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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CENTAUREA PRAECOX* 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 praecox* 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

Introduction

Various medicinal properties have been attributed to the natural herbs. Medicinal plants constitute the main source of new pharmaceutical and healthcare products (Ivanova *et al*., 2005). All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine,

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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 EF 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). *C. praecox* 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. praecox* as source of chemical compounds with effective antibacterial properties.

Keywords: *Centaurea praecox,* Phytochemical Screening, Antibacterial Activity, Gram Positive, Gram Negative

Particularly in Africa and Asia (Tsakala *et al*., 2006). Medicinal plants contain some compounds which have definite physiological actions on animals and humans as such they are potential sources of drugs because they produce secondary metabolites such as alkaloids, saponins, flavonoid, 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). The

Centaurea genus are used in traditional medicine for curing numerous human diseases (Dutta *et al*., 2013).

Large numbers of Centaurea species were investigated for antibacterial activities, antioxidant among many others. *Centaurea praecox* is an annual herb up to 60 cm high from a perennial, flowers in prickly heads at ground-level appearing before the vegetative shoots, from Mali to Northern and Southern Nigeria, and extending to Sudan. They have spiny-heads at ground-level which are apt to cause injury by piercing bare feet. 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 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. *praecox* extracts have not been reported previously. Thus, the aim of this study was to investigate the phytochemical constituents and antibacterial activity of C. *praecox* extracts with a view to establish the efficacy of the plant in search of antibacterial agents which had not been reported.

MATERIAL AND METHODS

Plant collection and extraction: The plant of *Centaurea praecox* was collected from Jabba Local government, Kaduna State of Nigeria in March, 2018. The identity of the plant was confirmed by the Department of Botany, Faculty of Life Science, Ahmadu Bello, University, Zaria,

Nigeria, where a voucher specimen number 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 gramnegative 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 37oC 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).

RESULTS AND DISCUSSION

Phytochemical and antibacterial screening: The phytochemical screening of *Centaurea praecox* 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. praecox* 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. praecox* 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: Phytochemical screening of *C. praecox*

	HF	DF	EF	MF
Tannins	-	-	+	+
Terpenes	+	+	+	+
Saponnins	-	+	+	+
Glycosides	-	+	+	+
Flavonoids	+	-	+	+
Anthraquinones	-	-	-	-

+ = 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		18	26	28	23	33	23
MRSA		0	24	27	22	24	31
E. coli		19	23	25	21	24	33
Candida albiccins		18	22	24	20		
Candida Stellatoidea		18	24	26	21		
S. typhi		17	26	28	22		

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).

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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