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Preliminary phytochemical screening, antimicrobial potentials and GC-MS analysis of *Secamone afzelli* Rhoem (Asclepiadaceae) leaves extracts

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ABSTRACT

Secamone afzelli is used ethno-medicinally as remedies for stomach, colic and gastro-intestinal tract infections. This study screened the hexane, ethyl acetate and ethanol extracts of *S. afzelli* leaves for phytocompounds, antimicrobial activity and identified bioactive compounds in the most active extract using Gas Chromatography Mass Spectrometry (GC-MS) analysis. *S. afzelli* (200mg) leave was extracted by soxhlet apparatus using three solvents (hexane, ethyl acetate and ethanol) and the phytochemicals determined. Antimicrobial susceptibility and minimum inhibitory concentrations (MICs) was determined by agar-diffusion and agar-dilution methods respectively against selected resistant bacterial and fungal clinical isolates. The most active of the three crude extracts was analysed by GC-MS for bioactive compounds identification. Presence of carbohydrates, anthraquinone, glycosides, proteins, tannin, phenolic compounds and steroids were determined. The three extracts (hexane, ethyl acetate, and ethanol extracts) had antibacterial (Range of inhibition zone = 10 to 18 mm and MICs 12.5 to 200 mg/mL) but no antifungal activity. The ethanol extract was the most active with 29 bioactive compounds including piperazine, phytol, leucine methyl ester, N-methyl-N-(1-methylethyl)-1Pentanamine, 7-propyl-pyrrolizin-1-thione and 4,5-dimethylnonane. The antibacterial activity and arrays of bioactive compounds detected in *S. afzelli* leaves suggest that the plant may be a good source of novel antimicrobial compounds.

Keywords: *Secamone afzelli*, antimicrobial activity, minimum inhibitory concentration, agar plate

Introduction

About three quarters of the World's population, particularly in the developed countries relies on plants derived herbal medicines for treatment and management of several illnesses (WHO, 2019). Majority of these herbal remedies have been highly efficient over the years in the treatment of allergic, metabolic and cardiovascular diseases (Igoli *et al*., 2005). The practice of traditional medicine is more common among the population living in the rural areas where modern health facilities are inadequate or are lacking (Gabhe *et al*., 2006). Several plant parts or whole plant are being used in folklore medicines to cure specific ailments in the past and even currently. Majority of these folklore medicines were proven scientifically to actually possess important bioactive compounds as their

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metabolites and thus are excellent sources of novel antimicrobial agents (Azaizeh *et al*., 2003; Saadabi, 2007; Venkataswamy *et al*., 2010). These plants used in folklore medicines usually serve as prototypes for the development of highly potent natural, semisynthetic or synthetic drug products usually having lesser toxicity compared to the existing ones and thus suitable and safer for human and animal use (Santos *et al*., 1995; Lewis and Ausubel, 2006). Drug products derived from plants are usually accepted for use because of their relative safety, availability and are cheaper than the synthetic alternatives (Iwu *et al*., 1999). Plants are known to make diverse secondary metabolites which are known to have biological and ecological functions such as establishment of antimicrobial, anticancer, antioxidant, anti-insect agents and mutualistic interaction with their environment (Ashour, 2008; Daise *et al*., 2008; Griffin *et al.,* 2015; Aljbory and Chen, 2018). Some of these bioactive agents have been isolated, characterized and find their use in health care system while a lot are yet to be discovered. Some notable secondary metabolites in plant extracts are alkaloids, terpenoids, flavonoids and many more (Chen *et al*., 2009; Zhao *et al.,* 2013). Presently, most synthetic drugs are gradually becoming ineffective in the treatment of some diseases including those that are used as drug of last resort (Mhondoro *et al*., 2019; Mahase, 2019). As a result of depletion in the availability and effectiveness of current synthetic antimicrobial agents, it is therefore essential to search for alternative compounds with better therapeutic action particularly against resistant strains of microorganisms, from plants and other natural sources. *Secamone afzelli* Rhoem, (Asclepiadaceae) is a climbing or scrambling shrub mostly found in the secondary forests in Ghana (Mensah *et al*., 2004; Selvi *et al*., 2007). Aqueous extract of the plant is usually used in the cleaning and washing of wounds to aid healing (Irvine, 1961). A search of literature showed little evidence of the scientific justification of the traditional uses of *S. afzelii* as antimicrobial agent. It has been shown that the plant has anti-oxidant activity and inhibited the peroxidation of bovine brain liposome. The compound contributing to the anti-oxidant activity was identified to be alpha-tocopherol (vitamin E) (Mensah *et al*., 2004).

In the present study, preliminary investigation on the antimicrobial activity of hexane, ethyl acetate and ethanol extracts of *S. afzelli* leaves against selected resistant bacteria and fungi isolates was done. In addition to this, presence of phytochemical and bioactive constituents in the most active extract was determined.

Materials and Methods

Collection, preparation and extraction of plant powdered samples

Fresh healthy leaves of *S. afzelii* were collected in the month of July, 2018 from Ogbomoso jungle in Oyo state, Nigeria and the identity confirmed at the Federal Research Institute of Nigeria (FRIN), Ibadan, Oyo-State. The voucher number for the specimen was FHI110361 and deposited in the Department of Pharmacognosy, University of Ibadan, Oyo State. The leaves were air-dried and the dried leaves powdered into coarse particles using mechanical grinder. Sequential extraction of the powdered samples was carried out using solvents in an increasing polarity. About 200 g of the dry leaf powder was sequentially extracted using hexane, ethyl acetate, and ethanol in Soxhlet apparatus. After about forty siphons of each solvent extraction step, the crude extracts were concentrated by evaporation using a rotary evaporator under reduced pressure (Murugan and Parimelazhagan, 2014). The concentrated extracts were lyophilized (freeze dried) to remove the remnant solvent (Cheaib *et al*., 2018). The yield of each extract was calculated and stored for further studies.

Qualitative Phytochemical Determination

The dried powdered sample and the three crude extracts were subjected to preliminary phytochemical tests using standard techniques as described by Trease and Evans (2002) to detect the presence of some secondary metabolites such as saponins, alkaloids, flavonoids, anthraquinone, Tannins and terpenoids.

Collection, authentication and antibiotic susceptibility profile of test isolates

Bacteria exhibiting multiple antibiotic resistance previously isolated from urine of patients diagnosed of urinary tract infection (UTI) (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and patient with diarrhea (*Salmonella typhi*) as well as fungi isolates (*Aspergillus niger, Candida albicans, Rhizopus* spp., and *Penicillium* spp.) reported to exhibit resistance to ketoconazole were collected from the Laboratory of the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan. Each isolate was authenticated by cultural and standard biochemical tests as described by Cowan *et al*. (2003) and were subjected to antibiotic susceptibility test to confirm their resistance profile. Amoxicillin-clavulanic acid (20/10µg), ceftriaxone (30µg), ceftazidime (30µg), cefuroxime (30µg), erythromycin (5µg), cloxacillin (5µg), ofloxacin (5µg) and gentamicin (10µg) were tested against the Gram-positive isolates while amoxicillin-clavulanic acid (20/10µg), cefuroxime (30µg), cefixime (30µg), ceftazidime (30µg), ofloxacin (5µg), ciprofloxacin (5µg), gentamicin (10µg) and nitrofurantoin (300µg) were tested against the Gram-negative bacteria. The fungi isolates were tested against ketoconazole (10µg). The results of the bacterial susceptibility tests were interpreted as sensitive or resistant following the Clinical Laboratory Standard Institute (CLSI) guidelines breakpoints (CLSI, 2017). A fungus with zone of inhibition <20mm is interpreted as resistant and when >20mm, susceptible. All the isolates were then stored on slants at 4ºC for further studies.

Antimicrobial screening of the crude extracts

The antibacterial and antifungal activity of the crude extracts of *S. afzelli* was determined by agar-diffusion method. Briefly, from the overnight cultures of the bacteria and 48 to 72 hours culture of the fungi, speck of each bacteria and fungi colony was taken using a sterile wire loop and separately dispersed in 10mL of normal saline, vortexed to form homogenised suspension and then further diluted to 0.5 Mac Farland standard with sterile normal saline. With the aid of a sterile cotton swab mildly soaked with the individual microbial suspensions, the isolates were inoculated on the surface of Mueller Hinton agar (for bacteria) and Sabouraud dextrose Agar (for fungi) by surface spreading. After the inoculation of the isolates and the agar surface dried, wells of approximately 6 mm in diameter and 2.5 mm deep were made on the surface of the solid medium using a sterile 6 mm cup-borer and the well labelled. Each well was filled with the reconstituted hexane, ethylacetate and ethanol crude extracts serially diluted to five different concentrations (10, 5, 2.5, 1.25 and 0.025 mg/mL) each with sterile distilled water. Sterile distilled water was used as negative control, while gentamicin (10µg/mL) and ketoconazole (10µg/mL) were used as positive control for bacteria and fungi respectively. The inoculated plates were maintained at room temperature for 2 hours enabling the diffusion of the extracts into the medium. Agar plates inoculated with the bacteria were incubated at 37^{o}C for 24 hours, while those inoculated with the fungi were incubated at 25°C for up to 48 hours. The antimicrobial activity tests were undertaken under aseptic conditions and in duplicates. With the aid of a transparent ruler the diameters of zones of inhibition around the wells were measured in millimeters (mm) and the average of the duplicate's measurements recorded.

Determination of Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentrations (MICs) of the three crude extracts against the test isolates were determined using the agar-dilution method as described by Akinpelu and Kolawole (2004) and Mushore and Matuvhunye (2013). Five dilutions of each of the plant extract was prepared by reconstituting 2 grams of each of the dried extract in 10 mL of sterile distilled water and serially diluted four times to give five different concentrations as stock solutions. From each of the five concentrations, 2 mL were added separately to 18 ml of pre-sterilized molten Mueller Hinton agar (for bacteria) or Sabouraud agar (for fungi), mixed appropriately and poured into respective Petri dishes to give the final concentrations of 200, 100, 50, 25, and 12.5mg/mL. They were then allowed to solidify under laminar flow prior to streaking with the aid of a sterile swab, the 24 hrs old bacterial and 48 to 72 hours old fungi suspension previously diluted and adjusted to 0.5 McFarland standard. The culture plates were incubated at 37°C for 24 hrs for bacteria and 25ºC for up to 72 hrs for fungi. After the stated time of incubation, the culture plates were examined for growth. The procedure was done in duplicates for each test organism. The lowest concentration of each extract that inhibited the growth of the test microorganism was recorded as the MIC.

Gas Chromatography-Mass Spectrometry (GC-MS) screening of the most active extract

The most active of the three extracts (ethanolic extract) was subjected to the GC-MS analysis using a Hewlett-Package 5890 Gas Chromatograph equipped and coupled to VG Analytical 70-250S Mass Spectrometer with a fused silica capillary CP-Sil 5CB column (30m x 0.25 mm ID x 1µm of capillary column). The analysis was done using Helium gas as carrier at flow rate of 1mL/min. The oven was set to an initial temperature of 90ºC, sustained for 2min and then heated at 10ºC/min to 270ºC and finally held isothermally for 15 min. At 70eV ionization voltage, a scan was applied covering a mass range from 36 to 600 amu for 0.6s. Major constituents of the analysed ethanolic extract were identified by matching their MS and retention index data with those of the standards with the aid of computer search on a NIST version 2.1 MS Library (Odumosu *et al*., 2016).

Results

The results of the phytochemical screening of *Secamone afzelli* leaves are presented in Table 1. The dried leaves and ethylacetate extract contain alkaloid, anthraquinone, flavonoid, saponins, terpenoids and tannins. The ethanol extract contains the same phytochemicals like the dried leaves but lack flavonoids and terpenoids while the hexane extract lacks saponins, terpenoids and tannins. The susceptibility test of the test isolates against selected antimicrobials showed that they are resistant strains as presented in table 2. The anti-microbial and antifungal studies on the extracts showed that they lack antifungal activity but possesses antibacterial activity as shown in Table 3. The zone of inhibition ranged from 10 to 18 mm with ethyl acetate and ethanol extract been active on *P. aeruginosa* only while the other isolates were susceptible to the three extracts. The MICs of the extracts against the test bacteria is presented in table 4. The MIC values ranged from 12.5 to >100mg/mL. The results of the GC-MS analysis of the ethanolic extract revealed the presence of 29 compounds (figure 1) most of which are organic acids and esters of decanoic acid derivatives such as dodecanoic acid, tetradecanoic acid, hexadecanoic acid methyl ester, 9,12-octadecadienoic acid (E,E)-, dodecanoic acid 1,2,3-propanetriyl ester and others (table 5). Other notable bioactive compounds detected are piperazine, phytol, leucine methyl ester, N-methyl-N-(1-methylethyl)-1Pentanamine,7-propyl-pyrrolizin-1-thione and 4,5-dimethylnonane.

Table 1: Preliminary Phytochemical Analysis of dried and extracted leaves of *Secamone afzelli* Rhoem

Extract	Alkaloid	Anthraquinone	Flavonoid	Saponins	Terpenoids	Tannins
Dried Leaves	+	+	+	+	+	+
Hexane	+	+	+	-	-	-
Ethyl acetate	+	+	+	+	+	+
Ethanol	+	+	-	+	-	+

Legend: + = Present, - = Absent

Table 2: Susceptibility profile of the test isolates

Test Isolates	AMC 20/10µg	CTX 30µg	CRO 30µg	CRX 30µg	CXM 30µg	OFL 5µg	CIP 5µg	ERY 5µg	GEN 10µg	CLX 5µg	NIT 300µg	KCZ 10µg
Gram-positive												
Staphylococcus aureus	R	R	R	R	R	S	S	R	I	R	S	ND
Bacillus. Subtilis	R	R	R	R	R	R	R	R	I	R	R	ND
Gram-negative												
Escherichia Coli	R	R	R	R	R	R	R	ND	R	ND	R	ND
Klebsiella pneumoniae	R	R	R	R	R	R	R	ND	R	ND	R	ND
Pseudomonas aeruginosa	R	R	R	R	R	R	R	ND	R	ND	R	ND
Salmonella typhi	R	R	R	R	R	I	I	ND	R	ND	R	ND
Fungi												
Aspergillus niger	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	R
Candida albicans	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	R
Rhizopus spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	R
Penicillium spp.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	R

Legend: ND = Not Done, R = Resistant, I = Intermediate, S = Sensitive

Table 3: Susceptibility study of the extracts of *Secamone afzelli* leaves against test isolates

Extract	Ext Conc (mg/ml)	*S. aureus*	*B. subtilis*	*E coli*	*K pneumoniae*	*P aeruginosa*	*S. typhi*	*A. niger*	*C. albicans*	*Rhizopus* sp	*P. notatum*
		Zone of growth inhibition (mm)									
HX	10	16	14	14	14	-	10	-	-	-	-
	5	14	12	18	12	-	12	-	-	-	-
	2.5	12	10	12	10	-	12	-	-	-	-
	1.25	10	-	10	10	-	-	-	-	-	-
	0.025	-	-	14	-	-	-	-	-	-	-
EA	10	14	16	14	14	14	14	-	-	-	-
	5	12	14	12	14	12	12	-	-	-	-
	2.5	10	12	10	10	10	10	-	-	-	-
	1.25	-	10	-	-	-	-	-	-	-	-
	0.025	-	-	-	-	-	-	-	-	-	-
ETH	10	16	16	14	18	14	16	-	-	-	-
	5	12	14	12	14	12	14	-	-	-	-
	2.5	10	10	10	12	10	12	-	-	-	-
	1.25	-	-	-	10	-	10	-	-	-	-
	0.025	-	-	-	10	-	-	-	-	-	-
GEN	10µg/mL	23	15	12	16	22	24	ND	ND	ND	ND
KET	10µg/mL	ND	ND	ND	ND	ND	ND	23	12	15	20
SDW	-	-	-	-	-	-	-				

Legend: HX -Hexane, EA- Ethyl acetate, ETH- Ethanol, GEN- Gentamicin, KET- Ketoconazole and SDW- Sterile Distilled Water, (-) = No zone of inhibition

Table 4: Minimum inhibitory concentration of *Secamone afzelli* leaves extract against the test bacteria

Microorganisms	**Minimum Inhibitory Concentrations (MICs) mg/mL**		
	Hexane Extract	**Ethyl acetate Extract**	**Ethanol Extract**
S. aureus	50	25	25
K. pneumoniae	50	50	12.5
B. subtilis	50	50	25
S. typhi	100	50	25
E. coli	50	50	50
P. aeruginosa	200	50	50

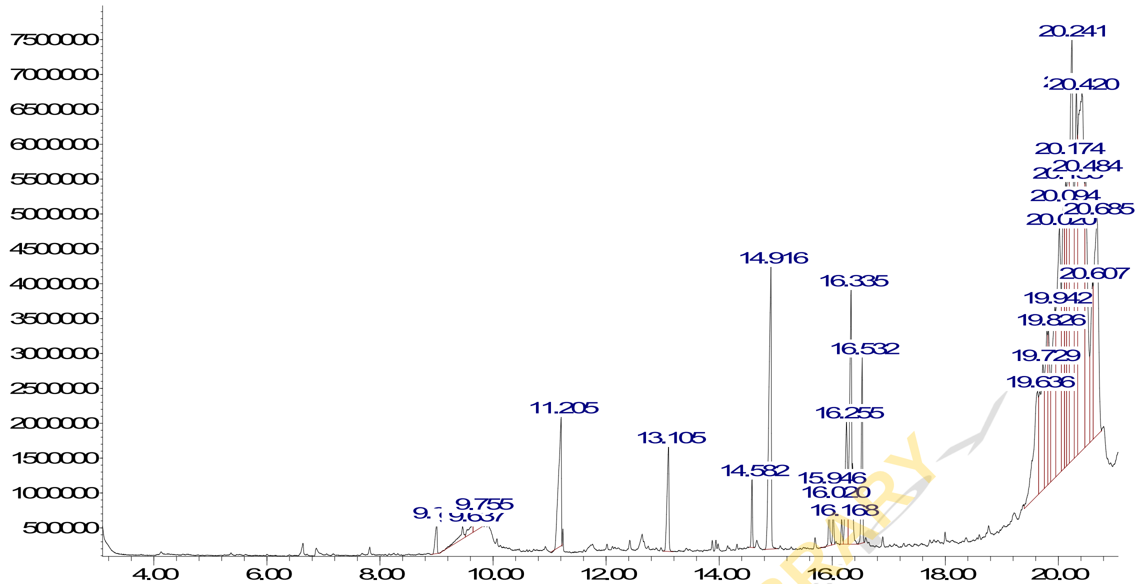

Figure 1: Total ion current chromatogram of *Secamone afzelli* leaves extract

Table 5: GCMS analysis showing the chemical constituents of ethanolic extract of *Secamone afzelli* leaves

Serial No.	Constituents	Retention time (min)	Peak area	Total (%)
1	n-Decanoic acid	9.006	10518689	0.410
2	1-Pentanamine, N-methyl-N-(1-methylethyl)-	9.463	9243622	0.360
3	L-Leucine, methyl ester	9.639	6773464	0.264
4	Piperazine	9.755	10869953	0.424
5	Dodecanoic acid	11.205	64540228	2.517
6	Tetradecanoic acid	13.105	35825257	1.397
7	Hexadecanoic acid, methyl ester	14.582	13699298	0.534
8	n-Hexadecanoic acid	14.916	117693193	4.590
9	9, 12-octadecadienoic acid, methyl ester, (E,E)-	15.946	11961678	0.466
10	Cis-13-octadecenoic acid, methyl ester	16.020	9123185	0.356
11	Phytol	16.168	5304896	0.207
12	9, 12-octadecadienoic acid (Z,Z)-	16.255	47874908	1.867
13	Cis-13-octadecenoic acid	16.335	106352733	4.148
14	Octadecanoic acid	16.532	45424187	1.771
15	Dodecanoic acid, 1,2,3-propanetriyl ester	19.636	91958728	3.586
16	Lauric anhydride	19.729	92718656	3.616
17	Pyrrolizin-1-thione, 7 propyl-	19.800	71248998	2.779
18	Nonane, 4,5-dimethyl-	19.826	58702206	2.289
19	Dodecanoic acid, 1,2,3-propanetriyl ester	19.942	105815977	4.127
20	Dodecanoic acid, 1,2,3-propanetriy	20.020	174300529	6.798
21	Lauric anhydride	20.094	110669867	4.316
22	Dodecanoic acid, 1,2,3-propanetriy	20.133	89542935	3.492
23	Dodecanoic acid, 1,2,3-propanetriy	20.174	119488317	4.660
24	Dodecanoic acid, 1,2,3-propanetriy	20.241	253626143	9.891
25	Dodecanoic acid, 1,2,3-propanetriy	20.321	166572130	6.496
26	Pyrrolizin-1-thione, 7-propyl-	20.420	357693055	13.950
27	Dodecanoic acid, 1,2,3-propanetriyl ester	20.484	139884900	5.455
28	Nonane, 4,5-dimethyl-	20.607	66290380	2.585
29	Lauric anhydride	20.685	170463809	6.648

Discussion

The result of the phytochemical analysis of the hexane, ethanolic and ethyl acetate extracts of the leave of *S. afzelli* revealed the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides (Table 1). However, in a similar work carried out by Herve *et al.* (2008), methanol extracts of both the leaves and stems of the plant showed the presence of all these metabolites except alkaloids. The difference in the result may be due to the solvent used for the extraction and the effect of due to the geographical location of the plant. The presence of these secondary metabolic components in the plant extract may be responsible for the ethnomedicinal claims including the antimicrobial activity observed in this study as each of the components identified has been previously recorded to have one therapeutic usage or another. Plants rich in saponins are known to be immune boosting and have anti-inflammatory properties (Kenner and Requena, 1996; Hassan *et al*., 2012; Khan *et al*., 2018). Alkaloids present in plants are well known for their medicinal values over the years. Their unique biological properties, particularly their cytotoxicity potential, (Nobori *et al*., 1994), tend to promote their antimicrobial property. Similarly, plants with tannins have antibacterial potentials due to the ability of the tannin to react with proteins to form stable water-soluble compounds thereby killing the microorganism by directly damaging its cell membrane (Buzzini *et al*., 2008). The antibacterial activities of alkaloids and flavonoids have been reported by Adesina *et al.* (2000) and Onwuliri and Wonany (2005).

The isolates used as test organisms in this study are multidrug resistant strains as shown in Table 2. The antimicrobial screening of *S. afzelli* against the test organisms showed varying diameter of zones of inhibition against the bacterial isolates at different concentrations for hexane extract (10 to 18 mm), ethyl acetate extract (10 to 16 mm), and ethanolic extract (10 to18mm). However, none of the extracts had activity against the fungal isolates used in this study (Table 3). This report does not totally agree with the report of Mansah *et al.* (2006) where it was recorded that the methanolic extract of the plant leaves and stems had weak activity against *S aureus* and *E coli* and no activity against *B subtilis* and *P aeruginosa*. It was also reported that the methanolic extract had activity against the yeasts but no activity against the dermatophytes. These discrepancies may be as a result of the solvent of extraction. Ethanol, hexane and ethyl acetate was used in this study while methanol was used by Mansah *et al.* (2006). Hence, the possibility that more of the active biological compounds against the bacteria may have been extracted by ethanol, hexane and ethylacetate compared with the methanol. None of the extracts in this study had activity against the fungal isolates including the yeast, *C. albicans*, as presented in Table 4, which is contrary to the report of Mansah *et al*. (2006) with respect to yeasts. This contradiction may also be solvent based or the ecological factors.

From Table 4, the minimum inhibitory concentrations (MICs) obtained showed that all the extracts were able to prevent the growth of the test bacterial isolates within an MIC range of 12.5 to 200mg/mL, with the lowest MIC value against the test bacterial exhibited by ethyl acetate (25mg/mL) and ethanol extracts (12.5mg/mL). The results of the GC-MS analysis (figure 1) revealed the presence of some bioactive compounds in the ethanolic extract. Among the notable bioactive compounds detected in this study are piperazine, a compound known to belong to the antihelminthic class, phytol, an acyclic diterpene usually used as precursor for production of vitamin E and K1, leucine methyl ester, a known lysosomotropic agent with immunomodulatory activity and thus very useful agent in cancer therapy, N-methyl-N-(1-methylethyl)-1-Pentanamine,7-propyl-pyrrolizin-1-thione and 4,5-dimethylnonane (Table 5).

Mansah *et al*. (2004) and Hougton *et al*. (2005) carried out a High-Performance Liquid Chromatography (HPLC) analysis of the methanolic extract of the plant and reported the presence of alpha-tocopherol, a flavonoid and an antioxidant commonly called vitamin E which was also detected in this study as phytol, a diterpene flavonoid which serve as a precursor for the synthesis of vitamin E.

Conclusions

This study has elucidated the various medicinal potentials of *Secamone afzelli* as a good source of bioactive substances with notable antimicrobial activity as well as anti-oxidant potentials. This thus justifies the ethno-medicinal uses of the plant in the treatment of several ailments. Although, weak antibacterial activity and non-susceptibility of the fungal isolates to the extracts was recorded in this study, the GC-MS detection of some potential antimicrobial compounds suggest that *S. afzelli* will be a good source of various potential antimicrobial and anti-oxidant precursors for the development of better and effective drugs.

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Competing interests

Authors have declared that no competing interests exist.

References

Adesina, S. K., Idowu, O., Ogundaini, A. O., Oladimeji, H., Olugbade, T. A. and Onawunmi, G. O. P. (2000). Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. *Phytoth. Res*. 14: 371-374.

Akinpelu, D. A. and Kolawole, D. O. (2004). Phytochemistry and antimicrobial activity of leaf extract of *Piliostigma thonningii* (*Schum*). *Sci. Focus*, 7: 64-70

Aljbory, Z. and Chen, M. S. (2018). Indirect plant defense against insect herbivores a review. *Insect Sci*. 25: 2–23.

Ashour, H. M. (2008). Antibacterial, antifungal, and anticancer activities of volatile oils and extracts from stems, leaves, and flowers of *Eucalyptus sideroxylon* and *Eucalyptus torquata*. *Cancer Biol Ther*. 7: 399–403.

Azaizeh, H., Fulder, S., Said, K. and Khalil, O. (2003). Ethno-botanical knowledge of local Arab practitioners in the middle eastern region *Fitoterapia*, 74: 98-108.

Buzzini, P., Arapitsas, P., Goretti, M., Branda, E, Turchetti, B., Pinelli, P., Ieri, F. and Romani, A. (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini-Rev. Med. Chem.* 8:1179-1187.

Cheaib, D., El Darra, N., Rajha, H. N., El-Ghazzawi, I., Maroun, R. G. and Louka, N. (2018). Effect of the extraction process on the biological activity of lyophilized apricot extracts recovered from apricot pomace. *Antioxidants*, 7(1): 11. doi:10.3390/antiox7010011.

Chen, F., Liu, C.J., Tschaplinski, T. J. and Zhao, N. (2009). Genomics of secondary metabolism in Populus: Interactions with biotic and abiotic environments. *Crit. Rev. Plant Sci*. 28: 375–392.

CLSI (2017). Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-seventh Edition. CLSI Supplement M100. Wayne, PA, USA.

Cowan, S. T., Steel K. J., Barrow, G. I. and Feltham, R. K. A. (2003). Manual for the identification of medical bacteria. Third Edition, Cambridge University Press, London.

Daíse, L. L., Alviano, D. S., Alviano, C. S. and Kolodziejczyk, P. P. (2008). Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. *Phytochemistry*, 69: 1732–1738.

Gabhe, S. Y., Tatke, P. A. and Khan, T. A. (2006). Evaluation of the immunomodulatory activity of the methanol extract of *Ficus benghalensis* roots in rats. *Indian J. Pharmacol.*38(4): 271-275.

Griffin, S. G., Wyllie, S. G., Markham, J. L. and Leach, D. N. (2015). The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *FlavourFragr. J.* 14: 322–332.

Hassan, H. S., Sule, M. I., Musa, A. M., Musa, K. Y., Abubakar, M. S. and Hassan, A. S. (2012). Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. *African Journal of Traditional, Complementary and Alternative Medicines*, 9(2), 250-255.

Hervé, Z., Charles, K., Anoubilé, B., Janat, M. B. and Yves, A. B. (2008). Phytochemical screening and determination of flavonoids in *Secamone afzelii* (Asclepiadaceae) extracts. *Afr. J. Pure Appl. Chem.* 2(8): 80-82.

Hougton, P. J., Hylands, P. J., Mensah, A. Y. and Hensel, A. (2005). In vitro tests and etnopharmacological investigations: Wound healing as an example. *J. Etnobotanique*, 100: 100-107.

Igoli, J. O., Ogaji, O. G., Tor-Anylin, T. A. and Igoli, N. P. (2005). Traditional medical practices amongst the Igede people of Nigeria. *Afri J Trad CAM*. 2(2): 134-152.

Irvine, F. R. (1961). Woody plants of Ghana with special reference to their uses. Oxford University Press, London.

Iwu, M. M., Duncan, A. R. and Okunji, C. O. (1999). New antimicrobials of plant origin: In J. Janick (ed). Prospective on new crops and new uses. ASHS press, Alexandria, V.A. Pp: 457-462.

Kenner, D. and Requena, Y. (1996). Botanical Medicine: A European Professional Perfective: Paradign Publication, Brookline, Massachusetts.

Khan, M. I., Ahhmed, A., Shin, J. H., Baek, J. S., Kim, M. Y., and Kim, J. D. (2018). Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study in vitro and in vivo. *Evidence-Based Complementary and Alternative Medicine*. 2018: 1 – 12. https://doi.org/10.1155/2018/3486106.

Lewis, K. and Ausubel, F. M. (2006). Prospects of plant derived antibacterials. *Nat. Biotechnol*. 24: 1504–1507.

Mahase, E. (2019). Superbug spreads in European hospitals as resistance to last resort antibiotics grows. *BMJ;* 366:14942. doi: https://doi.org/10.1136/bmj.l4942.

Mensah, A. Y., Houghton, P. J., Akyirem, G. N. A., Fleischer, T. C., Mensah, M. L. K., Sarpong, K., Adosraku, R. J. and Wiley & Sons, Ltd. (2004). Evaluation of the antioxidant and free radical scavenging proporties of *Secamone afzelii* Rhoem. *Phytotherapy Res.* 18: 1031-1032.

Mensah, A. Y., Houghton, P. J., Akyirem, G. N., Flerscher, T. C., Mensah, M. L., Sarpong, K., and Adosraku, R. (2006). In-Vitro evaluation of effects two Ghanaian plants relevant to wound healing. *Phytotherapy Res*. 20: 941-944.

Mhondoro, M., Ndlovu, N., Bangure, D., Juru, T., Gombe, N. T., Shambira, G., Nsubuga, P. and Tshimanga, M. (2019). Trends in antimicrobial resistance of bacterial pathogens in Harare, Zimbabwe, 2012–2017: a secondary dataset analysis. *BMC Infect Dis* 19(746): 1 - 9. https://doi.org/10 .1186/s12879-019-4295-6.

Murugan, R. and Parimelazhagan, T. (2014). Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from Osbeckia parvifolia Arn.–An in vitro approach. *Journal of King Saud University-Science*26(4), 267-275.

Mushore, J. and Matuvhunye, M. (2013). Antibacterial properties of *Mangiferaindica* on *Staphylococcus aureus*. *Afri. J. Cln. Exper. Microbiol*. 14(2): 62 – 74.

Nobori, T., Miurak, K., Wu, D. J., Takabayashik, L. A. and Carson, D. A. (1994). Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*, 368(6473): 753–756.

Odumosu, B. T., Salawu, O. T., Oyeyemi, I., Alabi, O. S., Rufai, T. R. and Odunukan, O. (2016). Bioactive constituents and antibacterial screening of two Nigerian plant extracts against selected clinical bacteria. *Nigerian Journal of Pharmaceutical Research,* 12(2): 123-133.

Onwuliri, F. C. and Wonany, D. L. (2005). Studies on the combined antibacterial action of Ginger (*Zingiber officinale* L.) and Garlic (*Allium sativum L.*) on some bacteria. *Nig. J. Bot*. 18: 224-228

Saadabi, A. M. (2007). An evaluation of *Lawsoniainermis Linn*. (Sudanese henna) leaf extracts as antimicrobial agent. *Res. J. Biol. Sci.* 2(4): 419-423.

Santos, P. R. V., Oliveira, A. C. X. and Tomassini, T. C. B. (1995). Controle microbiogico de productous fitoterapicos. *Rev. Farm. Bioquim*. 31: 35–38.

Selvi, S., Uma, D. P., Chinnaswamy, P., Giji, T. M. and Sharmila, S. P. (2007). Antibacterial efficacy and phytochemical observation of some indian medicinal plants. *Anc. Sci. Life*, 26(3): 16 – 22.

Trease, G. E. and Evans, W. C. (2002). Pharmacognosy. 15th Ed. London: Saunders Publishers.

Venkataswamy, R., Doss, A.,Sukumar, M. and Mubarack, H. M. (2010). Preliminary Phytochemical screening and antimicrobial studies of *Lantana indica* Roxb. *Indian J. Pharm. Sci*. 72(2): 229–231.

W.H.O (2019). WHO global report on traditional and complementary medicine 2019. Geneva: World Health Organization. Licence: CC BY-NC-SA 3.0 IGO. Available at: https://www.who.int/traditiona l-complementary-integrative-medicine/Who GlobalReportOnTraditionalAndComplementaryMedicine2019.pdf?ua=1.

Zhao, N., Wang, G., Norris, A., Chen, X. and Chen, F. (2013). Studying plant secondary metabolism in the age of genomics. *Crit. Rev. Plant Sci*. 32: 369–382.