



Antibacterial activities of leaf extracts of *Brassica oleraceae* var. *capitata*. (Brassicaceae) against multi-drug resistant clinical isolates in Maiduguri

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Abstract

Brassica oleraceae var. C. green cabbage, a herbaceous biennial plant with leaves that form a compact head, is an edible vegetable used historically as a medicinal herb for a variety of purported health benefits. The aim of the study was to evaluate the antibacterial activities of ethanolic and methanolic leaf extracts of *Brassica oleraceae* var. C. against clinical isolates of pathogenic bacteria (*S. aureus*, *E. coli*, *K. pneumonia* and *P. aeruginosa*) by agar well diffusion method. The extraction was carried out by cold maceration and qualitative phytochemical analysis was conducted. The phytochemical screening revealed the presence of cardiac glycosides, flavonoids, tannins, terpenoids and reducing sugars. The ethanolic and methanolic extracts demonstrated a concentration-dependent antibacterial activity against *S.aureus*, *E.coli*, and *P. aeruginosa*. In conclusion, the ethanolic and methanolic extracts of *B. oleraceae* demonstrated antibacterial activities and these findings could contribute to effective use of the plant.

Keywords: Antibacterial, *Brassica oleraceae*, Clinical isolates, Multi-drug resistant, Phytochemical constituents

Introduction

Medicinal plants have been an effective source of both traditional and modern medicines (Hafidh *et al.*, 2011) with about 80 % of rural population relying on herbal medicine for their primary health care (Okwori *et al.*, 2007). Herbal drugs are prescribed widely because of their effectiveness, less side effect and relatively low cost (Venkatesh *et al.*, 2003). Therefore, investigation of plants for their pharmacological values has become more important (Suba *et al.*, 2004).

Cabbage (*Brassica oleraceae* var. C.) is a leafy garden plant and is among the most important vegetables

consumed worldwide due to its availability in local markets and consumer preference. It is rich in phytochemicals such as flavonoids and glucosinolates (Taveira *et al.*, 2009; Solomon *et al.*, 2017). It is a good source of health promoting compounds that shows preventive effects against cancer, atherosclerosis, nephritis and diabetes mellitus (Taveira *et al.*, 2009). Similarly, few studies have highlighted the importance of the plant as potential source of antifungal agent (Hafidh *et al.*, 2011).

Glucosinolates and their break down products in cabbage have been reported to possess antimicrobial activity (Shofran *et al.*, 1998).

Increase in resistant strains of clinically important pathogens had led to the emergence of new bacterial strains that are Multi Drug Resistant (MDR) (Aibinu *et al.*, 2003) which are virtually common to most frequently used antibacterial drugs. Unaccessibility and high cost of new antibacterial has contributed to an increased rate of morbidity and mortality (WHO, 2000). Consequently, these led to increase in search for more effective antibacterial agents of plant origins (Pretorious *et al.*, 2003) for effective treatment of infections resulting from MDR strains of bacteria. Thus the study was carried out to evaluate the antibacterial activity of leaf extract of *Brassica oleraceae* var. C. against clinical isolates of MDR strains of pathogenic bacteria.

Materials and Methods

Collection and Identification of Brassica oleraceae var. C.

Brassica oleraceae var. C. (green cabbage) was purchased from Monday market, Maiduguri, Nigeria. The plant was identified and authenticated at the Department of Biological Sciences, Faculty of Science, University of Maiduguri. A voucher specimen (Voucher no 015) was prepared and deposited in Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics, College of Medical Sciences, University of Maiduguri, Nigeria.

Preparation of plant extract

The fresh leaves were washed with clean water and were dried in shade for 5 days. The dried leaves were pulverized with electric blender into homogenous texture. The leaf extract was prepared using the method of maceration. Twenty gram (20 g) of the powder was subjected to maceration using 500 mL each of ethanol and methanol. The solutions were allowed to stay for 72 hours with periodic thorough shaking. The solutions were filtered and the filtrates were evaporated in oven set at low temperature. The percentage yields were also determined using the formular below.

Percentage (%) yield = $\frac{\text{final weight (g)}}{\text{initial weight (g)}} \times 100$

Clinical isolates of pathogenic bacteria

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were obtained from the samples analyzed in the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH) between February and May, 2015. The isolates were identified via morphological features on culture plate and biochemical analysis. They were subjected to antibacterial sensitivity testing as described by Arora (2013). Bacteria with least resistance against three (3) antimicrobial drugs of different chemical classes were described as MDR and they were used for the present study.

Phytochemical screening

The ethanolic and methanolic extracts of *Brassica oleraceae* var. C. were subjected to preliminary phytochemical tests using standard techniques to detect the presence of tannins, flavonoids, saponins, alkaloids, steroids, phenols and glycosides, cardiac glycosides as described by Brain & Turner (1975); Vishnoi (1979); Trease & Evans (2002).

Determination of the antibacterial activity of the extracts

The antibacterial activity of the extracts were evaluated using agar well diffusion method as described by Norrel & Messley (1997) and Mbata *et al.* (2006) and the concentrations of the ethanolic and methanolic extracts used were 100, 250 and 1000 mg/ml. The inoculum containing 50 µl of the bacteria was swabbed on the plates with sterile swabs separately. The holes, 4 mm in diameter were punched with a sterile cork borer aseptically in the media. The holes were spaced such that each was at least 30 mm from each other and 4 mm from the edge of each plate. Different concentration of ethanolic and metholic plant extracts prepared were introduced into the bored agar well with a sterile syringe and filled just to the brim. The extracts were allowed to pre diffuse into the agar media at room temperature for 15 minutes before the plates were incubated at 37 °C for 48 hours. Ciprofloxacin a standard drug was used as positive control. Zones of inhibition were measured and recorded in mm. The diameter of zone of inhibition mean of two replicates ± SD as indicated by clear area which was devoid of growth of microbes was measured to determine antibacterial activity.

Data analysis

The data were expressed as mean \pm standard deviation. The analysis was done by one way analysis of the variance (ANOVA) using Statistical Package for Social Sciences version 21 (SPSS, 2006) followed by Student Newman-keul post hoc test. $P < 0.05$ was considered significant.

Results

The leaf extract

The yield of the ethanolic and methanolic extracts of *B. oleracea* var. *C.* appeared dark brown in colour, pasty and sticky. The assessment of the percentage yield of the extracts indicated that the methanolic and ethanolic leaf extracts yielded the same value of 18.75 % (3.75/20 g) w/w ($P > 0.05$).

Profile of the bacterial clinical isolates

The profile of the four (4) bacterial isolates used for the study is presented in Table 1. The *P. aeruginosa* and *E. coli* were resistance to at least 6 drugs.

The qualitative phytochemical profile of *Brassica oleracea* var. *C.*

The phytochemical profile of leaf extracts of *Brassica oleracea* is presented in Table 2. The results showed similarity in the phytochemical constituents of both the ethanolic and methanolic extract of *B. oleracea* which indicated the presence of cardiac glycosides, flavonoids, tannins and terpenoids.

Antibacterial activities of the leaf extracts of *Brassica oleracea* var. *C.*

Table 3 and 4 present the zones of inhibition indicating the antibacterial activities of varying concentrations (100, 250 and 1000 mg/ml) of the leaf extracts. The results revealed that the ethanolic and methanolic extracts of *B. oleracea* exhibited distinct zones of inhibition at all concentrations against *S. aureus* and *E. coli*. The extract only had activity against *Pseudomonas* at 250 and 1000 mg/ml. The methanolic extract of *B. oleracea* showed the widest zone of inhibition of 22.00 ± 0.1 against *S. aureus* at concentration of 1000 mg/mL of the extract. The ethanolic extract showed the highest inhibitory zone against *S. aureus* (19.00 ± 0.1) at 1000 mg/ml.

Table 1: Profile of the Multi-Drug Resistant Bacteria Clinical Isolates

Isolates	Antibiogram	
	Sensitive	Resistance
<i>S.aureus</i>	OFX, NA, PEF, CN, AU, CPX, S, CEP	SXT, PN, AM
<i>E.coli</i>	CN, NA, CPX	OFX, CN, PEF, AU, PN, CEP, SXT
<i>K. pneumoniae</i>	OFX, CN, C, PEF, PN, SXT, CEP	NA, S, AM
<i>P. aeruginosa</i>	CH, CPX, OFL	COT, NA, AM, AU, CN, ERY, CXC, NA

AM Amoxicillin; AU Augmentin; CN Gentamycin; CPX Ciprofloxacin; CXC Cloxacyclin; ERY Erythromycin; NA Nalidixic acid; S Streptomycin; SXT Cotrimoxazole

Table 2: Phytochemical profile of Leaf Extracts of *B. oleracea*

Phytochemical	Methanolic Extract	Ethanolic Extract
Cardiac glycoside		
Salkowski test	+	+
Lieberman test	+	+
Terpenoid	+	+
Saponin glycoside	-	-
Tannins	+	+
Soluble starch	-	-
Flavonoids	+	+
Alkaloid	-	-
Combined anthraquinone	-	-
Free anthraquinone	-	-

+ Present
- Absent

Table 3: Zones of inhibition (mm) produced by the methanol cabbage extract

Extracts concentrations (mg/ml) and Ciprofloxacin(μ g/ml)	Zones of inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
100	6.00 \pm 0.12	4.20 \pm 0.09	0.00 \pm 0.00	0.00 \pm 0.00
250	18.00 \pm 0.06	17.00 \pm 0.03	0.00 \pm 0.00	8.00 \pm 0.00
1000	22.00 \pm 0.06	20.00 \pm 0.07	0.00 \pm 0.00	15.00 \pm 0.00
Ciprofloxacin	18.00 \pm 0.00	19.00 \pm 0.00	15.00 \pm 0.00	12.00 \pm 0.00

Table 4: Zones of inhibition (mm) produced by the Ethanolic cabbage extract

Extracts concentrations (mg/ml) and Ciprofloxacin(μ g/ml)	Zones of inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>
100	4.90 \pm 0.08	4.30 \pm 0.02	0.00 \pm 0.00	0.00 \pm 0.00
250	16.00 \pm 0.09	16.00 \pm 0.02	0.00 \pm 0.00	8.00 \pm 0.00
1000	19.00 \pm 0.09	18.00 \pm 0.10	0.00 \pm 0.00	13.00 \pm 0.00
Ciprofloxacin	18.00 \pm 0.00	19.00 \pm 0.00	15.00 \pm 0.00	12.00 \pm 0.00

Discussion

Emergence of MDR among the most important bacterial pathogens has contributed to a clinical problem of increasing magnitude and significance in the treatment of infectious diseases. Therefore, it is important to develop new antibiotics with new mechanism of action to overcome these problems (Anjana *et al.*, 2009). In search for new antimicrobial drugs, the use of medicinal plants with antimicrobial activity has been proposed as an alternative complementary medicine in treatment of microbial infection (Nakamura *et al.*, 2004). In the present study, the antibacterial activity of leaf extracts of *B. oleracea* var. C. was demonstrated.

The phytochemical profiling of *B. oleracea* extract revealed the presence of flavonoids, tannins, terpenoid and cardiac glycosides. This is in agreement with Jasmine *et al.* (2013) who also reported the presence of flavonoids and tannins in *B. oleracea*. Flavonoids, tannins and terpenoid have been reported to possess antimicrobial activities and have been suggested to be responsible for antibacterial activity of most medicinal plants (Usman & Osuji, 2007).

According to the work done by Hafidh *et al.* (2011) in Selangor, Malaysia revealed that the plant extract has significant activity on *S. aureus*, *E. coli*, *K. pneumonia* and *P. aeruginosa*. The result is in agreement with present study that also reported activity against *S. aureus*, *E. coli* and *Pseudomonas*. However, in contrast to this previous study, our extracts did not produce activity against *K. pneumonia*. This could partly be due to the differences that exist in the climatic or

environmental conditions where the experiments were carried out and the MDR strain of bacteria used. Moreover red cabbage was used for their experiment, but in this present study, green cabbage was being used. Red cabbage is a rich source of phenolic compounds with anthocyanins being predominant over flavonoids compared to green cabbage.

According to the report of many studies conducted, it was suggested that the mechanism of resistance to almost all antibiotics are; the presence of an enzyme that inactivates the antimicrobial agent, the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent, a mutation in the antimicrobial agent's target which reduces the binding of the antimicrobial agent, post-transcriptional or post-translational modification of the antimicrobial agent's target which reduces binding of the antimicrobial agent, reduced uptake of the antimicrobial agent, active efflux which are non-drug specific proteins that can recognize and export a broad range of chemically and structurally unrelated compounds from bacteria without drug alteration or degradation, overproduction of the target of the antimicrobial agent and expression or suppression of a gene *in vivo* in contrast to the situation *in vitro* (Adwan *et al.*, 2010; Alemayehu & Serawit, 2015). The ethanolic and methanolic extracts tested in this study showed antibacterial activity against MDR strain of *S. aureus*, *E. coli* and *P. aeruginosa*. These results suggested that the mechanism of action of *B. oleracea* extracts may be inhibition of one or more above mentioned mechanism of resistance.

Anthocyanins from various coloured vegetables and fruits are documented to have antimicrobial, antineoplastic, antiatherogenic, lipid lowering, antidiabetic and anti-inflammatory properties, which are mainly due their potent anti-oxidant properties (Stintzing & Carle, 2004). This could explain why the work done by Hafidh *et al.* (2011) had more antimicrobial even on capsulated *K. pneumonia* when compared with this work.

In antibacterial susceptibility testing, the methanolic extract of *B. oleraceae* had significant *in vitro* antibacterial activity when compared with ethanolic extract ($P < 0.05$). From the results, Gram-positive bacteria were more susceptible to the extracts than the Gram-negative bacteria. Generally, Gram-negative bacteria are more prone to resistance antibiotics than Gram-positive bacteria. Gram-positive and Gram-negative bacteria possess cell wall that is made of peptidoglycan and phospholipid bilayer with membrane of proteins. In addition, the presence of a unique outer membrane with lipopolysaccharides, a thinner layer of peptidoglycan and a periplasmic space between the cell wall and the membrane in Gram-negative bacteria confers for this kind of bacteria higher resistance to lysozymes and antibiotic attacks (Aires *et al.*, 2009). However, these barriers generally allow the passage of low molecular weight (phyto)chemicals with lipophilic properties. Although the present study does not show the mechanism underlying the resistant behavior. The resistance may occur as a result of the differences in their cell wall composition. The efficacy of these extracts to exhibit antibacterial activity against the bacteria, suggested the presence of phyto-constituents with antibacterial compounds. In conclusion, the present study demonstrates that ethanolic and methanolic extracts of *B. oleraceae* contain phytochemicals with antibacterial activities against multi-drug resistant phenotypes of *S. aureus*, *E. coli* and *Pseudomonas*. This could serve as potential source of drug which can be used in the management of bacterial infections including MDR phenotypes. However, further investigations on the antimicrobial components of the cabbage leave should be done to provide pharmaceutical companies with a novel, cheap and effective antimicrobial agent.

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