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Preliminary Phytochemical Analysis and Antibacterial Studies on the ethylacetate soluble fraction of the whole plant of *Centaurea perrottetii* DC

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Abstract

The development of resistance by bacteria has necessitated continuous search for newer and safer antibiotics. *Centaurea perrottetii* have been used in the management of bacterial infections and other ailments in traditional medicine. The study aims to carry out the preliminary phytochemical screening and evaluation of the antibacterial activities of ethylacetate soluble fraction of the whole plant of *Centaurea perrottetii* on the test organisms *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive bacteria) and *Escherichia coli and Pseudomonas aeruginosa* (Gram negative bacteria). The phytochemicals screening of the ethylacetate soluble fraction revealed the presence of Alkaloids, Carbohydrates, Steroids, Flavonoids, and Tannins. The ethyl-acetate fraction of the plant exhibited moderate antibacterial activity against the Grams positive bacteria *S. aureus* and *B. subtilis* at all the test concentrations with zone of inhibition in the range of 4-12mm, but no inhibitory effect on the Grams negative bacteria (*E. coli* and *P. aeruginosa*). The standard antibacterial drug, Chloramphenicol had inhibitory activity against all the organisms with zones of inhibition in the range of 30 to 33mm. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) recorded for the ethyl acetate soluble fraction on *S. aureus* were 15mg/ml and 7.5mg/ml and on *B. subtilis* were 30 and 7.5mg/ml respectively. The low MIC value suggests that the extract has good antibacterial activity. This justifies the use of *Centaurea perrotettii* in ethno-medicine for the treatment of bacterial infections such as skin infections and wound healing.

Keywords: Centaurea perrottetii; Antibacterial activity; Ethylacetate Fraction

Introduction

The ability to introduce multi-drug resistance by different strains of pathogenic bacteria has been a thing of concern. This also has been a trigger for continuous search for new remedies from plant origin (Cowan, 1999; Abdullahi *et al*., 2015). This poses both economic and health challenges on the public around the globe (Franklin and Snow, 2005; Davies and Davies, 2010; Ijeh *et al.,* 2011; Dar *et al*., 2016). Plants have been known to be a major source of complex and highly structurally diverse chemical compounds, thus a potential source of new therapeutic compounds which have been reported in several research to possess significant antibacterial activity against a wide range of bacterial infections with little or no side effect (Joshua and Takudzwa, 2013; Kanth *et al*.,2016; Mabhiza *et al*., 2016). Compounds that are derived from plants had been reported to exhibit antibacterial activity by diverse mechanisms, thereby possessing clinical values in the treatment of infections caused by resistant strains of bacteria. The plant, *Centaurea perrottetii*, is known in northern Nigeria as Danya (Mudzengi *et al*.,2017). It is an erect shrub, 2 m high, found on hillside grassland in Guinea to Northern Nigeria, Western Cameroon and Central Africa to Angola belonging to the family, Asteraceae (Mudzengi *et al*.,2017; Sedar

et al., 2017). The plant has been reported to be used against malaria, and dysmenorrhea (Mudzengi *et al*.,2017; Sedar *et al*., 2017). This study is therefore aimed at evaluating the antibacterial activity of the ethylacetate soluble fraction of the whole plant of *Centaurea perrottetii* against some selected human pathogens for development of novel antibacterial agent.

Experimental

Collection and identification of plant material.

The whole plant material of *Centaurea perrottetii* was collected from Sokoto State, Northern-Nigeria during the rainy season. It was authenticated by Mal M.A. Salihu. A voucher specimen (No. UDUH/ANS/0034) was deposited at the herbarium for future reference.

Preparation of extract.

The whole plant was shade dried, pulverized, labelled and stored at room temperature in an airtight container prior to extraction. The powdered leaves (2100 g) were extracted with 70 % methanol using maceration method. The extract was evaporated *in vacuo* using rotary evaporator at 40 °C to obtain a gummy greenish product (147.84 g) subsequently referred to as the crude methanol extract (CME). The crude methanol extract (120 g) was suspended in 600 mL distilled water and successively extracted with the organic solvent of increasing polarity to obtain n-hexane (HF), chloroform (CF), ethylacetate (EF), n-butanol (BF) and the residual aqueous (AF) soluble fractions, respectively.

Preliminary phytochemical screening

The ethylacetate soluble fraction (3g) was taken and subjected to phytochemical screening to test for the presence of alkaloids, flavonoids, saponins, tannins, steroids/triterpenes, carbohydrate and lipid using standard procedures.

Test organisms.

The pathogenic microbes used in this study were obtained from the Department of Pharmaceutics Pharm-microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The organisms include four (4) bacterial species- *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*; which were maintained on nutrient agar slant in a refrigerator at 4 °C.

Antibacterial screening.

Antibacterial screening was carried out using the agar diffusion method as described by Wahyuningrum *et al.(2016)*. This was done by adding 0.1 mL of 1 mg/mL solution of the EF into a well bored by the use of a standard 6 mm cork-borer on a Mueller Hinton agar plate already seeded with the standard 0.5 McFarland turbidity of the test organisms. The plates were then incubated at 37 ^{0}C for 24 h after which the plates were observed for the zone of inhibition of growth. The total diameter of zone of inhibition was measured with a transparent ruler and the result recorded in millimeters. Chloramphenicol standard antibiotic discs as positive control was maintained.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Minimum Inhibitory Concentration (MIC) of the fraction against the pathogens was determined by the Broth dilution method using serially diluted ethylacetate fraction as previously described (Dar *et al.,* 2016). The fraction was serially diluted into different concentrations ranging from 1, 0.5, 0.25, 0.125 and 0.063 mg/mL in nutrient broth. Then, into each of the tubes containing the fraction, 0.1 mL of broth culture of the test organisms (1.5×10^{8} CFU/mL) was added and the tubes incubated at 37ºC for 18-24 h.

The MIC is the lowest or minimum concentration of the fraction in a tube with no visible growth of bacteria. Cultures from the tubes with no visible growth were sub-cultured into a fresh recovery media, Mueller Hinton agar, and incubated at 37°C for 24 h, after which the plates of the medium were observed for colony growth. The lowest concentration at which no colony/turbidity was observed was interpreted as Minimum Bactericidal Concentration (MBC).

Results

The result of the preliminary phytochemical screening of the ethylacetate soluble fraction of the whole plant of *Centaurea perrottetii* revealed

the presence of alkaloids, flavonoids, tannins, steroids and carbohydrates. The antibacterial screening revealed that the pathogens were moderately inhibited by the ethylacetate soluble fraction of *Centaurea perrottetii* with the exception of *Escherichia coli and Pseudomonas aeruginosa*. The fraction (EF) exhibited a moderate antibacterial activity with inhibition range of 4 – 12.4 mm. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) recorded for the ethyl acetate soluble fraction on *S. aureus* were 15mg/ml and 7.5mg/ml and on *B. subtilis* were 30 and 7.5mg/ml respectively, as indicated in (Tables 1 – 3).

Discussion

The rate at which life – threatening diseases due to resistant microorganisms has increased worldwide with possibility of an impending post-antibiotic era where common infections could kill (Steward *et al*., 2001; Toyang and Verpoorte, 2013; Ventola, 2015; Udochukwu *et al*., 2015). This research is therefore of importance as research effort was made to identify plant derived antibacterial agents with therapeutic value against some pathogens with least or no adverse effects. This is of utmost important in order to trigger the preservation, conservation and sustainable management of the plant for use in drug research and development (Ijeh *et al.,* 2011).

The results obtained from this study showed that the ethylacetate soluble fraction of the methanol whole plant extract of *Centaurea perrottetii* possess moderate antibacterial activities against the tested pathogens. The antibacterial activity recorded in this study may be due to the presence of these potent secondary metabolites. These phytochemicals have been demonstrated in several literatures to exert antimicrobial activities via a number of diverse mechanisms like intercalation of DNA, destruction of cell membrane, inactivation of microbial adhesions and enzymes (WHO, 2014; Yusuf *et al.,*2015; Waltrich *et al.,* 2015; Zulkipli *et al*.,2017). This validates the ethno-medicinal use of the plant in the management of bacterial infections.

Table 1: Zone of inhibition (mm) of the EF of *C. perrottetii* against the test microbes

Test microbes	Concentration (mg/mL)	Mean Zone of Inhibition mm)
Staphylococcus aureus	15.00	4.0±0.00
	20.00	9.0±0.00
	25.00	10.2±0.00
	30.00	12.4±0.00
Bacillus subtilis	15.00	4.2±0.00
	20.00	5.2±0.00
	25.00	5.6±0.00
	30.00	6.0±0.00
Escherichia coli	15.00	0.0±0.00
	20.00	0.0±0.00
	25.00	0.0±0.00
	30.00	0.0±0.00
Pseudomonas aeruginosa	15.00	0.0±0.00
	20.00	0.0±0.00
	25.00	0.0±0.00
	30.00	0.0±0.00

Note: 0 means no activity

Table 2: Minimum Inhibition Concentration of the fraction against the test microbes

Test Microbes	Concentration (mg/mL)
Staphylococcus aureus	15.00
Bacillus subtilis	30.00

Table 3: Minimum Bactericidal Concentration of the fraction against the test microbes

Test Microbes	Concentration (mg/mL)
Staphylococcus aureus	7.50
Bacillus subtilis	7.50

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