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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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ABSTRACT

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.[1] 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.[2] Studies have also emphasised on the impact of antimicrobial resistance on various outcomes, including mortality, morbidity, cost and lengthy hospitalisation.[3,4] Resistance of pathogens against conventional antibiotics has compelled users to probe for substitutes of conventional antimicrobials.[5] 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.[6,7] 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.[8]

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.[9,10] 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]

Vangueria has received scientific attention for its extensive ethnomedicinal applications worldwide. Generally cultivated for its sweet-sour fruits, this plant has also brought significant contribution in the African *Materia Medica* for its antimicrobial properties since time immemorial.[7] Preliminary *in vitro* study showed that VM possesses antimicrobial potential against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). *In vitro* data revealed the presence of a number of bio-constituents with pluri-potential mechanism of action which might be responsible for its medicinal virtues.[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: F101-01-000) 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). These steps were repeated multiple times for exhaustive maceration. The resultant sample was then lyophilized and the sticky material was stored in dark air-tight containers at −18 °C to be used later. Same procedure was used for the ripe fruit parts.

The most common method of using VM locally is in aqueous form, i.e. by boiling of the fruits and leaves.[14] Hence, the aqueous crude extract of VM was also prepared and evaluated for possible biological properties. The already processed fruit and leaves were subjected to reconstituted-boiling for few hours (200 mL sterile distilled water was added to 50 g of leaves powder). The sample powder was mixed and boiled until reduced to 1/4 of the original volume. After cooling, the extract obtained was filtered through sterile muslin cloth for removal of large unwanted material and then through sterile Whatman (Number 1) filter paper. 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-nitrophenyl)-5-phenyltetrazolium chloride) colourimetric assay with some modifications.[15–17] 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 70 μL of the respective stock solutions and 30 μL of antibiotics (Chloramphenicol, Ciprofloxacin 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

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