, antioxidant and antihemorrhoidal agents.^{30,31} Tannins were found to be present in all the extracts.

Alkaloids have been found to have antidiarrheal, antihypertensive, antimicrobial, anti-inflammatory and antifibrogenic effects.³² They are well known for their central nervous system activities as well,³³ and were detected in all the extracts.

Saponins, also found in all the extracts, are known to possess good selectivity against cancer cells.³⁴ They have been reported to exhibit antihypercholesterol, anti-inflammatory and cardiac depressant properties.³⁵

Acute toxicity

The results of the acute toxicity analysis of the extracts of MS, AC, AA and SN are presented in Table 2.

The results from the 1st investigation (Table 2) showed that there were no death occurrences in the animals (mice). This indicated that the extracts were not toxic at the dosage range tested (10–1,000 mg/kg). In the 2nd investigation, all the animals (mice) died as a result of the increase in concentration of the extracts administered, coordinating with the increased toxicity level of the extracts. This implies that the toxicity of the extracts is dose dependent. The 50% lethal dose (LD₅₀) value of the intraperitoneal administration of the extracts of MS, AC, AA and SN was found by graphical computation to be 1,275 mg/kg (Fig. 1). The animals thus have a very high resistance to the extracts, showing very high resistance to a wide range of microbial infections when fed with

Table 2. Investigation of acute toxicity of CH₃OH extracts of MS, AC, AA and SN on mice

Doses, mg/kg	1 st Investigation				Doses, mg/kg	2 nd Investigation			
	Mortality					Mortality			
	AC	MS	SN	AA		AC	MS	SN	AA
10	0/3	0/3	0/3	0/3	1,600	2/2	2/2	2/2	2/2
100	0/3	0/3	0/3	0/3	2,900	2/2	2/2	2/2	2/2
1,000	0/3	0/3	0/3	0/3	5,000	2/2	2/2	2/2	2/2

Mortality: number of animals which died/number of animals used. AA, *Aspilia africana*; AC, *Ageratum conyzoides*; MS, *Melanthera scandens*; SN, *Synedrella nodiflora*.

these plants. Daniel and Ekam reported that the ethanol extract of MS exhibited more toxic effect as compared to aqueous extract.³⁶ The toxic effect observed by these authors (LC₅₀ 173.78 µg/mL) may be due to the solvent ethanol, which is known to be quite toxic both to microbes and animals.

Antimicrobial sensitivity

The results of the antimicrobial sensitivity test of the CH₃OH extracts of MS, AC, AA and SN on different microorganisms are presented in Table 3.

As seen in Table 3, the extracts displayed a varying degree of activity against the microbes. MS was very active against *E. coli*, *C. sporogens*, *K. pneumonia* and *B. subtilis*, with zones of inhibition between 15 mm and 20 mm. It displayed moderate activity against other microbes (zones of inhibition between 10 mm and 14 mm), with the exceptions of *B. anthracis*, *B. stearothermophilus*, *C. pyrogenes* and *E. faecalis*, to which it was inactive. All Gram-negative microbes tested were responsive to the MS extract. AC showed very good activity against *P. vulgaris*, *B. anthracis*, *B. subtilis*, *S. aureus*, *B. polymyxa* and *E. coli* (15 mm to 22 mm zone of inhibition). It also demonstrated moderate activity against other microbes (10 mm to 14 mm zone of inhibition), with the exception of *B. stearothermophilus* and *P. fluorescens*, to which it was resistant. AA displayed good activity against four of the microbes (*B. cerus*, *E. coli*, *P. vulgaris* and *S. aureus* (17 mm to 15 mm zone of inhibition), moderately activity against others and resistance to *B. anthracis*, *K. pneumonia*, *P. fluorescens* and *E. faecalis*. SN, however, showed a broad spectrum of activity, being moderately active against most of the microbes and resistant only to *E. coli*. The *Bacillus* species were generally

responsive to all the extracts, with the exception of *B. anthracis* and *B. stearothermophilus*. Almagboul *et al.*³⁷ reported the inhibitory action of AC against *S. aureus*, *E. coli* and *B. subtilis*. The activity of *A. africana* against *B. subtilis*, *E. coli* and *S. aureus* had previously been reported by Adeniyi and Odufowora.³⁸ They found that the cold extracts showed better antibacterial activity than the soxhlet extract.

The results for minimum inhibitory concentration (commonly known as MIC) and minimal bactericidal concentration of the extracts are shown in Table 4. *B. anthracis*, *B. subtilis* and *P. vulgaris* displayed very high sensitivity against AA (MIC of 0.78), while *B. cerus*, *P. vulgaris* and *S. aureus* were equally sensitive to AA, and *E. coli* was similarly sensitive to MS. The activity of these Compositae species against a wide variety of microbes and very high tolerance to animal cells as indicated in toxicity study below might inform on their application as animal feed (we feed rabbits mainly with MS and SN) and inclusion in poultry feed formulation.⁵

GC-MS analysis results

The n-hexane fractions obtained from the fractionation of the crude extracts of MS, AC, AA and SN were analyzed by GC-MS. The compounds identified from the n-hexane fractions of the different plants are shown in Table 5. Hexadecanoic acid, octadecanoic acid, and/or their methyl esters/derivatives were present in all the extracts, and they have been reported previously to exhibit a variety of biological activities.³⁹ Similarly, the presence of these fatty acids in *Cola nitida* seed was implicated in the antimicrobial activity of the methanol extract of the seed.⁴⁰ Unsaturated long-chain hydrocarbons, such as tetradecane, have been found to exhibit antimicrobial activity against a number of human pathogens.⁴¹ These compounds were found in the n-hexane extracts of these plants, except for AC. However, phytol was identified only in the AA n-hexane extract. The presence of tetradecene in SN and

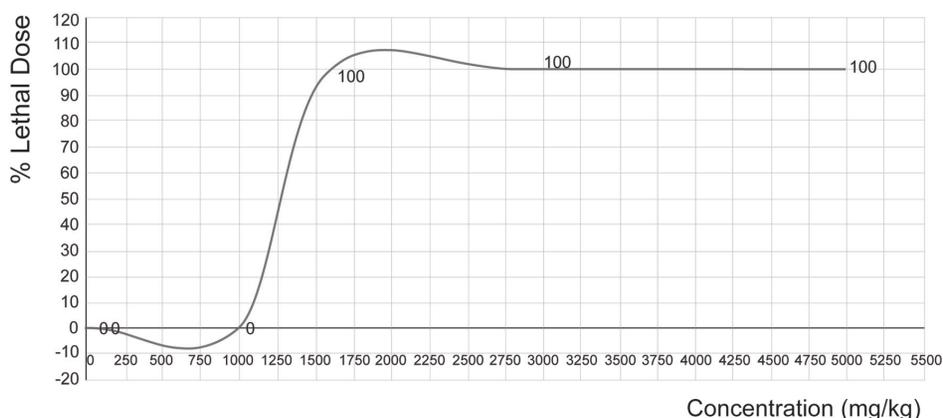


Fig. 1. Graphical computation of the acute toxicity (LD₅₀) of MS extract in mice. Abbreviation: MS, *Melanthera scandens*.

Table 3. Sensitivity pattern exhibited by CH₃OH extracts of MS, AC, AA and SN (at 25 mg/mL) against bacterial strains

Bacterial Strains	Zones of Inhibition (mm)				
	AC ^a	MS ^a	SN ^a	AA ^a	Strep. ^b
<i>Bacillus anthracis</i> (LIO) ^c	21	0	13	0	20
<i>Bacillus cereus</i> (NCIB 6349) ^c	14	10	11	17	21
<i>Bacillus polymyxa</i> (LIO) ^c	16	12	15	10	18
<i>Bacillus stearothermophilus</i> (NCIB 8222) ^c	0	0	10	12	19
<i>Bacillus subtilis</i> (NCIB 3610) ^c	18	15	13	11	20
<i>Clostridium sporogenes</i> (NCIB 8588) ^c	13	18	16	13	15
<i>Corynebacterium pyogenes</i> (LIO) ^c	14	0	9	12	18
<i>Staphylococcus aureus</i> (NCIB 8588) ^c	17	12	12	15	19
<i>Enterococcus faecalis</i> (LIO) ^c	12	0	14	0	16
<i>Escherichia coli</i> (NCIB 86) ^d	15	20	0	16	22
<i>Klebsiella pneumonia</i> (NCIB 418) ^d	10	18	10	0	16
<i>Pseudomonas fluorescens</i> (NCIB 3756) ^d	0	13	12	0	17
<i>Proteus vulgaris</i> (NCIB 67) ^d	22	14	14	16	22

0 = resistant; ^a25 mg/mL; ^bstreptomycin standard drug, 1 mg/mL; ^cGram-positive bacteria; ^dGram-negative bacteria. Abbreviations: AA, *Aspilia africana*; AC, *Ageratum conyzoides*; MS, *Melanthera scandens*; SN, *Synedrella nodiflora*.

AA corroborates our earlier finding that the essential oil from SN an AA contained n-tetradecane.⁵

Conclusions

The plants MS, AC, AA and SN belonging to the family *Asteraceae* are used in ethnomedicine for the treatment and prevention of stomach ulcer, sores, diabetes, cardiac troubles, and wound bleeding, among others.⁵ The presence of different classes of

phytochemicals in the leaves of the plants gives credence to their use in traditional medicine. *Bacillus* species (*B. cereus*, *B. polymyxa* and *B. subtilis*) were found to be generally responsive to the methanol extracts of these plants. MS was not active against four of the Gram-positive microbes: *B. anthracis*, *B. stearothermophilus*, *C. sporogens*, and *E. faecalis*. AC did not show any observable activity against *B. stearothermophilus* and *P. fluorescens*, representing Gram-positive and Gram-negative bacteria respectively. AA did not exhibit activity against two microbes: *B. anthracis* and *E. faecalis* (both Gram-positive). SN, on the other

Table 4. MIC and MBC exhibited by the extracts and streptomycin against susceptible bacterial strains

Organisms	AC		MS		SN		AA		STREP	
	MIC ^a	MBC ^a								
<i>B. anthracis</i> ^b	0.78	1.56	ND	ND	3.125	6.25	ND	ND	0.25	0.5
<i>B. cereus</i> ^b	3.125	6.25	6.25	12.5	6.25	12.5	0.78	1.56	0.06	0.13
<i>B. polymyxa</i> ^b	1.56	3.125	3.125	6.25	1.56	3.125	3.125	6.25	0.13	0.25
<i>B. stearothermophilus</i> ^b	ND	ND	ND	ND	6.25	12.5	1.56	3.125	0.06	0.13
<i>B. Subtilis</i> ^b	0.78	3.125	1.56	3.125	3.125	6.25	1.56	6.25	0.25	0.5
<i>S. aureus</i> ^b	1.56	3.125	1.56	3.125	3.125	6.25	0.78	3.125	0.25	0.5
<i>E. faecalis</i> ^b	3.125	6.25	ND	ND	1.56	6.25	ND	ND	0.03	0.06
<i>C. sporogenes</i> ^b	3.125	6.25	1.56	3.125	1.56	3.125	1.56	3.125	0.06	0.13
<i>C. pyogenes</i> ^b	1.56	3.125	ND	ND	6.25	12.5	3.125	6.25	0.25	0.5
<i>K. pneumonia</i> ^c	6.25	12.5	1.56	3.125	3.125	12.5	ND	ND	0.5	ND
<i>P. fluorescens</i> ^c	ND	ND	3.125	6.25	3.125	6.25	ND	ND	0.03	0.06
<i>P. vulgaris</i> ^c	0.78	1.56	3.125	6.25	1.56	3.125	0.78	1.56	0.5	ND
<i>E. coli</i> ^c	1.56	3.125	0.78	1.56	ND	ND	1.56	3.125	0.5	ND

^a(mg/ mL); ^bGram-positive; ^cGram-negative; ND: not done. Abbreviations: AA, *Aspilia africana*; AC, *Ageratum conyzoides*; MBC, minimal bactericidal concentration; MIC, minimum inhibitory concentration; MS, *Melanthera scandens*; SN, *Synedrella nodiflora*.

Table 5. Compounds identified from n-hexane fractions of MS, AC, AA and SN

Plant	Compounds
MS	4-tetradecene Methyl hexadecanoate Methyl-9,12-octadecadienoate 9,12,15-octadecatrien-1-ol 4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-eneoxide
AC	7-methoxy-2,2-dimethyl-2H-1-benzopyran 7-(1,1-dimethylethyl)-3,4-dihydro-1 (2H)-naphthalenone 5-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl-1H-inden-1-one 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-6-yl)ethanone Methyl hexadecanoate Methyl-9,12,15-octadecatrienoate Phytol
AA	4-tetradecene 2-tetradecene 1-nonadecene 2-pentadecanone-6,10,14-trimethyl Methyl hexadecanoate n-hexadecanoic acid Methyl-11,14-octadecadienoate Methyl -9,12,15-octadecatrienoate Methyl octadecanoate
SN	4-tetradecene 2-tetradecene 4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene 1-octadecene Octadecene Methyl hexadecanoate Methyl-2,4-di(pro-1-en-2-yl)-1-vinylcyclohexane 1-docosene Methyl- 9,12,15-octadecatrienoate

Abbreviations: AA, *Aspilia africana*; AC, *Ageratum conyzoides*; MS, *Melanthera scandens*; SN, *Synedrella nodiflora*.

hand, was active against all the microbes except *E. coli*, a Gram-negative bacteria.

The toxicity study showed that the CH₃OH extracts of MS, AC, AA and SN were not toxic at concentrations between 10 mg/kg and 1,000 mg/kg; however, at concentration above 1,600 mg/kg, the extracts became toxic. The LD₅₀ was determined to be 1,275 mg/kg. In practice, rabbits feed on MS and SN in preference to other members of the family (authors' personal observation); the animals live healthily when maintained on these plants solely. From the GC-MS analysis, MS and SN contain mainly long-chain unsaturated hydrocarbons and fatty acids, which might be responsible for immunity against diseases and microbial infections. This experiment indicated that a toxicity factor may not be the reason for the rabbits' preference for some members of this family as feed stock; however, difference in the chemical composition of the volatile constituents, which definitely impact the plants with different shades of aroma, might be responsible for the animals' relative preference.

Future research directions

The four plants under investigation are species of the Compositae family. They are utilized locally as alternative non-conventional rabbit feed. They have been reported to be biologically active in the treatment of a number of diseases. In this current study, we

found that the extracts are not toxic to animals at dose levels below 1,000 mg/kg body weight, and that they inhibit the growth of a broad range of microbes.

It is important to remember that the chemical analysis carried out was limited to low polar (volatile) components only. The activities of extracts generally depend on the collective chemical function of the various chemical constituents, including the non-polar, low/medium polar and the highly polar constituents.

There is, therefore, a need for chemists to probe further into the chemical constituents of these extracts, including the medium polar (dichloromethane) fraction and the highly polar (methanol) fraction. The process will involve both a qualitative approach (that is, identification of compounds using GC-MS) and a quantitative approach (requiring isolation of chemical compounds and their characterization using spectroscopic techniques).

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Originator of research idea and supervised the research work (JKA), research student on the work (COE), collaborator who as-

sisted in acquiring GC-MS data (DOM), microbiologist who assisted with the antibacterial experiment (DAA).

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