



Antimicrobial Activity of the n- butanol soluble fraction of the Aerial part of *Centaurea perrottetii* DC (Asteraceae) on Some Selected microorganisms

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ABSTRACT

Centaurea perrottetii is a herbaceous shrub that has been in use traditionally for the treatment of microbial infections and other infectious ailments. This study was aimed at evaluating the antimicrobial activity of n-butanol soluble fraction (obtained through maceration and liquid-liquid fractionation techniques) of the aerial part of *Centaurea perrottetii* against some pathogenic microbes which include *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Aspergillus niger*, *Trichophyton spp* and *Candida albicans*. Agar Disc diffusion and Nutrient Broth dilution techniques were employed. Susceptibility test results showed that the n-butanol fraction inhibited the growth of all the test organisms (bacteria and fungi) with a mean zone of inhibition range of 5-22 mm with the exception of *Staphylococcus aureus*, *Salmonella typhi* and *Aspergillus flavus*. The standard antibiotic, Ofloxacin had inhibitory activity against all the bacteria with a mean zone of inhibition range of 10-44 mm, while Fluconazole, the standard anti-fungal drug showed activity on the fungi species tested with the mean zone of inhibition between 34 – 37 mm. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal/Fungicidal Concentration (MBC) range for the n-butanol fraction was 0.31-20 mg/mL and 0.31-80 / 0.31-20 mg/mL, respectively. Preliminary phytochemical screening conducted using standard procedures revealed the presence of flavonoids, tannins, phenols saponins, glycosides and alkaloids in the n-butanol soluble fraction. The results of this study suggest that the plant contains bioactive constituent(s) with good antimicrobial activity and thus justifies the use of the plant for management of microbial infections.

Keywords: *Centaurea perrottetii*, Antimicrobial, n-butanol, fraction, phytochemical screening

INTRODUCTION

Antimicrobial agents are among the most commonly used and misused of all drugs (Mancuso *et al.*, 2021). Resistance to antimicrobial agents has become an increasingly important and pressing global challenge (Chinemerem *et al.*, 2022). According to World Health Organization (WHO), emergence and re-emergence of resistance to the available antimicrobial agents has of recent become a global challenge; this is majorly due to constant use (overuse) and misuse of antimicrobial agents (Majumder *et al.*, 2020; WHO, 2023). It has been reported that more than two (2) million people per year in the United States become infected with bacteria which are resistant to antibiotics, out of which 23,000 death were recorded to have occurred as a direct result of these infections, while others were reported to have died due to other complications related to antimicrobial resistance (Lindsay *et al.*, 2006; Mancuso *et al.*, 2021).

Several researches had showed that microbes will continue to develop resistance once exposed to treatment using antimicrobial agents, this brings about the continuous search for new, safer and effective antimicrobial drugs majorly from plant origin (Silver and Bostian, 1993; Mancuso *et al.*, 2021; Chinemerem *et al.*, 2022; Larsson and Flach, 2022).

The WHO, reported that, eighty (80) percent of the world population use medicinal plants (herbal medicines) as part of their primary health care (Hostettman and Marston, 2002; WHO, 2011). The presence of phytochemical constituents to a great extent in medicinal plants (herbs) has been the connection between their curative effect (properties) on man and animals (Martin *et al.*, 2006). *Centaurea perrottetii* belong to Asteraceae (alternative name: Compositae) family, and genus *Centaurea* which is composed of 350 and 600 species. Herbaceous thistle-like flowering plants; a flora of West Tropical Africa

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(Burkill, 2000); herb like of up to 18 inches high, with rather smaller heads and larger leaves; florets purple. Common names of *Centaurea perrottetii* include Danyii, Danar maguzawo (in Hausa language; Northern Nigeria) and star-thistle. It is used in traditional medicine to treat skin infections, for wound healings amongst others (Alebiosu *et al.*, 2019). Many species of *Centaurea* genus are used traditionally as medicines (Kargioğlu *et al.*, 2008).

Different chemical constituents with effective biological activities have also been reported from different species of this genus (Arif *et al.*, 2004; Csapi *et al.*, 2010; Erol-Dayi *et al.*, 2011 Aktumsek *et al.*, 2013); these include sesquiterpene lactones from *Centaurea zuccariniana* (Ciric *et al.*, 2012), flavonoids from *Centaurea scoparia* and *Centaurea acaulis* (Sabrina *et al.*, 2013; Sayed and Emadeldin, 2014). Preliminary phytochemical screening and antibacterial studies have been carried out on the ethylacetate soluble fraction of the whole plant of *Centaurea perrottetii* DC (Alebiosu and Umaru, 2023). A Quercetin-Derivative (amino-flavone) was reported to have been isolated from the n-hexane soluble fraction of the whole plant of *Centaurea perrottetii* (Celestina *et al.*, 2022). Also, isolation of Taraxasterol and Stigmasterol were reported from the chloroform fraction of the aerial part of *Centaurea perrottetii* (Aduku *et al.*, 2020). The isolation and structural elucidation of two methoxylated flavones from *Centaurea perrottetii* whole plant was also reported (Oluranti *et al.*, 2024).

This study was therefore undertaken to evaluate the antimicrobial activity of the n-butanol soluble fraction of the aerial part of *Centaurea perrottetii* against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Aspergillus niger*, *Trichophyton spp* and *Candida albicans* which are routinely implicated in gastrointestinal infections, oral diseases, wounds and other skin diseases in order to establish a scientific evidence for their traditional usage

MATERIALS AND METHODS

Collection and Identification of Plant material

The aerial part of *Centaurea perrottetii* was collected from Wamakko LGA, Sokoto State, Northern-Nigeria in July 2021. It was re-authenticated by a Botanist, Malam Salihu, at the Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria, (compared with the deposited specimen with voucher number UDUH/ANS/0034).

Preparation of the Extract

The aerial parts were air-dried for two (2) weeks, pulverized, labelled and stored in air-tight container. Powdered aerial parts of the plant (833.55 g) were continuously extracted with methanol by maceration for 7 days (1000 mL - three times); the extract was filtered and the filtrate was dried in-vacuo using rotary evaporator at 40 °C to afford a greenish product coded CME and was kept at room temperature prior to use.

Preliminary Phytochemical Screening

Portion (1g) of the fraction was subjected to phytochemical screening for the presence of secondary metabolites including, flavonoids, saponins, tannins, glycosides, phenols, steroids/triterpenes and alkaloids, following standard procedures (African Pharmacopoeia, 1985; Silva *et al.*, 1998)

Test Organisms

The microbes, Clinical isolates, were obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. All bacterial cultures were checked for purity and maintained in a blood agar slant while the fungi were maintained on a slant of Sabouraud Dextrose Agar (SDA). The microbes tested include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Aspergillus flavus*, *Aspergillus niger*, *Trichophyton spp* and *Candida albicans*.

Susceptibility Test

Preliminary antimicrobial activity of the n-butanol soluble fraction of the aerial part of *Centaurea perrottetii* was first determined through susceptibility test using agar diffusion method. The stock concentration of the fraction (80 mg/mL) was prepared with dimethyl sulfoxide (DMSO). Mueller Hinton agar, the growth medium, was prepared according to Manufacturer's instructions and sterilised for 15 minutes at 121 °C; it was poured into sterile petri dishes and allowed to cool and solidify. The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 hours at 37°C for the bacteria, while for fungi, Sabouraud Dextrose broth was used and incubated for 48 hours at 25°C. The sterile medium was seeded with 0.1 standard inoculum of the test microbe at 45°C, swirled gently and allowed to cool and solidify. Wells were bored into the solidified inoculated nutrient agar plates using cork borer of 6 mm diameter. The wells were filled with 0.1 mL (400 µg) solution of the fraction. Ofloxacin (5 µg) disc was also placed on the agar plates and served as the standard drug for bacteria. 1

hour was allowed for the fraction and the standard compound to diffuse into the agar after which the plates were incubated overnight at 37 and 25°C for bacteria and fungi, respectively. At the end of incubation period, diameter of inhibition zone was measured using transparent ruler and recorded. The zones of inhibition of microbial growth were tested in duplicates and the mean of the results was recorded in millimetres (mm).

Minimum Inhibitory Concentration (MIC)

The MIC of the n-butanol soluble fraction was carried out using Broth dilution method (Volekobia *et al.*, 2001). Mueller Hinton broth was prepared of which 10ml was dispensed into test tubes and sterilised at 121°C for 15 minutes and allowed to cool; MC-Farlands standard turbidity scale number 0.5 was prepared. Dilution of the organism suspension was done continuously using sterile normal saline until the turbidity marched that of Mc-Farland's scale by visual comparison at that point, the concentration of the test microbe was about 1.5×10^8 cfu/mL. Two-fold serial dilution of the fraction in the sterile broth was made to obtain the concentrations of 80, 40, 20, 10, 5, 2.5, 1.25, 0.63 and 0.31 mg/mL, then standard inoculum of the test microbe was then inoculated into the different concentrations of the fraction in the broth. The tubes were incubated at 37 °C for 24 h and 25 °C for 48 h for bacteria and fungi respectively after which the

plates were observed for turbidity (growth). The MIC was defined as the lowest concentration of the n-butanol soluble fraction inhibiting the visible growth of each micro-organism.

Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC)

The MBC/MFC was carried out to determine whether there is complete death of test microbes or just growth inhibition. Mueller Hinton agar broth was prepared, sterilized at 121°C for 15mins, and transferred into sterile petri dishes to cool and solidify. The contents of the MIC in the serial dilution were sub-cultured into the prepared medium and incubated at 37 °C for 24 hrs; the plates were observed for colony growth; the MBC/MFC was the plate with lowest concentration of the n-butanol soluble fraction in serial dilution without colony growth (Volekobia *et al.*, 2001).

RESULTS

Preliminary phytochemical screening

The Preliminary phytochemical screening of the n-butanol soluble fraction of the aerial part of *Centaurea perrottetii* revealed the presence of steroids, flavonoids, tannins, phenols, saponins, glycosides and alkaloids. The results of preliminary phytochemical tests conducted on the n-butanol soluble fraction of the aerial part of *Centaurea perrottetii* are presented in Table 1

Table 1: Preliminary Phytochemical Screening on the n-butanol soluble fraction of the aerial part of *C. perrottetii*

Constituents	Test	Inferences
Steroids & Triterpenes	Lieberman-Buchard	-
	Salkowski test	-
Alkaloids	Mayer's test	+
	Dragendorf's test	+
Flavonoids	Ferric chloride test	+
	NaOH test	+
Saponins	Frothing test	+
Tannins	Lead Sub-acetate	+
Glycosides	Fehling's test	+
Phenols	Ferric chloride test	+

Key: + presence of constituent.

Antimicrobial Activity

The results of susceptibility test, MIC and MBC/MFC have been summarized as shown on Tables 2, 3 and 4 respectively. Susceptibility test results showed that the n-butanol fraction inhibited the growth of all the test organisms (bacteria and fungi) with a mean zone of inhibition range of 5-22 mm with the exception of *Staphylococcus aureus*, *Salmonella typhi* and *Aspergillus flavus*. The standard antibiotic, Ofloxacin

had inhibitory activity against all the bacteria with a mean zone of inhibition range of 10-44 mm. The concentration ranges for the MIC and MBC/MFC were 0.31-20 mg/ml and 0.31-80 mg/ml, respectively.

Table 2: Zone of Inhibition (mm) of n-butanol soluble fraction of the aerial part of *Centaurea perrotteti* against the test microbes

Test organism	Concentration (mg/mL)				Ofloxacin	Fluconazole
	80	60	40	20		
<i>B. subtilis</i>	15	12	10	-	32	-
<i>E. coli</i>	15	13	-	-	10	-
<i>S. aureus</i>	-	-	-	-	52	-
<i>P. aeruginosa</i>	17	13	8	-	17	-
<i>K. pneumoniae</i>	16	14	12	5	33	-
<i>A. niger</i>	20	18	14	10	-	35
<i>A. Flavus</i>	-	-	-	-	-	34
<i>Trichophyto spp</i>	19	13	10	-	-	36
<i>C. albicans</i>	22	20	16	10	-	37

Keys: mean zone of inhibition measured in millimetre (mm); - = activity not detected

Table 3: Minimum Inhibitory Concentration of n-butanol soluble fraction of the aerial part of *Centaurea perrotteti* against the test microbes

Test microbes	Concentration (mg/mL)										G	S
	80	40	20	10	5	2.5	1.25	0.63	0.315			
<i>B. subtilis</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>E. coli</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>K. pneumoniae</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>A. niger</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>Trichophyton spp</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>C. albicans</i>	-	-	+	+	+	+	+	+	+	+	+	-

Keys: - = no turbidity (no growth), + = turbid (growth), G = growth control; S = Sterility control

Table 4: Minimum Bactericidal/Fungicidal Concentration of n-butanol soluble fraction of the aerial part of *Centaurea perrotteti* against the test microbes

Test microbes	Concentration (mg/mL)										G	S
	80	40	20	10	5	2.5	1.25	0.63	0.315			
<i>B. subtilis</i>	-	+	+	+	+	+	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	-
<i>P. aeruginosa</i>	-	+	+	+	+	+	+	+	+	+	+	-
<i>K. pneumoniae</i>	+	+	+	+	+	+	+	+	+	+	+	-
<i>A. niger</i>	-	-	+	+	+	+	+	+	+	+	+	-
<i>T. spp</i>	-	+	+	+	+	+	+	+	+	+	+	-
<i>C. albicans</i>	-	-	+	+	+	+	+	+	+	+	+	-

Key: - = no colony growth, + = colonies growth, G = growth control; S = Sterility control

DISCUSSION

C. perrottetii has been reported to be used for the treatment of skin infection, syphilis, for wound healing, among others. The outcome of the present study revealed the scientific basis of the traditional claim on the uses of the plant. After subjecting the aerial part of the plant to maceration and the crude extract to liquid – liquid fractionation using solvent of varied polarity, preliminary phytochemical screening of chemical constituents of the n-butanol soluble fraction revealed the presence of alkaloids, flavonoids,

tannins, glycosides and phenols. This study showed that the n-butanol soluble fraction of the aerial part of *C. perrottetii* exhibited activity against both fungi and bacteria (gram positive and gram negative). The resistance of *Staphylococcus aureus*, *Salmonella typhi* and *Aspergillus flavus* to the fraction means that it cannot be used to cure infections caused by these bacteria. The increased incidence of multidrug resistant bacteria strain has added yet another dimension to the already compounded nightmare of antimicrobial treatment. Paradoxically, the antibiotic

pipeline has almost run dry, with no new classes of agent expected to be in use in the next 20 years (Gould, 2007).

From the result of the phytochemical study, the fraction contains constituents which are mostly phenolic compounds. These phenolics are known to be synthesised by plants to act as defence against microbial infection. It is not surprising therefore, that they are effective *in-vitro*, against a wide range of microorganisms. Several classes of phenolics have been found to possess antimicrobial activities (Alves *et al.*, 2013).

Flavonoids on the other hand also possess antimicrobial activities among other several activities. Examples of flavonoids with antimicrobial activity include calodenin B, kaempferol, naringin, etc. Their ability to complex with bacterial cell wall and also ability to form complexes with soluble and extracellular proteins are probably the cause of their activity as antimicrobials (Cowan, 1999).

Several species investigated in the genus *Centaurea* have been found to contain phenolics with appreciable antimicrobial activities. For example, many species of *Centaurea* genus are used traditionally as medicines (Kargioğlu *et al.*, 2008).

The preliminary phytochemical screening and antibacterial studies carried out on the ethylacetate soluble fraction of the whole plant of *C. perrottetii* showed moderate antibacterial activities (Alebiosu and Umaru, 2023). However, the ability of the n-butanol soluble fraction of the aerial part of *Centaurea perrottetii* to possess antimicrobial activity could be attributed to the presence of the bioactive constituents found in the plant.

CONCLUSION

The results of the present study suggest that, the n-butanol soluble fraction of the aerial part of *C. perrottetii* contains bioactive constituents with promising antimicrobial and antifungal activity, thus, the use of the plant for treating microbial infections traditionally can be said to be valid and reliable.

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