

In vitro Anti-inflammatory and Antioxidant Activities as well as Phytochemical Content of the Fresh Stem Juice from *Montrichardia arborescens* Schott (Araceae)

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ABSTRACT

Background: *Montrichardia arborescens* Schott (Araceae) is traditionally used for treating, among others, inflammatory conditions, but so far without scientific evidence. In this study, the fresh stem juice was assessed for its anti-inflammatory and antioxidant activities and phytochemical content. **Methods:** The stem juice's anti-inflammatory activity was evaluated using a heat-induced bovine serum albumin (BSA) denaturation assay and a hypotonicity-induced human erythrocyte membrane lysis assay. Its antioxidant activity was determined by a DPPH free radical-scavenging assay and a FRAP assay. Diclofenac and Trolox were used as reference compounds. Its phytochemical content was qualitatively explored by standard methods. Data (means \pm SDs; $n \geq 3$) were evaluated for statistically significant differences ($p < 0.05$) using ANOVA with Bonferroni post hoc adjustments. **Results:** At $\geq 12.5\%$ (v/v), the stem juice inhibited BSA denaturation by 50%-75% and membrane lysis by roughly 90%. At 0.20-7.0% (v/v), it did not affect BSA denaturation but stabilized membranes by $> 50\%$. The juice scavenged DPPH free radicals at an EC_{50} of $2.0 \pm 0.2\%$ (v/v) and achieved at that dilution an antioxidant power of $2,616 \pm 16 \mu\text{M Fe}^{2+}$ equivalents. All the effects were in the range of those found with diclofenac 50 $\mu\text{g/mL}$ and Trolox 1.0-6.0 $\mu\text{g/mL}$. The stem juice contained phenolic compounds, flavonoids, tannins, alkaloids, saponins, and glycosides. **Conclusion:** The *M. arborescens* stem juice exhibited appreciable anti-inflammatory activities that might mainly be associated with the inhibition of the release of inflammatory mediators, along with notable antioxidant activity, which might be attributable to phytochemicals with known anti-inflammatory and antioxidant properties.

Key words: *Montrichardia arborescens* Schott, Stem juice, In vitro studies, Anti-inflammatory activity, Antioxidant activity, Phytochemical content.

INTRODUCTION

Inflammation is part of a complex physiological defense mechanism of the body against tissue damage caused, for instance, by irritation, injury, or infection.^{1,2} The aim of this phenomenon is the elimination of the harmful stimulus, the removal of necrotic cells and damaged tissues, and the initiation of repair and the restoration of the functions of the affected structures.^{1,2} Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to a local harmful stimulus such as a *Staphylococcus aureus* infection of the skin that results in a boil, and usually lasts only a few days.^{1,2} On the other hand, chronic or prolonged inflammation can last for months or years and is associated with considerable destruction of the affected tissues.¹⁻³ Chronic inflammation occurs, for instance, in allergies, chronic obstructive pulmonary disease, atherosclerosis, and rheumatoid arthritis,^{1,2} but also in non-communicable diseases such as diabetes mellitus and cardiovascular disease.¹⁻³

Inflammations are associated with the recruitment of specific immune cells (including neutrophils, basophils, mast cells, T-cells, and B-cells) from the blood into the injured tissues, the dilation of blood vessels, and the production of a variety of inflammatory mediators such as cytokines,

growth factors, eicosanoids like prostaglandins, complement and peptides.^{1,2} These substances are not only involved in the redness, swelling, and pain that are characteristic for an inflammation,^{1,2} but also in the generation of reactive oxygen-derived species (ROS).⁴ In the absence of counteracting antioxidants or radical scavengers, ROS can cause oxidative stress that contributes to the tissue injury at the site of inflammation.⁴ This occurs particularly in chronic inflammations.⁴

Inflammations are generally treated with compounds that reduce the symptoms and/or provide pain relief such as the analgesic paracetamol,⁵ nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin⁵ and cyclooxygenase inhibitors such as ibuprofen, naproxen, and celecoxib,^{6,7} and corticosteroids such as cortisol and dexamethasone.⁸ However, paracetamol has been associated with nausea, vomiting, and constipation⁵, NSAIDs with gastric ulceration, bleeding, asthma exacerbations, kidney damage, and an increased the risk of heart attack and stroke,^{6,7} and corticosteroids with Cushing's syndrome, adrenal suppression, hyperglycemia, dyslipidemia, cardiovascular disease, osteoporosis, psychiatric disturbances, and immunosuppression.⁸ These considerations indicate the need of at least equally efficacious but less toxic anti-inflammatory compounds.

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The mokomoko or mocou mocou *Montrichardia arborescens* (L.) Schott. is a member of the Araceae or arum family and is native to the West Indies and the tropical parts of the Americas including the Republic of Suriname.^{9,10} It is commonly found along river banks, swamps, and creeks with either salt-, fresh-, or brackish water.¹¹ The plant can reach a height of about three meters, a stem diameter of twenty-five centimeters, and leaf stalks of thirty centimeters long with triangular-shaped leaves of ten to thirty centimeters.¹¹ The leaves are a food source for animal species such as manatees.¹² Like all Araceae family members, *M. arborescens* is characterized by flowers on a cylindrical or ellipsoid inflorescence called a spadix that is partially enclosed in a curved leaf-like bract called a spathe.¹¹ The fruiting spadices produce large infructescences which contain about eighty edible yellow fruits¹¹ and have been consumed by hinterland peoples in times of food scarcity.¹³

The stem juice as well as slices and macerations of the stem from *M. arborescens* are traditionally used in Suriname and other South American and Caribbean countries to stop bleeding.^{9,10} The stem juice is also used as an aphrodisiac, either by applying it externally¹⁴ or by drinking it as a concoction prepared with parts of certain other plants.¹⁵ The leaf is included in herbal baths against rashes, itching, and burning of the skin⁹ as well as fever.¹⁰ And preparations from the stem and the leaf would treat coughs and colds, eyelid inflammation, rheumatism, diabetes mellitus, hypertension, tuberculosis, thrush, and warts.^{9,16} Phytochemical analyses of a leaf extract have demonstrated the presence of biologically active compounds such as alkaloids, flavonoids, saponins, and tannins.¹⁶ However, up to now, scientific data to support the traditional uses including those against inflammatory diseases, are scant and limited to indications for antidiabetic activity of a leaf extract in an α -amylase inhibition assay.¹⁶

For this reason, the current study has evaluated the fresh stem juice of the plant for its potential anti-inflammatory activity using two well-known *in vitro* assays. As oxidative stress has been associated with the development, progression, and complications of inflammatory diseases,⁴ antioxidants may provide protection from free radical-induced tissue damage during the inflammatory response.⁴ Therefore, the *M. arborescens* stem juice has also been assessed for its antioxidant potential using two common *in vitro* assays. In an attempt to relate the possible anti-inflammatory and antioxidant activities of the stem juice to its bioactive constituents, its phytochemical composition was also verified using a series of qualitative analyses.

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. In accordance with the International Union for Conservation of Nature's Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora, we ascertained that there were no restrictions for collecting this plant. The collections were monitored by the National Herbarium of Suriname (BBS), a division of our university that 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 plant was 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 cut into small pieces which were squeezed with a manual hand press juicer to obtain the juice. The liquid thus obtained was centrifuged for 5 min at 3,000 rpm to remove coarse particles. It was immediately used for experiments without further processing, and has been referred to as the fresh *M. arborescens* stem juice throughout this paper.

Drugs and chemicals

Bovine serum albumin (BSA), fraction V, was from Roche Diagnostics (Mannheim, Germany). Tris Base (99.8% purity) was from G-Biosciences (St. Louis, MO, USA). Acetic acid glacial (CH₃COOH) was from Solon Industrial Parkway (Solon, OH, USA). The non-steroidal anti-inflammatory drug diclofenac (75 mg/mL, as the sodium salt) was from Sandoz BV (Veluwezoom, Almere, The Netherlands). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) as well as iron (III) chloride hexahydrate (FeCl₃ · 6H₂O), iron (II) sulfate heptahydrate (FeSO₄ · 7H₂O), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and the water-soluble vitamin E analog Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was from AppliChem GmbH (Darmstadt, Germany). All other chemicals used were from our laboratory stock and were of the highest grade available.

Determination of anti-inflammatory activity by inhibition of heat-induced bovine serum albumin denaturation

The anti-inflammatory activity of fresh *M. arborescens* stem juice was determined using a heat-induced BSA denaturation assay.¹⁷ Thus, a stock solution of BSA 5% (w/v) was prepared in a mixture of Tris-acetate buffer 0.05 M, which was adjusted to pH 6.8 with acetic acid glacial. Several amounts of the stem juice were added to 100 μ L aliquots of the BSA stock solution and distilled water to give 1-mL samples that contained the stem juice at various dilutions. Protein denaturation was induced by heating the samples for 7 min at 70 °C in a water bath. After cooling to room temperature, the turbidity of the solutions was spectrophotometrically measured at a wavelength of 660 nm. Solutions containing distilled water instead of the stem juice served as controls, and values found with these preparations were taken to represent 100% protein denaturation. Samples without BSA were used as blanks. In parallel experiments, the anti-denaturing activity of diclofenac¹⁸ was used as a positive control. The percentage inhibition of denaturation accomplished by the stem juice dilutions or the diclofenac samples was calculated as follows:

$$\% \text{ inhibition of denaturation} = \left[\frac{\text{Abs}_{\text{control}} - (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})}{\text{Abs}_{\text{control}}} \right] \times 100\%$$

where Abs_{control} is the absorbance of the controls, Abs_{sample} that of the stem juice or diclofenac samples, and Abs_{blank} that of the blank.

Determination of anti-inflammatory activity by inhibition of hypotonicity-induced human erythrocyte membrane lysis

The anti-inflammatory activity of fresh *M. arborescens* stem juice was also determined by a method based on the inhibition of the lysis of erythrocyte membranes in a hypotonic solution.¹⁹ Thus, specifically for this purpose, blood was collected from healthy volunteers in tubes with 3.2%-buffered sodium citrate solution. The volunteers had not taken any NSAID for at least two weeks prior to the experiment. They had orally consented to donate blood after the purpose of the study had been explained to them. Authorization for the collection of blood was obtained from the Ethics Committee of our institution (Faculty of Medical Sciences, Anton de Kom University of Suriname). The blood samples were centrifuged for 5 min at 2,500 rpm, the pelleted erythrocytes were washed three times with isosaline (0.9% (w/v) NaCl

in distilled water) and reconstituted with isosaline to give a 10% (v/v) suspension. Next, 0.5 mL of *M. arborescens* stem juice at various dilutions was added to 0.5 mL of 10% (v/v) erythrocyte suspensions and 2 mL hyposaline (0.36% (w/v) NaCl in distilled water) to give samples with the stem juice at various end-concentrations. The mixtures were incubated for 15 min at 37 °C and then centrifuged for 5 min at 2,500 rpm, after which the hemoglobin content of the supernatant was spectrophotometrically determined at a wavelength of 560 nm. Controls consisted of 2.5 mL hyposaline and 0.5 mL of 10% (v/v) erythrocyte suspension without stem juice, and the spectrophotometric results with these samples were considered producing 100% hemolysis. Samples with the stem juice and erythrocytes in isosaline served as blanks. Again, diclofenac was used as a reference compound.¹⁸ The percentage of erythrocyte membrane stabilization was calculated as follows:

$$\% \text{ membrane stabilization} = 100 - [(Abs_{\text{sample}} - Abs_{\text{blank}} / Abs_{\text{control}}) \times 100],$$

where Abs_{sample} the absorbance of the stem juice or diclofenac samples, Abs_{blank} that of the blank, and Abs_{control} is that of the control.

Determination of antioxidant activity by the 1,1-diphenyl-2-picrylhydrazyl assay

Next, the *M. arborescens* stem juice was assessed for its antioxidant activity using a DPPH free radical scavenging assay.²⁰ Thus, 0.5 mL samples of the stem juice at various dilutions were mixed with 3 mL absolute ethanol and 0.5 mL DPPH solution of 0.5 mM in ethanol. After 90 min in the dark and at room temperature, the absorbance of the solutions was measured at a wavelength of 517 nm against blanks consisting of a mixture of 3.3 mL ethanol and 0.5 mL sample. The control solution consisted of 3.5 mL ethanol and 0.3 mL DPPH solution. The results were compared to those obtained with the positive control Trolox.²¹ The percentage antioxidant activity (AA %) of each dilution of the *M. arborescens* stem juice was determined using the formula:

$$AA \% = 100 - \{[(Abs_{\text{sample}} - Abs_{\text{blank}}) / Abs_{\text{control}}] \times 100\},$$

where Abs_{sample} was the absorbance of the stem juice or Trolox, Abs_{blank} that of the blank, and Abs_{control} that of the control. The absorbance values of the stem juice or Trolox dilutions were converted into AA % which and recorded. These values were then plotted against the corresponding concentrations to yield dose-response curves from which IC_{50} value were derived, i.e., the concentrations of the stem juice (in % (v/v) dilution) or Trolox (in µg/mL) accomplishing a 50% decrease in absorbance (i.e., a 50% decrease in the initial number of DPPH free radicals) when compared to untreated controls.

Determination of antioxidant activity by ferric-reducing antioxidant power

The antioxidant activity of the *M. arborescens* stem juice was also determined by a spectrophotometric ferric-reducing antioxidant power (FRAP) method.²² Thus, 3 mL of working FRAP reagent (daily freshly prepared) was mixed with 100 µL of *M. arborescens* stem juice at various dilutions and 1 mL distilled water. The FRAP reagent consisted of TPTZ 10 mM in HCl, $FeCl_3 \cdot 6H_2O$ 20 mM, and acetate buffer 300 mM pH 3.6 in the proportion of 1/1/10 (v/v/v). After thorough mixing and incubation for 30 min in the dark and at room temperature, absorbance values at a wavelength of 593 nm were recorded against blanks consisting of samples in which the stem juice samples were substituted by distilled water. The change in absorbance was directly related to the total reducing power of the electron-donating antioxidants present in the stem juice. This was estimated from a calibration curve constructed from the absorbance of different concentrations of $FeSO_4$ at 593 nm. Data were expressed as µM Fe^{2+} equivalents reduced. Again, Trolox was used as a reference compound.²¹

Qualitative phytochemical analyses

The fresh *M. arborescens* stem juice was qualitatively evaluated for its phytochemical constituents using standard methods of analysis.^{23,24}

The presence of phenolics and tannins in the stem juice was assessed by adding a few drops of 1% (v/v) $FeCl_3$ solution to 2 mL of the juice and the observation of the formation of a blue-black, blackish-brown, green, or blue-green precipitation.

In order to demonstrate the presence of flavonoids, 2 mL of the stem juice were mixed with 2 mL of 2.0% (w/v) NaOH. The appearance of a yellow color that disappeared upon the addition of 2 drops of diluted HCl would indicate the presence of flavonoids.

Testing for alkaloids was done by Wagner's method. Thus, 2 mL of the stem juice was mixed with 2 mL of 1% (v/v) HCl and gently heated. Wagner's reagent (2.5 g iodine (I_2) and 12.5 g of KI_2 in 250 mL of distilled water were then added to the mixture. The formation of a brownish to yellowish precipitate indicated the presence of alkaloids.

The Salkowski test was used to provide evidence for the presence of terpenoids. Thus, to 1 mL of the stem juice, 0.5 mL of chloroform was added, followed by a few drops of concentrated H_2SO_4 . A reddish-brown coloration of the interface would indicate the presence of terpenoids.

The presence of saponins was demonstrated with the frothing test. This involved the dilution of 2 mL of the stem juice with 6 mL of distilled water, and observing the formation of persistent bubbles or foam after vigorous shaking for 5 min.

To demonstrate the presence of anthraquinones, 2 mL of the stem juice was boiled with 4 mL concentrated H_2SO_4 and shaken well, after which 3 mL of chloroform were added to the mixture. The chloroform layer was then removed and mixed with an equal volume of 50% (v/v) diluted ammonia (1:1). A change in color would signify the presence of anthraquinones.

Testing for steroids was done according to the Liebermann-Burchard method. Thus, 5 mL chloroform was added to 1 mL of the stem juice which was followed by the addition of 5 mL concentrated H_2SO_4 . The appearance of a red color in the upper layer and a yellow with green fluorescence coloration in the H_2SO_4 layer would indicate the presence of steroids.

The Kellar-Kiliani test was used to identify (cardiac) glycosides in the stem juice. For this purpose, 2 mL were mixed with 2 mL acetic acid and 2 mL chloroform. After cooling down, 1 mL concentrated H_2SO_4 was added. The appearance of a green color would indicate the presence of aglycone fragments of glycosides. The formation of a reddish-brown color following the addition of 2 mL concentrated H_2SO_4 to 2 mL stem juice would suggest the presence of a steroidal moiety in (some of) the aglycones of the glycosides.

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

Anti-inflammatory activity of *M. arborescens* stem juice

The fresh stem juice of *M. arborescens* has been assessed for its anti-inflammatory activity at various dilutions using a heat-induced BSA denaturation assay¹⁷ and a hypotonicity-induced human erythrocyte

membrane lysis assay.¹⁹ The former method is based on the protein denaturation that occurs during inflammation and the prevention of this event by anti-inflammatory substances.¹⁷ The latter method relies on the stabilizing effect of anti-inflammatory drugs on the membranes of lysosomes resulting in the inhibition of the release of inflammatory mediators that is mimicked by assessing the stabilization of erythrocyte plasma membranes and the inhibition of hemolysis following exposure to hypotonicity.¹⁹

Evaluation of the stem juice at several dilutions showed a dose-dependent inhibition in both assays (Table 1). At the dilutions of 25% (v/v) and 50% (v/v), the inhibitory effects on heat-induced BSA denaturation ($69 \pm 4\%$ and $76 \pm 5\%$, respectively) did not differ statistically significantly from each other (Table 1). However, these effects were statistically significantly greater when compared to that accomplished by the dilution of 12.5% (v/v) ($48 \pm 2\%$ inhibition of BSA denaturation; $p = 0.0009$ and 0.0002 , respectively; Table 1). On the other hand, the stem juice countered hypotonicity-induced erythrocyte membrane lysis by roughly 90% not only at the dilutions of 25% (v/v) and 50% (v/v) but also at that of 12.5% (v/v) (Table 1). The degrees of inhibition ($92 \pm 8\%$, $93 \pm 7\%$, and $89 \pm 5\%$, respectively) did not differ statistically significantly from one another (Table 1). Furthermore, at dilutions between 0.5% (v/v) and 7.0% (v/v), the stem juice did not affect heat-induced BSA denaturation but still stabilized hypotonicity-treated erythrocyte membranes by more than 50% (Table 1). The inhibitory effects on erythrocyte membrane lysis caused by the stem juice at these dilutions did not differ statistically significantly from one another (Table 1). These observations suggest that the *M. arborescens* stem juice elicited a more pronounced anti-inflammatory effect in the

membrane lysis assay when compared to the protein denaturation assay (Table 1).

The use of diclofenac also led to dose-dependent anti-inflammatory effects (Table 1), reducing heat-induced BSA denaturation and hypotonicity-induced erythrocyte membrane lysis processes by $56 \pm 6\%$ and $62 \pm 2\%$, respectively, at the concentration of $50 \mu\text{g/mL}$ (Table 1). This was in the same range as that produced by 12.5% (v/v) diluted stem juice in the BSA denaturation assay, and by 0.5 - 7% (v/v) diluted stem juice in the membrane lysis assay (Table 1). Together, these findings validate the usefulness of both assays to assess the *M. arborescens* stem juice for its anti-inflammatory activity, indicate that this preparation displayed substantial anti-inflammatory activity, and support the above-mentioned suggestion that its anti-inflammatory effect manifested more in the membrane lysis assay than in the protein denaturation assay.

Antioxidant activity of *M. arborescens* stem juice

Next, the *M. arborescens* stem juice was evaluated for its antioxidant activity using a DPPH assay²⁰ and a FRAP assay.²² Both assays are based on electron transfer reactions which result in the reduction of an oxidant to give a colored substance that can spectrophotometrically be visualized.^{20,22} In the former case, the antioxidant(s) in a test sample donate(s) an electron or hydrogen radical to the stable and violet-colored DPPH free radical, turning it colorless to pale yellow.²⁰ In the latter case, the antioxidant(s) in a test sample donate an electron (at low pH) to the ferric (Fe^{3+}) ion in the colorless Fe^{3+} -TPTZ complex, reducing it to a ferrous (Fe^{2+}) ion in the Fe^{2+} -complex.²²

Table 1: Anti-inflammatory activity of *M. arborescens* stem juice and diclofenac as determined by a heat-induced BSA denaturation assay and a hypotonicity-induced erythrocyte membrane hemolysis assay. Values are means \pm SDs of at least three experiments performed in triplicate and have been compared for statistically significant differences using ANOVA with post hoc Bonferroni adjustments.

	% Inhibition of heat-induced BSA denaturation	% Stabilization of hypotonicity-induced erythrocyte membrane hemolysis
<i>M. arborescens</i> stem juice, 0.5% (v/v)	-	56 ± 2^a
1.0% (v/v)	-	58 ± 1^a
2.0% (v/v)	-	61 ± 2^a
5.0% (v/v)	-	67 ± 4^a
7.0% (v/v)	-	77 ± 1^b
12.5% (v/v)	48 ± 2^a	89 ± 5^b
25% (v/v)	69 ± 4^b	92 ± 8^b
50% (v/v)	76 ± 5^b	93 ± 7^b
Diclofenac, 25 $\mu\text{g/mL}$	34 ± 5^a	16 ± 8^a
Diclofenac, 50 $\mu\text{g/mL}$	56 ± 6^b	62 ± 2^b
100 $\mu\text{g/mL}$	72 ± 1^c	93 ± 1^c

Values followed by different letters in superscript are statistically significantly different from each other ($p \leq 0.0165$).

Table 2. Antioxidant activity of *M. arborescens* stem juice and Trolox as determined by a DPPH free radical scavenging assay and a FRAP assay. Values are means \pm SDs of at least three experiments performed in triplicate and have been compared for statistically significant differences using ANOVA with post hoc Bonferroni adjustments.

	DPPH free radical scavenging activity (% AA)	Ferric-reducing antioxidant power ($\mu\text{M Fe}^{2+}$ equivalents reduced)
<i>M. arborescens</i> stem juice 0.2% (v/v)	23 ± 4^a	130 ± 12^a
0.3% (v/v)	-	549 ± 23^b
0.6% (v/v)	-	887 ± 26^c
1.2% (v/v)	45 ± 3^b	$1,720 \pm 7^d$
2.0% (v/v)	-	$2,616 \pm 16^c$
3.5% (v/v)	83 ± 5^c	-
Trolox 1.0 $\mu\text{g/mL}$	52 ± 3^a	543 ± 51^a
3.0 $\mu\text{g/mL}$	74 ± 3^b	1089 ± 36^b
6.0 $\mu\text{g/mL}$	98 ± 2^c	$2,721 \pm 22^c$

Values followed by different letters in superscript are statistically significantly different from each other ($p \leq 0.005$).

Table 3: Presence (+) or absence (-) of several phytochemical classes in the fresh stem juice of *M. arborescens*.

Phytochemical constituent	Test	Observation	Inference
Phenolic compounds	FeCl ₃ test	Brownish-blue or black color	+
Tannins	FeCl ₃ test	Brownish-blue or black color	+
Flavonoids	NaOH test	(Disappearance of) Yellow color	+
Alkaloids	Wagner's test	Brownish precipitate	+
Terpenoids	Salkowski test	No reddish brown color	-
Saponins	Froth test	Formation of bubbles and persistent foam	+
Antraquinones	Borntrager test	No change in color	-
Steroids	Liebermann-Burchard test	<i>M. arborescens</i> stem juice is not soluble in chloroform	-
(Cardiac) Glycosides	Kellar-Kiliani test	Green and reddish-brown color	+

The *M. arborescens* stem juice elicited antioxidant activity at relatively high dilutions and displayed in both the DPPH assay and the FRAP assay a dose-dependent antioxidant activity (Table 2). Thus, at serial dilutions between 0.2% (v/v) and 3.5% (v/v), the DPPH scavenging antioxidant activities of the stem juice increased from 23 ± 4 to 83 ± 5%, and its antioxidant power from 130 ± 12 to 2,616 ± 16 μM Fe²⁺ equivalents reduced (Table 2). At *p* values ≤ 0.0022, the differences among these values were statistically significant (Table 2). Trolox 1.0 - 6.0 μg/mL accomplished a dose-dependent antioxidant activity in both the DPPH and the FRAP assay (Table 2), validating the use of these methods to assess the stem juice for its antioxidant activity. Furthermore, the antioxidant effects of the stem juice in the DPPH assay at 1.2% and 3.5% (v/v) were comparable to those caused by Trolox 1.0 - 6.0 μg/mL (Table 2). The same held true for the antioxidant effects of the stem juice at 0.3 - 3.5% (v/v) in the FRAP assay (Table 2).

The *M. arborescens* stem juice could be estimated to eliminate 50% of the DPPH free radicals (its EC₅₀ value) at the dilution of 2.0 ± 0.2% (v/v) (not shown). This was in close agreement with the results from a separate set of experiments showing that the stem juice indeed removed nearly half (40 ± 3%) of the DPPH free radicals at the dilution of 1.2% (v/v) (Table 2). Apparently, even when diluted roughly 50-fold, the *M. arborescens* stem juice was able to eliminate about half of the DPPH free radicals, indicating its potent antioxidant activity. Furthermore, at the dilution of 2% (v/v) - i.e., at its DPPH IC₅₀ value - the stem juice produced an antioxidant power of 2,616 ± 16 μM Fe²⁺ equivalents (Table 2). This was in the same range as the antioxidant power accomplished by Trolox 6.0 μg/mL (Table 2), confirming the substantial antioxidant activity of the *M. arborescens* stem juice seen with the DPPH assay. Importantly, as mentioned in the previous paragraph, at the rather high dilutions tested in the antioxidant assays, the stem juice stabilized hypotonicity-induced erythrocyte-membranes by at least 50% (Table 1).

Phytochemicals in *M. arborescens* stem juice

Pharmacologically active constituents in the *M. arborescens* stem juice were detected using standard chemical tests.^{23,24} Phytochemicals such as phenolic compounds and tannins, flavonoids, alkaloids, saponins, as well as (cardiac) glycosides were identified (Table 3). Terpenoids, anthraquinones, and phytosterols could not be detected (Table 3).

DISCUSSION

Preparations from parts of *M. arborescens* are traditionally used against, among others, inflammatory conditions^{9,10,16} but so far without sufficient scientific evidence. The current study presents pharmacological studies to support this suggestion, to our knowledge for the first time, showing meaningful anti-inflammatory activity of the fresh *M. arborescens* stem juice in two *in vitro* assays. This substance also displayed notable antioxidant activity in two *in vitro* assays. Qualitative phytochemical analyses confirmed the presence in the fresh stem juice of various

bioactive compounds with previously reported anti-inflammatory and antioxidant properties.

Evidence for anti-inflammatory activity of the fresh *M. arborescens* stem juice came from its ability to inhibit heat-induced denaturation of BSA and hypotonicity-induced lysis of human erythrocyte membranes in a dose-dependent fashion. The inhibitory effects of the stem juice were substantial, since they occurred at relatively high dilutions of the stem juice (≥ 50% inhibition at stem juice dilutions ≤ 12.5% (v/v)). That the potency of the stem juice could indeed be qualified as 'substantial' is supported by its comparable anti-inflammatory activity - even at the relatively high dilutions ≤ 12.5% (v/v) - as that found for diclofenac 50 μg/mL: this concentration of diclofenac was much higher than the peak plasma concentration of 1.5 μg/mL required for therapeutic efficacy after orally taking 50 mg of an enteric-coated tablet of diclofenac.²⁵ In other words, the stem juice seemed sufficiently potent to accomplish anti-inflammatory efficacy at even relatively high dilutions.

Furthermore, at the dilution of 12.5% (v/v), the *M. arborescens* stem juice inhibited BSA denaturation by about 50% but hypotonicity-induced erythrocyte membrane lysis by roughly 90%, and at 0.5% (v/v) - 7.0% (v/v) it did not affect heat-induced BSA denaturation but stabilized hypotonicity-treated erythrocyte membranes by more than 50%. This observation raises the possibility that the anti-inflammatory activity of the stem juice was mediated by both the inhibition of protein denaturation and the prevention of membrane lysis, but that it was mainly associated with the latter phenomenon. This suggests, by extension, that the anti-inflammatory activity of the *M. arborescens* stem juice was primarily based on the inhibition of the release of the lysosomal content of neutrophils at inflammation sites.

Clearly, this suggestion must be verified in future studies. However, the stabilization of hypotonicity-induced (or heat-induced) human erythrocyte membranes by plant preparations at lower concentrations than those required for the inhibition of albumin denaturation has been reported before. This has been found, for instance, for an aqueous leaf extract of the strawberry tree *Arbutus unedo* L. (Ericaceae),²⁶ a flavonoid-rich stem bark extract of the yellow flame *Peltophorum pterocarpum* (DC.) K.Heyne (Fabaceae),²⁷ and an ethanolic root extract of the piper chilli *Piper chaba* Trel. & Yunck. (Piperaceae).²⁸ The mechanisms underlying the anti-inflammatory activities may involve, among others, the inhibition of lipoxygenase activity, inducible nitric oxide synthase expression, cyclooxygenases-1 and -2 activities, and phospholipase A₂ activity (reviewed in references.²⁹⁻³¹ Obviously, these suggestions also need to be confirmed.

The fresh *M. arborescens* stem juice also displayed considerable antioxidant activity in a dose-dependent way in a DPPH and a FRAP assay. This was in line with the previously reported antioxidant activity of both polar and apolar stem and leaf extracts of the closely related plant species *M. linifera* in a DPPH assay.³² The notable potency of the antioxidant activity of the *M. arborescens* stem juice was underscored by its ability to eliminate 50% of the initial number of DPPH free

radicals even when diluted approximately 50-fold. At this dilution, the antioxidant power ($2,616 \pm 16 \mu\text{M Fe}^{2+}$ equivalents) was in the same range as that accomplished by Trolox $6.0 \mu\text{g/mL}$ ($2,721 \pm 22 \mu\text{M Fe}^{2+}$ equivalents). This value compared favorably with the antioxidant power considered high ($2,133 \pm 6.87 \mu\text{M Trolox}$ equivalents per 100 g dry weight) for cloves of *Syzygium aromaticum* (L.) Merr. & L.M.Perry, Myrtaceae) in a study where thirty-one other plants displayed an antioxidant power lower than $700 \mu\text{M Trolox}$ equivalents per 100 g dry weight.³³

Importantly, the *M. arborescens* stem juice elicited its antioxidant activities at the relatively high dilutions that were also able to stabilize the hypotonicity-induced erythrocyte membrane lysis. This suggests that both phenomena were important determinants of the anti-inflammatory activity of the stem juice. This has been reported before for, for instance, a hydroalcoholic extract from the fruit of the Indian gall nut *Terminalia chebula* Retz. (Combretaceae) that reduced both carrageenin-induced paw edema in laboratory rats and stabilized hypotonicity-treated human erythrocyte membranes while diminishing lipid peroxidation in the rats' liver and promoting scavenging of DPPH free radicals,³⁴ and a methanol extract from the rhizome and leaf of the oakleaf fern *Drynaria quercifolia* (L.) J.Sm. (Polypodiaceae) and fractions therefrom that stabilized erythrocyte membranes in hypotonic solution while exhibiting antioxidant activity in DPPH, FRAP, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays.³⁵

Furthermore, preparations from members of the plant family Araceae other than *M. arborescens* such as a 50% alcohol extract of the rhizome from the sweetflag *Acrous calamus* L.,³⁶ a methanol extract of the tuber from the whipcord cobra lily *Arisaema tortuosum* (Wall.) Schott,³⁷ and an ethanolic extract of the leaf from Solomon's lily *Arum palaestinum* Boiss.³⁸ also displayed both anti-inflammatory and antioxidant activities. Interestingly, in India^{36,37} as well as Jordan and Palestine,³⁸ these plants are also traditionally used for treating inflammatory conditions.

Qualitative phytochemical analyses of the fresh *M. arborescens* stem juice showed the presence of phenolic compounds such as flavonoids and tannins as well as alkaloids, saponins, and (cardiac) glycosides. This was in accordance with previous data, mentioning the presence of flavonoids, tannins, alkaloids, and saponins in *M. arborescens* leaf¹⁶ and of alkaloids, flavonoids, coumarins, tannins, triterpenes, p-hydroxybenzaldehyde, and steroids in stem and leaf of *M. linifera*.^{32,39-44} Several studies have reported that these compounds - either from various plant parts or as ingredients of plant extracts or enriched herbal preparations - exerted both anti-inflammatory and antioxidant activities in various *in vitro*, cell culture, and animal models (see, for instance, references).⁴⁵⁻⁵⁰

Summarizing, the results from the current study provide evidence that the fresh *M. arborescens* stem juice possesses considerable membrane-stabilizing and antioxidant activities, and contains phytochemicals that may be associated with these activities. These data support the ethnopharmacological use of *Montrichardia* spp. preparations against inflammatory conditions.^{9,10,16} The precise phytochemical nature of the active ingredient(s) in the stem juice, its/their mechanism(s) of action, and its/their specific target(s) in the inflammatory cascade must be determined in future studies. These studies must also use animal models in order to have a better representation of the clinical setting and to address the safety of the stem juice.

CONCLUSIONS

The fresh stem juice of *M. arborescens* exhibited appreciable *in vitro* anti-inflammatory and antioxidant activities and contained phytochemicals known to possess these properties, including phenolic compounds such as flavonoids and tannins as well as alkaloids, saponins, and (cardiac) glycosides. These findings support the traditional use of this

preparation for treating, among others, inflammatory conditions, and speak in favor for further studies to validate its efficacy as an anti-inflammatory therapy.

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CONFLICTS OF INTEREST

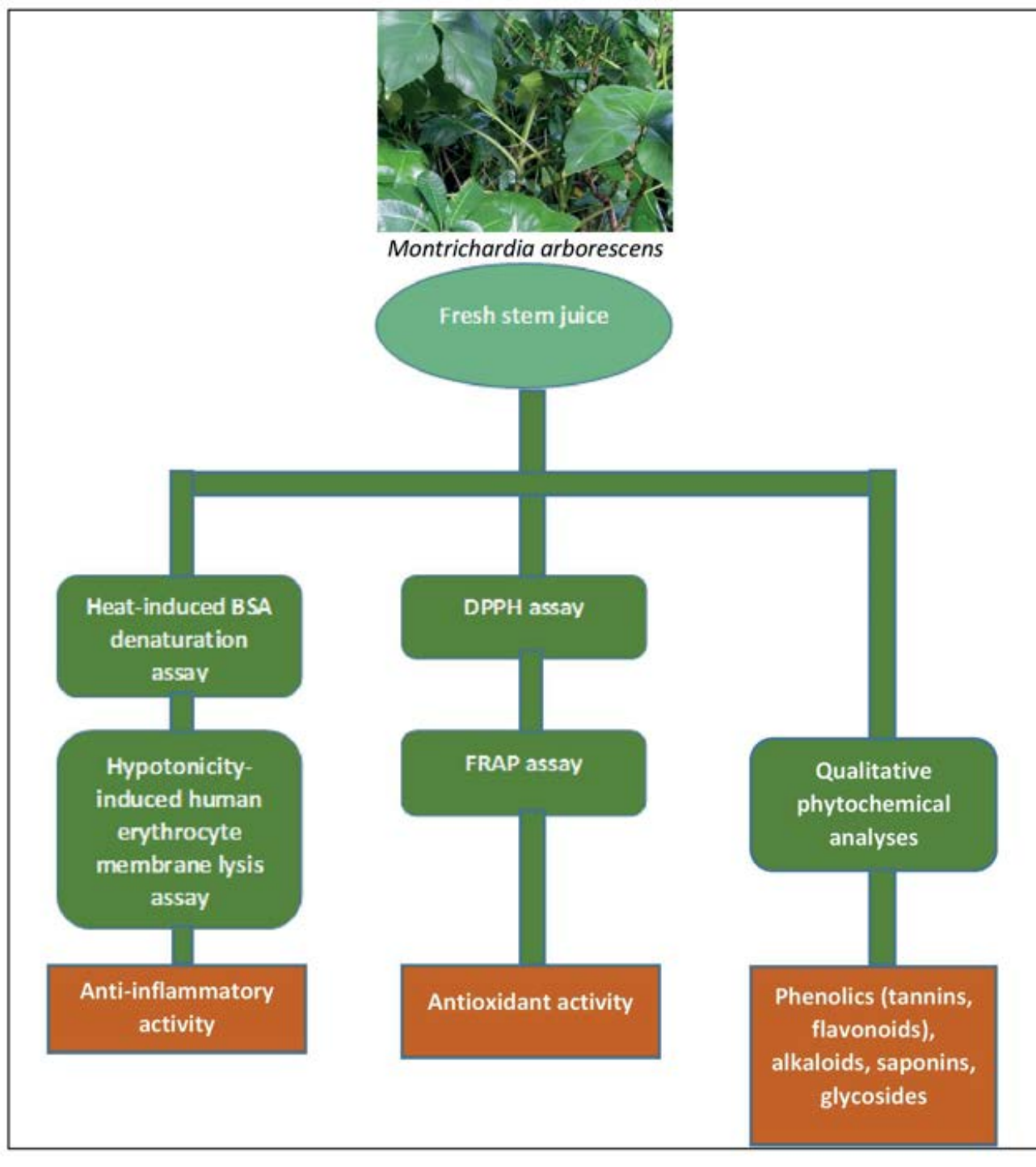
The author reports no conflicts of interest in this work.

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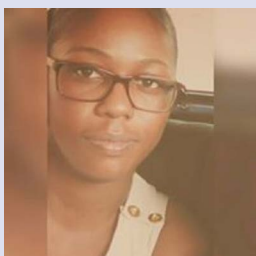
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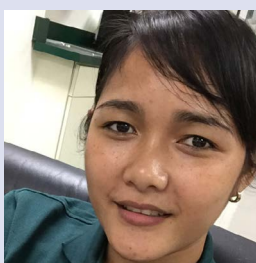
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