Original article

Antibacterial and antibiotic potentiating activity of Vangueria madagascariensis leaves and ripe fruit pericarp against human pathogenic clinical bacterial isolates

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A B S T R A C T

This study aimed to assess the antibacterial and antibiotic potentiating property of Vangueria madagascariensis (VM) (fruit and leaf extracts) against 10 clinical isolates. A microdilution broth susceptibility assay for bacteria was used for the determination of the minimum inhibitory concentration (MIC) and associated with antibiotics to evaluate any synergistic effect. VM extracts were found to potentiate the activity of 3 conventional antibiotics. Chloramphenicol and Ciprofloxacin showed no activity against Acinetobacter spp. but when mixed with VM (in a ratio of 50% VM extracts: 30% antibiotic), showed potentiating effect. The methanolic fruit extract at lower concentration of Chloramphenicol (30%) gave better synergistic effect (MIC = 3.75 µg/mL) as compared to 50% (MIC = 12.5 µg/mL). With Gentamicin, no activity was detected with leaf decoction but other extracts (methanolic leaf/fruit extract and fruit decoction) showed enhancement (MIC = 0.47, 7.5 and 15 µg/mL respectively). Interestingly, Chloramphenicol showed no activity against MRSA, but when mixed with VM, produced low MICs (<0.39 –0.78 µg/mL with 50% antibiotic and from <0.47 to 0.94 µg/mL with 30% antibiotic). Combining Gentamicin with VM extracts showed an enhancement in the potentiating activity against MRSA. In conclusion, the observed antimicrobial property of VM tend to suggest a promising alternative and complementary strategy to manage bacterial infections and hence can open new avenues for further research using traditional medicinal food plant.

1. Introduction

It is common knowledge that the last few decades have experienced a growing public health challenge in relation to the control and management of infectious diseases and microbial resistance to existing therapies. Indeed, recent studies by the World Health Organisation (WHO) tend to confirm that new resistance mechanisms have emerged, making the latest generation of antibiotics virtually ineffective. Studies have also emphasised on the impact of antimicrobial resistance on various outcomes, including mortality, morbidity, cost and lengthy hospitalisation. Resistance of pathogens against conventional antibiotics has compelled users to probe for substitutes of conventional antimicrobials. To this effect, traditionally used medicinal herbs and food plants have attracted much interest in the scientific community as potential alternative antimicrobial agents. Indeed, the nutraceutical value and functional importance of food plants have received much attention as supported by the growing number of publication during the last past decades emphasizing on the property of food plants for their diversified health benefits and potential clinical applications. Health experts are now recognizing that a synergism of drug therapy and nutrition might give optimum results in the fight against existing and emerging diseases. Indeed, the WHO estimates that 70–80% of the world population relies on traditional remedies including the use of medicinal food plants as primary health care to manage and treat various diseases. Vangueria madagascariensis (VM) J.F. Gmelin. (Rubiaceae) is a native medicinal food plant from Africa that naturally grows along the river banks of forests and volcanic ash soils throughout Africa and Asia. This perennial food plant is in common use in the

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Republic of Mauritius, India, Northern Australia, Singapore and Trinidad.10,11 The genus ‘Vangueria’ is derived from the Madagascan vernacular name ‘voa-vanguier’. Other common local vernacular names include ‘Voavanga’ and ‘Vavandrika’ in Madagascar; ‘Vavang’ and ‘Vavangue’ in Mauritius, Madagascar and Seychelles as well as ‘mviru’ or ‘muiru’ in Swahili. Common English names of VM are Spanish-tamarind, or tamarind-of-the-Indies.11,13 In the light of the above, the present study was designed to further investigate into the antibacterial and antibiotic-potentiating property of VM against clinical pathogenic isolates. It is anticipated that the present work might establish important baseline data on the antibacterial property of VM as a traditional food which can open new avenues for research and bio-product development to manage infectious diseases.

2. Materials and methods

2.1. Collection and preparation of plant samples

Fresh leaves and ripe fruits of VM were collected from the northern parts of Mauritius and authenticated by a local botanist. Samples were thoroughly washed under running tap water, followed by distilled water and patted dry on the same day of collection to remove any undesired substances and kept at 4 °C until further processing.

Fresh leaves were cut and air dried under shade till a constant mass was obtained. Direct sunlight and temperatures above 40 °C were avoided during drying. The dried leaves were grinded in a clean electrical food grinder to a fine homogenized powder and stored in dark air-tight containers at –4 °C.

The ripe fruits were cut into small pieces using sterile scalpel. The seeds were discarded and the pericarp pieces were stored at –18 °C for 48 h until they became brittle. Then the pericarp was lyophilized (Modulo Edwards: F101-000) for 24 h. The samples were then homogenized to a fine powder using an electrical food grinder and stored in dark air-tight containers at –18 °C to be used later.

Methanol (500 mL of 70%) was added to 50 g of leaves, mixed and covered with aluminium foil. The mixture was left for 24 h at room temperature with frequent mixing and then filtered. The filtrate was subjected to Rotary Vacuum Evaporator – in vacuo (Stuart RE100). The resultant sample was again lyophilized and stored in dark air-tight containers at –18 °C to be used later. Same procedure was adopted to process the fruits. The percentage yield was calculated.

2.2. Microdilution broth susceptibility assay

The microorganisms used in the present investigation included human pathogenic clinical bacterial isolates (Acinetobacter spp., E. coli, Enterococcus faecalis, Klebsiella spp., Proteus spp., Pseudomonas aeruginosa, S. aureus, Streptococcus group A, Streptococcus group B, and Methicillin-resistant S. aureus [MRSA]) obtained from Central Laboratory, Victoria Hospital, Candos, Mauritius.

A microdilution broth susceptibility assay for bacteria was used, as described previously for the determination of the minimum inhibitory concentration (MIC) in 96-well microplates with INT (2-(4-iodophenyl)-3-(4-nitropheno)-5-phenyltetrazolium chloride) colourimetric assay with some modifications.17–19 The procedure involves the transfer of 1 mL of fresh inoculums of microorganisms aseptically to 50 mL of peptone broth. These yielded 106 CFU/mL. Sterile peptone broth (100 μL) was then transferred aseptically into each well. Respective stock solutions (100 μL) and control antibiotics (Chloramphenicol and Gentamicin at 2 mg/mL) were transferred to each of the first 3 wells of the first row of the 96-microtitre plate. Double dilution was carried out from the first to last row and the last remaining was discarded. Inoculum (100 μL) was added to each respective well. The plates with bacteria were then incubated for 24 h at 37 ± 1 °C. After incubation, 40 μL of INT at concentration 0.2 mg/mL was added to each well. The plates were further incubated for 30 min at 37 °C. Viable bacteria reduce the yellow dye to pink. Well with no pinkish red colour was taken to be the actual MIC.

2.3. Antibiotic potentiating assay

Extracts showing significant bacterial activities as compared to the positive control in the previous microdilution broth susceptibility assay were associated with conventional antibiotics in view of evaluating any possible synergistic effect.20 106 CFU/mL of microorganisms as described in the microbroth technique was used in the antibiotic potentiating assay. Sterile peptone (100 μL) broth was then transferred aseptically into each well. A final combination of 70 μL of the respective stock solutions and 30 μL of antibiotics (Chloramphenicol, Ciprofloxacine and Gentamicin at concentration of 2 mg/mL) were transferred to each of the first 3 wells of the first row of the 96-microtitre plate. In the second 3 wells, 50 μL of the respective stock solutions and 50 μL of antibiotics were added. The third and the fourth 3 wells were used as control and blank respectively. Double dilution was carried out from the first to last row. Inoculum (100 μL) was added to each respective well. The plates were then incubated for 24 h at 37 ± 1 °C. After incubation, 40 μL of INT at concentration 0.2 mg/mL was added to each well. The plates were further incubated for 30 min at 37 °C. A colour change from yellow to pink indicates bacterial growth.

3. Results and discussion

3.1. Antimicrobial activity

Table 1 shows results obtained for the antimicrobial property of VM extracts against the tested clinical isolates. The MIC recorded ranged between <0.10 to 6.25 mg/mL. MIC <0.20 mg/mL was recorded against E. faecalis, Streptococcus group A and B using VM methanolic leaf extract. The fruit decoction extract showed...
comparable activity as the positive control against Streptococcus group A (both MICs of <0.10 mg/mL). The MIC recorded was 6.25 mg/mL against Enterococcus faecalis. Interestingly, VM extracts (both fruit and leaf) showed activity against MRSA and being more potent that the antibiotic Chloramphenicol.

Differences in antimicrobial activity of the different parts of VM against the clinical isolates are related to differences in their bioactive phytochemical composition. Available reports tend to show that alkaloids and flavonoids are the responsible compounds for the antimicrobial activities in higher plants. Moreover, it was also claimed that secondary metabolites such as tannins and other compounds of phenolic nature are classified as active antimicrobial compounds. Interestingly, we previously reported the preliminary phytochemical screening of VM which showed the presence of phenols and flavonoids. Therefore, the presence of these phytochemicals could to some extent justify the observed antimicrobial activities in the current study. Moreover, as the current antimicrobial study was done using the aqueous preparation as locally prescribed by the traditional healers, therefore these results would tend to support the way people use VM as a herbal remedy.

### Table 1
MIC of VM extracts using the microbroth technique.

| Test microorganisms          | MIC (mg/mL) | Controls | VM extracts |
|------------------------------|-------------|----------|-------------|
|                              |             | Gentamicin | Chloramphenicol | Methanol | Fruit | Decotion | Leaf | Fruit |
| Acinetobacter spp.           | 0.10        | –         | –           | 3.13     | 6.25  | 3.13     | –    | 1.56   |
| Escherichia coli             | 0.03        | <0.01     | 3.13       | 6.25     | 3.13  | 0.78    |
| Enterococcus faecalis        | 0.05        | <0.01     | <0.20      | 3.13     | 3.13  | 1.33    |
| Klebsiella spp.              | –           | 0.10      | 3.13       | 3.13     | 6.25  | 1.56    |
| Proteus spp.                 | –           | 0.10      | 3.13       | 3.13     | 6.25  | 1.56    |
| Staphylococcus aureus        | 0.05        | –         | 3.13       | 3.13     | 1.56  | 1.56    |
| Streptococcus group A        | <0.01       | <0.01     | <0.20      | 0.78     | 0.78  | <0.10   |
| Streptococcus group B        | 0.05        | –         | <0.20      | 1.56     | 0.78  | 0.78    |
| Methicillin-resistant Staphylococcus aureus (MRSA) | 0.05 | – | 6.25 | 3.13 | 6.25 | 1.56 |

*No. of replicates (n = 3) for each sample.*

### Table 2
Potentiating activity using 50% antibiotics with 50% VM extracts.

| B | Control (MIC μg/mL) | Chloramphenicol (CL) | Ciprofloxacin (CIP) | Gentamicin (GEN) |
|---|---------------------|----------------------|---------------------|-----------------|
|   |                     | Methanol* | Decotion* | Methanol* | Decotion* | Methanol* | Decotion* |
| 1 | –                   | 1562.50 (3125) | 6250 (<48.83) | <97.66 (<97.66) | <97.66 (390.63) | 1562.5 (6250) | – (6250) |
|   | –<0.78              | 1562.50 (6250) | 781.25 (6250) | 1562.5 (<97.66) | 97.66 (<48.83) | – (6250) | – (6250) |
|   | 2                   | 3.13 50 | 1250 (6250) | 1250 (50) | 1562.5 (6250) | 781.25 (6250) | – (6250) |
|   | 5                   | 100    | 6250 (6250) | 1250 (3125) | 1562.5 (6250) | 781.25 (6250) | – (6250) |
|   | 6                   | 50     | 6250 (6250) | 1250 (3125) | 1562.5 (6250) | 781.25 (6250) | – (6250) |
|   | 7                   | <0.78  | 6250 (6250) | 1250 (3125) | 1562.5 (6250) | 781.25 (6250) | – (6250) |
|   | 8                   | 6.25   | <97.66 (6250) | 1562.5 (6250) | <97.66 (6250) | <97.66 (6250) | <97.66 (6250) |
|   | 9                   | 0.78   | <97.66 (195.3) | 1562.5 (6250) | <97.66 (6250) | 6.25 (25) | <25 (90) |

No. of replicates (n = 3) for each sample.

*Concentration of; leaf extract (fruit extract) [antibiotic in leaf extract (antibiotic in fruit extract)]. B; Bacteria; (1) Acinetobacter spp; (2) Escherichia coli; (3) Enterococcus faecalis; (4) Klebsiella spp.; (5) Proteus spp.; (6) Staphylococcus aureus; (7) Streptococcus group A; (8) Streptococcus group B; (9) Methicillin-resistant Staphylococcus aureus (MRSA). (--), No activity detected.
With Ciprofloxacin, methanolic leaf extract and decoction at 30% concentration lowered the MIC to <0.23 μg/mL as compared to that of 50% (<0.39 μg/mL). With Gentamicin, no activity was detected with leaf decoction but other extracts (leaf/fruit methanolic extracts and fruit decoction) showed antimicrobial enhancement (MIC of 0.47 μg/mL, 7.5 μg/mL and 15 μg/mL respectively) at 30% antibiotic.

Chloramphenicol showed a decrease or no activity against E. coli. With Ciprofloxacin, there was an increase in potentiating activity of the latter from 50% (<0.39 μg/mL) to 30% (<0.23 μg/mL) when combined with VM extracts. At 50% and 30% Gentamicin, no activity was detected with methanolic fruit extract and leaf decoction but an increase in potentiating activity was observed with methanolic leaf extract and fruit decoction from <0.39 μg/mL to <0.23 μg/mL.

Chloramphenicol at both 50% and 30% concentration showed a decrease in activity against E. faecalis (i.e. an increase in MIC value) as compared to the control. Potentiating activity was depicted in methanolic leaf extract in 50% (<0.39 μg/mL) and 30% (<0.23 μg/mL) concentrations with Ciprofloxacin. All extracts were found to potentiate the antimicrobial activity except fruit decoction at 50% Gentamicin.

Chloramphenicol at 50% concentration showed a potentiating activity of <0.39 μg/mL against Klesbiella spp. Other extracts were found to decrease the activity as compared to the control. Using 50% Ciprofloxacin and Gentamicin, potentiating activity was observed for the leaf decoction only. The other extract either decreased the MIC level or remained unchanged. In the 70% antibiotic (Ciprofloxacin and Gentamicin) assay, a significant potentiating activity was recorded with the exception of methanolic fruit extract and fruit decoction.

Potentiating activity was recorded against Proteus spp. using any 3 antibiotics and either VM decoction or methanolic extracts with the only exception of methanolic leaf/fruit and leaf decoction combined with Gentamicin. Using 50% Gentamicin and VM extracts, antibacterial activity against S. aureus was enhanced with the exception of fruit decoction combined with Ciprofloxacin and Gentamicin which showed no change. Using the 30% antibiotics with VM extracts, potentiating activities were observed and showed that increasing the concentration of VM greatly enhanced the antimicrobial activities.

| B | MIC (μg/mL) | Control | Chloramphenicol (CL) | Ciprofloxacin (CIP) | Gentamicin (GEN) |
|---|-------------|---------|---------------------|---------------------|-----------------|
|   |             | CL | Methanol* | Decoction* | Methanol* | Decoction* | Methanol* | Decoction* |
| 1 | –           | 100 | 17500 (2187.5) | 17500 (58.36) | <136.72 (4375) | <136.72 (4375) | 273.44 (4375) | – (4375) |
|   |             |      | [15 (3.75)] | [30 (0.23)] | [-0.23 (7.5)] | [-0.23 (15)] | [0.47 (7.5)] | [– (15)] |
| 2 | <0.78       | 25  | 8750 (1093.75) | 568.88 (1093.75) | <136.72 (<136.72) | <136.72 (<88.36) | <136.72 (<–) | <-88.36 |
|   |             |      | [15 (1.86)] | [0.94 (3.75)] | [-0.23 (<0.23)] | [-0.23 (<2.23)] | [-0.23 (<–)] | [-<0.23] |
| 3 | <0.78       | 131 | 1903.75 (2187.5) | 2187.5 (2187.5) | <136.72 (2185) | 373.45 (4375) | <136.72 (4375) | 546.88 (2187.5) |
|   |             |      | [1.86 (3.75)] | [3.75 (7.5)] | [-0.23 (3.75)] | [7.5 (15)] | [0.47 (7.5)] | [0.94 (7.5)] |
| 4 | <0.78       | 25  | 273.44 (2187.5) | 1093.75 (4375) | 1093.75 (2187.5) | 1093.75 (2187.5) | 2187.5 (7) | 2187.5 (7) |
|   |             |      | [0.47 (3.75)] | [1.86 (15)] | [1.86 (7.5)] | [1.86 (7.5)] | [-7.5] | [-7.5] |
| 5 | 100         | –   | 1903.75 (4375) | 4375 (2187.5) | 273.44 (8750) | 136.72 (90375) | 136.72 (90375) | – (–) |
|   |             |      | [1.86 (7.5)] | [7.5 (7.5)] | [0.47 (15)] | [-<0.23 (3.75)] | [-<0.23 (–)] | [-<0.23] |
| 6 | –           | 50  | 1903.75 (2187.5) | 1093.75 (2187.5) | <136.72 (4375) | <136.72 (4375) | 2187.5 (4380) | 2187.5 (4380) |
|   |             |      | [1.86 (3.75)] | [1.86 (7.5)] | [-0.23 (7.5)] | [-0.23 (7.5)] | [1.86 (7.5)] | [-0.23 (7.5)] |
| 7 | <0.78       | 0.78 | <0.78 | 273.44 (237.44) | 273.44 (546.88) | <136.72 (<136.72) | <136.72 (<237.44) | <136.72 (7) |
|   |             |      | [0.23 (0.47)] | [0.47 (1.86)] | [-0.23 (<0.23)] | [-0.23 (0.94)] | [-0.23 (0.94)] | [-0.23 (0.94)] |
| 8 | –           | 625 | 273.44 (546.88) | 273.44 (86.36) | <136.72 (237.44) | 373.45 (109375) | <136.72 (109375) | 1093.75 (109375) |
|   |             |      | [0.23 (0.94)] | [0.47 (0.23)] | [-0.23 (0.47)] | [7.5 (3.75)] | [-0.23 (1.86)] | [1.86 (3.75)] |
| 9 | –           | 0.78 | 273.44 (546.88) | 568.88 (273.44) | 273.44 (2187.5) | – (468.88) | <136.72 (<–) | <136.72 (<–) |
|   |             |      | [0.47 (3.75)] | [0.94 (0.94)] | [1.86 (7.5)] | [-1.86] | [-<0.23 (<–)] | [-<0.23 (<–)] |

No. of replicates (n = 3) for each sample.

* Concentration of leaf extract (fruit extract) [antibiotic in leaf extract (antibiotic in fruit extract)], B: Bacteria; (1) Acinetobacter spp.; (2) Escherichia coli; (3) Enterococcus faecalis; (4) Klesbiella spp.; (5) Proteus spp.; (6) Staphylococcus aureus; (7) Streptococcus group A; (8) Streptococcus group B; (9) Methicillin-resistant Staphylococcus aureus (MRSA); (–), No activity detected.
perturbation by these lipophilic components could be involved as part of the observed antibacterial action. This could be partly due to a hydrophobic nature of some phytochemicals from VM extracts. Interestingly, it has already been reported that the extract from plants can also act by improving the penetration of antibiotics in cells via membrane alteration.\(^2\,^{19}^{19},\)\(^{20}\) Also, these phytochemicals can interact with the double lipid layer of the cellular membrane and affect the respiratory chain and energy production. It may also increase the permeability to antibiotics leading to the suspension of vital cellular activity or interfering with enzymatic activity.\(^2\,^{11}\)

4. Conclusion

In conclusion, VM leaf and fruit extracts showed potent antibacterial and antibiotic potentiating activity against tested antibiotics in vitro. The observed antimicrobial property and antibiotic potentiating activity of VM tend to suggest a promising alternative and complementary strategy to manage bacterial infections and hence can open new avenues for further research using traditional medicinal food plant. The current study also tend to advocate the need for continuing screening for antimicrobial agents from local medicinal food plants of Mauritius and results of this study can be cited as evidence for antimicrobial property and antibiotic potentiating activity of VM. However, the exact mechanism of antibacterial effects of VM needs to be further examined for potential health uses and toxicological studies need to be carried out to evaluate its safety.

Conflict of interest

All authors associated with this manuscript have no conflicts of interest to declare.

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