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(RESEARCH ARTICLE)



Antimicrobial and antioxidant activities of the fruits of bemavo, a variety of *Ravenala madagascariensis* Sonn. (Strelitziaceae)

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Abstract

This work was designed to evaluate the antibacterial and antioxidant activities of the fruit extracts from bemavo, a variety of *Ravenala madagascariensis*. Methanolic extracts from pericarp (PME), seed (SME) and aril (AME) were tested against 5 Gram positive and 6 Gram negative of pathogenic bacteria using disk diffusion and microdilution methods. At 1000 μ g/ml, all extracts prevented selectively the growth of at least 5 of the tested bacteria with Inhibition Zone Diameter (IZD) ranking from 8.5 mm to 19 mm. The best activity was recorded with PME which was active against 81.8 % of the studied bacteria with 63.6 % of IZD higher than 14 mm: 15 mm on *Yersinia enterocolitica, Salmonella enterica, Shigella flexneri*, and *Listeria monocytogenes*, 16 mm on *Staphylococcus aureus*, 18 mm on *Enteroacter aerogenes*, and 19 mm on *Vibrio fischeri. Clostridium perfringens* and *Bacillus cereus* were resistant to the three extracts. The great majority (97 %) of MCI recorded were $\leq 1000 \ \mu$ g/ml, 84.8 % were $\leq 500 \ \mu$ g/ml, 12.1 % between 500 μ g/ml and 1000 μ g/ml. *Enterobacter aerogenes* and *Clostridium perfringens*, bacteriostatic against *Salmonella enterica* and *Bacillus cereus* and bactericidal or bactericidal against the remaining strains. The antioxidant activity of extracts was determined by the method using free radical scavenging against DPPH. Compared with ascorbic acid (IC₅₀=7.320 μ g/ml), SME (IC₅₀=0.568 μ g/ml) and AME (IC₅₀=3.792 μ g/ml) were 12.9 and 6.5 times more active respectively. Phenolic compounds present in three extracts could be responsible for antimicrobial and antioxidant activities of the bemavo fruit methanol extracts.

Keywords: *Ravenala madagascariensis*; Bemavo; Strelitziaceae; Antibacterial; Antioxidant; Disk Diffusion; MIC, MBC, DPPH.

1. Introduction

Infectious diseases remain the main cause of the high mortality rates recorded in the developing nations. Resistance of bacterial pathogens to antibacterial drugs is a global concern. The number of multi drug resistant microbial strains and the appearance of the strains with reduced susceptibility to antibiotics are continuously increasing.

Many diseases such as development of cell injury, aging, cardiovascular and neurodegenerative diseases, autoimmune disorders, rheumatoid arthritis and cancer are related to overproduction of free radicals and reactive oxygen species (ROS) [1, 2, 3].

World Health Organization (WHO) reported that about 80 % of the globe population rely on plants or its derivative products for the treatment of various sicknesses [4]. Search for new drugs with low cost, more potential, without adverse effects is being pursued in several laboratories all around the world. Bioactive secondary metabolites produced by plants have proven to possess a wide range of therapeutic effects for human diseases [5]. Considerable attention has been devoted to medicinal plants with antimicrobial and antioxidant properties [5, 6].

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With the richness of its biodiversity and the high level of its endemism (80 %), Madagascar flora is a potential source of medicinal plants. *Ravenala madagascariensis* (Strelitziaceae) is one of those endemic medicinal plants. Also known as the traveler's palm or traveler's tree, this plant has been considered as an iconic symbol of Madagascar [7]. It is naturalized in the Mascarene Islands and widely planted as an ornamental tree throughout the tropics [8, 9]. In Madagascar, *Ravenala madagascariensis* is a multi-purpose plant, used primarily for construction (house, roof, fence etc.) but also as food, tools and medicines [10, 11]. It is traditionally used to treat some diseases like, diabetes, inflammations, arterial hypertension, tooth decay [12, 13] and wounds [14]. Investigations on *Ravenala madagascariensis* from other countries showed that aerial parts exhibited antimicrobial activity) [15, 16], leaf extract has antioxidant activity [17]. However, it is noteworthy to emphasis that, in Madagascar, there are 4 varieties of the *Ravenala* genus (Malama, Hiranirana, Bemavo and Horonorona) which are different by their macromorphological characters, growth habits and habitat preferences [12, 18, 19]. However, in these works hereabove cited, the name of the variety was not mentioned.

The objective of this study was to evaluate the antibacterial and the antioxidant potential of the fruits of bemavo, a variety of *Ravenala madagascariensis*.

2. Material and methods

2.1. Plant material

Bemavo is a variety of *Ravenala madagascariensis* growing in open areas between 200 and 600 m of altitude [8]. It is single-stemmed, without offshoots at the base and with yellow leaf sheaths that was at the origin of its vernacular name (figure 1).



Figure 1 Ravenala madagascariensis Sonn: a) whole plant; b) fruits; c) Pericarp; d) aril; e) seeds.

Source: the authors

Fruits were harvested in September 2016 in Antsirinala-Moramanga (18°53'49.6"S, 048°08'59.4"E, altitude 940 m), in the eastern region of Madagascar, 100 km from Antananarivo. The botanical identification of the plant was made by Rakotoarisoa S.E. on May 2009 and voucher specimens were conserved at the Tsimbazaza herbarium under SNGF 2241.

Fruits were dried in the shade until their dehiscence. The fruits parts (pericarp, aril and seed) were separately ground into fine powders and then stored at -20 °C.

2.2. Bacterial strains

Eleven pathogenic strains supplied by the National Center for Application of Pharmaceutical Research (CNARP) including 5 Gram (+) and 6 Gram (-) were used (Table 1).

Table 1 Bacterial strains used

Strains		References
GRAM (-)	Enterobacter aerogenes	ATCC 13048
	Yersinia enterocolitica	ATCC 23715
	Salmonella enterica	ATCC13076
	Shigella flexneri	ATCC12022
	Escherichia coli	ATCC 25922
	Pseudomonas aeruginosa	ATCC 10145
GRAM (+)	Clostridium perfringens	ATCC 25923
	Staphylococcus aureus	ATCC11632
	Listeria monocytogenes	ATCC19114
	Vibrio fischeri	ATCC 49387
	Bacillus cereus	ATCC 14579

2.3. Preparation of the methanolic extracts

In a Soxhlet apparatus, exhaustive extractions were successively carried out with hexane, ethyl acetate and methanol from 100 g of pericarp, aril and seed powder. All solvent extracts were evaporated to dryness using a rotary evaporator at 40 °C under reduced pressure. Methanolic extracts of pericarp, (PME), aril (AME) and seeds (SME) were used for all tests.

2.4. Phytochemical screening

The qualitative chemical analysis of the different fruit extracts was carried out according to the methods used by Firdouse *et al.* [20].

2.5. Antibacterial assays

Antibacterial activities were assessed by measuring the Inhibition Zone Diameter (IZD) using the disk diffusion method [21, 22] and determining the Minimum inhibitory (MIC) and the Minimum bactericidal concentration (MBC) using the microdilution method [23].

PME, AME and SME extracts dissolved in Dimethyl sulfoxide (DMSO) 10 % were used for all antibacterial assays.

2.5.1. Determination of IZD

Two (2) ml of inoculum, equivalent to 0.5 Mc Farland (10^8 CFU/ml), were spread on the surface of a solid medium of Mueller-Hinton Agar. Sterilized filter paper disks (6 mm diameter) (BioMérieux, REF54991) were impregnated with 10 µl of each extract. The concentration of each extract was 1 mg/ml /disk which is a concentration generally used to assess the antibacterial potential of plant extracts [24]. Impregnated disks were placed on the agar surface and incubated at 37 °C. After 24 h of incubation, IZDs were measured and results were interpreted according to the IZD scale of Ponce *et al.* [25] and Celikel and Kavas [26]: bacteria are not sensitive for IZD \leq 8 mm; sensitive for 9 mm \leq IZD \leq 14 mm; very sensitive for 15 mm \leq IZD \leq 19 mm and extremely sensitive for IZD \geq 20 mm. Imepenem was used as reference antibiotic and the experiments were carried out in triplicate.

2.5.2. Determination of MIC, MBC and MBC/MIC

Increasing concentrations ranging from 15.625 to 8000 μ g/ml were prepared by twofold dilution series of each extract. 100 μ l of each concentration were poured into 96 wells microplates containing 95 μ l of Mueller-Hinton broth (MHB) and 5 μ l of inoculum (standardized at 0.5 Mc Farland). A positive control (bacterial culture) and a negative control (medium culture) were also analyzed. After 24 h of incubation, the MIC of each extract was determined after adding 40 μ l of 0.2 mg/ml of Tri-Methyl Tetrazolium Chloride (MTT) and a second incubation for 30 min at 37 °C. Viable bacteria reduced the yellow color of MTT to purple. MIC was the lowest extract concentration showing no color change.

For the MBC determination, 5 µl of the solution concentration corresponding to the MIC were transferred on to Muller-Hinton agar plate and incubated at 37 °C for 24 h. MBC was the lowest concentration showing no bacterial growth.

The MBC/MIC ratio was calculated for each extract. Extract was bactericidal for MBC/MIC \leq 4 and bacteriostatic for MBC/MIC >4 [27, 28].

2.6. Determination of antioxidant activity

The antioxidant capacity of PME, SME and AME, was evaluated by the method using free radical scavenging against DPPH (2, 2-Diphenyl-1-Pycryl Hydrazyl) [29]. One (1) ml of 0.004 % DPPH in methanol was added to 1 ml of methanol extract at different concentrations ranking from 0 to 120 μ g/ml depending on the extract. In the case of free scavenging activity, the purple DPPH was reduced to a yellow compound. After 30 min of incubation at room temperature and in the dark, the optical density was measured at 517 nm with a spectrophotometer using methanol sample as blank. A mixture of 1ml of DPPH and 1 ml of methanol was used as negative control and ascorbic acid as positive reference. Assays were made in triplicate.

The decrease of the optical density was then converted into percentage of inhibition (I %), according to the equation hereafter:

Free scavenging activity (I %) =
$$100 \text{ X} \frac{\text{Abs DPPH} - \text{Abs assay}}{\text{Abs DPPH}}$$

With, Abs DPPH: Absorbance of DPPH at 517 nm.

Abs assay: Absorbance of extract solution in methanol at 517 nm

The IC_{50} (in µg/ml) which is the extract concentration inducing the 50 % loss of DPPH antioxidant activity was determined by linear regression of I % values. The lower the IC_{50} value the higher the extract antioxidant activity [30].

2.7. Statistical analysis of data

The results obtained by the disk diffusion method were expressed as mean values \pm standard deviations of three separate replicates.

One way analysis of variance (ANOVA) followed by Newman Keuls comparison test with statitcf software MS-DOS 6.21 was used for statistical analysis. Statistical estimates were done with a confidence interval of 95 %.

3. Results

3.1. Extraction yield

Methanol extraction yields of *R. madagascariensis* Sonn. were 0.70 % for pericarp, 10.23 % for seeds and 7.60 % for aril.

3.2. Phytochemical results

The chemical families present in the PME, SME and AME are shown in Table 2.

Chemical groups	Tests	PME	SME	AME
Flavonoids	Willstätter	-	+	+
Leucoanthocyanins	Bate-Smith	+	+	+
	FeCl ₃	+	+	+
Tannins and Polyphenols	Gelatin 1 %	+	+	+
	Gelatin -Salt 10 %	+	+	+
	Liebermann- Burchard	-	+	+
Triterpenes and Steroids	Salkowski	+	+	+
Deoxyoses	Keller-Kiliani	-	+	+
Quinones	Borntrager	-	-	-
Saponins	Foam test	-	-	-
Iridoids	Hot HCl	-	-	
	Wagner	-	-	-
Alkaloids	Dragendorff	-	-	-
	Mayer	-	-	-
+: positive test; -: negative test				

Table 2 Phytochemical screening of PME, SME and AME

Leucoanthocyanins, tannins, polyphenols and steroids were detected in the three extracts. Flavonoids, triterpenes and desoxyoses were present in SME and AME but not in PME. Quinones, saponins, irridoids and alkaloids were not found in the three extracts.

3.3. Antimicrobial activity

As shown in Table 3, active IZD ranked from 8.5 to 19 mm.

Table 3 IZD of PME, SME, AME (1 mg/ disk) and Imipenem (10 µg/ml) against 8 bacterial strains

Bacteria strains		Inhibition Zone Diameter (mm)			
		РМЕ	SME	AME	Imipenem
	Yersinia enterocolitica	15±0	9±1	10±0	32±0
	Salmonella enterica	15±0.20	10±0	12.5±0.50	33±1
GRAM (-)	Shigella flexneri	15±1	8.5±0.50	10±0	33±1
	Escherischia coli	11±1	6±0	6±0	34±0
	Pseudomonas aeruginosa	12±0	6±0	6±0	18±0
	Clostridium perfringens	8±1	6±0	6±0	31±0
	Staphylococcus aureus	16±0	7±1	7.5±0.41	45±4
GRAM (+)	Listeria monocytogenes	15±0	9±0	12±0	30±0
	Vibrio fischeri	19±1.52	10±0	11.5±0.41	28±2
	Bacillus cereus	6±0	7±1	7±1	34±0

Values are averages of three separate replicates ± standard deviations

PME, SME and AME displayed antibacterial activity with selective effects. They were active against both Gram (+) and Gram (-) germs. *Clostridium perfringens* and *Bacillus cereus* were the only resistant strains to the three extracts.

PME was proved to be the most efficient extract: it was active against 9 of the 11 bacteria tested (81.8 %) with IZD ranking from 11 mm (*Escherichia coli*) to 19 mm (*Vibrio fischeri*) and the majority of IZD values (63.6 %) was higher than 14 mm.

AME showed a moderate activity against 5 of the 11 bacteria tested (45.6 %) with IZD ranking from 10 mm (*Yersinia enterocolitica* and *Shigella flexneri*) to 12.5 mm (*Salmonella enterica*). SME was the least effective extract: against the 5 sensitive bacteria, IZD values ranked from 8.5 mm (*Shigella flexneri*) to 10 mm (*Salmonella enterica* and *Vibrio fischeri*). The three extracts were less efficient than reference antibiotic Imipenem at 10 μ g/ml.

The values of MIC, MBC and the MBC/MIC ratios are presented in Table 4.

All the three extracts prevented the growth of the 11 bacteria tested. *Enterobacter aerogenes* was the most sensitive to the three extracts. The great majority of MIC values ranked from 100 μ g/ml to 1000 μ g/ml: 84.8 % were \leq 500 μ g/ml, 12.1 % between 500 μ g/ml and 1000 μ g/ml and 3.1 % higher than 1000 μ g/ml.

The effect of PME, SME and AME was bactericidal (MBC/MCI \leq 4) against *Salmonella enterica* and *Bacillus cereus*, bacteriostatic (MBC/MCI > 4) against *Enterobacter aerogenes* and *Clostridium perfringens* whereas variable according to the extract against the remaining strains.

Table 4 MIC, MBC and MBC/MIC ratio of PME, SME and AME on Gram (+) and Gram (-) strains

STRAINS		EXTRACT	MIC	MBC	MBC/MIC
		PME	125	>8000	>4
	Enterobacter aerogenes	SME	125	>8000	>4
		AME	125	>8000	>4
		PME	750	8000	>4
	Yersinia enterocolitica	SME	250	250	1
		AME	250	250	1
		PME	250	250	1
	Salmonella enterica	SME	500	1000	2
CRAM (_)	_	AME	375	375	1
ukam (-)		PME	250	250	1
	Shigella flexneri	SME	375	8000	>4
		AME	500	500	1
		PME	250	1000	4
	Esherischia coli	SME	500	>8000	>4
		AME	500	>8000	>4
		PME	250	1000	4
	Pseudomonas aeruginosa	SME	500	>8000	>4
		AME	1000	8000	>4
		PME	350	>8000	>4
(Clostridium perfringens	SME	250	8000	>4
		AME	250	>8000	>4
		PME	500	>8000	>4
	Staphylococcus aureus	SME	390	6250	>4
		AME	2000	8000	4
	Listeria monocytogenes	PME	250	250	1
		SME	187.5	8000	>4
		AME	500	8000	>4
		PME	500	500	1
	Vibrio fischeri	SME	250	>80000	>4
		AME	250	8000	>4
		PME	350	500	1.42
	Bacillus cereus	SME	1000	2000	2
		AME	1000	1000	1

3.4. Antioxidant activity

As ascorbic acid, PME, SME and AME had concentration dependent effects on the reduction of DPPH (Figure 2) and showed a highly significant (p= 0.0000) antioxidant activity.



Figure 2 Inhibition percentage (I%) of DPPH in the presence of different concentrations of PME, SME, AME and ascorbic acid (AC ASC), p= 0.0000.

The IC₅₀ values, determined from the linear curve of free scavenging activity I % in terms of extract concentration in μ g/ml, are presented in Table 5.

Table 5 IC_{50} of Ascorbic acid, AME, SME and PME

Extracts	Ascorbic acid	PME	SME	AME
IC50 % in µg/ml	7.320	8.925	0.568	3.792

In comparison with ascorbic acid which is a pure product, AME and SME were more active and PME was somewhat less active. SME had the highest antioxidant capacity with an IC_{50} 12.9 times less than ascorbic acid 6.7 and 15.7 times than AME and PME respectively.

4. Discussion

Preliminary phytochemical screening revealed that all fruit parts from the bemavo variety of *R. madagascariensis* contained the same chemical families (phenolic compounds and triterpenes). However, it did not provide information about the number, the nature and the respective amounts of each chemical group in each fruit part. These phytochemicals were also present in other parts (leaves and roots) of *R. madagascariensis* the variety name of which is unknown [31, 3, 16].

All the fruit extracts showed selective inhibitory activity against the tested bacterial strains. However in some cases, the disk diffusion and the microdilution assays gave different results. Bioactive compounds probably diffused little or not at all in solid medium. With disk diffusion method, PME was by far the most efficient extract. It clearly differed from SME and AME by the number of susceptible strains (9 of 11) and the inhibition level. However, with microdilution method there was no much difference between the 3 extracts. That might be due to the fact that PME contained active molecule(s) absent or in low amounts in AME and SME. In liquid medium, the activity of these molecules might be prevented by antagonist interactions which were low or non-existent in solid medium.

With disk diffusion *Vibrio fischeri* (IZD = 19 mm) was the most susceptible to PME, *Salmonella enterica* (IZD = 12.5 mm) to AME and *Salmonella enterica* and *Vibrio fischeri* (IZD = 10 mm) for SME. *Enterobacter aerogenes* (MIC = 125 µg/ml) was the most sensitive to the three extracts with microdilution assay. According to Fabry *et al.* [32], with MIC values below 8 mg/ ml, PME, SME and AME were considered having noteworthy antibacterial activity against all the tested bacterial strains. In reference to the more restrictive scale of Dalmarco *et al.* [33], 85 % of the bemavo fruit extracts had moderate (CMI \leq 500 µg/ml) and 12 % low (500 µg/ml < CMI \geq 2000 µg/ml) antibacterial activity.

Comparison of our results with those of other authors was not easy because the extracts, the antibacterial assays and the bacterial strains used were different. PME, SME and AME (great majority of CMI \leq 1 mg/ml) were by far more efficient than leaf extract from *R. madagascariensis* of Nigeria (75 mg/ml \leq CMI \geq 150 mg/ml) [16]. They also were more active (9 mm \geq IZD \leq 29 mm) than leaf extract from Bangladeshi *R. madagascariensis* (IZD < 9 mm) [15].

The effect nature of PME, SME and AME depended upon the bacteria but bactericidal activity (MBC/MCI >4) was the most recorded.

All extracts exhibited a highly significant (p = 0.0000) scavenging activity against DPPH. In comparison with ascorbic acid (IC₅₀ = 7.320 μ g/ml) used as reference antioxidant, the bemavo fruit extracts displayed high antioxidant activities: PME (IC₅₀ = 8.925 μ g/ml); SME (IC₅₀ = 0.568 μ g/ml) and AME (IC₅₀ = 3.792 μ g/ml), the highest scavenging activity against the 1,1-dipheny1–2-picrylhydrazyl radical,

Comparison of the IC_{50} of PME, SME and AME with those of other plant crude extracts determined by DPPH assay is shown in Table 6.

	Table 6 IC50 of PME, SME	, AME and other	plant crude extracts	determined by	v DPPH assay
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Plant source	Organs	IC ₅₀	Reference
Ravenala madagacariensis	Fruit parts	0.562 - 9.125 μg/ml	-
Garcinia multiflora	Root	16 μg/ml	[1]
Dalbergia sissoo	Stem bark	23.63 µg/ml	[34]
Kyllinga nemoralis	Whole plant	90.94 µg /ml	[3]
Uapaca togoensis	Trunk bark	4.758 mg/ml	[35]
Stevia rebaudiana	Leaves	268.31 - 340.12 mg/ml	[30]

According to the literature, the antioxidant and antimicrobial activities were correlated with phenolic compounds [36, 6]. The antibacterial and antioxidant activities of the bemavo fruit could therefore be induced by those secondary metabolites present in its pericarp, aril and seeds.

Further chemical and biological investigations will still be needed to determine the number and the identities of the active principles responsible for the antibacterial and the antioxidant properties.

5. Conclusion

In conclusion, all fruit parts of *R. madagascariensis* possessed an antibacterial activity which confirmed its traditional uses as antiseptics and to treat diarrhea and tooth decay. They could constitute sources of natural antibacterial agents and antioxidants to treat diseases related to oxidation stress.

Investigations on the other Ravenala varieties, Malama, Hiranirana and Horonorona are ongoing.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interests.

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