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Research Article

The Antibacterial Effect of the Leaf Extract of *Buchholzia coriacae (Capparidaceae)* on Gram-Negative Nasal Isolates

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Abstract: This study was aimed at determining the antibacterial effect of the leaf extract of *Buchholzia coriacea* (Wonderful Kola) on nasal isolates. Nasal isolates were collected from one hundred students of the Delta State Polytechnic, Ogwash-uku, Delta State. The antimicrobial activity of the methanolic extract of the leaves was assessed against nasal isolated microorganisms *(E.coli, Citrobacter species, Klebsiella species,* and *Proteus species)* using the agar well diffusion method. Inhibition zones and Minimum Inhibitory Concentration (MIC) were used as indicators of antimicrobial activity. The extracts inhibited the growth of the bacterial isolates in a concentration dependent manner with MICs of 39.81mg/ml, 69.18mg/ml, 79.43mg/ml and 97.7mg/ml *(E. coli, Citrobacter species, Klebsiella species* and *Proteus species)* respectively. Phytochemical screening reveals the presence of secondary metabolites; tannins, saponins, cardiac glycosides, purines, reducing sugars, flavonoids, steroids, alkalloids and phlobatannins. The result indicates promising antibacterial potential of *B. coriacea* leaf extracts and hence could be considered for pharmaceutical and medicinal purposes. Characterization of the bioactive components of *B. coriacea* leaves could be used in the development of drugs for the treatment of bacterial related infections.
Keywords: Bochholzia coriacea, Antibacterial effect, Leaf Extract, Traditional medicine.

INTRODUCTION

Traditional medicine is widespread throughout the world and it can be described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation, verbally or written[1]. Medicinal plant has been defined by World Health Organization (WHO) consultative group as any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [2]. For many years medicine depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs come into use and in many instances, there are carbon copies of chemicals identified in plants [3]. In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredient is extracted, refined and made ready for consumption while in traditional medicine a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food [3].

Buchholzia coriacea E. (Capparidaceae) is a forest tree with large, glossy, leathery leaves and conspicuous cream white flowers in racemes at the end of the branches. The plant is easily recognized by the compound pinnate leaves and the long narrow angular fruits containing large, usually aligned seeds. In Nigeria the plant has various common names including; "Uke" (Ibo), 'Ovu' (Bini), and 'Aponmu' (Akure). *B. coriacea* is found widely distributed in other African countries such as Ivory Coast and Gabon [4]. The plant's fruit is about 5 inches long and 2 - 3 inches in diameter and resembles avocado pear, yellowish when ripe with a yellow flesh containing a few large, blackish seeds about 1 inch long. They are edible and taste peppery. It has been used for years to meet a variety of illnesses; since it has been used continually over many generations it is likely that the leaf actually has an effect against illnesses. The leaves and stem bark of *Buchholzia coriacea* in various formulations, decoctions and concoction exhibit antihelmintics, antimicrobial and cytotoxicity effects on micro-organisms[5,6].

Buchholzia coriacea leaves are large, obovate, oblanceolate to elliptic, shortly acuminate or acute at apex, cuneate at base, 15-30×5-11 cm, thinly coriaceous, glabrous, with very prominent midrib below; it has about 10 lateral nerves, each running directly into the one above and forming distinct loops close to the margin, its stalk is about 10-15 cm long,

swollen for about 1 cm at both ends and pale green. The plant parts commonly eaten are the seeds which are either cooked or eaten raw. It is a brain food which promotes memory. It's also useful in the treatment of hypertension and also prevents premature aging; it has also been claimed in Africa that wonderful kola *(B. coriacea)* has the ability to stop migraine headache on the forehead for about 10 minutes.

In Ghana fresh bark of the plants are used for earache [7]. Despite the various reports, information on the anti- bacterial properties of the leaf of the plant on nasal isolates is scarce. The study was therefore undertaken in order to evaluate the antibacterial activities of the crude extracts of the leaves of *B. coriacea* on some Gram-negative nasal isolate.

Fig-2: *Buchholzia coriacae* leaves and nuts (Wonderful kola)

MATERIALS AND METHOD

The study was conducted from May 2013 to July 2013 on 100 students of Delta State Polytechnic, Ogwuash-uku. Nose swabs of 100 students were collected using sterile swab-sticks and the samples were stored in the refrigerator at 4^{o}C to avoid contamination from the environment before they were used. Media used include MacConkey agar (Fluka), Nutrient agar (Micromaster), Mannitol salt agar (Titan Biotech), Nutrient broth (Titan Biotech), Peptone water (Titan Biotech), Muller-Hinton agar (Oxoid.) Reagents include Kovac's reagent (Park), Phenol red (Kem Light), Glucose, Dextrose, Sucrose, and Lactose (Fisher.)

Bacteriology

The collected specimens were inoculated on nutrient agar for growth using a standard procedure [8]. The culture plates were incubated at 35-37°C for 24 hours and observed for growth through formation of colonies with different colours and morphology. Different growth patterns and colours obtained were further sub-cultured in nutrient agar for discrete colonies for another 24 hours at $35\text{-}37^{0}$C. The organisms were then further subjected to identification test such as Gram staining, morphology on media, motility tests, growth on selective media, lactose, sucrose, glucose fermentation, and biochemical tests such as indole test and hydrogen sulphide test.

Preparation of Plant Leaf Extract

The plant leaves were collected from Orerokpe, in Okpe Local Government Area of Delta State and identified at the Department of Pharcognocy, Faculty of Pharmacy, Delta State University, Abraka; Delta State. The plant leaves were dried under room temperature to prevent denaturing of the active constituents of the plant. Thereafter the leaves were blended to small particles with the aid of an electric blender (Binatone,China) and was extracted using soxhlet extraction method with methanol (50%) as the extracting solvent.

Preparation of Plant Crude Extract for Sensitivity Testing

The plant extract was reconstituted by dilution with methanol (50%) to various concentrations of 50%, 40% , 30%, 20%, and 10% (500, 400, 300, 200, 100mg/ml respectively) as described by Akujobi et al, [9] and used for antibacterial susceptibility testing.

Phytochemical Screening of The Plant Extract

Phytochemical screening was carried out using the method of Trease and Evans [10] . 5g of the plant

extract was dissolved in 50ml of water in a beaker as a stock solution of the plant extract from where different volumes of the extract were taken for different phytochemical screening; tannin, saponin; flavonoid, steroids, terpenoids, cardiac glycosides, alkaloids, purine, reducing sugars and phlobatannin.

Preparation of Isolates For Sensitivity Testing

The pure isolates were inoculated in nutrient broth at 37°C for 24 hours. The turbidity of the actively growing broth culture was adjusted to obtain turbidity optically comparable to that of 0.5McFarland standard, from where the organisms were collected for preparation of plates for sensitivity testing.

Preparation of Plates for Zone of Inhibition

The agar well diffusion method was employed in the assay. 24ml of sterilized Mueller-Hinton agar was seeded with 1ml of the microorganisms, mixed and poured into a Petri dish to solidify. A flamed sterilized cork borer of 5mm diameter was used to make wells in the agar. With the aid of a sterile pipette, two drops of the extract, was dispensed into the wells depending on the concentrations of the plant extracts indicated on different wells. Methanol (50%) and ciprofloxacin (2mg/ml) was used as negative and positive control respectively. The antibacterial agents were allowed to diffuse for 30 minutes and the plates were incubated at 37°C for 24 hours. The zones of inhibition were measured in millimeters (mm) using a ruler after 24 hours of incubation and recorded.

Determination of minimum inhibitory concentration (MIC)

A graph of the diameter of inhibition zones of the plant extract was plotted against the log of concentration. The MIC of the particular organism was then calculated as the antilog of the X-intercept from the line obtained[11].

RESULTS AND ANALYSIS

A total of 21 gram negative organisms were isolated from the one hundred (100) nose swabs and identified.

Table-1: Showing results of the identification test of bacteria nasal isolates.

Isolate	Gram staining(Morphology)	Color(Mac Conkey)	Lactose	Sucrose	Glucose	Indole	Motility	H_2S
1	Negative (rod)	Pinkish	+	+	+	+	+	-
2	Negative (rod)	Pinkish	+	+	+	+	+	-
3	Negative (rod)	Yellowish	-	+	+	+	+	+
4	Negative (rod)	Pinkish	+	+	+	+	+	-
5	Negative (rod)	Yellowish	+	+	+	+	+	+
6	Negative (rod)	Yellowish	-	+	+	+	+	+
7	Negative (rod)	Yellowish	-	+	+	+	+	+
8	Negative (rod)	Yellowish	-	+	+	+	+	+
9	Negative (rod)	Yellowish	-	+	+	+	+	+
10	Negative (rod)	Yellowish	-	+	+	+	+	+
11	Negative (rod)	Yellowish	-	+	+	+	+	+
12	Negative (rod)	Pinkish	+	+	+	+	+	-
13	Negative (rod)	Pinkish	+	+	+	+	+	-
14	Negative (rod)	Pinkish	+	+	+	+	+	-
15	Negative (rod)	Pinkish	+	+	+	+	+	-
16	Negative (rod)	Pinkish	+	+	+	+	+	-
17	Negative (rod)	Pinkish	+	+	+	+	+	-
18	Negative (rod)	Pinkish	+	+	+	+	+	-
19	Negative (rod)	Pinkish	+	+	+	-	-	-
20	Negative (rod)	Pinkish	+	+	+	+	+	-
21	Negative (rod)	Pinkish	+	+	+	+	+	-

Table-2: Showing the isolates and the identified organisms

Isolate	Organism
1	*E.coli*
2	*E.coli*
3	*Proteus Vulgaris*
4	*E.coli*
5	*Citrobacter spp*
6	*Proteus Vulgaris*
7	*Proteus Vulgaris*
8	*Proteus Vulgaris*
9	*Proteus Vulgaris*
10	*Proteus Vulgaris*
11	*Proteus Vulgaris*
12	*E.coli*
13	*E.coli*
14	*E.coli*
15	*E.coli*
16	*E.coli*
17	*E.coli*
18	*E.coli*
19	*Klebsiella pneumonia*
20	*E.coli*
21	*E.coli*

Table-3: Showing the number of the identified organisms and the percentage of occurrence

Bacteria	**Number**	**Percentage**
Citrobacter spp	1	4.76
E.coli	12	57.14
Klebsiella spp	1	4.76
Proteus vulgaris	7	35
Total	21	100

Table-4: showing the result for the antimicrobial activity of the leaf extract *Buchholzia coriacae* on Gram-negative nasal isolates.

Isolate	**mean zone of inhibition(mm)**						
Conc. of extract(mg/ml)	**500**	**400**	**300**	**200**	**100**	**+ve control**	**-ve control**
E.coli	15	13	10	8	7	32	0
E.coli	15	13	11	9	7	30	0
Proteus Sp	10	8	7	5	4	29	0
E.coli	9	9	8	5	4	32	0
Citrobacter Sp	10	9	8	6	4	28	0
Proteus Sp	11	10	9	5	4	26	0
Proteus Sp	12	10	8	7	4	27	0
Proteus Sp	12	10	7	6	5	32	0
Proteus Sp	10	8	7	5	4	28	0
Proteus Sp	11	9	6	5	4	33	0
Proteus Sp	10	8	7	6	4	27	0
E.coli	10	9	7	6	4	36	0
E.coli	16	13	12	9	7	36	0
E.coli	14	13	12	8	5	37	0
E.coli	9	7	7	5	3	27	0
E.coli	15	11	10	7	7	28	0
E.coli	15	13	9	7	5	35	0
E.coli	14	12	11	10	9	35	0
Klebsiella Sp	16	13	12	11	5	27	0
E.coli	15	13	13	12	8	34	0
E.coli	16	11	13	13	7	28	0

Table-5: Showing the minimum inhibitory concentration of the isolates

Isolate	Organism	Minimum Inhibitory Concentration(MIC)
18	*E.coli*	39.81mg/ml
5	*Citrobacter species*	69.18mg/ml
19	*Klebsiella species*	79.43mg/ml
6	*Proteus* vulgaris	97.7mg/ml

Table-6: Phytochemical analysis of the dried leaf extract of *Buchholzia coriecea*

Secondary Metabolite	Results
Tannin	+
Saponin	+
Flavonoid	+
Steroid	+
Terpenoid	+
Cardiac glycoside	+
Alkaloid	+
Purine	+
Reducing sugar	+
Phlobatannin	+

(+) Present (-) Absent

DISCUSSION AND CONCLUSION

From the result of the biochemical tests carried out it is observed that *E.coli* is more predominant among the organisms isolated from the nose followed by *proteus species*, while *citrobacter species* and *klebsiella species* occurred equally.

Results of preliminary phytochemical screening of the leaf extracts of *B. coriacea* are shown in Table-6. Results showed the presence of Tannin, saponin, flavonoids, steroidal, terpenoids, cardiac glycosides, alkaloid, Purine, reducing sugar and phlobatannin. These results are in agreement with similar studies by Ajaiyeoba et al.[5].

Table-4 shows the results of antimicrobial effects of leaf extracts of the plant against the test bacteria. The leaf extract of the plant showed some level of activity against all the test organisms by inhibiting their growth. This suggests that the extract contains antimicrobial substances which are responsible for its activity. The effect of the plant extract varies from one microorganism to another. The activity of the plant extract was concentration dependent, increasing with increasing concentration. However, *Escherichia coli* were more susceptible to the extract than the rest of the microorganisms. Although in all, the antimicrobial activities of ciprofloxacin were significantly higher than the plant extract.

CONCLUSION

Buchholzia coriacea has a very promising use as an anti-bacterial agent and for the treatment of infections caused by the test organisms used- *Escherichia coli, proteus species, citrobacter species and klebsiella species.* Work done [12] on the seed extract has shown promising results on pathogenic bacteria.

For the usage of this medicinal plant (*Buchholzia coriacea*) to be fully maximized, it is expected that all parts of the plant should be properly assayed in the laboratory and further research is needed to optimize the effective use of this agent in clinical practice.

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