Antibacterial isobiflavonoids from *Ochna pulchra* Hook. root bark

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Abstract

A phytochemical investigation of *Ochna pulchra* root bark, used in folk medicine to treat microbial infections in parts of Botswana, led to the isolation of six known compounds, namely: afzelone D (**1**), calodenone (**2**), 7-hydroxy-3-(1-(2- hydroxy-4-methoxyphenyl)-3,3-bis(4-methoxyphenyl)-1-oxopropan-2-yl)-4*H*-chromen-4-one (**3**), 4-hydroxy-3- methoxybenzoic acid (**4**), methyl 2-(3,4-dihydroxyphenyl acetate) (**5**), and stigmasterol (**6**). The structures of these compounds were determined by comparing their spectroscopic data (1D and 2D NMR) with those reported in the literature. Compound **2** (MIC = 0.18 ± 0.01 mg/mL) showed better activity than the positive control, ampicillin (MIC = 0.26 ± 0.03 mg/mL), against *P. aeruginosa*. *In silico* molecular docking simulations using Autodock Vina performed on compounds **1** & **2**, showed binding energies and the inhibition constants (K_i) of **1** (-8.72 kcal/mol; 0.41 µM) and **2** (- 8.66 kcal/mol; 0.45 µM) against the *LasR* protein of *P. aeruginosa,* comparable to those of ciprofloxacin (-8.29 kcal/mol; 0.842 µM) and penicillin (-8.62 kcal/mol; 0.484 µM). Furthermore, compounds **1** (-7.31 kcal/mol; .40 µM) and **2** (-6.50 kcal/mol; 17.08 µM) had better binding affinity and inhibition constants (K_i) than ciprofloxacin (-5.82 kcal/mol; 54.33 µM) against the OmpK36 protein of *K. pneumoniae*, suggesting that *O. pulchra* is a source of bioactive compounds with antibacterial potential.

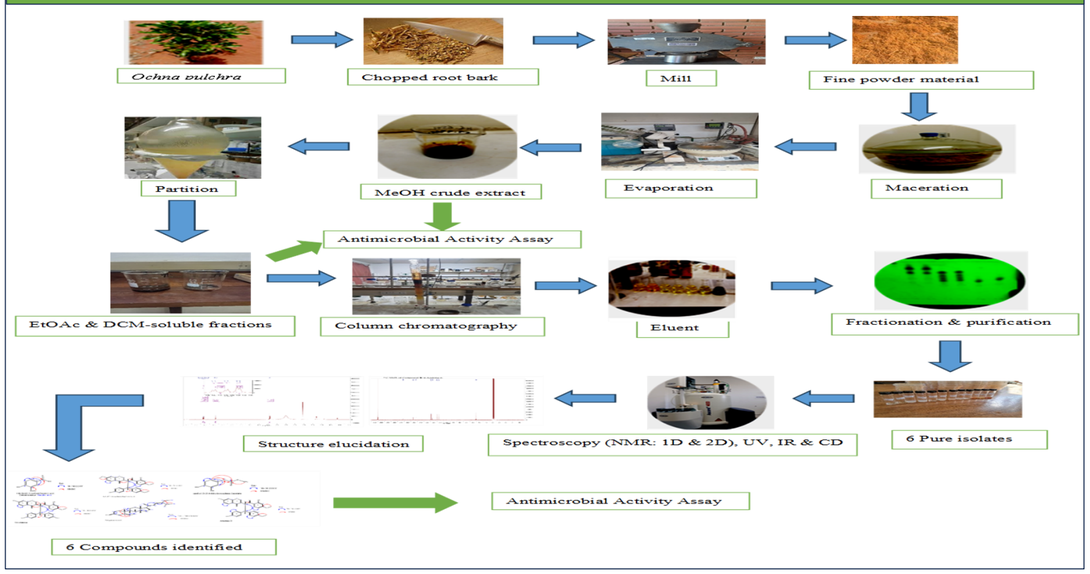
Introduction

Antimicrobial resistance (AMR) occurs when changes in microorganisms such as bacteria, viruses, fungi, and parasites cause the drugs used to treat infections to become less effective making infections more difficult or impossible to treat, thus increasing the risk of disease spread, severe illness, disability and death (Murray et al., 2022).

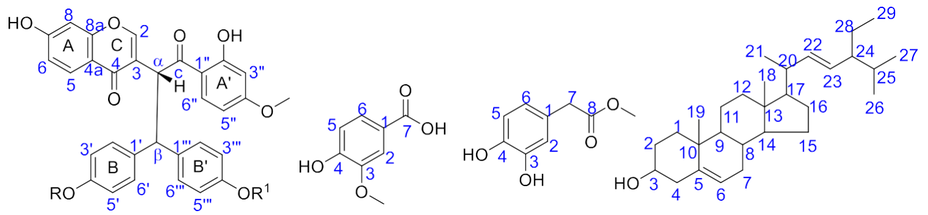
Bacterial AMR was directly responsible for **1.27 million** deaths globally in 2019 and contributed to **4.95 million** deaths, and this number is projected to increase to **10 million** deaths per year by 2050 (Ahmed et al., 2024).The World Bank estimates that AMR could result in US$1 trillion additional healthcare costs by 2050 and US$1 trillion to US$3.4 trillion gross domestic product (GDP) losses per year by 2030. Therefore, there is an urgent need to discover novel antibiotics and explore alternative treatment modalities against AMR derived from plant-based medicines.

Ochna species are known to metabolize flavonoids, especially biflavonoids which are polyphenolic dimers of flavonoid units connected in a symmetrical or unsymmetrical manner, through an alkyl (C-C) or alkoxy (C-O-C)-based linker, and are also reported to possess antibacterial, anti-inflammatory, antimalarial and cytotoxic activities (Band et al., 2012).

Methodology



Results and Discussion



2 R = R^1 = H
1 R = H, R^1 = CH_3
3 R = R^1 = CH_3

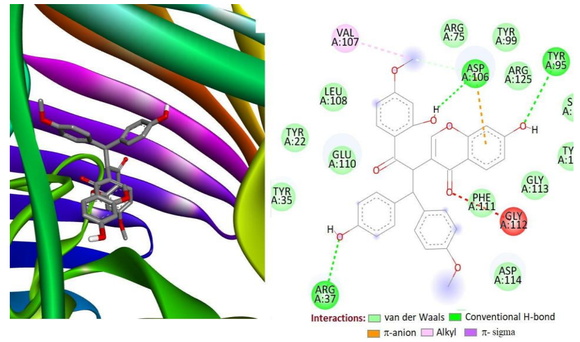
4 **5** **6**

In vitro antibacterial activity of compounds **1** and **2** against *P. aeruginosa*, *K. pneumoniae*, and *E. faecalis*

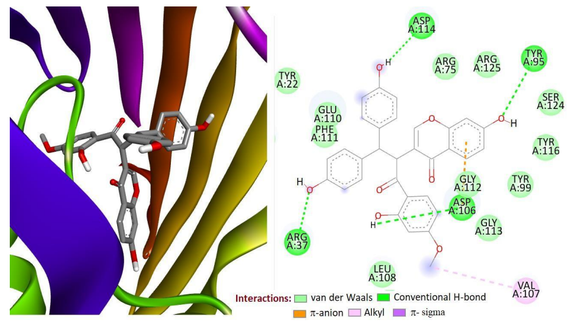
Compound	Minimum Inhibitory Concentration (mg/mL)		
	P. aeruginosa	*K. pneumoniae*	*E. faecalis*
1	0.18 ± 0.01	1.07 ± 0.19	0.30 ± 0.06
2	1.08 ± 0.08	0.69 ± 0.02	0.11 ± 0.04
20% DMSO	-	-	-
Ampicillin	0.26 ± 0.03	0.28 ± 0.01	0.02 ± 0.01

Molecular Docking Scores

Compounds	Lowest binding energy (kcal/mol)	Inhibition constant (K_i, in µM)
	against *P. aeruginosa LasR* (PDB: 2uv0)	
1	-8.72	0.41
2	-8.66	0.45
Ciprofloxacin	-8.29	0.84
Penicillin	-8.62	0.48
	against *K. pneumoniae* (PDB: 6rd3)	
1	-7.31	4.40
2	-6.50	17.08
Ciprofloxacin	-5.82	54.33
Penicillin	-7.56	2.90



Binding interactions of **1** against OmpK36 from *K. pneumoniae* (PDB: 6rd3).



Binding interactions of **2** against OmpK36 from *K. pneumoniae* (PDB: 6rd3).

Conclusion

Compound **2** (MIC = 0.18 ± 0.01 mg/mL) showed better activity than the positive control, ampicillin (MIC = 0.26 ± 0.03 mg/mL), against *P. aeruginosa*. The binding energies of **2** (-8.66 kcal/mol) and 8 (-8.72 kcal/mol), and the inhibition constants (Ki) of **2** (0.45 µM) and **1** (0.41 µM) against the LasR protein of *P. aeruginosa* were comparable to those of ciprofloxacin (-8.29 kcal/mol; 0.84 µM) and penicillin (-8.62 kcal/mol; 0.48 µM). Furthermore, compounds **2** (-6.50 kcal/mol; 17.08 µM) and **1** (-7.31 kcal/mol; 4.40 µM) had better binding affinity and inhibition constants (Ki) than ciprofloxacin (-5.82 kcal/mol; 54.33 µM) against the OmpK36 protein of *K. pneumoniae*. These results suggest that *O. pulchra* is a source of bioactive compounds with antibacterial potential.

Acknowledgement

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