Trop J Nat Prod Res, July 2019; 3(7):231-234 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of *Nauclea diderrichii* (De Wild.) Merrill Leaf Extracts and Fractions against Some Clinical Bacterial Isolates from Urinary Tract Infections

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ARTICLE INFO

Article history:
Received 22 June 2019
Revised 01 August 2019
Accepted 05 August 2019
Published online 25 August 2019

ABSTRACT

Urinary tract infections (UTIs) constitute considerable health and economic burdens globally. The advent of resistant strains of uropathogens coupled with high rate of recurrent UTIs has heightened the precarious situation. Consequently, research efforts are being directed towards alternative treatment solutions. This study investigated the potential of *Nauclea diderrichii* leaf extracts and fractions as antibacterial agents against some clinical uropathogen isolates.

Chloroform, acetone and methanol extracts of dried leaf of *N. diderrichii*, obtained by cold maceration, were evaluated for antibacterial activity against five different species (2 strains each) of clinical uropathogenic bacterial isolates from UTI; namely: *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter feacalis* and *Pseudomonas aeruginosa*. The secondary metabolites present in these extracts were investigated by qualitative phytochemical screening. The most active extract was subjected to chromatographic fractionation, while the minimum inhibitory concentrations (MICs) of the fractions were determined.

Chloroform extract exhibited activity against all the strains with inhibition zone diameters of 10 mm – 19 mm, while methanol and acetone extracts showed some activity against some of the organisms with inhibition zone diameters of 10 mm – 18 mm and 12 mm – 16 mm, respectively. Phytochemical screening revealed the presence of saponins, alkaloids, anthraquinones, terpenoids, cardiac glycosides, phenolics and flavonoids. Fractionation of the chloroform extract yielded three fractions, with MICs: 0.78125 – 25 mg/mL, fraction C; 1.5625 – 12.5 mg/mL, fraction B; and 1.5625 – 25 mg/mL, fraction A.

This study confirmed that *N. diderrichii* leaf contains bioactive compounds against UTI-causing uropathogens.

Keywords: *Nauclea diderrichii*, Urinary tract infections, Uropathogens, Chromatographic fractionation, Secondary metabolites.

Introduction

Urinary tract infections (UTIs) represent bacterial incursion into various parts of the urinary tract including the kidney (pyelonephritis), ureter (ureteritis), urinary bladder (cystitis) and urethra (urethritis). They are now one of the commonest microbial infections globally constituting a significant burden in terms of morbidity and medical costs.[1] While UTIs, complicated or uncomplicated, may be caused by a number of uropathogens which include (but not limited to) *Escherichia coli* (one of the predominant causative agent), *Staphylococcus saprophyticus*, *Klebsiella* species, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Enterococcus species*.[2] However, with the emergence of resistant strains of uropathogens coupled with the high rate of recurrent UTIs, research efforts are being directed towards alternative treatment options for UTIs.[3] One of this alternative treatment options include the use of medicinal plants and herbal preparations, some of which act by direct antibacterial activity or antiadhesive activity.[4] Medicinal plants – rich in bioactive principles - are used in ethnomedicine in different countries to treat different ailments including bacterial infections, pain management and as antiparasitic agents.[5] Apart from their use as herbal preparations and extracts remedies, these plants are potential sources of new antibacterial agents.

Nauclea diderrichii (De Wild.) Merrill (Rubiaceae) is a widely cultivated, tropical, evergreen tree grown in West and Central Africa. The plant is traditionally used as a tonic, as chewing stick and in treatments of toothaches, dental caries, septic mouth, fever, diarrhoea, dysentery, measles, stomach ache and indigestion. The root is credited with diuretic property and used for the treatment of anaemia. Bark infusions and decoctions are used to treat gonorrhoea, as a vermifuge, and against parasites and other tropical diseases.[6,7] Phytochemical study of *N. diderrichii* showed that the stem bark and leaf contain alkaloids, flavonoids, terpenes, saponins and glycosides.[8] The alkaloids include simple *β*-carbolines (desoxycordifolinic acid), simple pyridines, and indole-pyridines (naucledine, naucleverine).[9,10]

While, there are sufficient documented ethnopharmacological information on the usage of herbal preparations and remedies from *N. diderrichii* against infectious diseases;[7] however, scientific reports on the antimicrobial activity are very scanty and general, with a large number of ethnomedicinal claims remaining unverified. This study, therefore, investigated the potentials of *N. diderrichii* leaf extracts and fractions as antibacterial agent against some UTIs-causing pathogens.

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Citation: Aderibigbe SA, Alabi OS, Yekini QA. Evaluation of *Nauclea diderrichii* (De Wild.) Merrill Leaf Extracts and Fractions against Some Clinical Bacterial Isolates from Urinary Tract Infections. Trop J Nat Prod Res. 2019; 3(7):231-234. doi.org/10.26538/tjnpr/v3i7.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Collection and Preparation of Plant Material

Fresh *N. diderrichii* leaves were collected from Botanical Garden, University of Ibadan, South-West, Nigeria. The plant was identified, and Herbarium specimen was deposited at Forestry Research Institute of Nigeria, Ibadan (Voucher No.: FHI 111889). The leaves were air-dried at room temperature over two weeks, and milled into powder using laboratory blender (Elgento-125, China).

Collection and Characterization of Test Bacteria Species

Pure cultures of the bacterial organisms (5 different species) were obtained from urine samples of patients diagnosed as having UTIs at the Medical Laboratory, Microbiology Unit, University College Hospital, Ibadan, Nigeria. The bacterial species, which were assigned number codes for identification purpose, include: *Escherichia coli* 001 and 007, *Klebsiella pneumoniae* 001 and 003, *Pseudomonas aeruginosa* 001 and 003, *Enterococcus faecalis* 005 and 010, and *Proteus mirabilis* 004 and 006 strains. The organisms were confirmed using various standard biochemical tests such as indole, sugar fermentation, catalase, oxidase, citrate utilization and urease test.[11]

Gradient Extraction of Plant Material

The powdered plant materials (1.06 kg) were defatted with 3 L of *n*-hexane for 24 h and dried. The marc was then extracted twice for 24 h by cold maceration using 3 L each of chloroform, acetone, and methanol successively. The extracts were filtered into clean containers, concentrated and evaporated to dryness *in vacuo* at 40ºC. The dried extracts were subjected to qualitative phytochemical analysis to determine the phytochemical constituents present in them following standard procedures.[12]

Antibacterial evaluation of the extracts

Two concentrations (100 mg/mL and 50 mg/mL) of each of the crude extracts were prepared by dissolving appropriate weights (1000 mg and 500 mg, respectively) in 10 mL methanol, and were evaluated for antibacterial activity by agar diffusion method using Mueller Hinton agar. Briefly, the agar was prepared according to the manufacturer's procedure, sterilized in an autoclave at 121^{o}C for 15 min and the cool molten agar (45^{o}C) was then poured into sterile Petri-dishes to set. The surface of the already set Mueller Hinton agar plates were dried in an ethanol disinfected incubator at 45^{o}C for 30 min and inoculated with 0.1 mL McFarland standard of the suspension of each of the test microorganism (*E. coli*, *K. pneumoniae*, *E. faecalis*, *P. aeruginosa*, and *P. mirabilis*) separately by surface spreading using sterile cotton swabs. Wells were then made aseptically with an 8 mm sterile cork borer and 0.1 mL of each of the test extract concentrates (100 mg/mL and 50 mg/mL) were introduced into the wells. The extract solution was allowed to diffuse into the medium for 1 h before being incubated aerobically for 24 h at 37°C. The antimicrobial activity of the extracts was measured (in millimeters) as the mean diameter of the resulting growth inhibition zones, and compared with the growth inhibition zones of gentamicin, 100 mg/mL (positive control) and methanol (negative control).

Chromatographic Fractionation of Chloroform Extract

Based on the result obtained from the antibacterial evaluation of the extracts, the most active extract was subjected to chromatographic fractionation. Briefly, chloroform extract (0.5 g) was dissolved in chloroform (10 mL) and adsorbed with 2 g of silica gel 60 G (Merck, Germany). The air-dried, adsorbed silica gel was packed on to a sintered glass funnel already pre-packed with 8 g of silica gel. The set up was then eluted using negative pressure provided by a vacuum line with three different solvent systems (50 mL each) of increasing polarity [A: *n*-hexane /ethyl acetate (70:30); B: *n*-hexane /ethyl acetate (20/80); and C: ethyl acetate/methanol (50/50)]. Whenever a given solvent system was added, the packed silica gel was allowed to dry before the next solvent system was applied. The fractions (A, B, and C) were collected into separate, clean glass bottles. More samples of the extract were subjected to chromatographic fractionation following the procedure earlier outlined until a total of 10 g of the extract was fractionated. At the end, the fractions were concentrated by rotary evaporation, and then analyzed for their phytochemical constituents following standard procedures.[12]

Determination of Minimum Inhibitory Concentration of Chloroform Fractions

The minimum inhibitory concentrations (MIC) of the fractions obtained from chromatographic fractionation of the chloroform extract were determined by serial agar plate dilution method. Stock solutions of the fractions were prepared, each at a concentration of 25 mg/mL. This was achieved, for example, by dissolving 1.0 g of fraction A in chloroform (2 mL). 1 mL of this was transferred into a 19 mL molten nutrient agar to give a final concentration of 25 mg/mL. From the stock solutions, serial lower dilutions for the fractions were made as follows: 12.5, 6.25, 3.125, 1.5625, and 0.78125 mg/mL. Each bottle containing 18 mL of nutrient agar was melted and cooled to between 45 – 50°C; and 2 mL of each of the concentration was added. The bottles were mixed properly, poured into Petri-dishes and the agar allowed to set. The set agar plates were divided into ten compartments with board marker and labeled. The bacteria were then streaked accordingly and the plates incubated at 37°C for 24 h. [13]

Results and Discussion

Globally, Urinary tract infections still constitute a substantial public health burden. The situation is being exacerbated with the advent of resistant organisms and high frequency of recurrent UTI infection.[3,14] One of the alternative strategies under intense consideration is exploration of medicinal plants for their potential anti-UTI activity. It is envisaged that secondary metabolites present in medicinal plants could provide prophylactic and curative treatments, as well as help to curtail or slow down resistance development.[15,16] From the study, the chloroform extract gave a percentage yield of 3.28% w/w, while that of acetone and methanol were 5.22% w/w and 7.70% w/w, respectively. The gradient solvents extraction was used since it could afford the relative extraction of the varying secondary metabolites present in the powdered plant material based on their polarity and affinity for the individual solvent. The chromatographic fractionation of the chloroform extract resulted into three fractions. The percentage yields obtained were: fraction A - 52.86% w/w, fraction B - 22.18% w/w, and fraction C - 18.02% w/w. The result of the qualitative phytochemical analysis of the extracts and the fractions is shown in Table 1, indicating the presence of saponins, phenolics, flavonoids, cardiac glycosides, terpenoids, alkaloids, and anthraquinones as secondary metabolites in the powdered leaf material.

The results of the antibacterial evaluation are presented in Table 2. Chloroform extract at 100 mg/mL exhibited highest activity against all the bacterial strains with inhibition zone diameter of (10 mm − 19 mm), while with methanol (10 mm − 18 mm), and acetone (12 mm − 16 mm) extracts some of the bacterial were resistant. Chloroform extract showed maximum zone of inhibition (19 mm) against *P. mirabilis* 004 and minimum inhibition (10 mm) against *E. coli* 001 and *K. pneumoniae* 001. Methanol extract showed maximum inhibition (18 mm) against *P. mirabilis* 004 and minimum inhibition (10 mm) against *E. faecalis* 010 and *K. pneumoniae* 001. Acetone extract showed maximum inhibition (14 mm) against *E. coli* 007 and *P. aeruginosa* 001 and minimum inhibition (12 mm) against *K. pneumoniae* 001. The results of the MIC determination of the fractions (A, B and C) are shown in the Table 3. Fraction C gave an MIC range of 0.78125 – 25 mg/mL, fraction B gave MIC range of 1.5625 – 12.5 mg/mL, and fraction A gave MIC range of 1.5625 – 25 mg/mL.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Qualitative phytochemical evaluation of the *Nauclea diderrichii* leaf extracts and chloroform fractions (A, B, and C) indicating the various secondary metabolites present.

Phytochemical constituents	Methanol extract	Acetone extract	Chloroform extract	Fraction A	Fraction B	Fraction C
Saponins	+	-	+	-	+	+
Phenolics	+	+	-	-	-	-
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	-	-	+	+	+	+
Terpenoids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Anthraquinones	+	+	+	+	+	+

+, Present; −, Absent.

Table 2: Antibacterial activity of the solvent extracts of *Nauclea diderrichii* leaf against clinical uropathogenic bacterial isolates

Bacteria strains	Inhibition Zone Diameters (mm)							
	ME		AE		CE		Controls	
	100 mg/mL	50 mg/mL	100 mg/mL	50 mg/mL	100 mg/mL	50 mg/mL	Positive	Negative
***E. coli* 001**	NG	NG	NG	NG	10	10	14	13
***E. coli* 007**	13	13	16	14	17	15	17	NG
***P. aeruginosa* 001**	NG	NG	NG	NG	11	10	11	11
***P. aeruginosa* 003**	13	13	14	14	15	14	14	15
***E. faecalis* 005**	NG	NG	NG	NG	13	12	15	NG
***E. faecalis* 010**	10	10	NG	NG	11	11	20	14
***K. pneumoniae* 001**	10	10	13	12	14	10	13	13
***K. pneumoniae* 003**	NG	NG	NG	NG	11	10	11	NG
***P. mirabilis* 004**	18	18	NG	NG	19	15	NG	18
***P. mirabilis* 006**	15	13	NG	NG	15	14	14	NG

Note: ME, Methanol extract; AE, Acetone extract; CE, Chloroform extract; NG, No Growth.

Table 3: Minimum inhibitory concentration (MIC) for chloroform fractions A, B and C

Bacteria strains	MIC (mg/mL)		
	Fraction A	Fraction B	Fraction C
***E. coli* 001**	6.25	6.25	1.5625
***E. coli* 007**	25	12.5	12.5
***P. aeruginosa* 001**	6.25	6.25	12.5
***P. aeruginosa* 003**	25	12.5	25
***E. faecalis* 005**	12.5	12.5	12.5
***E. faecalis* 010**	6.25	6.25	0.78125
***K. pneumoniae* 001**	12.5	12.5	25
***K. pneumoniae* 003**	25	12.5	25
***P. mirabilis* 004**	6.25	6.25	0.78125
***P. mirabilis* 006**	1.5625	1.5625	0.78125

From this study, the antibacterial evaluation of the solvent extracts revealed chloroform extract as showing the highest activity among the three extracts. The chloroform extract exhibited antibacterial activity against all the uropathogenic strains used in this study unlike acetone and methanol extracts where some level of resistance was observed. Determination of the MICs of chloroform fractions showed fractions C to be the most active against *P. mirabilis* 004 and 006, *E. faecalis* 010 MIC of 0.78125 mg/mL, and *E. coli* 001 with MIC of 1.5625 mg/mL; while fraction B and A were most active against *P. mirabilis* 006 with MIC of 1.5625 mg/mL. This observed activity can be attributed to the various secondary metabolites present in these fractions. Studies have shown this kind of compounds as possessing antimicrobial properties, eliciting their activity through different mechanisms including biofilm inhibitory activity and efflux inhibitory activity.[17,18]

There are a few reports on the antimicrobial effects of *N. diderrichii* in literature. Khan *et. al* (2017), reported that chloroform fraction obtained from a methanol soluble extract of the plant stem-bark gave the best antimicrobial activity with an MIC of 1.25 µg/mL,[19] while Akunne *et. al* (2017) showed an ethyl acetate fraction, obtained from methanol-dichloromethane (1:1) root-bark extract, having the least MIC of 0.63 mg/mL against *S. aureus* and A*spergillus flavus*.[20] In an investigation of plants consumed by chimpanzees, Ahoua *et. al* (2015) reported the least MICs of 0.094 mg/mL (*Staphylococcus aureus* CIP 4.83), 0.188 mg/mL (*Staphylococcus aureus* sensitive); and 0.375 mg/mL (*Staphylococcus aureus* sensitive and *P. aeruginosa* CIP 103467) for methanol and dichloromethane leaf extracts of *N. diderrichii*, respectively, with no activity against *E. coli* (*E. coli* CIP 54127AF and *E. coli* ATCC 25925).[21] In the current study, however, the chloroform extract and fractions thereof showed activity against the *E. coli* strains used. This disparity may be due to different strains of *E. coli* used in the studies.

Conclusion

This study represents the first report on the activity of *N. diderrichii* leaf extracts against uropathogenic bacterial isolates. The result

suggests that the leaf of *N. diderrichii* contains bioactive secondary metabolites against UTI-causing pathogens, and could find application in development of plant-based remedies to treat UTIs. Further efforts would be targeted at isolating these bioactive secondary metabolites.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors would like to thank Prof Adegoke O.A. of the Department of Pharmaceutical Chemistry, University of Ibadan for his helpful suggestions in the preparation of the manuscript.

References

1. Barber AE, Norton JP, Spivak AM, Mulvey MA. Urinary tract infections: current and emerging management strategies. Clin Infect Dis. 2013; 57(5):719-724.
2. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. Am J Med. 2002; 113(suppl 1A):14S-19S.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015; 13(5):269-284.
4. Rafsanjany N, Sendker J, Lechtenberg M, Petereit F, Scharf B, Hensel A. Traditionally used medicinal plants against uncomplicated urinary tract infections: are unusual, flavan-4-ol- and derhamnosylmaysin derivatives responsible for the antiadhesive activity of extracts obtained from stigmata of *Zea mays* L. against uropathogenic *E. coli* and benzethonium chloride as frequent contaminant faking potential antibacterial activities? Fitoterapia 2015; 105:246-253.
5. Oreagba IA, Oshikoya KA, Amachree M. Herbal medicine use among urban residents in Lagos, Nigeria. BMC Compl Altern Med. 2011; 11:117.
6. Di Giorgio C, Lamidi M, Delmas F, Balansard G, Ollivier E. Antileishmanial activity of quinovic acid glycosides and cadambine acid isolated from *Nauclea diderrichii*. Planta Med. 2006; 72(15):1396-1402.
7. Haudecoeur R, Peuchmaur M, Pérès B, Rome M, Taïwe GS, Boumendjel A, Boucherle B. Traditional uses, phytochemistry and pharmacological properties of African Nauclea species: a review. J Ethnopharmacol. 2018; 212:106-136.
8. Agnaniet H, Mbot EJ, Keita O, Fehrentz J-A, Ankli A, Gallud A, Garcia M, Gary-Bobo M, Lebibi J, Cresteil T, Menut C. Antidiabetic potential of two medicinal plants used in Gabonese folk medicine. BMC Compl Altern Med. 2016; 16(1):71.
9. Adeoye AO, Waigh RD. Secoiridoid and triterpenic acids from the stems of *Nauclea diderrichii*. Phytochem 1983; 22(4):975-978.
10. Lamidi M, Ollivier E, Faure R, Debrauwer L, Nze-Ekekang L, Balansard G. Quinovic acid glycosides from *Nauclea diderrichii*. Phytochem 1995; 38(1):209-212.
11. Cheesbrough M. District laboratory practice in tropical countries. (Part 2). Cambridge: University Press; 2000. 188-189 p.
12. Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan: Spectrum Books Limited; 1993. 261-268 p.
13. CLSI - Clinical and Laboratory Standards Institute, Guidelines. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa; 2011.
14. Ozumba UC. Increasing incidence of bacterial resistance to antibiotics by isolates from the urinary tract. Nig J Clin Pract. 2005; 8(2):107-109.
15. Albrecht U, Goos K, Schneider B. A randomised, double-blind, placebo-controlled trial of a herbal medicinal product containing *Tropaeoli majoris* herba (Nasturtium) and *Armoraciae rusticanae* radix (Horseradish) for the prophylactic treatment of patients with chronically recurrent lower urinary tract infections. Curr Med Res Opin. 2007; 23(10):2415-2422.
16. Lüthje P and Brauner A. Novel strategies in the prevention and treatment of urinary tract infections. Pathogens 2016; 5(13):1-14.
17. Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics. Fut Microbiol. 2012; 7(8):979-990.
18. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999; 12(4):564-582.
19. Khan M, Amupitan J, Sudi I. Antimicrobial activity and isolation of methylated flavanone from methanol stem-bark extract of *Nauclea diderrichii*. Int J Biochem Res Rev. 2017; 18(2):1-10.
20. Akunne TC, Obi BC, Akpa PA, Sunday O, Anaenugwu AJ. Antibacterial, antifungal and wound healing potentials of extract and fractions of *Nauclea diderrichii* root bark. Int J Trad Compl Med. 2017; 2(1):1-8.
21. Ahoua ARC, Konan AG, Bonfoh B, Koné MW. Antimicrobial potential of 27 plants consumed by chimpanzees (Pan troglodytes verus Blumenbach) in Ivory Coast. BMC Compl Altern Med. 2015; 15:383.