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Full Length Research Paper

Phytochemical composition and antimicrobial effects of *Corchorous olitorius* leaf extracts on four bacterial isolates

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The phytochemistry and antimicrobial potential of *Corchorus olitorius* leaf extracts on four bacterial isolates was investigated using both agar diffusion and tube dilution methods. Aqueous and methanolic extracts were tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus*. Extracts concentration of 500, 125, 125 and 62.5 mgml^{-1} were used while tetracycline was used as the standard drug. The phytochemical investigation revealed the presence of hydrocyanin, cardiac glycosides (+++) and tannins (+), flavonoids (++), anthraquinones (++) and saponins (++). Agar and tube dilution tests of both aqueous and methanolic extracts indicated that the extracts had antimicrobial activities against the four bacterial isolates, though the methanolic extracts had wider diameter of inhibition and activity indices than the aqueous extracts. Susceptibility increased with concentrations and highest susceptibility was observed against *E. coli* and other isolates at 500 mgml^{-1}. The extracts exhibited the high antimicrobial activity (Activity Index, A.I with respect to the standard drug used). This could be adduced to the presence of phytochemical constituents and can be of prophylactic importance. Its highest potency against *E. coli* justifies its therapeutic use by traditional healers in South-Western Nigeria for gastroenteritis with good results.

Key words: Phytochemistry, activity index, antimicrobial activity, *Escherichia coli*, gastroenteritis

INTRODUCTION

Corchorus olitorius L. which belongs to the family Tiliaceae and is known as a fiber crop, jute is also a medicinal vegetable. It has been given various names in various languages such as Red Jute, Tossa Jute, Tussa Jute, Jew's Mallow (Potherb), Bush Okra, West African Sorrel in English; Tosha Pat, Deshi Pat, Meetha (Sweet) Pat in Bangla; Janascha Kashto, Singin in Hindi; Nalta, Lif Khaysha in Arabic; Jute Roax/Rouge, Corète Potagèr (Potherb), Feuilles Lalo/Lalou (Potherb)in French; Langkapsel-Jute in German and Almindelig Jute in Danish (http://www.pfaf.org/cgibin/pfaf/arr_html?Corchorus+olitorius).

It has been reported to be demulcent, deobstruent, diuretic, lactagogue, purgative, and tonic, tussa jute is a folk remedy for aches and pains, dysentery, enteritis, fever, dysentery, pectoral pains, and tumors (Duke and Wain, 1981; List and Horhammer, 1979). The leaves have been variously used in folk medicines for ascites, pain, piles, tumors cystitis, dysuria, fever, and gonorrhea while the cold infusion is said to restore the appetite and strength.

Various such plants have been reported to have antimicrobial activity. A study reported the presence of antifungal activity in different extracts of the leaves (Masoko et al., 2005). They are important because microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). The increase in resistance of microorganisms encourages scientists to search for new antimicrobial substances from various sources including medicinal plants (Karaman et al., 2003).

The four isolates in this research are aetiologies of one of these aforenamed clinical cases or the other. For instance, *Escherichia coli* is known for enteritis, *Salmone-*

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lla typhi for typhoid fever, *Klebsiella pneumoniae* for pneumonia and *Staphylococcus aureus* is frequently connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis and furuncle etc. (Komolafe and Adegoke, 2008). This study therefore was aimed at studying the phytochemistry and antimicrobial activity of *C. olitorius* that made it of such diverse therapeutic and prophylactic use.

MATERIALS AND METHODS

Collection of samples and test organisms

The plant leaves were collected from nearby farmland in Itu Local Government Area of Akwa Ibom State. The leaves were identified by Mr. Bala of Faculty of Pharmacy, University of Uyo. The pathogenic microorganisms were collected at University of Uyo Medical Centre, Uyo. They (*E. coli, K. pneumonia, S. typhi* and *S. aureus*) were recharacterized and reidendified using microbiological procedures of cowan and steel (2004). These are microscopy and biochemical tests.

Phytochemical analysis of the *Corchorious olitorius* leaf extracts

Phytochemical analysis of the plant extracts were carried out in order to confirm the presence of alkaloids (by Dragendorff's and Mayer's test), cardiac glycosides (by Liberman's test, Salkowski test and Keller-Killani test), Anthraquinones (Borntrager's test). Saponins, tannins and phlobatannins were also analysed using standard phytochemical methods as described by Sofowora (1993), Culei (1982) and Trease and Evans (2002).

Extracts preparation

Exactly 70 g of the plant leaves were dried in the oven at 70°C for 4 h and then grinded in sterile mortar and pestle to fine particles. The methanolic extraction in absolute methanol [1:2 (w/v)] was carried out in an orbital shaker (Stuart Scientific, U.K) at 30°C for 3 days, filtered with Whatman No. 1 filter paper (W and R Balson Ltd, England) and evaporated to dryness at 70°C in water bath. The dried extract was reconstituted in distilled water in the ratio 1:2 (w/v) after which it was sterilized by membrane filteration technique and the sterile extracts were stored at 5°C in the refrigerator until required for use (Babalola, 1988).

For the aqueous extraction, forty two grams of the plant leaves was grinded into powder after drying. It was weighed into eylenmeyer flask and to this was added 500 ml of distilled water. This was heated to boil using hot plates. The mixture was stirred at regular (3 - 5 min) interval for 1 h. Then it was filtered with Whatman No 1 filter paper (W and R Balson Ltd, England) after 24 h. The filterates were then filter-sterilized using a membrane filter of pore sized 0.45 cm diameter (millipores corp, England). The sterile extracts were refrigerated at 5°C until required for use.

Antimicrobial susceptibity test

The spreading method of Cruickshank et al. (1980) and dose (agar) diffusion method were used. Twenty four hours old cultures of the organisms to be tested were used. A loopful of the cultures was uniformly spread over the surface of a sterile Muller-Hilton agar with a sterile bent rod. The extracts were diluted to obtain different concentration of 500, 250, 125 and 62.5 mg/ml using sterile peptone water. Various concentrations of the prepared extracts were used to fill hole bored by 5 mm cork borer in the inoculated agar. The plates were made in triplicate with one for the test organism- tetracycline, standard drug. All plates were incubated at 37°C for 24 h. The diameter of the zones of inhibition in the triplicate plates was measured by calculating the difference between core borer (5 mm) and the zone (diameters) of inhibition (Hewitt and Vincent, 1989). The activity indices, designated as AI, were calculated as the division of zone of inhibition of the extract by that of the standard drug (Singh et al., 2002; Adebayo and Adegoke, 2008, 2009).

Tube dilution method

The extracts were diluted into different concentrations of 500, 250, 125 and 62.5 $mgml^{-1}$ with sterile peptone water in test tubes. Methanol and water were used as the control. To each of the dilution was added 0.2 ml broth culture of the test organism. The tubes were incubated at 37°C for 24 h after which turbidity reading was taken using turbidiometer. Extracts added with peptone water served as control

RESULTS

Characterization and identification

The results of the recharacterization and reidentification of the isolates from various clinical cases are depicted in Table 1. The organisms were identified as *E. coli, K. pneumoniae*, *S. typhi* and *S. aureus*.

Phytochemistry of the plant extracts

The phytochemistry revealed that contains hydrocyanin and cardiac glycosides in large quantity and trace of tannins, appreciable quantities of flavonoids, anthraquinones and saponins.

Antimicrobial activity

Both crude metanolic and aqueous forms of the extracts of *C. olitorius* exhibited varying degree of antimicrobial activities against the test organisms. On the general note, metanolic extracts exhibited higher degree of antibacterial activities than the aqueous extracts at extract concentrations of 500, 250, 125 and 62.5 $mgml^{-1}$. At 62.5 $mgml^{-1}$, crude ethanolic extract had higher antibacterial activity with mean zones of inhibition 8.4 ± 0.2 (A.I = 1.14) and 7.0 ± 0.6 (0.93) than crude aqueous extract with mean zones of inhibition 7.2±0.5 (A.I=0.96) and 6.3 ± 0.1 (A.I = 0.84) against *S. aureus* at concentrations of 500 and 250 $mgml^{-1}$ respectively. This higher sensitivity and activity index by methanolic extracts continued through to the least concentration of 62.5 $mgml^{-1}$ used where the methanolic extracts had mean zone of inhibition 5.0 ± 0.3 (A.I = 0.67) compared to 4.0 ± 0.1 (A.I = 0.53) by aqueous extracts.

Similar trend was observed against *E. coli, S. typhi and*

Table 1. Characterization and identification of the isolates.

Cell Shape	Characteristics on EMB	Gram Stain	Indole Test	Motility	Coagulase Test	Catalase Test	citrate	Urease	methyl red	vogesprosk	Carbohydrate fermentation				Organisms
											Glu	Dext	Gal	Lac	
Cocci in chain		+	ND	-	+	+					AG	A	A	A	*S. aureus*
Rods	Metallic sheen	-	+	-	-	+	-	-	-	+	AG	A	A	A	*E. coli*
Rods		-	ND	-	-	+	+	+	-	+	AG	A	-	A	*K. Pneumoniae*
Rods		-	ND	+	-	+					AG	A		-	*S. typhi*

Key: A = Acid, AG = Acid and Gas, + = Positive, - = Negative, ND = Not Done.

Table 2. Zones of inhibition (mm) of the plant extracts on the bacterial isolates.

Bacterial Isolates	500 mgml^{-1}				250 mgml^{-1}				125 mgml^{-1}				62.5 mgml^{-1}			
	M	A.I	A	A.I	M	A.I	A	A.I	M	A.I	A	A.I	M	A.I	A	A.I
S. aureus	8.4±0.2	1.14	7.2±05	0.96	7.0±0.6	0.93	6.3±0.1	0.84	6.0±1.0	0.80	5.0±0.5	0.67	5.0±0.3	0.67	4.0±0.1	0.53
E. coli	10.0±04	1.22	9.5±0.2	1.16	8.0±0.1	0.98	7.3±0.5	0.89	7.5±0.2	0.91	6.2±0.3	0.76	6.0±0.2	0.73	5.6±0.4	0.68
K. Pneumoniae	8.1±0.2	1.04	7.0±0.3	0.90	7.0±0.2	0.98	6.0±0.3	0.77	6.0±0.2	0.77	5.2±0.4	0.67	5.0±0.1	0.64	4.2±0.2	0.54
S. typhi	8.0±0.3	1.01	6.3±0.1	0.80	7.0±0.8	0.89	5.0±0.2	0.63	6.3±0.4	0.80	4.0±0.1	0.50	5.0±0.5	0.63	3.0±0.1	0.38

Key: M = methanolic extracts, A = Aqueous extracts, A.I = Activity Index w.r.t. Tetracycline

K. pneumoniae. At extract concentration of 500 mgml^{-1} against *E. coli* for instance, the mean zones of inhibition of 10.0 ± 0.4 (A.I = 1.22) and 9.5 ± 0.2 (AI = 1.16) for methanolic and aqueous extracts respectively, while mean zones of inhibition of 6.0 ± 0.2(A.I = 0.73) and 5.6 ± 0.4(A.I = 0.68) was observed for methanolic and aqueous extracts respectively at 62.5 mgml^{-1}. The mean zones of inhibition of 8.1 ± 0.2 (A.I = 1.04) and 7.0 ± 0.3 (A.I = 0.90) at 500 mgml^{-1}, 7.0 ± 0.2 (A.I = 0.98) and 6.0 ± 0.3 (A.I = 0.77) at 250 mgml^{-1}, 6.0 ± 0.2 (A.I = 0.77) and 5.2 ± 0.4 (A.I = 0.67) at 125 mgml^{-1}, and 5.0 ± 0.1 (A.I = 0.64) and 4.2 ± 0.2 (A.I = 0.54) at 62.5 mgml^{-1} for methanolic and aqueous extracts in that order against *K. Pneumoniae*, as such higher mean zone was also observed against S. typhi. Equal or sometimes higher activities were observed at concentration of 500 mg/ml, 250 mg/ml by the crude methanolic extracts than the standard drug, tetracycline. Hence, the activity index, A.I ≥ 1 against *S. aureus*, *E. coli, S. typhi and K. pneumoniae*. Consistently high activity indices were observed against the etiology of pneumonia at crude concentration of 250 and 125 mg/ml, though varying by both metanollic and aqueous extracts of *C. olitorius* (Table 2). The high activity indices were enduring with decrease in concentration from 500 to 62.5 mg/ml. Just low reduction in activities was observed as the crude extract concentrations were reduced gradually from 500 to 62.5 mg/ml in both the agar diffusion set up (Table 2) and tube dilution method (Table 3). The same trend of activity in agar dilution was equally observed in tube dilution method. Methanolic extract inhibited the growth of the four bacteria the lower turbidity than the aqueous extracts. For instance at 500 mg/ml, the turbidity readings nephelometric unit were 1.13, 2.42, 3.02 and 3.75 for crude metanolic extracts, while the reading for crude aqueous extracts were 2.03, 3.45, 4.10 and 3.79 against *S. aureus*, *E. coli, K. pneumoniae* and *S. typhi* respectively. The slight higher potency observed in methanol (control) than water was expected due to antimicrobial activity of alcohol in general. (Table).

Table 3. Antimicrobial activity assay with tube dilution method by using nephelometric turbidity unit.

Bacterial Isolates	500 mgml^{-1}		250 mgml^{-1}		125 mgml^{-1}		62.5 mgml^{-1}	
	M	A	M	A	M	A	M	A
S. aureus	1.13	2.03	2.67	2.79	3.96	3.55	4.80	4.20
E. coli	2.42	3.45	3.10	3.97	4.15	4.58	5.50	5.75
K. Pneumoniae	3.02	4.10	3.65	4.52	4.50	5.30	4.90	6.60
S. typhi	3.75	3.79	4.28	3.83	5.52	4.50	6.10	4.91
Control	4.05	4.00	3.97	3.79	5.29	4.80	5.56	4.57

Key. M = methanolic extracts, A = Aqueous extracts.

DISCUSSION

The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants (Benjamin et al., 1980; Adebayo and Adegoke, 2008). These agents were alkaloids, saponins, flavonoids, tannins, anthraquinone and cardiac glycosides (Sofowora, 1980, 1993). Research work revealed that tannins from the barks, roots etc of many plants are used to treat cells that have gone neoplastic (Duke and Wain, 1981). It is obviously interesting to observe the result of high antibacterial effects of both metanollic and aqueous extracts the four potential pathogens of public health importance. *S. aureus*, no doubt is frequently connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis, furuncle etc. It is also frequently involved in both nosocomial and community acquired infection. The successful inhibition of this bacteria and its contemporary aetiology of gastroenteritis (*E. coli*) is a good development, especially when we consider the record of resistance to various conventional antibiotics by them over the years (Wiedemann, 1996; Voss, 1996 and Ayliffe, 1997). The plant can be therefore taken for prophylactic purpose by people living with HIV/AIDS against infections involving these two isolates.

C. olitorius is consumed in the Western Nigeria as vegetable and as such is believed to have prophylactic effect against enteric fever and gastroenteritis. Similarly in this research, the extracts showed appreciable level of potency against the commonest aetiology of enteric fever and both methanolic and aqueous extracts could be put into fixed dosage combination use to treat the infection. This probably explains why enteric fever is less prevalent in the South Western Nigeria than South Southern Nigeria. Records have it that the enteric fever had mortality rate of 10 - 15% in developing countries (Brooks et al., 2004). This is already in use by the traditional medicine practitioners in Western Nigeria. By virtue of high activity indices above unitary value even in crude forms, the extracts have more promising therapeutic advantages than the likes of tetracycline and other broad spectrum antibiotics to which the test bacteria had developed resistance.

In conclusion, this finding justifies the traditional use of this plant, *C. olitorius* for prophylactic and therapeutic purposes. The findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs.

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