Journal of Advances in Microbiology

14(1): 1-6, 2019; Article no.JAMB.45824
ISSN: 2456-7116

Antimicrobial Activities of Novel Xylopic Acid Derivatives

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Authors' contributions

This work was carried out in collaboration between all authors. Author WK designed and supervised the study. Author JPF carried out experimental work, literature search and wrote first draft of the manuscript, and author RKA co-supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2019/45824
Editor(s):
(1) Dr. Simone Aquino, Professor, Universidade Nove de Julho, São Paulo, Brazil.
Reviewers:
(1) Vivek Kumar Singh, Public Health and Infectious Disease Research Ceter (PHIDReC), Nepal.
(2) Rao Ane Silva Siqueira, Federal University of Campina Grande, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/28104

Short Research Article

Received 23 September 2018
Accepted 18 December 2018
Published 03 January 2019

ABSTRACT

Xylopic acid is one of the most abundant constituents in *Xylopia aethiopica*. Various studies have shown that the compound possesses a broad spectrum of antimicrobial activity.
In this study, the antimicrobial activities of novel ester, amide and de-acetyl derivatives of xylopic acid were investigated by determining their minimum inhibitory concentrations (MIC).
The broth dilution method using microtitre plate was employed in the antimicrobial assay.
The ester derivatives were the most active, with MIC values of up to 160µg/mL. The benzyl amide and the ester of de-acetyl xylopic acid generally exhibited lower antimicrobial activity with MICs of up to 320µg/mL
All the synthesized derivatives showed good antimicrobial activity and proved more active than the parent xylopic acid against the test organisms (*Staphylococcus aureus*, *Streptococcus pyrogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*).

Keywords: Xylopic acid; ester; amide; antimicrobial; minimum inhibitory concentration; broth dilution.

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1. INTRODUCTION

Xylopia aethiopica has over the years played a pivotal role in traditional medicine with regards to the treatment of various infections. One key constituent that makes *Xylopia aethiopica* an effective antimicrobial agent is xylopic acid [1]. It has been identified as one of the most abundant constituents in most parts of the plant and occurs as a white crystalline solid [2]. Various *in vitro* studies have revealed that xylopic acid possesses very good antimicrobial properties. Boakye-Yiadom et al. showed that xylopic acid possesses a broad spectrum of antimicrobial activity against gram positive and gram negative bacteria, and fungi [3]. Davino et al. also reported that xylopic acid and its epimer, acetylgrandifloric acid exhibit significant antimicrobial activities against various microorganisms with MIC greater than or equal to 250µg/mL [4]. In spite of all these findings, investigating antimicrobial activities of derivatives of xylopic acid has received little attention, particularly how these biological properties compare with the parent xylopic acid.

In recent times, pathogens have developed an inherent ability to adopt mechanisms of resistance against most antimicrobial agents. Typically, ATP-dependent efflux pumps have been among the main mechanisms by which microorganisms like *P. aeruginosa* prevent the accumulation of effective concentrations of antibiotics at molecular target sites [5,6]. In gram-positive bacteria such as *S. aureus,* transporters of the Major Facilitator Superfamily (MFS); QacA, QacB, NorA, and NorB have been found in strains causing hospital-acquired infections, and conferring resistance to several previously effective antibiotics [6]. Also, intercellular communication systems called Quorum Sensing have been known to play a key role in antimicrobial resistance [7-11]; the bacteria within a colony express an autoinducer molecule that binds to transmembrane or intracellular binding sites. Generally, gram-negative bacteria employ acylated homoserine lactone autoinducers [10], while most gram-positive bacteria depend on modified peptide autoinducers to exhibit bacterial resistance [12]. A typical example of Quorum Sensing virulence is *S. aureus* based toxic shock syndrome. Another prominent means of antimicrobial resistance is Horizontal Gene Transfer (HGT) which occurs mostly as a result of quorum sensing. HGT mechanisms basically ensure persistence as they provide a counterpoint to the host's adaptive immune response [13]. All these challenges have necessitated the need to search for newer and more effective antimicrobials.

Synthetic modification of parent natural product became a popular method for discovering antibacterial agents after the innovative chemical alterations of naturally occurring aminoglycosides and tetracyclines [14]. It was proven that catalytic hydrogenation of streptomycin resulted in a new derivative dihydrostreptomycin, which exhibited similar antibacterial properties as streptomycin but was chemically more stable [15]. Again, cephalothin is a first-generation cephalosporin which is very active against Gram-positive bacteria but only moderately active against Gram-negative bacteria. However, through derivatization, it has been possible to synthesize compounds that possess broader spectrum of activity and better pharmacological properties [16]. Our investigations therefore seek to determine the antimicrobial activities of derivatives of xylopic acid obtained *via* slight synthetic modifications, and compare these activities with that of the parent xylopic acid. Such findings, we believe could lead to the improvement of antimicrobial properties of xylopic acid and also aid in the fight against continuous microbial resistance.

2. EXPERIMENTAL DETAILS

2.1 Isolation and Synthesis

The isolation of xylopic acid and the synthesis of derivatives which were all undertaken at the Department of Pharmaceutical Chemistry, KNUST, and characterization of the derivatives have been described in detail in our previous report [2], and generally follow conditions illustrated in Scheme 1.

Table 1 shows the physical data of xylopic acid and the derivatives synthesized.

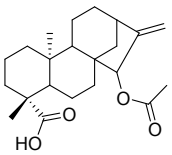
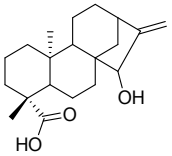
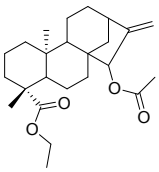
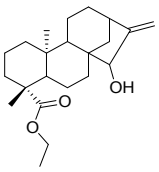
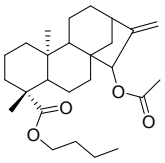
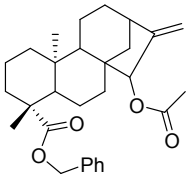
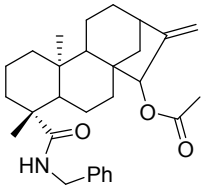
2.2 MIC Determination

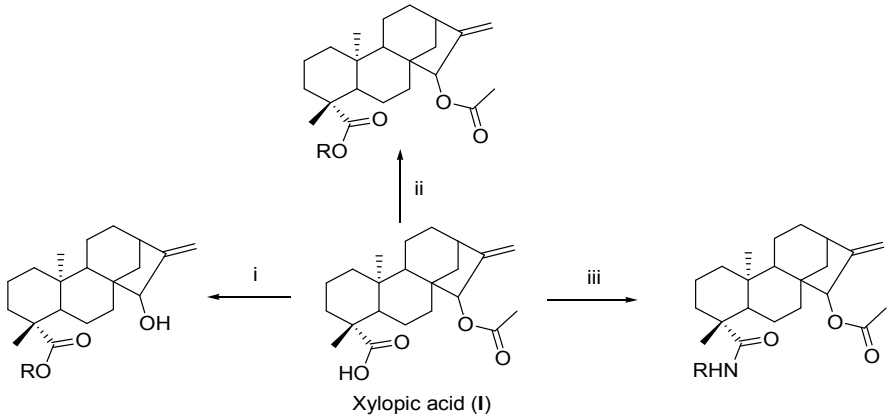
The broth dilution method was employed in determining the antimicrobial activities of our compounds, with Minimum Inhibitory Concentration (MIC) being the key determinant in this study. Sterility and growth (negative controls) as well as positive control (using cefuroxime axetil) experiments were performed. The microorganisms used for the antimicrobial assay were cultures of two Gram-positive

(*Staphylococcus aureus* and *Streptococcus pyrogenes)* and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)* bacteria and a fungus (*Candida albicans*), all of which were obtained from the department of Pharmaceutical Microbiology, Faculty of Pharmacy and Pharmaceutical sciences, KNUST. The microorganisms were sub-cultured in sterile nutrient broth and incubated at 37˚C (25˚C for the fungus) for 18-24 hours. All microbial experiments were conducted in the department of Pharmaceutical Microbiology, KNUST.

Eight concentrations of each test compound (1000, 800, 500, 400, 250, 200, 100 and 10µg/mL) were prepared as aqueous solutions (methanol: water, 50:50) by serial dilution.

Table 1. Physical data of various compounds

Compound	Code	Mol. Weight (g/mol)	Melting pt. (^{o}C)	Rf
	**1**	360.49	264-265	Pet:EtOAc (9:1) 0.51
	**2**	318.45	204-206	Pet:Et_2O (2:1) 0.29
	**3**	388.54	134-135	Pet:Et_2O (2:1) 0.75
	**4**	346.50		Pet:EtOAc (9:1) 0.48
	**5**	416.21		Pet:Et_2O (2:1) 0.77
	**6**	450.61	114-115	Pet:Et_2O (2:1) 0.81
	**7**	449.62		Pet:Et_2O (2:1) 0.68



Xylopic acid (**I**)

Scheme 1. i) H_2SO_4, Solvent Reflux. ii) Na_2CO_3, R-X, DMSO 80˚C iii) HBTU, Et_3N, RNH_2, DCM RT

100µL of sterile broth was dispensed into visibly labelled wells of a 96-well micro titre plate by means of a micropipette. 80µL of each concentration of the test was dispensed into an appropriate well in each case. Finally, 20µL of each test organism was dispensed into the appropriately labelled well with each species in a separate micro plate. The plates were subsequently covered, labelled and incubated at 37 ˚C (25 ˚C for the plate containing the fungus) for 20 hours. The resultant concentrations of the antimicrobial agents obtained after inoculation were 400, 320, 200, 160, 100, 80, 40 and 4µg/mL respectively. MTT was used in the determination of the MIC values. The experiment was performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Results

As shown in Table 2, Xylopic acid 1, exhibit higher antimicrobial activity against the gram negative microorganisms (*Escherichia coli* and *Pseudomonas aeruginosa*) in our investigations. The ester derivatives of xylopic acid 3, 4, 5, and 6 did not show significant difference in antimicrobial activity as indicated by the MIC values. The deacetylated derivatives of xylopic acid 2, also possess activity comparable to that of the ester derivatives. The amide derivative 7, proved to be the least active among all the synthesized compounds.

3.2 Discussion

Antimicrobial properties of xylopic acid are well established. The results in Table 2, indicate that derivatization of xylopic acid does not result in a loss of antimicrobial activity. The improved antimicrobial activity observed for these derivatives suggest that the esters and amides, due to added lipophilicity, are readily transported across the cell membranes of the microorganisms. Upon reaching the cell, hydrolytic enzymes could then hydrolyse the compound to re-generate xylopic acid, which then inhibits the growth of the microorganism. These derivatives may possibly be acting as prodrugs.

It was interesting to note that xylopic acid **1** seem less active compared with its de-acetylated derivative 2 against most of the test organisms. For instance as indicated in Table 2, MIC of xylopic acid 1 for *Streptococcus pyrogenes* is 320 µg/mL, while MIC of 2 was measured to be 100 µg/mL. Also, 1 has MIC of 200 µg/mL for *Pseudomonas aeruginosa,* whereas de-acetylated derivative 2 showed MIC of 100 µg/mL against the same organism. Although further studies are needed to fully understand this outcome, it may be necessary to assume that polar and steric effects may be contributing to this difference in antimicrobial activities between 1 and 2.

The ethyl ester 3, butyl ester 5 and benzyl ester 6 derivatives of xylopic acid showed no significant difference in antimicrobial activity against the various microorganisms as observed from the MIC values, with 100 µg/mL for *Staphylococcus aureus*, and 160 µg/mL for *Candida albicans* in all cases. This may imply that the lipophilic properties of the various analogues in this study do not impact significantly on activity.

Table 2. Minimum inhibitory concentrations (MIC) of xylopic acid and its derivatives

	MIC (µg/mL)							
Organism	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Cefuroxime axetil**
Staphylococcus aureus	320	100	100	160	100	100	200	100
Streptococcus pyrogenes	320	100	100	100	100	100	200	100
Escherichia coli	200	100	100	100	100	100	200	100
Pseudomonas aeruginosa	200	100	100	160	160	100	200	80
Candida albicans	320	160	160	200	160	160	200	100

Key: 1 = xylopic acid; 2 = deacetyl xylopic acid; 3 = ethyl ester; 4 = deacetyl ethyl ester; 5 = butyl ester; 6 = benzyl ester; 7 = benzyl amide.

The benzyl ester 6 proved more active than the corresponding benzyl amide 7. This is revealed by the MIC of 100 µg/mL for 6 compared to 200 µg/mL for 7, for most of the organisms. This is indicative of amide bonds being generally more stable than ester linkages and as a result, the actions of hydrolase occur more readily in the benzyl ester, thus possibly releasing high levels of xylopic acid within the cell of the microorganism even at lower concentrations of the ester compared to the amide.

4. CONCLUSION

Our findings confirm previous studies that showed xylopic acid to possess a broad spectrum of antimicrobial activity. The synthesized derivatives tested proved more active than xylopic acid, confirming the importance of natural product sources as potential drugs. It is noteworthy that most of the synthesized derivatives exhibited antimicrobial activity comparable to that of the positive control (cefuroxime axetil).

Generally, most of the derivatives exhibited lower antifungal activity compared to the antibacterial activity. Cefuroxime axetil was most active against *P. aeruginosa* and *C. albicans* (MIC: 80µg/mL and 100µg/mL respectively) but showed comparable antibacterial activity with most of synthesized derivatives. Increasing the carbon chain of the esters did not significantly affect their antimicrobial activity, this is advantageous, as smaller molecules could be sought after in order to maintain molecular weight acceptability in potential drug design. Also the fact that most of the synthesized compounds compare largely in terms of antimicrobial activity with the control drug (cefuroxime axetil) suggests that xylopic acid derivatives possess much promise as potential antimicrobial agents. However, it will be important that further investigations are focused on elucidating the mechanism of action of xylopic acid as an antimicrobial agent. This will give investigators better understanding of this natural product and its analogues.

ACKNOWLEDGEMENTS

We would like to thank members of staff of the Department of Pharmaceutical Chemistry, KNUST for their support. We also acknowledge members of staff at the Department of Pharmaceutical Microbiology, KNUST for their assistance with the microbial work. We are also grateful to Dr Coura Diene for her support with this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/28104