

## Evaluation of the antibacterial, antibiofilm, and cytotoxic activities of *Peltophorum africanum* leaf methanolic extract

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### ABSTRACT

The modern era has used new phytochemical compounds to face the problem of antimicrobial resistance all over the world. The present study aimed to evaluate the antimicrobial and antibiofilm activity of *Peltophorum africanum* leaf methanolic extract against pathogenic isolates. To achieve this, clinical isolates of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* were obtained from Al-Demerdash Hospital where their antimicrobial resistance profiles were assessed according to CLSI guidelines using Kirby Bauer disc diffusion method. The calculated minimal inhibitory concentrations (MIC) of the crude extract against the collected isolates ranged between  $23 \pm 1.1$  and  $7.8 \pm 0.9$  with the highest observed activity against MRSA isolates. MIC was determined by the agar well diffusion. Antibiofilm activity was evaluated using the crystal violet method against three strong biofilm producers and showed that the extract exhibited no antibiofilm activity. The paper also investigated its anticancer potential against the human colon cancer cell (Caco-2) line. In conclusion, it can be inferred that; *Peltophorum africanum* leaf methanolic extract has a good potential to be used in treating infections caused by methicillin-resistant *Staphylococcus aureus* with probably a high safety profile.

**Keywords:** multidrug resistance, coumarins, anti-MRSA, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, herbal remedies.

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### 1. Introduction

Growing antimicrobial resistance represents a threat to public health with the urge to find solutions and alternatives to antibiotics with antimicrobial activity. Among the pathogens of high concern are *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Acinetobacter baumannii* has emerged as a major cause of hospital-acquired infections, primarily affecting immunocompromised individuals in intensive care units along with *Pseudomonas aeruginosa*, a notoriously difficult-to-treat pathogen due to its

intrinsic resistance mechanisms and ability to acquire additional resistance mechanisms. In Egypt, the prevalence of drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* has become a serious concern, leading to increased morbidity and mortality [1, 2]. Another high-risk pathogen is methicillin-resistant *Staphylococcus aureus* (MRSA), a strain of *Staphylococcus aureus* that has developed resistance to multiple antibiotics, including methicillin and other commonly used antibiotics. In Egypt, MRSA infections have been reported in healthcare settings and the community. The

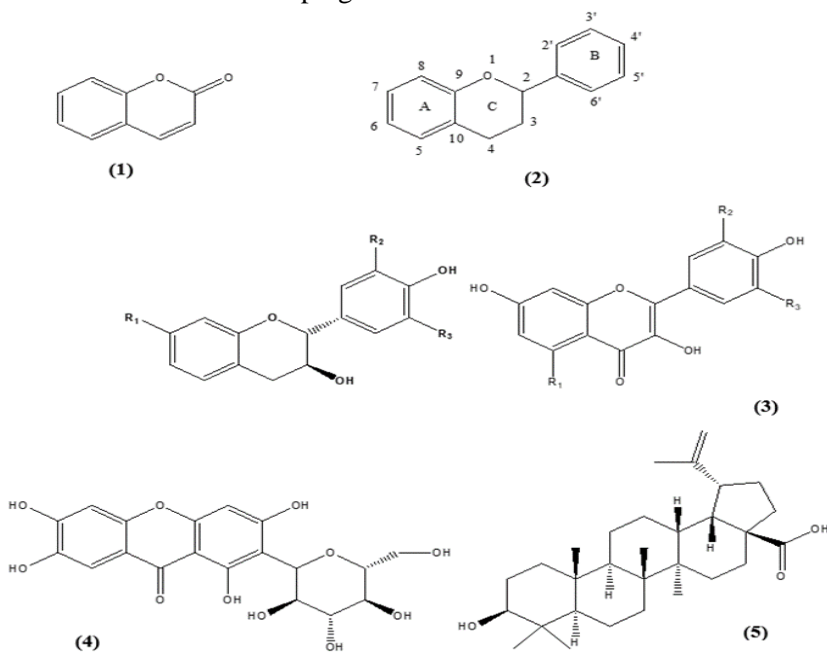
emergence of MRSA strains resistant to multiple antibiotics has led to increased morbidity, mortality, and healthcare costs [3].

Plants have long been recognized as a valuable source of metabolites and natural compounds with antimicrobial properties. Throughout history, various cultures have utilized medicinal plants to treat infections and diseases by producing a wide array of bioactive compounds such as flavonoids, alkaloids, terpenoids, and phenolic compounds, which possess antimicrobial activity. Extracts derived from plants have long been studied for their antimicrobial activity in search of promising compounds.

Herbal medicines have been used to treat diseases and have been part of human habitation for thousands of years. Herbal medicines are an integral part of the culture and tradition of the rural population in developing countries. As a result, the integration of traditional medicine into primary health care is on the rise. Developing

natural products (NPs) as antimicrobial treatments to address complex infections caused by multi-drug resistant pathogens has lately gained increasing interest. Today, a great percentage of the population in the developing world, particularly in countries where modern drugs are not affordable or not accessible or unacceptable, rely on traditional herbal remedies [4].

*Peltophorum africanum*, which is related to the Fabaceae family, is a tree species native to Africa, commonly known as the African wattle. Different studies reported that various parts of this plant have antibacterial, antiviral, antioxidant, and anthelmintic activity [5]. The leaves of the *Peltophorum africanum* were reported to contain different valuable groups of phytochemical constituents (Fig. 1) as coumarins (1), flavonol and flavanol glycosides (2, 3), xanthenes (4), and terpenoids (5) [6-8].



**Fig. 1.** Different valuable groups of phytochemical constituents reported in *Peltophorum africanum*. (1) Basic structure of coumarin reported in *P. africanum*. (2) Basic structure and numbering system of flavonoids reported in *P. africanum*. (3) Basic structure of Flavanols reported in *P. africanum*. (4) Structure of xanthone mangiferin reported in *P. africanum*. (5) Structure of terpenoid betulinic acid reported in *P. africanum*

Flavonoids are a type of natural compounds that play a critical role in preventing and treating various diseases, including infectious diseases caused by a variety of pathogenic bacteria [9]. Flavonoids, the key type of phenolic compounds, are plant secondary metabolites found in aglycone or glycosidic form. Flavonoids have a standard structure where two phenyl rings (A and B) are connected to a heterocyclic ring (C) which contains embedded oxygen, abbreviated as C6-C3-C6. They often have hydroxylation at positions 5 and 7 on the A ring as well as oxidation at 3', 4' or 3'', 4'', and 5'' positions on the B ring due to their biosynthesis routes [10].

Coumarin derivatives are reported to have potent inhibitory effects on various bacteria including MRSA [11]. This tree has gained attention recently for its antimicrobial properties. Some previous studies have demonstrated its inhibitory effects against various microorganisms [12, 13]. However, it is worth noting that the specific antimicrobial activity of *Peltophorum africanum* against methicillin-resistant *Staphylococcus aureus* has not been specifically tested. Accordingly, this study aimed to assess the antimicrobial and antibiofilm activities of the crude methanolic extract of the leaves of *Peltophorum africanum* against multidrug-resistant (MDR) pathogens such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* as well as against MRSA.

## 2. Materials and Methods

### 2.1. Chemicals and media

Muller-Hinton agar (MHA) and tryptic soy broth (TSB) were purchased from HiMedia, USA. Antibiotic discs were purchased from Bioanalyse, Turkey. Roswell Park Memorial Institute medium (RPMI) was a product of Merck, Germany. Fetal bovine serum (FBS) was a product of Sigma-Aldrich, Munich, Germany.

### 2.2. Bacterial isolates

#### 2.2.1. Standard bacterial reference strains

*Staphylococcus aureus* ATCC 43300 was used as a strong biofilm producer for testing the antibiofilm activity of the prepared extract.

#### 2.2.2. Clinical pathogenic isolates

Clinical isolates of the pathogens: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* were obtained from the discharged clinical specimens of Al-Demerdash Hospital Microbiology laboratory in Cairo, Egypt. The collected isolates were coded Acin 1, 2, 3 and Ps 1, 2, and MRSA 1, 2, respectively.

#### 2.2.3. Other clinically strong biofilm producers' pathogenic isolates

A strong biofilm producer methicillin-resistant *Staphylococcus aureus* (MRSA) was recovered from wound exudate and coded MRSA 95.

Another strong biofilm producer *Pseudomonas aeruginosa* was recovered from a urine specimen of a patient suffering from urinary tract infections (UTIs) and coded P50.

### 2.3. Antimicrobial susceptibility profiling of the clinical isolates

The resistance profile of the collected clinical isolates against different antibiotics was evaluated using the Kirby-Bauer disc diffusion method as described by the CLSI guidelines (Clinical and Laboratory Standards Institute, (CLSI M100-Ed29, 2019). The Inoculum was first prepared by suspending one or two freshly isolated colonies (grown overnight on MHA) of the grown test isolates in saline. Then, turbidity was adjusted to 0.5 McFarland. The antibiotics tested against each isolate – shown in **Table 1** – were also selected according to the guidelines of CLSI. The susceptibility of each tested isolate against each antibiotic was then documented as susceptible (S), intermediate (I), or resistant (R).

**Table 1. List of antibiotics used in antimicrobial susceptibility testing, their concentrations, and their sources**

Antibiotic tested	Concentration (amount per disc in µg)	Source
Cefoxitin (FOX)	30	Bioanalyse <sup>®</sup> , Turkey
Amikacin (AK)	30	Oxoid <sup>®</sup> , UK
Imipenem (IPM)	10	Oxoid <sup>®</sup> , UK
Gentamicin (CN)	10	Oxoid <sup>®</sup> , UK
Ceftriaxone (CRO)	30	Bioanalyse <sup>®</sup> , Turkey
Trimethoprim/sulfamethoxazole (SXT)	25	Bioanalyse <sup>®</sup> , Turkey
Meropenem (MEM)	10	Oxoid <sup>®</sup> , UK
Doxycycline (DO)	30	Bioanalyse <sup>®</sup> , Turkey
Tetracycline (TE)	30	Bioanalyse <sup>®</sup> , Turkey
Azithromycin (AZM)	15	Oxoid <sup>®</sup> , UK

## 2.4. Plant material and preparation of crude methanolic extract

### 2.4.1. Preparation of the plant extract

Around 150 g of fresh leaves of *Peltophorum africanum* were collected from the El-Orman botanical garden in Giza, Egypt, and authenticated by taxonomist specialist Tereize Labib. Extraction with methanol was performed using the traditional method: the leaves were washed, air-dried, milled, and macerated in neat methanol at room temperature till exhaustive extraction. The extract was filtered and concentrated under reduced pressure to obtain a brownish extract. This was refrigerated till further investigations.

### 2.5. Testing the antimicrobial activity of the extract and determination of the minimum inhibitory concentration (MIC)

This was performed by the agar diffusion method as described by the CLSI guidelines (CLSI M100-Ed29, 2019). Freshly isolated colonies grown overnight on Muller-Hinton agar were suspended in saline, their turbidity adjusted to 0.5 McFarland and used to make surface inoculation on MHA plates with sterile swabs. Afterward, a cork borer was used to punch wells into the agar. Wells were then filled with different dilutions of the extract and incubated overnight at 37 °C. The inhibition zones formed, displaying no visible growth (if any) were measured to calculate the MIC. The experiment was carried out in triplicate and the mean and standard deviation were calculated.

### 2.6. Antibiofilm activity of the extract

The antibiofilm activity was performed by mixing 100 µL of the tested extract (1/2 MIC)

with 100  $\mu\text{L}$  of freshly prepared bacterial suspension (count adjusted to  $10^6$  CFU/mL) in each well of 96 well sterile flat-bottom microtiter plate. The bacterial suspensions were prepared in tryptic soy broth (TSB) supplemented with 1% glucose. Six replicates were performed. Growth control and sterility control wells were performed. Methanol was used as a solvent for the extract and its activity was assessed to ensure it did not have antibiofilm activity. Incubation was done for 24 h at 37 °C without shaking. Then, the well contents were removed, wells were washed using sterile phosphate-buffered saline (PBS), and the formed biofilm was stained with crystal violet solution (0.1 %). The plate was left to dry at 50 °C and 100  $\mu\text{L}$  of glacial acetic acid (33%) solution (El Nasr Pharmaceutical Chemicals Co. (ADWIC), Egypt) was transferred to the wells to solubilize the adhered biofilms. Optical densities were then measured at 600 nm [14, 15]. Percentages of biofilm inhibition were calculated as follows: first, mean optical densities of growth control ( $\text{OD}_{\text{GC}}$ ), sterility control ( $\text{OD}_{\text{SC}}$ ), and test extract ( $\text{OD}_{\text{test}}$ ) wells were measured. Growth control ( $\text{OD}_{\text{GC}}$ ) and sterility control ( $\text{OD}_{\text{SC}}$ ) refer to control prepared by using bacterial suspension with methanol and control prepared using an empty medium, respectively, and represent 100% biofilm and 0% biofilm, respectively. Second, biofilm growth in the presence of the tested extract was calculated.

Biofilm growth% =  $100 \times (\text{OD}_{\text{test}} - \text{OD}_{\text{SC}}) / (\text{OD}_{\text{GC}} - \text{OD}_{\text{SC}})$  [16].

Finally, the biofilm inhibition percentage was calculated.

Biofilm inhibition% =  $100 - \text{Biofilm growth\%}$  [16].

## 2.7. Cytotoxic activity of the extract

The cytotoxic effect of the extract was assessed using Sulforhodamine B (SRB) assay against the colorectal adenocarcinoma derived

from the human colon; Caco-2 cell line as described by [17, 18]. The cell line was supplied by the Advanced Pharmaceutical Research and Experimental Unit, Faculty of Pharmacy, Ain Shams University.

### 2.7.1. Maintenance of the Caco-2 cell line

Maintenance of the cell line was performed using RPMI 1640 culture medium, supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL) at 37 °C in a humidified carbon dioxide incubator (5%  $\text{CO}_2$ ).

### 2.7.2. Compound preparation

A measured volume of the methanolic extract was evaporated and dissolved in 10% DMSO (vol/vol).

### 2.7.3. Testing the cytotoxicity of the extract using SRB assay

The Caco-2 monolayer was prepared as follows. Cells were trypsinized and suspended in RPMI to a density of  $10^4$  living cells/mL. This was determined by using a hemocytometer. One hundred microliter aliquots were transferred to the wells of 96-well sterile clear polystyrene tissue-culture plates. Incubation was done under the previously mentioned conditions for 24 h.

One hundred microliters of RPMI medium and 100  $\mu\text{L}$  of the extract were added to each well. For the determination of half maximum inhibitory concentration ( $\text{IC}_{50}$ ), a two-fold serial dilution of the extract was prepared. A total of 12 dilutions were used to calculate the  $\text{IC}_{50}$  of the extract. Following 72 h incubation, the cells were observed for morphological changes between treated and untreated cells (control wells) using an inverted microscope. Then cell monolayers were fixed using cold 10% (wt/vol) trichloroacetic acid (TCA). The plate was washed and air-dried at room temperature.

Each well was stained with 100  $\mu\text{L}$  of 0.057% (wt/vol) SRB at room temperature for 30 min and

then rinsed with 1% (vol/vol) acetic acid. The plate was dried at room temperature. To solubilize the protein-bound dye, 200  $\mu$ L of 10 mM Tris base solution (pH 10.5) was added to each well. Measurement of the optical densities (OD) was done at 545 nm using a microplate reader (BioTek Instruments, Vermont, USA). The cytotoxicity of the tested extract was determined by comparing the optical densities of the treated cells versus those of the untreated cells. Calculation of IC<sub>50</sub> was carried out using GraphPad Prism software, version 5.00 (GraphPad Software, Inc. La Jolla, CA, USA). A curve was plotted between the different extract concentrations and the percentage of growth inhibition.

Half-maximal inhibitory concentration (IC<sub>50</sub>) is the concentration at which the test agent caused 50% growth inhibition.

$$\text{Cytotoxicity \%} = [1 - [\text{OD}_{545} \text{ test} / \text{OD}_{545} \text{ control}]] \times 100$$

**Table 2. The resistance profile of the tested isolates**

Antibiotic tested	Acin 1	Acin 2	Acin 3	Ps 1	Ps 2	MRSA 1	MRSA 2
Cefoxitin (FOX)	R	R	R	S	S	R	R
Amikacin (AK)	S	S	S	S	S	NA*	NA*
Imipenem (IPM)	S	R	S	I	R	NA*	NA*
Gentamicin (CN)	I	R	S	R	R	NA*	NA*
Ceftriaxone (CRO)	R	R	I	I	S	NA*	NA*
Trimethoprim/sulfamethoxazole (SXT)	R	R	R	R	R	R	R
Meropenem (MEM)	NA*	NA*	NA*	S	S	NA*	NA*
Doxycycline (DO)	NA*	NA*	NA*	NA*	NA*	S	R
Tetracycline (TE)	NA*	NA*	NA*	NA*	NA*	S	S
Azithromycin (AZM)	NA*	NA*	NA*	NA*	NA*	R	R

\*NA: not assessed

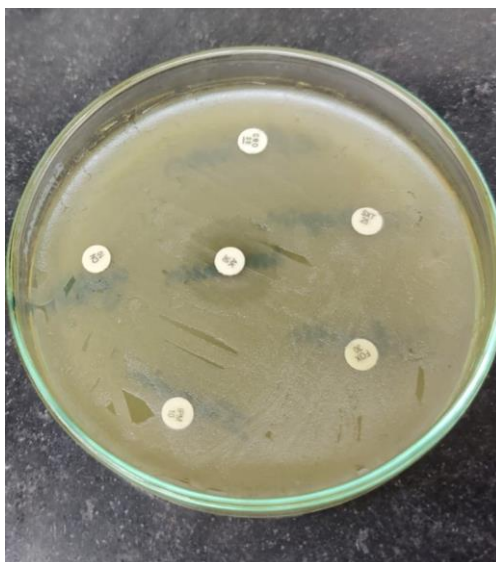
## Statistical analysis

All experiments were performed in triplicates whenever possible. Mean and standard deviation were calculated. Data was analyzed using Graph pad Instant-3 software (Graph Pad Software Inc., USA).

## 3. Results

### 3.1. Antimicrobial susceptibility of the collected isolates

Results shown in **Table 2** revealed that all the recovered isolates were resistant to at least two or more tested antibiotics of different classes indicating they are multidrug-resistant isolates. All the tested isolates were resistant to Trimethoprim/sulfamethoxazole. Amikacin was active against all the tested *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates. One isolate- Acin 2- was sensitive only to Amikacin (**Fig. 2**). Tetracycline was the only antibiotic to be active on the two tested MRSA isolates.



**Fig. 2.** Plate showing the resistance profile of *Acinetobacter baumannii* (Acin2) isolate against tested antibiotics where the isolate displayed sensitivity only to Amikacin (30  $\mu\text{g/mL}$ ).

### 3.2. Antimicrobial activity of the crude methanolic extract

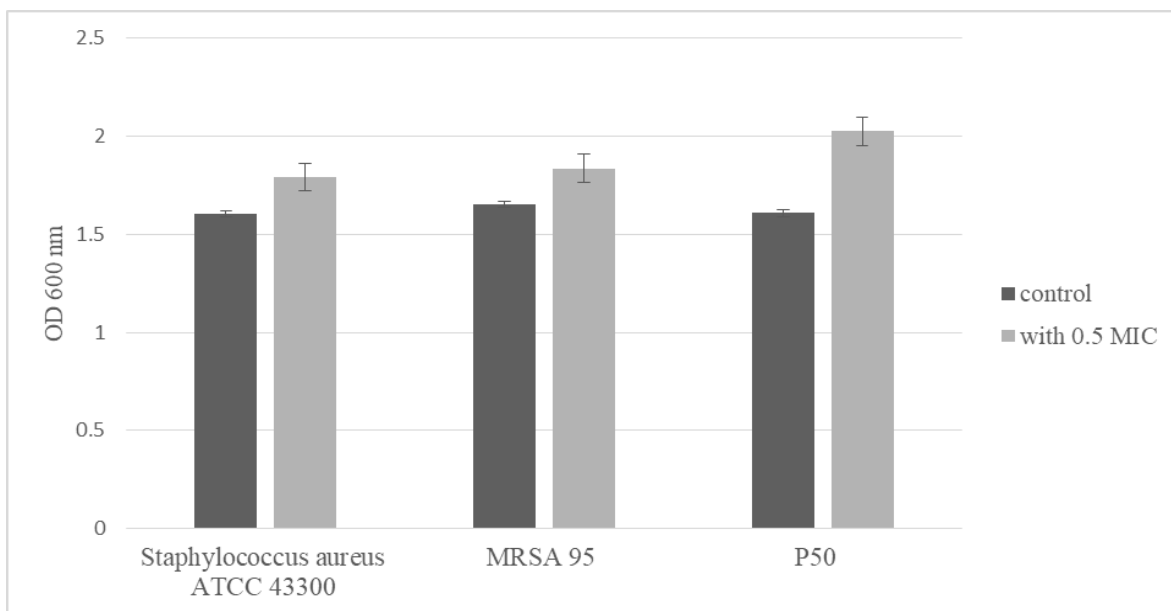
Results showed that the crude methanolic extract of *Peltophorum africanum* displayed antimicrobial activity against all the tested isolates. Calculated MIC shown in **Table 3** ranged between  $23 \pm 1.1$  and  $7.8 \pm 0.9$  with the highest observed activity against MRSA isolates and the least against *Acinetobacter baumannii*.

### 3.3. Antibiofilm of the crude methanolic extract

The leaf methanolic extract used in this study showed no antibiofilm activity (0% or less inhibition) against three tested strong biofilm producers (**Fig. 3**). The extract resulted in -0.95, -0.83, and -0.99 % biofilm inhibition by *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* MRSA 95 and *Pseudomonas aeruginosa* P50, respectively. Lacking inhibitory activity (0% or less inhibition) indicated the inability to prevent biofilm attachment.

**Table 3.** The calculated MIC of the leaf methanolic extract of *Peltophorum africanum* against the tested isolates

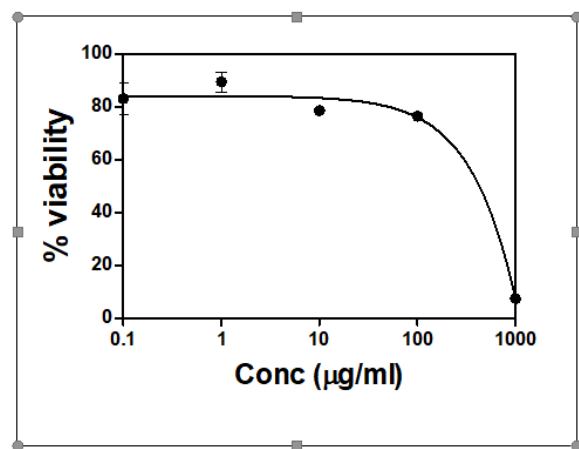
Isolate code	MIC ( $\mu\text{g/ mL}$ ) $\pm$ SD of the extract
Acin 1	$18 \pm 3.5$
Acin 2	$23 \pm 1.1$
Acin 3	$16 \pm 0.7$
Ps 1	$10 \pm 0.82$
Ps 2	$13 \pm 2$
MRSA 1	$9.1 \pm 1.4$
MRSA 2	$7.8 \pm 0.9$



**Fig. 3.** Optical densities of the formed biofilms in the presence of the methanolic extract versus the optical densities of control biofilms.

### 3.4. Testing the cytotoxicity of the extract using SRB assay

The SRB assay revealed a lack the cytotoxic activity of the extract against the Caco-2 cell line as the IC<sub>50</sub> of the methanolic extract was more than 1,000 µg/mL. The extract exhibited neither *in vitro* cytotoxic activity nor damage to the cancer cell line indicating its safety on this cell line (Fig. 4).



**Fig. 4.** Effects of different concentrations of *Peltophorum africanum* methanolic leaf extract on the viability of the Caco-2 Cell line. Values represent the mean of experiments.

### 4. Discussion

The current situation of Antimicrobial resistance (AMR) is a major global health challenge that continues to grow in urgency and complexity. Urgent efforts are required globally to combat AMR, of which the development of new treatment options represents one important approach. The advantage of using plant-derived antimicrobial agents lies in their diverse chemical structure, which often exhibits complex modes of action, making it more challenging for microorganisms to develop resistance. Additionally, plant-based antimicrobials are generally considered safer and more environmentally friendly than synthetic counterparts.

Different research has reported the powerful antibacterial activity of flavonoids which were previously identified in different organs of *Peltophorum africanum*. Flavonoids are polyphenolic compounds that have been suggested to tackle the growth of a wide range of pathogenic microorganisms owing to their different pharmacological mechanisms of action.



However, only a few previous studies have tested the activity of *Peltophorum africanum* extracts against MDR pathogens. Hence, the purpose of this study was to test the activity of methanolic leaf extract of *Peltophorum africanum* against MDR clinical isolates of MRSA as well as *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.

Clinical isolates of the tested pathogens collected from Al-Demerdash Hospital all displayed significant AMR displayed to their ability to resist different antibiotics belonging to different classes. This result comes following several other studies [1-3] and raises the alarm about the increasing resistance of such pathogens in Egypt. Upon testing the activity of the prepared extract against the collected clinical MDR isolates, it was found that it successfully inhibited the growth of all the tested isolates indicating its promising antimicrobial activity. A previous study reported that the hydroxylation at the 5 and 7 positions of the A ring and 4'-hydroxylation of the B ring are important for eliciting an in-vitro anti-MRSA activity [19]. The hydroxylation of C5, C7, C3', and C4'; as well as the prenylation at C6 or geranylation were reported to increase bacterial inhibition of the flavonoids. Methoxylation at C3' and C5 on the other hand has been reported to decrease flavonoids' antibacterial action which was found to be in accordance with most of the flavonoid structures previously identified in *P. africanum*. Also, the antimicrobial activity could be derived from the phenolic compounds identified previously in *P. africanum* extract including flavonoids like rutin and quercetin previously identified in *P. africanum* [20, 21] and non-flavonoid phenolic compounds like benzenoids [22] through inhibiting cell wall biosynthesis, efflux pumps, and key bacterial enzymes like urease and dihydrofolate reductase in MRSA and *Acinetobacter baumannii* [23-25].

Despite displaying a promising antimicrobial activity, the tested extract did not show any antibiofilm activity. On the contrary, there was a relative increase in biofilm formation in the tested isolates. Further investigation is still required to explore the reason for such a finding, but it might be attributed to the presence of certain metabolites or the formation of conditioning films for microbial adhesion as previously reported in a past study [26]. It is also worth noting that there was not any study that tested the antibiofilm activity of the *P. africanum* extract from different organs of the plant, so this is the first study to assess the antibiofilm activity of the *P. africanum* leaf extract which revealed that there was no activity on biofilm formation.

Although previous studies on other extracts of *P. africanum* reported it has potent cytotoxic activity against breast (MCF-7), cervical (HeLa), and colon (HT-29) cancer cell lines [20, 27, 28], the methanolic leaf extract tested in this study did not show any cytotoxic activity on the Caco2 cancer cells. Testing its activity against normal cell lines is still required to guarantee it is potentially safe to be used. Assessing its cytotoxic activity against other cell lines is still required to determine if it has any cytotoxicity or not at all.

### Conclusions and Future Perspectives

The methanolic extract of *Peltophorum africanum* displayed potential antibacterial activity against all the tested pathogens *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and MRSA with the lowest recorded MIC against MRSA. As far as the authors are aware, this is the first time that the antibacterial activity of *Peltophorum africanum* methanolic leaf extract against MRSA has been elucidated. The studied extract also showed to be probably safe and therefore can be further tested on normal cells and developed to control MDR pathogenic infections, especially those caused by MRSA.

However, further studies are still needed to adequately determine the antibacterial activity against other bacterial species and to evaluate the antioxidant and cytotoxic activity of the extract against other cell lines to ensure its safety and also assess its clinical efficacy through *in vivo* studies and clinical trials on humans.

### Declarations

#### Consent to publish

All authors have read and agreed to the published version of the manuscript

#### Ethics approval and consent to participate

Not applicable.

#### Availability of data and material

All data generated or analyzed during this study are included in this published article.

#### Conflict of Interest

The authors assert that there are no conflicts of interest.

#### Funding Statement

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#### Authors Contribution

M. Sakr designed the study. Y. Elkholy and M. Sakr conducted the experiments, analyzed the data, and wrote the manuscript. S. Abdel-ALL conducted the phytochemical experiments, analyzed the data, and wrote the manuscript. All the authors revised and approved the final manuscript.

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### 5. References

1. Salama S, Hadhoud A, Lotfy W, Anis R. Prevalence. Antimicrobial resistance pattern of *Acinetobacter baumannii* isolates from intensive care units in Zagazig University Hospitals. *Microbes Infect Dis* 2021; 2(3):550-557. doi: 10.21608/mid.2021.57273.1110
2. Al-Orphaly M, Hadi HA, Eltayeb FK, Al-Hail H, Samuel BG, Sultan AA, et al. Epidemiology of Multidrug-Resistant *Pseudomonas aeruginosa* in the Middle East and North Africa Region. *mSphere* 2021; 6(3):e00202-21. doi: 10.1128/mSphere.00202-21.
3. Azzam A, Khaled H, Mosa M, Rafeay N, AlSaifi M, Elsisy S, et al. Epidemiology of clinically isolated methicillin-resistant *Staphylococcus aureus* (MRSA) and its susceptibility to linezolid and vancomycin in Egypt: a systematic review with meta-analysis. *BMC Infect Dis* 2023; 23:263. <https://doi.org/10.1186/s12879-023-08202-2>
4. Ekor, M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol* 2014; 4:66193. doi: 10.3389/fphar.2013.00177.
5. Mongalo, N. *Peltophorum africanum* Sond [*Mosetlha*]: A review of its ethnomedicinal uses, toxicology, phytochemistry and pharmacological activities. *J Med Plants Res* 2013; 7(48): 3484-3491. doi: 10.5897/JMPR2013.5302
6. Khattab A and Nassar M. Phytochemical and molluscicidal studies on *Peltophorum africanum* and *Sesbania sesban*. *Bull Natl Res Cent (Egypt)* 1998; 23(4): 401-407
7. Mebe PP and Makuhunga P. 11-(E)-p-coumaric acid ester of bergenin from *Peltophorum africanum*. *Phytochem* 1992; 31(9): 3286-3287. [https://doi.org/10.1016/0031-9422\(92\)83499-O](https://doi.org/10.1016/0031-9422(92)83499-O)

8. Theo A, Masebe T, Suzuki Y, Kikuchi H, Wada S, Obi CL, et al. *Peltophorum africanum*, a traditional South African medicinal plant, contains an anti HIV-1 constituent, betulinic acid. *Tohoku J Exp Med* 2009; 217(2): 93-99. DOI: 10.1620/tjem.217.93
9. Karak P. Biological activities of flavonoids: An overview. *Int J Pharm Sci Res* 2019; 10(4): 1567-1574. doi: 10.1155/2013/162750
10. Kumar S and Pandey AK. Chemistry and biological activities of flavonoids: an overview. *Sci World J* 2013; 2013:162750. doi: 10.1155/2013/162750.
11. Hu C-F, Zhang PL, Sui YF, Lv JS, Ansari MF, Battini N, et al. Ethylenic conjugated coumarin thiazolidinediones as new efficient antimicrobial modulators against clinical *methicillin-resistant Staphylococcus aureus*. *Bioorg Chem* 2020; 94: 103434. DOI: 10.1016/j.bioorg.2019.103434
12. Mboweni HF. Antimicrobial. Cytotoxic and preliminary phytochemical analysis of four medicinal plants and their formulation (Doctoral dissertation). 2018. <http://hdl.handle.net/11602/1096>
13. Munodawafa T, Chagonda LS, Moyo SR. Antimicrobial and Phytochemical Screening of some Zimbabwean Medicinal Plants. *J Biol Active Prod Nature* 2013; 3(5-6):323330, DOI: 10.1080/22311866.2013.782759
14. Piechota M, Kot B, Frankowska-Maciejewska A, Gruzewska A, Woźniak-Kosek A. Biofilm Formation by Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus* Strains from Hospitalized Patients in Poland. *Biomed Res Int* 2018;2018: 4657396. doi: 10.1155/2018/4657396.
15. Dakheel KH, Abdul Rahim R, Neela VK, Al-Obaidi JR, Hun TG, Yusoff K. Methicillin-Resistant *Staphylococcus aureus* Biofilms and Their Influence on Bacterial Adhesion and Cohesion. *Biomed Res Int* 2016;4708425(10):2016: 4708425. <https://doi.org/10.1155/2016/4708425>
16. Haney EF, Trimble MJ, Hancock REW. Microtiter plate assays assess antibiofilm activity against bacteria. *Nat Protoc* 2021;16(5):2615-32. <https://doi.org/10.1038/s41596-021-00515-3>
17. Vichai V and Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc*. 2006;1: 1112–1116. <https://doi.org/10.1038/nprot.2006.179>
18. Tolba MF, Esmat A, Al-Abd AM, Azab SS, Khalifa AE, Mosli HA, et al. Caffeic acid phenethyl ester synergistically enhances docetaxel and paclitaxel cytotoxicity in prostate cancer cells. *IUBMB Life* 2013; 65(8):716-29. doi: 10.1002/iub.1188.
19. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, et al. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *J ethnopharmacol* 1996; 50(1): 27-34. [https://doi.org/10.1016/0378-8741\(96\)85514-0](https://doi.org/10.1016/0378-8741(96)85514-0)
20. Ebada S, Ayoub, N, Singab, AN, Azizi, M. Phytochemicals from *Peltophorum africanum* Sond.(Fabaceae) with promising hepatoprotective activity. *Pharmacogn Mag* 2008; 4(16): 287-293.
21. El Sherbeiny A, El Ansari MA, Nawwar MA, El Sayed NH. Flavonol glycosides and flavonol glucoside gallates from *Peltophorum africanum*. *Planta Medica*

- 1977; 32(06): 165-170. doi: 10.1055/s-0028-1097578
22. Bam M, Ferreira D and Brandt EV. Novel cyanomaclurin analogue from *Peltophorum africanum*. *Phytochem* 1988; 27(11):3704-3705. [https://doi.org/10.1016/0031-9422\(88\)80808-2](https://doi.org/10.1016/0031-9422(88)80808-2)
23. Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, et al. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiol Res* 2017; 196: 44-68. DOI: 10.1016/j.micres.2016.12.003
24. Khare T, Anand U, Dey A, Assaraf YG, Chen ZS, Liu Z, et al. Exploring phytochemicals for combating antibiotic resistance in microbial pathogens. *Front Pharmacol* 2021;12: 720726. <https://doi.org/10.3389/fphar.2021.720726>
25. City N. HPLC analysis of two major flavonoids and antimicrobial effectiveness against t Multi-Drug resistant bacteria of different parts of *Khaya senegalensis*. *J Pharm Med Sci* 2024; 4(1):32-39. doi: 10.21608/AJPMS.2023.196073.119
26. Olawuwo OS, Famuvide IM and McGaw LJ. Antibacterial and antibiofilm activity of selected medicinal plant leaf extracts against pathogens implicated in poultry diseases. *Front Vet Med* 2022, 9:820304. <https://doi.org/10.3389/fvets.2022.820304>
27. Okeleye BI, Mkwetshana NT, Ndip RN. Evaluation of the antibacterial and antifungal potential of *Peltophorum africanum*: toxicological effect on human Chang liver cell line. *Sci World J* 2013; 2013: 878735. <https://doi.org/10.1155/2013/878735>
28. Hordyjewska A, Ostapiuk A, Horecka A, Kurzepa J. Betulin and betulinic acid: Triterpenoids derivatives with a powerful biological potential. *Phytochem Rev* 2019;18: 929-951. <https://doi.org/10.1007/s11101-019-09623-1>