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Antibacterial activity of organic compounds from the leaves of *Alpinia oxyphylla* on multi-drug resistant bacteria isolated from patients with urinary tract infections

Fapetu Kemi Busayo [1, 2, *], Ojo-Bola Oluwatosin [3], Odeyemi Adebowale Toba [4], Oguntunnbi Damilola Esther [3] and Hui-Qin Chen [2]

[1] *School of Life Sciences, Hainan University, Haikou, Hainan 570228, P.R. China.*

[2] *Hainan Key Laboratory of Research and Development of Natural Product from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan 571101, P.R. China.*

[3] *Federal Teaching Hospital, Ido, Ekiti State Nigeria.*

[4] *Landmark University SDG groups 2 and 12; Microbiology Department, Omu-Aran, Kwara State, Nigeria.*

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Abstract

Background: *A. oxyphylla* which is a significant component of traditional Chinese medicine have being reported by many studies to be of significant impact in cure for many infectious diseases because of their high pharmacological activities. In this study, the effect of organic compounds nootkatone, chrysin, tectochrysin, alpha-tocotrienol, oxyphyllol C, sitosterol and epicatechin extracted from the leaves of *Alpinia oxyphylla* were tested against multi-drug resistant bacteria *Escherichia coli, Staphylococcus aureus*, *Klebsiella aerogenes* and Proteus vulgaris; isolated from patients with urinary tract infections.The results showed that the bacteria were resistant to the commonly prescribed antibiotics and the multi-drug resistance among them was also high. The Multi drug resistance analysis showed that 13(76.47%) of the *E.coli* showed Multi drug resistance, however 10(76.92%) and 9(69.23%) of the multi-drug resistance *E.coli* were susceptible to nootkatone and chrysin respectively at the concentration of 30µg/ml. 100% of *K. aerogenes* showed multi-drug resistance meanwhile they showed the highest susceptibility to chrysin and nootkatone with values 5(71.43%) and 4(57.14%) respectively. 4(66.67%) of *S. aureus* showed multi drug resistance however 4(66.67%) *of them were also susceptible* to chrysin and nootkatone while 5(83.33%) of P. vulgaris showed multi drug resistance and 100% of them were susceptible to nootkatone and chrysin also at the concentration of 30µg/ml. Nootkatone and chrysin showed a high susceptibility to the multi drug resistance bacteria isolated from patients with urinary tract infections. The leaves of *A. oxyphylla* can be potential sources for extraction of compounds to combat the increasing menace of multi drug resistance bacteria associated with urinary tract infections.

Keywords: *Alpinia oxyphylla*; Nootkatone; Chrysin; Multi-drug resistant bacteria; Urinary tract infection

1. Introduction

Urinary tract infections (UTIs) are among the most common infections worldwide with an estimated annual incidence of more than 150 million cases worldwide (Stamm and Norrby, 2001). They are usually caused by a variety of uropathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, various other Enterobacteriaceae, as well as *Staphylococcus saprophyticus* (Mazzariol, 2017). The effectiveness of antibiotics in the treatment of UTI cannot be denied, however, resistance has being reported over the years and also some adverse side effects such as damage in intestinal micro floral. Multi-drug resistance bacteria associated with UTI have long been a problem in hospitals and other healthcare facilities where patients are routinely exposed to bacterial pathogens and antibiotics (Madrazo *et al.*, 2021). They have become a serious global health problem associated with serious consequences and increase in the mortality and morbidity rates has led to a need for alternative effective interventions (Huh *et al.*, 2020). There is a

*Corresponding author: Fapetu Kemi Busayo

growing interest in the search for natural therapies for UTI prevention and treatment to face the increasing rate of MDR bacterial associated with these infectious diseases.

Alpinia oxyphylla which is a significant component of traditional Chinese medicine have being reported by many studies to be of significant impact in cure for many diseases and infections (Li *et al*., 2013). This is mainly because they have a wide range of pharmacological activities, the ability to regulate the immune system, as well as less toxic, highly effective against certain bacteria (Ghosh and Rangan, 2013).Studies have shown that *A. oxyphylla* is rich in chemical compounds such as nootkatone, chrysin, myternal, valencene etc (Xu *et al.,* 2009, Ying *et al.,* 2021) which have been described to possess certain bioactivities such as antibacterial (Bei-Bei *et al*., 2010, Zhang *et al*., 2018).Some reports have shown that some of these organic compounds isolated from *A. oxyphylla* showed broader anti-microbial spectrum activities (Lu *et al*., 2013). However there are no studies on their effect on multi-drug resistant bacteria isolated from patients with UTI. No studies have reported the effect of the main compounds on multi-drug resistant bacteria isolated from patients with UTIs. Therefore this study is focused on the effect of compounds isolated from the leaves of *A. oxyphylla* on multi-drug resistant bacteria isolated from patients with UTI.

2. Methods

2.1. Collection of organic compounds

The natural compounds isolated from *A. oxyphylla* were all gotten from Chinese academy of tropical agricultural sciences, Haikou. The compounds were nootkatone, chrysin, tectochrysin, alpha-tocotrienol, oxyphyllol C, sitosterol, Epicatechin

2.2. Collections and processing of urine samples

Urine samples were collected from 41 patients visiting the Federal Teaching Hospital, Ido Ekiti Nigeria, between December 2022 and January 2023. Consent was gotten from patients suspected of UTIs before collection of blood samples. Patients were orally taught on how to provide an adequate midstream urine sample and clean-catch midstream urine was collected from them. The samples were collected in clean sample bottles and refrigerated at 4°C. Cystine Lactose Electrolyte Deficient (CLED) agar were used for urinary culture according to Mackey *et al*., 1965. A loopful of the sample was streaked on the agar plates and incubated in ambient air at 35 ± 2°C for 18 to 24

h. Culture media were purchased from Merck (Sigma-Aldrich, Supelco, Poland). Plates with colony counts of ≥ 105 CFU/ml were considered significant and reported as positive for UTI. The colonies were further identified using gram stain and biochemical tests; catalase, indole, citrate and oxidase (Cheesbrough, 2000).

2.3. Antibiotics susceptibility test

Commercially prepared antibiotic discs (Oxoid, Hampshire, UK) were employed. The antibiotics and concentration (in µg) were as follows: Imipenem (10µg), ciprofloxacin (10µg), gentamycin (30 µg), cefotaxime (30µg), meropenem (30µg), cotrimoxazole (25µg), ofloxacin (5µg), ceftazidine (30 µg). Antibiogram was performed for all isolates using the disk diffusion method and the results were interpreted using the criteria of the Clinical Laboratory Standard Interpretation (CLSI) (2012). Each isolate was subcultured on different Nutrient agar plates (Oxoid) for revitalisation. A colony of the organism was picked and transferred to a tube containing 2 mL nutrient broth and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was adjusted to the optical density of a MacFarland turbidity standard of 0.5 to standardise the inoculum size. The inoculum was swabbed on the surface of Mueller Hinton agar plate. The discs were aseptically placed on the surface of the agar and the plates were incubated at 37 0C for 24 hours. After incubation the diameter of zone of inhibition was measured in millimeter and bacteria were classified as sensitive (S), intermediate (I) and resistant (R) according to CLSI (2012) guidelines. All the tested isolates were classified into MDR, defined as non-susceptibility to at least one agent in three or more classes of antibiotics.

2.4. Organic compounds susceptibility test

The Pure isolated compounds from *A. oxyphylla* were tested against the isolated bacteria. Sterile paper discs 6 mm in diameter were placed on a petri dish and a pipette gun was used to draw 30µg of 1mg/mL of each pure compound dissolved in methanol. The discs were allowed to dry for about 1 hour until the methanol was completely evaporated. Antibiogram was performed for all isolates using the disk diffusion method as described above. Disks saturated with 30µL of pure methanol were used as a negative control.

3. Results

A total no of 41 samples collected from the patients were tested for UTI, among which 35 yielded growth. 20(57.14%) of them were female between the ages 21-45years while 15(42.85%) were male between the ages 32- 61years. The following bacteria were identified: *E.coli, K. aerogenes, S. aureus, and P. vulgaris*. *E.coli* was discovered to be the most common cause of UTI with 17(48.57%), followed by *K. aerogenes* 7(20%) then *S. aureus* 6(17.1%) and *P. vulgaris* 5(14.29%) (Table 1).

Table 1 Profile of bacteria isolated from the urine samples of patients

Organisms	Total no of isolates	% occurrence
E. coli	17	48.57
K. aerogenes	7	20.0
S. aureus	6	17.1
P. vulgaris	5	14.3
Total	35	100

3.1. Antibiotic resistance of bacteria isolated from the urine

The results of the antibiotics tests show that 16(94.11%) of *E.coli* were resistant to ciprofloxacin; 15(88.24%) were resistant to cotrimoxazole and ofloxacin; 14(82.35%) were resistant to ceftazidine, 7(41.18) cefuroxime, 5(29.4%) gentamycin, and 2(11.77%) were resistant to meropenem. There was no resistance to imipramine. 100% of *K. aerogenes* were resistant to ofloxacin, 6(85.71%) cefuroxime, cotrimoxazole and ceftazidine;3(42.86%) were resistant to ciprofloxacin;1(14.23%) were resistant to meropenem, and there was no resistance to imipramine and gentamycin. 5(83.33%) of *S. aureus* were resistant to gentamycin, cefuroxime and ofloxacin, 4(66.67%) were resistant to ceftadine; 3(50%) were resistant to cotrimoxazole;1(16.67%) were resistant to ciprofloxacin; and no resistance to imipramine and meropenem. 5(100%) of *P. vulgaris* were resistant to cotrimoxazole and ofloxacin,4(80%) were resistant to ciprofloxacin and ceftazidine, 3(60%) were resistant to cefuroxime, 1(20%) were resistant to gentamycin, and there was no resistance to imipramine and meropenem (Table 2).

3.2. Multiple antibiotics resistance pattern of bacteria isolated from the urine

The results of the MDR analysis showed that 13(76.47%) of the *E.coli* showed MDR, 100% of *K. aerogenes* showed multi-drug resistance, 4(66.67%) of *S. aureus* also showed MDR and 5(83.33%) of *P. vulgaris* showed MDR (Table 3) to different classes of antibiotics.

Table 2 Antibiotics resistance of the bacteria isolated from the urine

	Antibiotics							
Samples	**IMI**	**CIP**	**GEN**	**CEFO**	**MER**	**COT**	**OFL**	**CEFT**
E.coli(n=17)	0	16	5	7	2	15	15	14
K.aerogenes (n=7)	0	3	0	6	1	6	7	6
S.aureus(n=6)	0	1	5	5	0	3	5	4
P.vulgaris(n=5)	0	4	1	3	0	5	5	4

Note: IMI: imipramine (10μg), CIP: ciprofloxacin (10μg), GEN: gentamycin (30μg), CEFO: cefoxime (30μg):, MER: meropenem (30μg), COT:cotrimoxazole (25μg), OFL: ofloxacin (10μg) , CEFT: ceftazidine (30 μg).

Table 3 Analysis of the Multi-drug resistance pattern of the bacteria from the urine

Samples	*E.coli*(n=17)	*K.aerogenes*(n=7)	*S.aureus*(n=6)	*P.vulgaris*(n=6)
1	CIP/GENT/OFL/CEF	CIP/CEFO/COT/OFL/CEF	CEFO/COT/OFL/CEF	GENT/CEFO/COT/OFL/CEF
2	CIP/GENT/CEFO/COT/OFL/CEF	CIP/COT/OFL/CEF	CEFO/COT/OFL/CEF	GENT/CEFO/OFL/CEF
3	CIP/GENT/CEFO/COT/	CIP/GENT/CEFO/COT/OFL/CEF	CEF/MERO/COT/OFL	CIP/GENT/CEFO/COT/OFL/CEF
4	CIP/GENT/COT/OFL/CEF	CIP/CEFO/COT/OFL/CEF	CEFO/COT/OFL/CEF	GENT/CEFO/COT/OFL/CEF
5	CIP/COT/OFL/CEF	CIP/COT/OFL/CEF	-	GENT/CEFO/OFL
6	CIP/COT/OFL/CEF	CIP/CEFO/COT/OFL/CEF	-	-
7	CIP/COT/OFL/CEF	CIP/GENT/CEFO/COT/OFL/CEF	-	
8	CIP/CEFO/COT/OFL/CEF	-	-	-
9	CIP/CEFO/COT/OFL/CEF	-	-	-
10	CIP/MERO/COT/OFL/CEF	-	-	-
11	CIP/COT/OFL/CEF	-	-	-
12	CIP/GENT/CEFO/MERO/COT/OFL/CEF	-	-	-
13	CIP/COT/OFL/CEF	-	-	-

3.3. Susceptibility of MDR bacteria to organic compounds isolated from *A. oxyphylla*

The results of testing the compounds on multi-drug resistant bacteria showed that 10 (76.92%) of the MDR *E.coli* were susceptible to nootkatone, 9(69.23%) were susceptible to chrysin, 6(46.15%) were susceptible to tectochrysin, 4(30.77%) were susceptible to oxyphyllol C, Alpha-tocotrienol and sitosterol. Also, 5(71.43%) of MDR *K. aerogenes* were susceptible to chrysin, 4(57.14%) were susceptible to nootkatone and 2(28.57%) were susceptible to epicatechin. 4(66.67%) of MDR *S. aureus* were susceptible to chrysin and nootkatone, while 2(33.33%) were susceptible diaryheptanoids, tectochrysin and epicatechin. Furthermore, 5(100%) of MDR *P. vulgaris* were susceptible to chrysin and nootkatone, 2(33.33%) were susceptible to sitosterol and 1(16.67%) were susceptible to tectochrysin and epicatechin.

Table 4 Antibacterial activity of organic compounds isolated from *A. oxyphylla* on Multi-drug resistant bacteria isolated from urine

Samples(30µg/ml)	*E.coli*(n=13)	*K.aerogenes* (n=7)	*S. aureus* (n=6)	*P.vulgaris* (n=5)
OxyphyllolC	4	0	0	0
Chrysin	9	5	4	5
Alpha-tocotrienol	4	0	0	0
diaryheptanoids	0	0	2	0
Nootkatone	10	4	4	5
Techtochrysin	6	0	2	1
sitosterol	4	0	0	2
Epicatechin	0	2	2	1

n=Total no of isolates

4. Discussion

A. oxyphylla is an important Chinese traditional medicine (Liu *et al*., 2008) that has been widely used as a medicinal herb for centuries because of their high pharmacological activities. *A. oxyphylla* contain compounds such as nootkatone, valencene and chrysin which have being reported to possess anti-bacteria effect against bacteria such as *Staphylococcus aureus*, *bacillus subtilis*, *E.coli* and *Aspergillums niger* (Luo *et al*., 2011, Yamaguchi 2019).

Urinary tract infection is a severe public health problem and is caused by a range of bacteria such as *E.coli*, K. *aerogenes, P. mirabilis, E. faecalis and S. aureus* (Foxman 2014, Nielubowicz and Mobley, 2010). In this study, *E.coli, S. aureus, K. aerogenes and P. vulgaris* were the bacteria associated with urinary tracts infections and they all exhibited a significant level of multi-drug resistant to the commonly prescribed antibiotics (Majumder, 2022). The increasing rate of antimicrobial resistance among these bacteria is a threat that greatly elevates the economic burden of these infections (Gomila, 2018). In this study, there is high resistance of the isolated bacteria to the commonly prescribed antibiotics. 76.4% of the *E. coli* associated with UTI showed MDR, The increase rate of MDR *E.coli* has being a concern over the years (Ramírez-Castillo *et al*., 2018). However, the results in this study showed that there is a high susceptibility to nootkatone and chrysin. Also, multi-drug resistance of *K. aerogenes* associated with UTI was also discovered in this study, as100% of them showed MDR (Ning *et al*., 2022). Meanwhile the test of compounds on these bacteria showed that they were also highly susceptible to chrysin and nootkatone.83.3% of the *P. vulgaris* isolated in this study also showed MDR however they were highly susceptible to nootkatone and chrysin. Moreover chrysin and nootkatone were also highly susceptible to *Staphylococcus aureus* which also exhibited MDR.

Chrysin and nootkatone isolated from *A. oxyphylla* in this study showed significant susceptibility to the MDR bacteria; *E.coli, S. aureus, K. aerogenes and P. vulgaris* associated with UTI. Nootkatone and chrysin have being reported by some studies to be highly abundant in the leaves of *A. oxyphylla* (Li *et al*., 2021). The antibacterial effect of these compounds have also being reported by several studies (Yamaguchi, 2021, alawode *et al*., 2021), however no studies yet have reported their effect on MDR bacteria isolated from the urine. Other organic compounds such as oxyphyllol C, epicatechin, diaryheptanoids, tectochrysin and sitosterol also showed certain level of susceptibility to the MDR bacteria associated with UTI. This shows that *A. oxyphylla* is medicinal and contain organic compounds that can be a good alternative to combat MDR bacteria isolated from patients with UTI. Although the mechanisms of action of these organic compounds were not discovered in this study however as studies are currently looking for alternatives to presently available antibiotics, researches can begin to point towards *A. oxyphylla* as potential sources for extraction of organic compounds to combat the increasing menace of MDR bacteria associated with UTI.

5. Conclusion

A. oxyphylla contain several compounds especially nootkatone and chrysin which are highly effective against MDR bacteria isolated from patients with UTI. This can be potential sources to combat the increasing menace of MDR of bacteria associated with UTI.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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