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Original Article

In vitro Platelet Aggregation-Stimulatory, Antibacterial, and Cytotoxic Activities of the Fresh Stem Juice from *Montrichardia arborescens* Schott (Araceae) – Evidence for Wound Healing-Stimulatory Properties

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ABSTRACT

Background: *Montrichardia arborescens* is traditionally used for wound healing, but without scientific evidence. In this study, the fresh stem juice was assessed for its platelet aggregation stimulatory and antibacterial activities and its safety.

Methods: The stem juice was evaluated at various dilutions for platelet aggregation-stimulatory activity in human platelet rich plasma (PRP) using norepinephrine as a positive control; antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* (gram-positive bacteria) as well as *Escherichia coli* and *Pseudomonas aeruginosa* (gram-negative bacteria) in a broth microdilution method using tetracycline as a reference compound; and absence of substantial cytotoxicity against cultured CHO cells in an MTT assay. Data (means ± SDs; n ≥ 3) were evaluated for statistically significant differences ($p < 0.05$) using ANOVA with Bonferroni's adjustments. **Results:** The stem juice stimulated platelet aggregation to nearly 100% at 50% (*v/v*) dilution and about 50% at 12.5% (*v/v*) dilution. It did not affect the growth of *E. coli* and *P. aeruginosa* at 50% (*v/v*) but partially inhibited that of *S. aureus* at 37.5% (*v/v*) and that of *S. pyogenes* completely at 12.5% (*v/v*). These effects were validated by those found with epinephrine 20 µM and tetracycline 10-60 µg/mL. CHO cell survival was only 3 ± 1% with the 50% (*v/v*) diluted stem juice but 64 ± 2% with the 12.5% (*v/v*) dilution. **Conclusion:** The *M. arborescens* stem juice exhibited substantial platelet aggregation-stimulatory and antibacterial activity at 12.5% (*v/v*) dilution which seemed safe to the normal tissues. These findings confirm its traditional use for wound healing.

Key words: *Montrichardia arborescens* Schott, Stem juice, *In vitro* studies, PRP, Platelet aggregation stimulation, Broth microdilution, Preliminary Antibacterial activity, MTT assay, Lack of cytotoxicity.

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INTRODUCTION

The mokomoko or mocou mocou *Montrichardia arborescens* (L.) Schott. (Araceae) is native to the tropical parts of the Caribbean, Central America, and South America including the Republic of Suriname.[1,2] It is frequently found in coastal areas, often forming dense stands along riverbanks, in swamps and creeks, and in brackish and fresh water estuaries.[3] The plant grows up to 3 meters tall, has an erect, woody stem with a diameter of up to 30 centimeters at the base that is covered with recurved prickles, and carries alternate, arrow-shaped leaves of 30 x 25 centimeters on leaf stalks with a length of roughly 30 centimeters.[1-3] The fruiting spadices are characteristic for the family Araceae and produce large infructescences that contain about eighty soft, egg-shaped fruits of about 2.5 centimeters in diameter[1-3] which have served as a food source for hinterland peoples in times of scarcity.[4]

Preparations from parts of *M. arborescens* are commonly used in various traditional medical practices throughout its natural range. The milky stem juice, slices or macerations from the stem or from a shoot, or a dressing made from crushed young shoots, are traditionally used to stop the bleeding of, and disinfect deep cuts and bruises as well as snakebites, scorpion stings, and stingray punctures.[1,2,5] Furthermore, decoctions and infusions from various other parts of the plant are used (both internally and externally) for treating elevated blood sugar levels; hypertension; tuberculosis; fever, coughing, and colds; rheumatism; rashes, itching, and burning of the skin; eyelid inflammation; thrush and warts; to stimulate diuresis; and as an aphrodisiac.[1,2,5-9]

Phytochemical analyses of extracts of the leaf and the stem juice of *M. arboresc*ens have demonstrated the presence of biologically active compounds such as alkaloids, flavonoids, tannins, saponins, carotenoids, cardiac glycosides, steroids, triterpenes, amino acids, proteins, and anthracenosides.[9-11] So far, only a handful of pharmacological studies has been carried out with this plant, reporting α-amylase inhibitory activity of a leaf extract,[9] and anti-inflammatory and antioxidant activities of the stem juice.[11] These observations speak in favor of the traditional uses of *M. arborescens* preparations against hyperglycemia and (other) conditions associated with inflammatory processes and oxidative stress.[1,2,5-9] So far, however, there are no scientific data to support the traditional use of the stem juice for treating wounds.

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A wound can be described as the loss or the disruption of the cellular, anatomical, and functional continuity of living tissues as a result of trauma.[12] The body deals with a wound by initiating a cascade of sequential but overlapping biochemical and cellular events to repair the injured tissues.[12] This involves a hemostasis stage when platelets aggregate to form a fibrin clot to stop the bleeding, an inflammatory stage to remove bacteria and cell debris and stabilize the wound, a proliferation stage to rebuild the wound area with new granulation tissue and vascularize the newly added materials, and a maturation stage to remodel and strengthen the newly formed tissues.[12] The orderly and timely manifestation of these events is imperative to speedily restore the anatomic and functional integrity of the injured site,[12] preventing the development of chronic wounds such as diabetic, vascular, and pressure ulcers.[13]

The stages of the wound healing process must proceed in the proper sequence and must continue for a specific duration and intensity.[12] For instance, inflammation is essential for the initiation of tissue repair, but should not continue excessively and/or prolonged in order to avoid the development of chronic wounds and unnecessary scaring.[14] The same holds true for the production of reactive oxygen species (ROS) during the inflammatory stage.[15] Low levels of ROS help protect the tissues from infection and trigger cell surviving signals for effective wound healing, but excessively high levels produce oxidative stress and cell damage, dysregulating the healing process.[15]

We previously reported that the fresh *M. arborescens* stem juice elicited encouraging anti-inflammatory and antioxidant activities *in vitro*, even when substantially diluted.[11] Thus, at dilutions of 12.5% (*v/v*) and greater, the stem juice inhibited the denaturation of bovine serum albumin by 50 to 75% and stabilized hypotonicity-induced human erythrocyte membrane lysis by more than 50%, and also scavenged about 50% of the free radicals generated from 2,2-diphenyl-1-picrylhydrazyl (DPPH) and achieved an antioxidant power of 2,616 ± 16 µM Fe^{2+} equivalents in a ferric-reducing antioxidant power (FRAP) assay.[11] These effects occurred in the physiologically feasible ranges of those achieved with the non-steroidal anti-inflammatory drug diclofenac 50 µg/mL and the antioxidant standard Trolox 1.0-6.0 µg/mL, a vitamin E analog.[11]

Anti-inflammatory and antioxidant activities are essential properties of wound healing-stimulatory (plant-derived) substances.[16,17] Two other important characteristics of such substances are meaningful platelet aggregation-stimulatory and antibacterial activity.[16,17] In the current study, the *M. arborescens* stem juice was evaluated for the latter properties using platelet-rich plasma (PRP) prepared from blood from healthy volunteers[18] and a broth microdilution method.[19] In addition, the stem juice was assessed for its safety by ascertaining its lack of significant cytotoxicity against cultured Chinese hamster ovary (CHO) cells.[20] This cell line is commonly used in biological, medical, and industrial research for tests on genotoxicity, cytotoxicity, and clonogenic cell death.[20] The results from the current and our previous study[11] are discussed.

MATERIALS AND METHODS

Plant material

Specimens of *M. arborescens* were collected in a remote, rural area at the outskirts of Suriname's capital city Paramaribo owned by our university (GPS coordinates 5.8129° N, 55.2175° W) that, to the best of our knowledge, had not been treated with herbicides and pesticides for at least the preceding six months. *M. arborescens* is not classified as a threatened, vulnerable, or endangered species by the International Union for Conservation of Nature and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. The collections were monitored by the National Herbarium of Suriname (BBS), a division of our university, which is in the possession of a collection permit from the Department for Nature Conservation from the Surinamese Ministry of Physical Planning, Land and Forestry Management. The collected plant specimens were authenticated by a staff member from the BBS, and voucher specimens were prepared and stored at the BBS for future reference (reference number UVS 19257).

The collected specimens of *M. arborescens* were first thoroughly washed with running tap water to remove adhering soil particles and other dirt, and twice with distilled water. After air drying, the stems were separated and cut into small pieces which were squeezed with a manual hand press juicer to obtain the juice. The liquid thus obtained was cleaned up by centrifugation for 5 min at 3,000 rpm to remove coarse particles, referred to as the fresh *M. arborescens* stem juice, and immediately used for experiments without further processing.

Drugs and chemicals

Hank's Balanced Salt Solution without Ca^{2+} and Mg^{2+} (HBSS-/-), fetal calf serum, and ethylenediaminetetraacetic acid-trypsin (EDTA-trypsin) were purchased from the American Type Culture Collection (Rockville, MD, USA). Epinephrine was from Fluka Biochemika (Buchs SG, Switzerland). Mueller-Hinton broth was from Becton Dickinson (Sparks, MD, USA), 0.5 McFarland standards from Remel Inc (Lenexa, KS, USA), and tetracycline from Rosco Diagnostica (Taastrup, Denmark). Penicillin, streptomycin, and amphotericin B were from Corning (Manassas, VA, USA), RPMI-1640 medium from Mediatech, Inc. (Manassas, VA, USA), and *L*-glutamine from Amresco LLC (Solon, OH, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was from Merck (Darmstadt, Germany), and cell culture-grade dimethylsulfoxide (DMSO) from VWR International LLC (West Chester, PA, USA). All other chemicals used were from our laboratory stock and were of the highest grade available.

Determination of platelet aggregation stimulatory activity of *M. arborescens* stem juice

The *M. arborescens* stem juice was assessed for its stimulatory activity on platelet aggregation according to a previously described 96-well plate-based aggregometry method[18] with some modifications. Thus, 10 mL-samples of venous blood were collected from healthy volunteers using siliconized syringes and 3.2% (*w/v*) sodium citrate anticoagulant tubes. The volunteers had not taken any medication for at least two weeks prior to the experiments, no coffee on the day of the blood collections, and had fasted for at least 4 h before donating blood. They had orally consented to participate in the current study after its purpose had been explained to them. Authorization for the collection of the blood samples had been obtained from the Ethics Committee of our institution.

The blood samples were centrifuged for 5 min at 200 x g and at room temperature to pellet the erythrocytes, which were discarded. The supernatants were transferred to sterile 15-mL tubes and centrifuged for 8 min at 1,000 x g and at room temperature. The upper one-third of the then obtained supernatant provided platelet-poor plasma (PPP) and the lower two-thirds together with the pellet gave platelet-rich plasma (PRP). The presence of platelets in the PRP, and their absence in the PPP, was verified by inspection under a phase-contrast microscope.

The PRP suspensions were diluted with sterile HBSS-/- to densities of 3.0×10^8 cells per mL after counting using a hemocytometer, and 100-µL aliquots were transferred into the wells of 96-well microplates. Control wells contained 100 µL PPP. Triplicate PRP- and PPP-containing wells were exposed for 30, 60, and 120 min to serial dilutions of the *M. arborescens* stem juice prepared with HBSS-/-. Incubations were in final volumes of 200 µL per well, at a temperature of 37 °C, and under continuous agitation. At the end of the incubations, the absorbance

of each of the wells was spectrophotometrically determined at a wavelength of 630 nm using an ELx800 TM Absorbance Microplate Reader (BioTek, Winooski, VT, USA). Wells containing HBSS-/- alone served as blanks. The platelet agonist epinephrine at the concentration of 20 µM in 200 µL HBBS-/- was used as a reference compound[18].

An increase in platelet aggregation following the addition of a stimulatory compound is reflected in an increase in light transmittance of the PRP with respect to that of untreated PRP and, accordingly, a decrease in light absorption. Thus, the percentage aggregation due to the *M. arborescens* stem juice samples or epinephrine was calculated using the formula:

$$\% \text{ platelet aggregation} = (A_{\text{treated PRP}} - A_{\text{treated PPP}})/(A_{\text{untreated PRP}} - A_{\text{untreated PPP}}) * 100\%$$

where $A_{\text{treated PRP}}$ is the absorbance at 630 nm of the PRP-containing wells exposed to the stem juice or epinephrine, $A_{\text{treated PPP}}$ that of the corresponding PPP-containing wells exposed to the stem juice or epinephrine, and $A_{\text{untreated PRP}}$ and $A_{\text{untreated PPP}}$ those of PRP- and PPP-containing wells that were only exposed to HBSS-/-.

Preliminary assessment of antibacterial activity of *M. arborescens* stem juice

A preliminary assessment of the antibacterial activity of the fresh *M. arborescens* stem juice was carried out using a broth microdilution assay.[19] The method involves the preparation of a liquid broth medium containing the test compound at various concentrations, into which a defined inoculum of bacteria was inoculated which was subsequently incubated and observed for growth inhibition.[19] The bacterial strains used were the gram-positive strains *Staphylococcus aureus* and *Streptococcus pyogenes* and the gram-negative strains *Escherichia coli* and *Pseudomonas aeruginosa* from the culture collection of the Department of Medical Microbiology of the Academic Hospital Paramaribo, Paramaribo, Suriname. The strains were cultured on Mueller-Hinton agar at 37°C overnight.

Based on the absorbance at a wavelength of 580 nm, the bacterial suspensions were standardized to 1 - 2 x 10^8 colony-forming units (CFU) per mL (0.5 McFarland standard) using HBSS-/- and diluted to 5 x 10^5 CFU/mL for use in the assay. Triplicate microtiter wells received 50 µL of this inoculum, 50 µL Mueller-Hinton broth, and 100 µL of the *M. arborescens* stem juice at various dilutions in HBSS-/-. Growth controls (50 µL bacterial suspension + 50 µL broth + 100 µL HBSS-/-) and sterility controls (100 µL + 100 µL HBSS-/- but no bacteria) were also incorporated in the experiments. Tetracycline 10 and 60 µg/mL was used as positive control. The final volume of each well was 200 µL. All samples had been sterilized through 0.46-µm filters as necessary. The contents of each well were thoroughly mixed, after which they were incubated for 24 h at 37 °C.

At the end of the incubations, bacterial growth was evaluated by measuring the turbidity of each well at a wavelength of 580 nm using an ELx800 TM Absorbance Microplate Reader (BioTek, Winooski, VT, USA). The absorbance values obtained were expressed relatively to that obtained for a Mc Farland standard (0.5%) that was taken to correspond to 1.5 x 10^8 CFU/mL.[19]

Assessment of cytotoxicity of *M. arborescens* stem juice against CHO cells

Finally, the *M. arborescens* stem juice was assessed for its potential cytotoxicity against cultured CHO cells. The cells had kindly been provided by dr. J.K. Wickleffe (Global Environmental Health Sciences, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA) and were originally from the American Type Culture Collection (Rockville, MD, USA). They were cultured in complete medium consisting of RPMI-1640 medium containing *L*-glutamine 2% (*w/v*) and supplemented with fetal calf serum 10% (*v/v*). The cells were maintained in 25-cm^2 culture flasks, at a temperature of 37 °C, a minimum relative humidity of 95%, and an atmosphere of 5% CO_2 in air.

For experiments, exponentially growing CHO cells were detached from the culture flasks using EDTA-trypsin. Triplicate cultures of CHO cells were inoculated in 96-well microplates at densities of 3 x 10^3 cells per 100 µL complete medium per well and allowed to stabilize for 24 h. The next day, the cell cultures were exposed to the *M. arborescens* stem juice at serial dilutions and in the presence of penicillin 100 IU/mL, streptomycin 100 µg/mL, and amphotericin B 5 µg/mL. Incubations were for three days and in final volumes of 200 µL per well.

At the end of the incubations, cellular responses were assessed with a MTT assay.[21] Briefly, the culture media were carefully aspirated from the cell cultures, which were then incubated for 3 h at 37 °C and in a dark environment in the presence of 90 µL serum-free medium and 10 µL filter-sterilized MTT solution of 5 mg/mL in phosphate-buffered saline. Next, the formazan crystals formed from the MTT (by the living /surviving cells) were solubilized with 100 µL of DMSO per well under continuous shaking for 15 min while the microplates were wrapped in aluminum foil.

The absorbance values of the colored formazan product in each well were then measured using an ELx800 TM Absorbance Microplate Reader (BioTek, Winooski, VT, USA) at a wavelength of 560 nm, corrected for background absorption (which was determined from wells containing medium alone, or stem juice-containing medium alone, but no cells) and semi-logarithmically plotted against the corresponding stem juice dilutions. All these values had first been corrected for non-specific interference (caused by plate variability, fingerprints, etc.) as indicated by the reference absorbance values at 650 nm. From the resulting dose-response curves, the stem juice dilution accomplishing 50% cytotoxicity was derived (the IC_{50} value), as well as the cytotoxicity of the stem juice at dilutions of 50, 37.5, 25, and 12.5 % (*v/v*).

Data processing and statistics

All experiments have been carried out at least three times in triplicate. Data (means ± SDs) have been compared using one-way ANOVA applying the Bonferroni post-hoc multiple comparison test and taking *P*-values < 0.05 to indicate statistically significant differences.

RESULTS

Platelet aggregation stimulatory activity of *M. arborescens* stem juice

The fresh stem juice from *M. arborescens* was assessed for its platelet aggregation-stimulatory activity at various dilutions and for various periods of time using PRP prepared from human blood.[18] The method is based on the decrease in the absorbance of PRP treated with a platelet aggregation-stimulatory substance with respect to that of untreated PRP at a wavelength of 630 nm[18]. The decrease in absorbance of the PRP caused by the platelet-stimulatory compound epinephrine (20 µM) served as a positive control.[18]

As shown in Table 1, the use of epinephrine 20 µM led to a decrease in the absorbance of the PRP of 70 to 80% after 30, 60, and 120 min. Thus, this compound indeed stimulated platelet aggregation, validating the usefulness of the assay to evaluate the *M. arborescens* stem juice for its platelet aggregation-stimulatory activity. Exposure of the PRP to the *M. arborescens* stem juice at the dilution of 50% (*v/v*) for either 30, 60, or 120 min led to a decrease in its absorbance of nearly 100% (Table 1). This suggests that the 50% (*v/v*) diluted stem juice maximally stimulated platelet aggregation. When the stem juice was diluted

Table 1. Platelet aggregation-stimulatory activity of *M. arborescens* stem juice and epinephrine as determined by the absorbance of PRP at a wavelength of 630 nm with respect to that of untreated PRP, taking the decrease in absorbance as a measure for platelet aggregation-stimulatory activity. Values are means ± SDs of at least three experiments performed in triplicate and have been compared for statistically significant differences using ANOVA with post hoc Bonferroni adjustments.

Treatment	% decrease in absorbance of PRP at 630 nm		
	30 min	60 min	120 min
Epinephrine 20 µM	82 ± 9	77 ± 7	69 ± 8
M. arborescens stem juice 50.0% (*v/v*)	95 ± 3[a]	91 ± 4[a]	93 ± 8[a]
M. arborescens stem juice 37.5% (*v/v*)	82 ± 3[b]	75 ± 2[b]	68 ± 1[b]
M. arborescens stem juice 25.0% (*v/v*)	70 ± 4[c]	58 ± 1[c]	52 ± 4[c]
M. arborescens stem juice 12.5% (*v/v*)	58 ± 2[d]	46 ± 6[d]	44 ± 9[d]

Values followed by different letters in superscript are statistically significantly different from each other ($p < 0.01$).

Table 2. Antibacterial activity of *M. arborescens* stem juice and tetracycline as determined by the number of colony-forming units (CFU)/mL produced after 24 h in a broth microdilution assay. Values are means ± SDs of at least three experiments performed in triplicate and have been compared for statistically significant differences using ANOVA with post hoc Bonferroni adjustments.

Treatment	CFU/mL			
	S. pyogenes (gram+)	S. aureus (gram+)	E. coli (gram-)	P. aeruginosa (gram-)
Growth control	$> 10^5$	10^5	$> 10^5$	$> 10^5$
M. arborescens stem juice 50.0% (*v/v*)	0	10^4 - 10^5	$> 10^5$	$> 10^5$
M. arborescens stem juice 37.5% (*v/v*)	0	10^4 - 10^5	$> 10^5$	$> 10^5$
M. arborescens stem juice 25.0% (*v/v*)	0	$> 10^5$	$> 10^5$	$> 10^5$
M. arborescens stem juice 12.5% (*v/v*)	0	$> 10^5$	$> 10^5$	$> 10^5$
Sterility control	0	0	0	0
Tetracycline 10 µg/ mL	0	10^4 - 10^5	10^4 - 10^5	$> 10^5$
Tetracycline 60 µg/ mL	0	10^4 - 10^5	10^4 - 10^5	$> 10^5$

Table 3. Cytotoxicity of *M. arborescens* stem juice towards Chinese hamster ovary cells as determined by an MTT assay. Values are means ± SDs of at least three experiments performed in triplicate and have been compared for statistically significant differences using ANOVA with post hoc Bonferroni adjustments.

	% cell viability
M. arborescens stem juice 50.0% (*v/v*)	3 ± 1[a]
M. arborescens stem juice about 37.5% (*v/v*)	24 ± 2[b]
M. arborescens stem juice about 25.0% (*v/v*)	42 ± 1[c]
M. arborescens stem juice 21 ± 1% (*v/v*)	50[d]
M. arborescens stem juice about 12.5% (*v/v*)	64 ± 2[e]

Values followed by different letters in superscript are statistically significantly different from each other ($p < 0.01$)

to 37.5% (*v/v*), 25% (*v/v*), or 12.5% (*v/v*), the absorbance of the PRP decreased in a proportionate manner at all three periods of exposure (Table 1). The absorbance values caused by the different dilutions differed statistically significantly differences from each other (Table 1; p values < 0.01; ANOVA). Importantly, at the dilution of 12.5% (*v/v*), the stem juice had still retained about 50% of its platelet aggregation-stimulatory activity (Table 1), emphasizing the substantial potency of this substance.

Antibacterial activity of *M. arborescens* stem juice

Next, the *M. arborescens* stem juice was assessed for its antibacterial activity against both gram-positive (*S. pyogenes* and *S. aureus*) and gram-negative (*E. coli* and *P. aeruginosa*) bacteria using a broth microdilution method. Bacterial growth was assessed after 24 h incubation based on changes in turbidity at a wavelength of 580 nm. The stem juice was evaluated at the dilutions of 50, 37.5, 25, or 12.5% (*v/v*). Tetracycline 10 and 60 µg/mL was used as positive control. The results from these studies are given in Table 2.

The stem juice completely inhibited the growth of *S. pyogenes* at all tested concentrations including the lowest (12.5% *v/v*), while that of *S. aureus* was partially inhibited at 37.5% (*v/v*) and 50.0% (*v/v*). However, the growth of *E. coli* and *P. aeruginosa* remained unaffected even at 50.0% (*v/v*). Tetracycline (10 and 60 µg/mL) exhibited a comparable spectrum of activity: complete inhibition of the growth of *S. pyogenes*, partial inhibition of that *S. aureus* and *E. coli*, and no inhibition of that of *P. aeruginosa*. These findings suggest that the *M. arborescens* stem juice elicited selective antibacterial activity against gram-positive bacteria, with a potency profile that mirrored that of tetracycline.

Cytotoxicity of *M. arborescens* stem juice towards CHO cells

Finally, the *M. arborescens* stem juice was evaluated for its cytotoxicity against cultured CHO cells. This was done by exposing CHO cell cultures to serial dilutions of the stem juice and determining their viability after 3 days using an MTT assay.[21] The results from these experiments are given in Figure 1 and Table 3.

The viability of the cells decreased in a dose-dependent manner with increasing degree of dilution of the stem juice (Figure 1). Applying linear regression to the dose-response curve, the half maximum inhibitory dilution (ID_{50}) of the stem juice (*i.e.*, the dilution that elicited 50% cytotoxicity) was estimated at 21 ± 1% (*v/v*) (Figure 1; Table 3). In the same way, the proportion of surviving cells after exposure to stem juice dilutions of 12.5, 25.0, 37.5, and 50.0% (*v/v*) was estimated at 64 ± 2, 42 ± 1, 24 ± 2, and 3 ± 1%, respectively (Table 3). These values differed statistically significantly from each other (p values < 0.01;

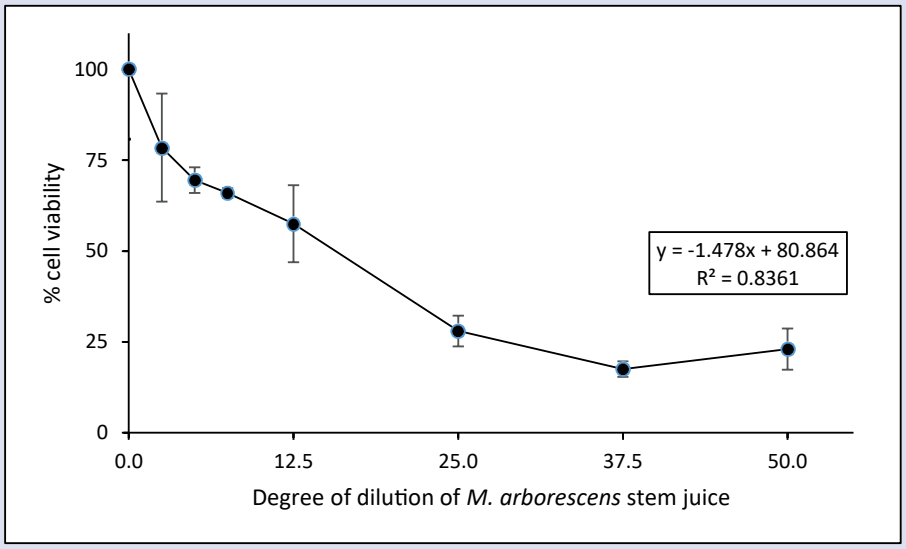

Figure 1. Inhibition of proliferation of cultured Chinese hamster ovary cells after exposure for 3 days to serial dilutions of the stem juice of *M. arborescens*. Data points are means ± SDs (vertical bars; n ≥ 3).

ANOVA). Thus, the stem juice was substantially more cytotoxic when more concentrated, but only lowly cytotoxic at the higher dilutions that accomplished substantial platelet aggregation and antibacterial effects. This suggests that the stem juice is safe to the normal tissues.

DISCUSSION

Wound healing-stimulatory substances including those derived from plants, should accomplish at least four pharmacological activities, namely platelet stimulatory, antimicrobial, anti-inflammatory, and antioxidant activities.[16,17] We previously reported that the fresh stem juice from *M. arborescens* exhibited meaningful anti-inflammatory activity in a hemolysis inhibition assay and a protein denaturation inhibition assay, as well as substantial antioxidant activity in a DPPH assay and a FRAP assay.[11] In the current study, we found evidence that the stem juice, in addition, stimulated the aggregation of platelets in PRP, and elicited partial antibacterial activity (*i.e.*, against gram-positive strains). Moreover, these activities - as well as the anti-inflammatory and antioxidant activities[11] - occurred at dilutions of the stem juice that were only slightly cytotoxic to cultured CHO cells. Together, these findings strongly suggest that the *M. arborescens* stem juice possesses encouraging wound healing-stimulatory properties and is safe to use, supporting its traditional use for managing various types of wounds.[1,2,5]

The current data provide, to our knowledge for the first time, scientific evidence for platelet aggregation stimulatory activity of *M. arborescens*. This activity has also not been reported for *M. linifera* (Schott), the only other species in the genus *Montrichardia*, that has rather extensively been evaluated for a variety of pharmacological activities.[22] However, the relative handful of investigations with members of the Araceae family other than those in the genus *Montrichardia* reported platelet aggregation inhibitory activity rather than platelet aggregation stimulatory activity. This held true for, among others, the sweet flag *Acorus calamus* L., the butanolic fraction from a methanolic rhizome extract of which strongly inhibited arachidonic acid-induced platelet aggregation.[23] On the other hand, members of plant families other than the Araceae elicited considerable platelet stimulation in animal models.[24-28] Examples are parts of the papaya *Carica papaya* L. (Caricaceae), the sweet potato *Ipomoea batatas* (L.) Lam. (Convolvulaceae), the sessile joyweed *Alternanthera sessilis* (L.) R.Br. ex DC. (Amaranthaceae), and the asthma-plant *Euphorbia hirta* L. (Euphorbiaceae).[24-28]

The discrepancies among the current study and those mentioned above, may tentatively be attributed to the presence of distinct biologically active ingredients in the different plants which may have elicited dissimilar effects on platelet aggregation. For instance, the Solomon's lily *Eminium spiculatum* (Blume) Kuntze (Araceae) contains the flavonoids luteolin, luteolin-7-O-glucoside, and vitexin which inhibited ADP and collagen-induced platelet aggregation in PRP[23,29]. On the other hand, cuminaldehyde (in, among others, cumin, eucalyptus, and myrrh), eugenol (in, among others, clove buds, cinnamon bark, and turmeric), as well as ursolic acid and its isomer oleanolic acid (in, among others, olive oil as well as rosemary and sage) stimulated platelet aggregation in various *in vitro* assays.[30,31]. One should also take into account that most investigations about the effects of test compounds on platelets were not concerned with the stimulation of platelet aggregation but with the prevention of this phenomenon in platelet-related cardiovascular diseases, focusing on compounds with anti-platelet aggregation effects.[18] Thus, more comprehensive studies of (plant-derived) substances - including the *M. arborescens* stem juice - dealing specifically with the stimulation of platelet aggregation are needed.

As far as we know, the current study is also the first to report on a potential antimicrobial activity of the stem juice of *M. arborsescens*, although apparently only against gram-positive bacteria. A study with an alcoholic leaf extract of *M. linifera* also reported a greater effect against gram-positive bacteria (*Enterococcus faecalis*, *S. aureus*, and *Staphylococcus epidermidis*) when compared to gram-negative bacterial strains[32]. However, another study with this plant found the opposite, *i.e.*, a (relatively small) inhibitory effect against the gram-negative bacterial strain *Aeromonas hydrophila*[33]. And still another study recorded antibacterial effects against the gram-positive *S. aureus* as well as the gram-negative *E. coli*[22]. Markedly, several members of the Araceae in genera other than *Montrichardia* (such as taro in the genus *Colocasia*, elephant's ear in the genus *Alocasia*, and water lettuce in the genus *Pistia*) were also active against both gram-positive and gram-negative bacteria.[34-36]

These differences in antibacterial activity are also difficult to explain. It is possible that the pharmacologically active constituent(s) in the aqueous *M. arboresens* stem juice do(es) not readily interact with the lipopolysaccharide-containing outer membrane of gram-negative bacteria[37], providing a tentative explanation for the far lesser

susceptibility of these strains to the antibiotic effects of the stem juice when compared to gram-positive bacteria. However, the passage of molecules through surface bacterial structures is also determined by various other physico-chemical properties such as chemical structure, size, and charge.[38] Therefore, establishing the chemical nature of the pharmacologically active ingredient (s) in the stem juice and its/their contribution to the antibacterial effect, is warranted. Still, the apparent antibacterial activity of the *M. arborescens* stem juice against *S. pyogenes* and *S. aureus* noted in the current study is encouraging when considering the involvement of these commensals of the human microbiota in, among others, wound infections.[39]

Another meaningful finding of the current study was the absence of a substantial effect of the *M. arborescens* stem juice on the viability of cultured CHO at the dilutions that stimulated platelet aggregation and eliminated the gram-positive bacteria, *i.e.*, at ≤ 12.5% (*v/v*). Notably, at these dilutions, the stem juice was previously found to accomplish meaningful anti-inflammatory and antioxidant activities.[11] Thus, the stem juice seemed to be safe to the normal tissues at relatively low concentrations that were still capable of eliciting meaningful platelet stimulatory, antibacterial, anti-inflammatory, and antioxidant activities. This is important, since the pure, undiluted *M. arborescens* stem juice has been associated with the development of dermatitis in nurserymen who had come into contact with the plant.[40] This may be attributable to the greater degree of acridity and causticity of the pure, undiluted stem juice, and is in accordance with the current results showing clear cytotoxicity of the more concentrated stem juice towards the CHO cells.

Summarizing, the results from the current study suggest that the fresh stem juice from *M. arborescens* achieved platelet-aggregation stimulatory and (partial) antibacterial activities at dilutions that were probably safe and were previously found to produce meaningful anti-inflammatory and antioxidant activities.[12] Thus, the *M. arborescens* stem juice may fulfill four key criteria for wound healing stimulatory activity assigned to natural phytomedicines, namely platelet stimulatory, antibacterial, anti-inflammatory, and antioxidant activities.[16,17] These observations support the traditional use of this preparation for wound healing[1,2,5] and suggest that its pharmacologically active ingredient(s) interfere(s) with events occurring in the hemostatic and inflammatory stage of the wound healing process.[12]

Interestingly, the results from preliminary studies in our laboratory indicated that the *M. arborescens* stem juice at the dilution of 12.5% (*v/v*) did not affect scratch-wound closure of monolayers of human umbilical vein endothelial cells and did not stimulate the formation of capillary-like structures by these cells when seeded on an artificial basement membrane (not shown). These observations tend to exclude an effect of the stem juice on cell migration in the proliferation phase,[12] speaking in favor of an event in (an)other stage(s) of the wound healing process (such as the hemostatic and inflammatory stage).[12] However, a very recent study with ethanolic extracts from the stem and the petioles of *M. linifera*[41] reported an increase in motility and proliferation of cultured fibroblasts in a scratch-wound closure and a bromodeoxyuridine cell proliferation assay. These observations support that the *M. linifera* preparations act in the proliferation phase of the wound healing process.[12]

The reasons for these differences are again unclear and emphasize the need for more comprehensive preclinical experiments to identify the precise mechanism(s) of action of the *M. arborescens* stem juice as well as the chemical nature of its active ingredient(s). Eventually, studies with animal models and human volunteers must corroborate the clinical usefulness of wound healing stimulatory preparations containing the *M. arborescens* stem juice. Notwithstanding, the current data together with those previously published[11] support the ethnopharmacological use of the *M. arborescens* stem juice for wound healing.[1,2,5] This finding is important for populations with limited access to western medicinal facilities such as those living in the hinterlands of Suriname and other parts of the Americas.

CONCLUSIONS

The fresh stem juice of *M. arborescens* exhibited appreciable *in vitro* platelet aggregation stimulatory activity as well as activity against (some) gram-positive bacterial strains. These effects occurred at dilutions that were lowly cytotoxic to CHO cells and previously found to exhibit notable anti-inflammatory and antioxidant activities. Together, these findings support the traditional use of this preparation to stop the bleeding of and disinfect various types of wounds, and justify efforts aimed at its development as a herbal wound healing-stimulatory substance.

LIST OF ABBREVIATIONS

BBS: National Herbarium of Suriname

CFU: colony-forming units

CHO cells: Chinese hamster ovary cells

DMSO: dimethylsulfoxide

EDTA: ethylenediaminetetraacetic acid-trypsin

HBSS-/-: Hank's Balanced Salt Solution without Ca^{2+} and Mg^{2+}

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

PPP: platelet-poor plasma

PRP: platelet-rich plasma

DECLARATION

Part of this work has been presented as part of a series of guest lectures within the context of the annual summer schools of the School of Public Health of the University of Pittsburgh (Pittsburgh, PA, USA) held in July 2024 and May 2025 at the Faculty of Medical Sciences of the Anton de Kom University in Paramaribo, Suriname (Mans DRA. *Plant-based traditional medicine in Suriname - background, rationale, and product development*).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of the Faculty of Medical Sciences of the Anton de Kom University of Suriname, Paramaribo, Suriname, has approved and authorized the study.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they do not have competing interests.

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AUTHORS' CONTRIBUTIONS

DM designed the experiments, oversaw the work, interpreted the experimental data, and wrote the manuscript. TM assisted with the microbiological studies and helped interpret the data from these studies. PF, MD, and JP carried out the practical work and helped with the interpretation of the experimental data. VHM, EPFIj and WJTS helped with the interpretation of the experimental data and reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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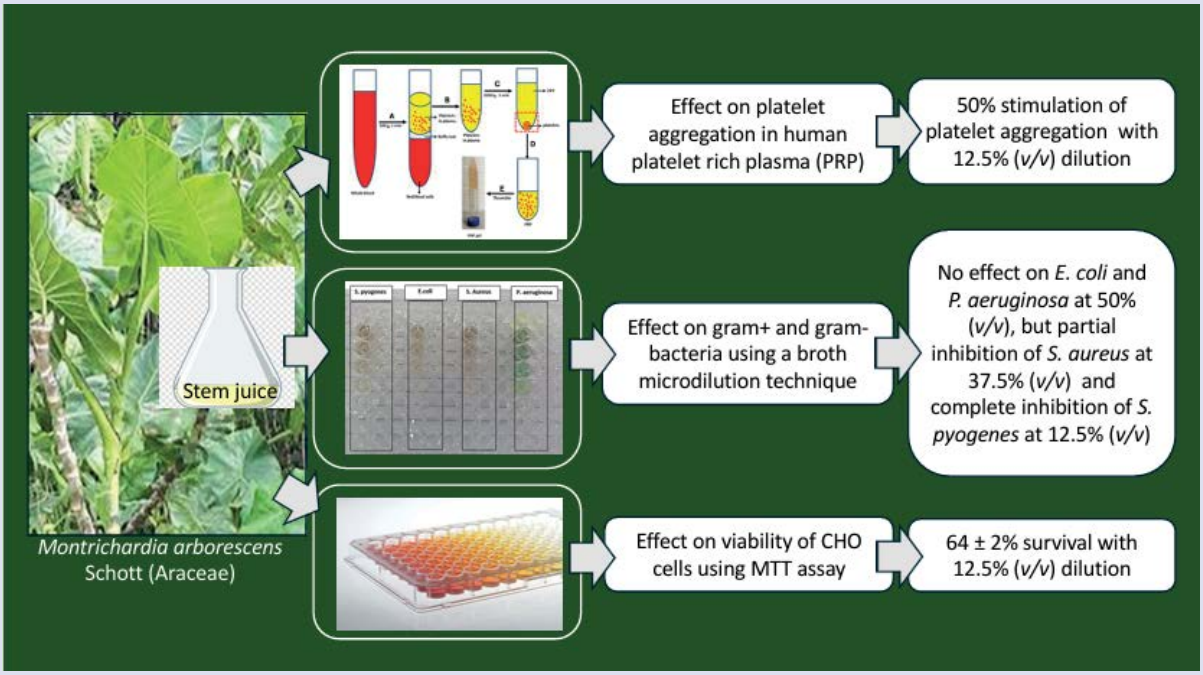
GRAPHICAL ABSTRACT



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