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Phytochemical Screening and Antibacterial Activity of *Centaurea senegalensis* growing in Nigeria

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ABSTRACT: The increasing prevalence of drug resistant pathogens in developing countries has necessitated research for effective therapeutic agents from plants. This study was designed to evaluate the antibacterial properties of *Centaurea senegalensis* and investigate the phytochemical constituents. The plant sample was extracted using methanol and subjected to successive partition with n-hexane, dichloromethane and ethyl acetate. The methanol extract (ME), hexane (HF), dichloromethane (DF) and ethylacetate (EF) fractions were subjected to antibacterial screening on selected gram-positive and gram-negative bacteria using agar well and micro broth dilution methods. The antibacterial efficacies of extracts showed varying zones of growth inhibitions (15-31 mm). The DF fraction was effective on Methicillin resistant *Staphylococcus aureus* (MRSA), and Vancomycin resistant *enterococci* (VRE) with interesting activity (28 mm, MIC 12.5 $mgmL^{-1}$). However, the EF was most effective fraction against gram-negative bacteria such as *Escherichia coli* (31 mm, MIC, 12.5 $mgmL^{-1}$). Chemical composition of bioactive fraction was determined using gas chromatography-mass spectrometry (GC-MS). The compounds detected were largely natural acetylenes such as 1, 5-heptadien-3-yne (23.2%), 2-hexyne-1-ol (12.3%), 2-methyl-1,5-hexadien-3-yne (6.6%) and 5-methyl-1-hexyn-3-ol (2.1%) as most abundant phytoconstituents identified. *C. senegalensis* fractions have demonstrated effective activity on both gram-positive and gram-negative bacteria, which might be attributed to acetylene derived natural compounds. Our findings have shown the importance of *C. senegalensis* as source of chemical compounds with effective antibacterial properties.

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Plants are potential sources of drugs because they synthesize secondary metabolites such as alkaloids, flavonoid, saponins, tannins and sesquiterpenoids among others. These metabolites are responsible for wide range of biological and pharmacological properties for therapeutic use as medicines. Several indigenous plants are used in traditional medicine across Africa and many parts of the world. Some of these herbal recipes are known for several decades in the treatment of illness or management of human and animal diseases (Mishra *et al*., 2013). Plants of the Centaurea genus are used in traditional medicine for treatment of numerous human diseases (Dutta *et al*., 2013).

Several *Centaurea* species especially from Turkey (Boga *et al*., 2016), Egypt (Mogahannem *et al*., 2016) Kosovo (Haziri *et al*., 2017) and Greece (Ciric *et al*., 2011) were investigated for antibacterial activities among many others. *Centaurea senegalensis* [DC] grows annually as herb of the Sahel from Mauritania to Nigeria and Western Cameroun extending to Sudan. The plant has branched leaves with rough surface about 1.2 ft high with stem up to 2-inch long (Hutchison, 1963). It is commonly known as "Dayi" in Hausa language used widely in Northern Nigerian traditional medicine for treatment of infectious diseases, stomach ache, swellings, oedema, arthritis and pain (Burkill, 1985). Previous phytochemical screening on *C. senegalensis* methanol extract has resulted to isolation and characterization of flavonoids such as 6-hydroxykaempferol, 6-methoxykaepferol, eupalitin and jaceosidin in addition to 7-hydroxy-3, 5, 6, 8, 4'-pentamethoxyflavone and 7, 4'-dihydroxy-3, 5, 6, 8-tetramethoxyflavone (Aqil *et al*., 1998). The Centaurea genus is rich in flavonoids and sesquiterpene lactones as main chemical constituents (Bruno *et al*., 2013).

However, phytochemical and antibacterial studies on *C. senegalensis* extracts have not been reported previously. Thus, the aim of this study was to investigate the antibacterial activity of *C. senegalensis* extracts and determine the chemical constituents using gas chromatography-mass spectrometry (GC-MS) with a view to establish the efficacy of the plant in search of antibacterial agents which had not been reported hitherto.

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MATERIAL AND METHODS

Plant collection and extraction: The plant sample was collected at Funtua (11°32N 7°19E and 11.533°N 7.317°E), Katsina state, in November 2017. It was authenticated at the Department of Botany, Ahmadu Bello University, Zaria, where a Voucher number 2052 was deposited. The plant was air-dried and pulverized using mortar and pestle. The pulverized plant (980 g) was subjected to extraction with methanol (2L) at room temperature. The sample was filtered and concentrated using a rotary evaporator under reduced pressure to yield 274.6 g (5.9%) of the crude methanol extract. The crude extract (160 g) was suspended in distilled water and partitioned successively to give the n-hexane (26.31 g), dichloromethane (20.50 g) and ethyl acetate (14.45 g) respectively.

Phytochemical screening: Phytochemical screening of *Centaurea senegalensis* crude extract/fractions was carried out to detect the presence of secondary metabolites by qualitative chemical tests as reported by Prabhu (2009).

*Antibacterial activity determination***:** The microorganisms used include gram-positive: *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, Vancomycin resistant *enterococci* (VRE) and gram-negative bacteria: *Shigella dysenteriae*, *Salmonella typhi* and *Escherichia coli* were obtained at the Department of Microbiology, Ahmadu Bello University Teaching Hospital, Shika, Zaria. The isolates were purified on nutrient agar (OXOID) plates and characterized using standard microbiological and biochemical procedures (Cowan and Steel, 1974; McFaddin, 1977). The antimicrobial screening of the extract was carried out using agar well diffusion method. Sterile Mueller Hinton's agar plates were flooded with 0.1 mL of the standardized bacterial suspensions. These were streaked uniformly on the surface of the culture media. Wells of 6 mm diameter were punched on each plate with sterile cork borer. The compound was dissolved in dimethyl sulfoxide (DMSO). About 0.1 mL of the extract and fractions at 200 $mgmL^{-1}$ was added to each well and allowed to stay for about 1hr to enhance diffusion through the media. The plates were incubated (inverted) aerobically at 37°C for about 18-24 hr. At the end of the incubation period, the diameters of the zones of inhibition of growth were measured using a transparent ruler and recorded. The compounds were tested in duplicates and mean zones of inhibition were calculated (Akerele *et al.*, 2011).

Minimum inhibitory concentration (MIC): The MIC was determined using broth dilution method as reported by Vellokobia *et al.* (2001). Two-fold serial dilution of the extract and fractions were made to obtain 100 $mgmL^{-1}$, 50 $mgmL^{-1}$, 25 $mgmL^{-1}$, 12.5 $mgmL^{-1}$ and 6.25 $mgmL^{-1}$. About 0.2 mL suspension of standard inoculum of each organism was inoculated to the different concentrations of the extract and fractions. The extract and fractions were then incubated at 37°C for 24 hr after which they were observed for inhibition of growth. Inhibition of growth was indicated by a clear solution. The MIC is defined as the least concentration of the compound inhibiting the visible growth of each organism.

Minimum bactericidal concentration (MBC): The contents of the MIC tubes and the preceding tubes in the serial dilution were sub-cultured into appropriately labelled nutrient agar plates by dipping a sterile wire loop into each tube and streaking on the surface of each agar plate respectively. The plates were then incubated at 37°C for 24 hr after which they were observed for colony growth. The lowest concentration of the subcultures with no growth was considered to the minimum bactericidal concentration of the extracts (Abdullahi *et al.*, 2011).

Gas chromatography–Mass Spectrometry (GC-MS): The GC-MS analysis on *Centaurea senegalensis* (EF) fraction was carried out on an Agilent Technologies (6890 Series) GC coupled with a (5973 Series) Mass Selective Detector. It was equipped with an Agilent HP-5MS capillary column (0.25 µm film thickness) with dimensions 30 m (length) × 0.25 µ I.D). The sample ionization energy of 70eV for GC-MS detection was used. Helium was used as the carrier gas at a pressure of 60 kPa, with the oven temperature programming at 100°C (for 2 min) to 280°C (for 30 min) at a ramping rate of 4°C per min. A 2.0 µl diluted sample was manually injected at 280°C with a split ratio of 1:50. The system software was driven by Agilent Chemstation software. The relative percentage of each component was calculated by comparing its average peak to the total areas. The identification of the various compounds was carried out by comparison of their mass spectra with those of authentic samples or those obtained from isolated pure compounds in our laboratory. The NIST/NBS 2005 mass spectral database of the GC-MS system was also used to identify some compounds whose structures were confirmed by published data (Yang *et al.,* 2010).

RESULTS AND DISCUSSION

*Phytochemical and antibacterial screening***:** The phytochemical screening of *Centaurea senegalensis* extract and fractions showed the presence of

flavonoids, saponins, terpenes and tannins (Table 1). These phytochemicals have been detected in several Centaurea species as biomarker molecules, where several flavonoids and sesquiterpene lactones were reported to have been isolated (Bruno *et al*., 2013). Centaurea species are widely used as herbal recipes across different cultures due to medicinal values attributed to secondary metabolites found in them. Previous studies on antibacterial activity of Centaurea species have shown the effects of secondary metabolites on various human pathogenic bacteria (Hardoim *et al*., 2015). In the present study, *C. senegalensis* methanol extract (ME), dichloromethane (DF) and ethyl acetate (EF) fractions demonstrated antibacterial activity with varying zones of growth inhibition (11-31 mm). The DF was effective on MRSA, VRE and *S. dysenteriae* with 28 mm growth inhibition. The efficacy of DF against resistant bacteria such as MRSA indicated significant potential antibacterial compounds therein. However, EF showed the highest growth inhibitions (31 mm) against *E. coli* (Table 2). It is interesting to note that gram-negative bacteria such as *E. coli* have inherent resistance to drug molecules probably due to peptidoglycan cell wall structure (Ifantis *et al*., 2013). Nevertheless, our findings agree with the report of Erecevit and Kurbag (2017) on the antibacterial activity *Centaurea variegata* methanol extracts with growth inhibition against *S. aureus* (23 mm) and *E. coli* (28 mm). It is remarkable that *C. senegalensis* extracts possess compounds with antibacterial activity due to chemical characteristics based on the extractants used. Previous study on various plant extractants used for antibacterial screening has revealed that the most effective extractant is acetone followed by ethylacetate (Eloff, 1998). However, *C. senegalensis* methanol extract (ME) ethylacetate fraction (EF) contain strong bioactive compounds. This point to the fact that the plant has medicinal value as antibacterial phytomedicine.

Table 1: Percent extracted and phytochemical screening of *C. senegalensis*

	HF	**DF**	**EF**	**ME**
Percent extracted (%)	16.40	12.81	14.45	5.90
Flavonoids	-	+	+	+
Glycosides	+	+	+	+
Saponins	-	-	+	+
Terpenes	+	+	+	+
Anthraquinone	-	-	-	-
Tannins	-	-	+	+

+ = present, - = absent, HF = hexane fraction, DF = dichloromethane fraction, EF = ethyl acetate fraction and ME = methanol extract.

Table 2: Antibacterial activity of *C. senegalensis* extract and fractions

Pathogens	Zone of inhibition (mm)						
	Gram	HF	DF	EF	ME	CFX	ERT
S. aureus	+	13	13	21	16	33	23
MRSA	+	18	28	26	23	24	31
VRE	-	27	28	24	26	31	-
B. subtilis	+	16	11	17	20	-	29
E. coli	-	15	25	31	20	24	33
S. typhi	-	17	26	28	22	-	27
S. dysenteriae	-	21	28	24	28	33	-

(-) No inhibition zone, MRSA = Methicillin resistant Staphylococcus aureus, VRE = Vancomycin resistant enterococci, CFX = Ciprofloxacin, ERT = Erythromycin, HF = hexane fraction, DF = dichloromethane fraction, EF = ethyl acetate fraction and ME = methanol extract

The minimum inhibitory concentration (MIC) describes the activity against test bacteria in terms of concentration. The lower MIC values indicates effective concentration that inhibits bacterial growth and hence the strength of antibacterial activity. The MIC values reported in this study varies across the extractants, but it was observed that both extract and fractions demonstrated lower MIC (12.5 $mgmL^{-1}$) against VRE. Similarly, MIC values reported against MRSA and *S. dysenteriae* by DF and EF (Table 3) indicated the potency of *C. senegalensis* extract and fractions as antibacterial agent. The lower MIC of *C. senegalensis* extracts on test organisms were found to surpass the MIC of *Centaurea helenoides* extract on *B. subtilis* (>1000 mg/mL) and *Centaurea appendicigera* extract on *S. aureus* (>1000 mg/mL) as reported by Buruk *et al*. (2006). However, acetone extracts of Centaurea species had demonstrated effective MIC on *S. aureus* for *Centaurea lycopifolia* (250 µg/mL) and *Centaurea balsamita* (170 µg/mL) as reported by Boga *et al*.,(2016). The minimum bactericidal concentration (MBC) describes the lethal concentration of extracts on the bacteria. Thus, the lowest MBC indicates effective antibacterial property. The lower MBC (25 $mgmL^{-1}$) reported in this study showed effective activity of all extract and fractions on VRE and *S. dysenteriae*. Similarly, lower MBC effects

(25 mgmL^{-1}) were observed on MRSA by the DF and EF (Table 3).

Chemical composition of bioactive fraction: Chemical composition of bioactive fraction (EF) of *C. senegalensis* determined using GC-MS has indicated 18 chemical compounds representing 80.5% of the identified components (Table 3). These compounds were detected to be largely vinylic and acetylenic compounds derived from natural polyacetylenes. Previous study reported the isolation of thirty-five acetylenes from leaves and roots of *Centaurea montana* (Christensen and Lam, 1991). These natural products are found in Asteraceae family and were reported for anti-inflammatory, anti-tumor and antibacterial efficacies (Konovalov, 2014). In this study, 1, 5-heptadien-3-yne (23.2%), in addition to 2-hexyne-1-ol (12.3%), 1, 2-dimethylcyclohexane (7.9%), 2, 3, 4, 5-tetrahydro pyridine (7.0%), 6-methyl-4-undecene (6.9%), 2-methyl-1, 5-hexadoene-3-yne (6.6%), 2-octene (3.7%) and 5-methyl-1-hexyn-3-ol (2.1%) were detected as major compounds (Figure 2). Although several other components including 1-(2-butenyl-1-yl)-Aziridine, N-prop-2-enylacetamide, 3-methyl-2, 5-dihydrofuran, (E)-2-butenyl cyclopropane and 3-methyl-1, 4-pentadiene were also detected in minor quantities. However, acetylenic derivatives alone constituted about 45.3% of the total components identified. Thus, the broad-spectrum antibacterial effects of EF fraction might be explained from the point of acetylenic compounds perhaps in synergy interaction with other components (Figure 1). The identification of acetylenic components from *C. senegalensis* fraction is an important milestone in the search for antibacterial agent from the plant.

Table 3: The MIC and MBC of *C. senegalensis extract* and fractions

MIC/MBC (mgmL-1)	Test organisms						
	S. aureus	MRSA	VRE	*B. subtilis*	*E. coli*	*S. typhi*	*S. dysenteriae*
HF	50/100	25/50	12.5/25	50/100	50/100	12.5/50	25/25
DF	50/100	12.5/25	12.5/25	100/100	12.5/50	12.5/50	12.5/25
EF	25/50	12.5/25	12.5/25	50/100	12.5/50	12.5/50	12.5/25
ME	50/100	25/50	12.5/25	25/50	25/50	25/100	12.5/25

MRSA = Methicillin resistant *Staphylococcus aureus*, VRE = Vancomycin resistant *enterococci*, *Salmonella typii*, *Shigella dysenteriae*, HF = hexane fraction, DF = dichloromethane fraction, EF = ethyl acetate fraction and ME = methanol extract.

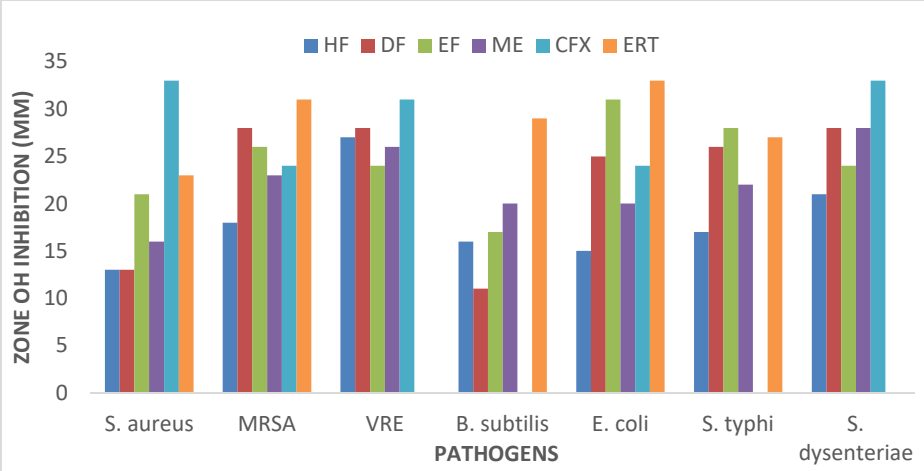

Fig 1: Antibacterial activity of *C. senegalensis* extract and fractions

HF: hexane fraction,DF: DCM fraction, EF: ethyl acetate fraction, ME: methanol extract; CFX: Ciprofloxacin, ERT: Erythromycin

Conclusion: Centaurea senegalensis is used in traditional medicine against infectious diseases. The phytochemical and antibacterial efficacy of the plant was evaluated. The ME, HF and EF fractions demonstrated effective growth inhibition on both gram-positive and gram-negative bacteria, with ethylacetate fraction (EF) being the most active antibacterial fraction, which contains largely vinylic and acetylenic compounds detected by GC-MS. These findings have shown the importance of *C. senegalensis* as a source of chemical compounds with effective antibacterial properties.

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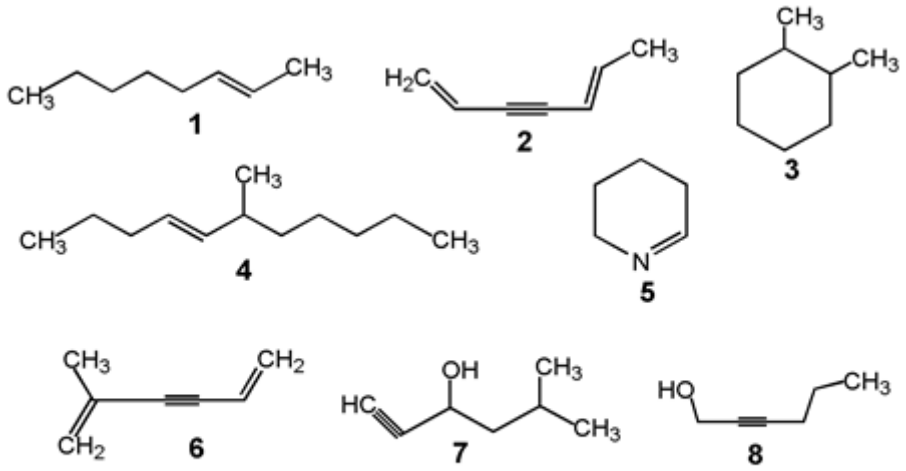
Table 4: GC-MS analysis of *C. senegalensis* ethyl acetate fraction (EAF)

s/no.	Names of compound	*RT	Composition (%)
1	2-octene	3.271	3.73
2	1,5-heptadien-3-yne	3.564	23.22
3	Cyclohexane-1,2-dimethyl-	3.711	7.90
4	Aziridine-1-(2-buten-1-yl)-	3.821	1.90
5	4-undecene-6-methyl-	4.040	6.93
6	Acetamide-N-2-propynyl	4.150	1.41
7	3-Aminipropionitrile	4.590	0.93
8	Pyridine-2,3,4,5-tetrahydro	4.737	7.00
9	Furan-2,5-dihydro-3-methyl	5.140	1.67
10	1,5-hexadien-3-yne-2-methyl	5.616	6.57
11	(E)-2-butenylcyclopropene	6.055	1.51
12	5-methyl-1-hexyn-3-ol	6.422	2.13
13	1,4-pentadien-3-methyl	7.008	1.05
14	Aziridine-2-methyl	8.217	0.60
15	2-propenitrile	8.876	0.64
16	Aminoacetonitrile	19.171	0.52
17	2-propenitrile	58.663	0.48
18	2-hexyne-1-ol	61.301	12.28
Total composition (%)		**80.5**	

Compounds' retention times (min) as eluted from DB-5MS column, compounds identified (%) = 80.5, RT = retention time.



(1) 2-octene (3.7%), **(2)** 1, 5-heptadien-3-yne (23.2%), **(3)** 1, 2-dimethyl-cyclohexane (7.9%), **(4)** 6-methyl-4-undecene (6.9%), **(5)** 2, 3, 4, 5-tetrahydro pyridine (7.0%), **(6)** 2-methyl-1, 5-hexadiene-3-yne (6.6%), **(7)** 5-methyl-1-hexyn-3-ol (2.1%), **(8)** 2-hexyne-1-ol (12.3%)

Fig 2: Major compounds identified from *C. senegalensis* ethyl acetate fraction (EF) by GC-MS

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