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Possible involvement of pro-angiogenic activities in the traditional use of the stembark of *Oenocarpus bacaba* Mart. (Arecaceae) for wound healing in the Republic of Suriname (South America)

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Abstract

Angiogenesis is an important part of the wound healing process. In this study, the aqueous extracts from seven plant species that are used for wound care in the Republic of Suriname (South America), were evaluated at sub-toxic concentrations for their stimulatory effects on the closure of scratch-wounds in cultured human umbilical vein endothelial cells (HUVECs), the formation of capillary-like structures by these cells, and the growth of sub-intestinal blood vessels in developing Tg (fli1a: EGFP) y1/+ zebrafish embryos. Sub-toxic extract concentrations were about one-third of IC_{50} values in HUVECs which were established using a sulforhodamine B assay after a 3-day exposure period. Data were expressed relatively to those found with untreated controls and considered statistically significantly different from each other when p values < 0.05 (ANOVA). When compared to untreated controls, the *Oenocarpus bacaba* stembark extract decreased HUVEC scratch-wound areas by about 30%; increased tube length, number of branching points, and number of loops formed by HUVECs by 50-70%, and increased total sub-intestinal blood vessel length in the zebrafish embryos by about 30%. The extracts from *Morinda citrifolia* (leaf), *Luffa acutangula* (fruit juice), *Momordica charantia* (leaf), *Psidium guajava* (leaf), *Cecropia peltata* (branch tops), and *Spondias mombin* (leaf) had no statistically significant effect on any of these variables. These observations suggest that the *O. bacaba* sample, unlike the other samples, possessed pro-angiogenic properties which may be involved in its beneficial effects in wound healing. Future studies should more elaborately evaluate these plants in order to definitely establish their therapeutic value in wound healing.

Keywords: Medicinal plants; Suriname; Wound healing; HUVECs; Scratch-wound closure; Capillary-like structure formation; Zebrafish embryos; Sub-intestinal blood vessel length

1. Introduction

The ancient wisdom of traditional healers about plants with medicinal properties has proven invaluable for the development of novel, breakthrough medicines [1]. For instance, the centuries-long use of the foxglove *Digitalis purpurea* L. (Plantaginaceae) against cardiac dropsy led to the development of the cardiac glycoside digoxin [2]. The traditional use of the French lilac *Galega officinalis* L. (Fabaceae) against the symptoms of diabetes mellitus since the Middle Ages was at the basis of the development of the oral hypoglycemic biguanide metformin [3]. The ancient use of the tubers of yam species in the genus *Dioscorea* (Dioscoreaceae) for birth control gave phytosteroid sapogenins such as diosgenin which serve as the raw materials for 95% of all steroidal drugs on the market including oral contraceptives [4]. And the use of the Indian plant *Psoralea corylifolia* L. (Fabaceae) against skin disorders in the Ayurveda and the

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Unani systems of medicine gave the furocoumarin psoralen, a component of PUVA (psoralen and ultraviolet A) for treating, among others, psoriasis, eczema, vitiligo, and graft-versus-host disease [5].

As there is reasonable certainty that humans have suffered all types of lesions since their existence (including open and closed wounds as well as clean, contaminated, infected, and colonized wounds [6, 7]), it is safe to say that the management of wounds is probably as old as humanity [8]. Thus, traditional medical sources may also prove useful to identify and develop substances for treating wounds and stimulating wound healing. Indeed, reports from all parts of the world have described a large variety of traditional modalities for wound care including the use of plants. A few examples are preparations from thirty-six plant species belonging to twenty-two families including the Euphorbiaceae, Asteraceae, and Solanaceae in Oyo State, Nigeria [9]; those from *Moringa oleifera* Lam. (Moringaceae) and *Aloe vera* (L.) Burm.f. (Asphodelaceae) in Indian Ayurveda and Unani [10]; and those from 305 plant species in Latin American traditional medicine [11].

The Republic of Suriname is located at the north-eastern coast of South America near the Atlantic Ocean. Despite the availability of modern and accessible health care throughout the entire country, the use of traditional (mainly plant-based) medicines is very common in all layers of the population [12]. An estimated 800 of the roughly 5,000 higher plant species in Suriname are used for medicinal purposes [13, 14]. And about 70 of the 800 medicinal plants (around 9%) are used for managing wounds, *i.e.*, for the stimulation of the healing of several types of wounds, as a wound dressing, for infected sores and wounds, and/or as an antiseptic [13, 14]. Unfortunately, the scientific evidence to support these uses is so far limited to only a few studies (see, for instance, references [15, 16]). This is probably attributable to the highly complex nature of the wound healing process that comprises four sequential and partially overlapping steps, namely hemostasis, inflammation, proliferation, and maturation and remodeling [17]. Hemostasis involves the formation of a fibrin clot by the aggregation of thrombocytes; during inflammation, bacteria and cell debris are removed by white blood cells; in the proliferation phase, the closing wound is rebuilt with new granulation tissue, vascularized by infiltrating blood vessels, and covered by epithelial cells; and during maturation and remodeling, newly formed collagen adds tensile strength to the wound area [17].

The orderly and timely manifestation of these processes is imperative to restore the anatomic and functional integrity of the injured site [17], and failure at any stage may result in the development of chronic wounds [18]. Chronic wounds are wounds that do not heal spontaneously within three months, such as diabetic, vascular, and pressure ulcers [18]. These lesions are mainly managed by several forms of debridement and the use of antiseptics and antibiotics, as well as wound support with proper dressings until the wound area has closed [19]. More recently, several research efforts have been directed at the stimulation of the vascularization of such chronic wounds during the proliferation phase by using, among others, pro-angiogenic growth factors and cytokines [19]. This has resulted in the development of recombinant human platelet-derived growth factor (PDGF), also known as platelet-derived growth factor BB and marketed as becaplermin (Regranex®) that is indicated for treating the symptoms of diabetic neuropathic ulcers [20]. PDGF probably signals through the cell-surface tyrosine kinase receptors PDGFRα and PDGFRβ to up-regulate the production of vascular-endothelial growth factor (VEGF), inducing angiogenesis and modulating the proliferation and recruitment of perivascular cells [20].

However, the use of three or more tubes of becaplermin has been associated with some serious side-effects including the risk of cancer [21]. This indicates a need of less harmful and at least equally efficacious alternatives, and has prompted efforts dedicated to the identification and development of wound healing substances from plant origin that may specifically stimulate angiogenesis [22]. A few examples emerging from these efforts are the ginsenosides from the Korean ginseng *Panax ginseng* C.A.Mey. (Araliaceae) [23], β-sitosterol from *A. vera* [24], as well as crude extracts from the sea-buckthorn *Hippophae rhamnoides* L. (Elaeagnaceae) [25] and the female ginseng *Angelica sinensis* (Oliv.) Diels (Apiaceae) [26].

With this background, it was decided to assess seven plants that are commonly used in Surinamese traditional medicine for wound care (Table 1) for their potential stimulatory effect on angiogenesis. Thus, extracts from parts of the plants were evaluated at lowly cytotoxic concentrations for their stimulatory effects on scratch-wound closure by cultured human umbilical vein endothelial cells (HUVECs) [27] and capillary-like structure formation by these cells [28], as well as on the growth of sub-intestinal blood vessels in developing zebrafish embryos [29]. The results from these studies provide indications about the involvement of angiogenesis in the presumed wound healing potential of the plants.

2. Material and methods

2.1. Plant material

Table 1 lists the plants and plant parts investigated in the current study. The plants were collected in rural areas around Suriname's capital city Paramaribo that had been free from herbicidal or pesticidal use for at least the preceding six months. The plant collections took place in close collaboration with the National Herbarium of Suriname (BBS) that is in the possession of a collection permit from the Surinamese Ministry of Physical Planning, Land and Forestry Management. At the time of the collections, none of the plants were on the International Union for Conservation of Nature's Red List of endangered or threatened species [30]. The collected samples were authenticated with the help of published data on regional flora [13, 14] and by comparing voucher specimens with identified herbarium collections at the BBS. The collected plant parts were thoroughly washed with distilled water, dried in open air, washed again, and extracted with water as indicated in Table 1. This was based on information from traditional healers about the way they process the plant parts, *i.e.*, by extracting, brewing, or boiling them with water in order to obtain medicinal teas, infusions, or decoctions. The plant extracts were filtered, freeze-dried, divided in aliquots of 0.5 g, and stored at -20 °C until experiments.

Table 1 Relevant information about the plants investigated in the current study. All reference vouchers have been stored at the National Herbarium of Suriname (BBS) at the Anton de Kom University of Suriname, Paramaribo, Suriname

Plant species (Family) (vernacular names in English; Surinamese)	Plant part used and extraction procedure	References
Oenocarpus bacaba Mart. (Arecaceae) (turu palm; kumbu)	Macerated stembark extracted with water for 2 h at 100 °C	Wound dressing; bleeding stopper [13]
Morinda citrifolia L. (Rubiaceae) (noni; didibri apra)	Macerated leaves extracted with water for 2 h at 45 °C	Joint pains [13]; abcesses, burn wounds, swelling [14]
Luffa acutangula L. (Cucurbitaceae) (ridged gourd; sukwa)	Fruit juice collected at room temperature	Internal bleedings [13]
Momordica charantia L. (Cucurbitaceae) (bitter melon; sopropo)	Macerated leaves extracted with water for 2 h at 45 °C	Ulcers and sores [13]; bruises [14]
Psidium guajava L. (Myrtaceae) (guava; guyaba)	Macerated leaves extracted with water for 2 h at 45 °C	Ulcers (due to leishmaniasis) [13]; abscesses and inflammations [14]
Cecropia peltata L. (Urticaceae) (trumpet tree; uma busipapaya)	Macerated branch tops extracted with water for 1 h at 60 °C	Cuts and warts [13]; cuts [14]
Spondias mombin L. (Anacardiaceae) (hog plum; mope)	Macerated leaves extracted with water for 1 h at 60 °C	Mouth sores and ulcers [14]

2.2. Studies with HUVEC cultures

2.2.1. Human umbilical vein endothelial cells and maintenance

HUVECs were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were maintained in vascular cell basal medium (ATCC; Rockville, MD, USA) supplemented with endothelial cell growth kit-VEGF (ATCC; Rockville, MD, USA) in 25-cm^2 flasks at a temperature of 37 °C, a minimum relative humidity of 95%, and an atmosphere of 5% CO_2 in air. Only exponentially growing cells were used for experiments.

2.2.2. Determination of cytotoxicity of plant extracts

HUVECs were detached from the culture flasks using ethylenediaminetetraacetic acid - trypsin (Corning; Manassas, VA, USA). Triplicate cultures of HUVEC were inoculated in 96-well microplates at densities of 4 x 10^3 cells per 100 µL vascular cell basal medium (ATCC; Rockville, MD, USA) supplemented with endothelial cell growth kit-VEGF (ATCC;

Rockville, MD, USA) and allowed to stabilize for 24 h. The next day, the cell cultures were exposed to the plant extracts at final concentrations of 0 to 3,000 µg/mL in the presence of 100 IU/mL penicillin, 100 µg/mL streptomycin, and 5 µg/mL amphotericin B (Corning; Manassas, VA, USA). 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 the sulphorhodamine B (SRB) assay [31]. Briefly, the cell cultures were fixed *in situ* with 10% (*w/v*) trichloroacetic acid (VWR International LLC; West Chester, PA, USA) and stained with SRB 0.4% (*w/v*) (Biotium, Inc.; Hayward, CA, USA) in 1% (*v/v*) acetic acid (AMRESCO LLC; Solon, OH, USA). Unbound SRB was removed with 1% (*v/v*) acetic acid, and cell-bound SRB was solubilized with 10 mM Tris Base pH 10.5 (Mediatech, Inc.; Manassas, VA, USA). Absorbance values at a wavelength of 515 nm were measured with a microplate reader, corrected for background absorption, and plotted against extract concentrations. Background absorption was determined from control wells which had received either medium alone or plant extract-containing medium, but no cells. Dose-response profiles were constructed from which IC_{50} values were derived, *i.e.*, extract concentrations resulting in 50% inhibition of cell growth when compared to untreated controls.

2.2.3. Assessment of wound gap areas in HUVEC monolayers exposed to plant extracts

Triplicate cultures of HUVECs were inoculated in 96-well microplates at densities of 6 x 10^3 cells per 100 µL vascular cell basal medium (ATCC; Rockville, MD, USA) supplemented with endothelial cell growth kit-VEGF (ATCC; Rockville, MD, USA) and allowed to grow to 90% confluence. Next, the medium was removed and a scratch representing a wound gap was applied in the center of the cultures using a sterile pipette tip [27]. The detached cells were carefully removed, and the cultures were exposed to lowly cytotoxic concentrations of the plant extracts - *i.e.*, at final concentrations corresponding to one-third of their respective IC_{50} values - and in the presence of 100 IU/mL penicillin, 100 µg/mL streptomycin, and 5 µg/mL amphotericin B (Corning; Manassas, VA, USA). Incubations were for 16 h and in final volumes of 200 µL per well. At the end of the incubations, the samples were photographed and wound gap areas were determined with the WimScratch Image Analysis platform (Ibidi GmbH, München, Germany). Data were expressed as percentages with respect to controls (wound gap areas determined in HUVEC cultures exposed to medium alone).

2.2.4. Assessment of capillary-like structure formation of HUVECs

Capillary-like structure formation of HUVECs was performed as described with some modifications [28]. Ninety-six-well culture plates were coated with 50 µL of Cell Matrix Basement Membrane Gel (ATCC® ACS-3035™; ATCC; Rockville, MD, USA) and allowed to solidify at 37 °C for 30 min. Then, triplicate cultures of HUVEC were seeded onto the artificial basement membrane at densities of 6 x 10^3 cells in 100 µL vascular cell basal medium (ATCC; Rockville, MD, USA) supplemented with endothelial cell growth kit-VEGF (ATCC; Rockville, MD, USA) and allowed to attach. Ninety minutes later, the cell cultures were exposed to the plant extracts at final concentrations that again corresponded to one-third of their respective IC_{50} values and in the presence of 100 IU/mL penicillin, 100 µg/mL streptomycin, and 5 µg/mL amphotericin B (Corning; Manassas, VA, USA). Incubations were for 6 h and in final volumes of 200 µL per well. At the end of the incubations, the samples were photographed and total tube length, numbers of branching points, and numbers of loops produced in the presence of the plant extracts were determined with the WimTube Image Analysis platform (Ibidi GmbH, München, Germany) and expressed relatively to those found for controls, *i.e.*, HUVECs exposed to medium alone.

2.3. Studies with zebrafish

2.3.1. Tg (fli1a: EGFP) y1/+ zebrafish and maintenance

The zebrafish (*Danio rerio*) strain Tg (fli1a:EGFP)y1/+ was from Zebrafish International Resource Center (Eugene, OR, USA). The fli1 promoter of this transgenic zebrafish line stimulates the expression of enhanced green fluorescent protein (EGFP) in endothelial cells, enabling visualization of blood vessel development throughout embryogenesis [32]. The fish were maintained under standard laboratory conditions using a light schedule of 14 h on and 10 h off, and at a temperature of 28 °C [33]. They were fed three times daily using a combination of dry food and freshly hatched brine shrimp (Ocean Star International, Snowville, UT, USA) [33]. For experiments, fertilized fish eggs were harvested shortly after the light was turned on and kept in Hank's buffer consisting of NaCl 0.137 M, KCl 5.4 mM, Na_2HPO_4 0.25 mM, KH_2PO_4 0.44 mM, $CaCl_2$ 1.3 mM, $MgSO_4$ 1.0 mM, and $NaHCO_3$ 4.2 mM.

2.3.2. Assessment of total sub-intestinal blood vessel length of zebrafish embryos exposed to plant extracts

At 8 hours post-fertilization (hpf) [29], the eggs were exposed to the same concentrations of the extracts which had been found to be lowly cytotoxic to the HUVECs after they had been dissolved in Hank's solution containing 0.1% (*v/v*) DMSO (Corning; Manassas, VA, USA). At 30 hpf, the embryos were removed from the chorion and allowed to swim freely

in the plant extract-containing medium [29]. At 96 hpf [29], the sub-intestinal vessels of the fish were visualized with an Axiovert 40 CFL fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany) and photographed. Total sub-intestinal vessel length following each treatment was determined with the Axiovision 4.8.1 Image Acquisition and Management Software for Light Microscopy (Carl Zeiss AG, Oberkochen, Germany) and expressed relatively to that found for untreated fish.

2.4. Statistics

All experiments have been carried out at least three times in triplicate. Results presented are means ± SDs. P values < 0.05 were taken to indicate statistically significant differences according to ANOVA.

3. Results

3.1. Cell culture studies

3.1.1. Cytotoxicity of plant extracts and determination of lowly cytotoxic concentrations

The cytotoxicity of aqueous extracts from parts of *O. bacaba* (stembark), *M. citrifolia* (leaf), *L. acutangula* (fruit juice), *M. charantia* (leaf), *P. guajava* (leaf), *C. peltata* (branch tops), and *S. mombin* (leaf) against cultures of HUVEC was determined after 3 days exposure to serial dilutions of the extracts using the SRB assay [31]. The IC_{50} values derived are given in Table 2. The *O. bacaba* and *P. guajava* samples had the greatest cell growth-inhibitory activity, displaying IC_{50} values of about 70 µg/mL (Table 2). Conversely, the *M. citrifolia* and *C. peltata* samples inhibited HUVEC proliferation at IC_{50} values in excess of 1,000 µg/mL (Table 2). The IC_{50} value for the *S. mombin* extract was about 300 µg/mL, and those for the *L. acutangula* and *M. charantia* extracts were 420 to 440 µg/mL (Table 2). Based on these findings, the subsequent experiments with the plant extracts were carried out at concentrations that were roughly 3 times lower than the IC_{50} values, in order to avoid too much background cell kill.

Table 2 IC_{50} values ± SDs (µg/mL) of plant extracts in HUVECs after three days exposure

Plant species (plant part)	IC_{50}values (µg/mL)
O. bacaba (stembark)	69 ± 13
M. citrifolia (leaf)	> 1,000
L. acutangula (fruit juice)	423 ± 61
M. charantia (leaf)	443 ± 33
P. guajava (leaf)	74 ± 17
C. peltata (branch tops)	> 1,000
S. mombin (leaf)	289 ± 23

3.1.2. Effects of plant extracts on scratch-wound areas in HUVECs

Next, the plant extracts were evaluated for their effects on the closure of wound gap areas in HUVEC monolayers [27] at the lowly cytotoxic concentrations of about one-third of their IC_{50} values. The scratch-wound area in HUVEC monolayers formed in the presence of the *O. bacaba* extract (25 µg/mL) had decreased to roughly three-quarters of that seen in the controls (Table 3). On the other hand, the scratch-wound areas formed in the presence of the samples from *M. citrifolia* (400 µg/mL), *L. acutangula* (150 µg/mL), *M. charantia* (150 µg/mL), *P. guajava* (25 µg/mL), *C. peltata* (400 µg/mL), and *S. mombin* (100 µg/mL) did not differ statistically significantly from the controls (Table 3). These observations suggest that the *O. bacaba* extract, unlike the other preparations, had stimulated HUVEC wound gap closure, eliciting pro-angiogenic effects.

Table 3 Scratch-wound areas in HUVEC monolayers following exposure for 16 h to the plant extracts at about one-third of their IC_{50} concentrations with respect to those found for untreated controls. The latter values were set at 100%. Data are means ± SDs of at least three independent experiments performed in triplicate

Plant species (concentration used)	Relative scratch-wound areas
O. bacaba (25 μg/mL)	71 ± 8[1]
M. citrifolia (400 μg/mL)	93 ± 23
L. acutangula (150 μg/mL)	156 ± 33
M. charantia (150 μg/mL)	105 ± 52
P. guajava (25 μg/mL)	115 ± 22
C. peltata (400 μg/mL)	116 ± 58
S. mombin (100 μg/mL)	129 ± 48

[1]Statistically significantly different from control (p value < 0.01, ANOVA)

3.1.3. Effects of plant extracts on capillary-like structure formation by HUVECs

The plant extracts were also evaluated at the same sub-toxic concentrations as those mentioned above for their effects on the formation of capillary-like structures by HUVECs [28]. As shown in Table 4, total tube length, the number of branching points, and the number of loops formed by HUVECs exposed to the *O. bacaba* extract (25 μg/mL) were approximately 1.5 times those formed by the untreated control cells (Table 4). Conversely, none of the other plant extracts exhibited statistically significant effects on HUVEC capillary-like structure forming activity when compared to the control (Table 4). These observations provided support for the pro-angiogenic properties of the *O. bacaba* sample and the absence of these properties of the other samples.

Table 4 Total tube length, number of branching points, and number of loops of capillary-like structures formed by HUVECs following exposure for 6 h to the plant extracts at about one-third of their IC_{50} concentrations with respect to those found for untreated controls. The latter values were set at 100%. Data are means ± SDs of at least three independent experiments performed in triplicate

Plant species (concentration used)	Relative tube length	Relative number of branching points	Relative number of loops
O. bacaba (25 μg/mL)	145 ± 26[1]	162 ± 9[1]	178 ± 34[1]
M. citrifolia (400 μg/mL)	73 ± 43	82 ± 75	116 ± 79
L. acutangula (150 μg/mL)	89 ± 17	76 ± 32	105 ± 40
M. charantia (150 μg/mL)	103 ± 1	102 ± 10	103 ± 18
P. guajava (25 μg/mL)	97 ± 2	95 ± 9	109 ± 38
C. peltata (400 μg/mL)	92 ± 35	103 ± 8	122 ± 30
S. mombin (100 μg/mL)	100 ± 8	99 ± 20	92 ± 33

[1]Statistically significantly greater than control (p value < 0.01; ANOVA)

3.2. Zebrafish studies

3.2.1. Effects of plant extracts on sub-intestinal blood vessel length in zebrafish embryos

Using the same concentrations as those applied in the *in vitro* studies mentioned above, the plant extracts were assessed for their effects of the formation of sub-intestinal blood vessels in developing zebrafish embryos [29]. The total sub-intestinal vessel length of fish treated with the *O. bacaba* extract (25 μg/mL) was about one-third greater than that found for untreated controls (Table 5). Thus, this sample seemed to have stimulated the formation and proliferation of blood vessels, supporting its pro-angiogenic properties noted in the *in vitro* experiments. In contrast, the extracts from *M. citrifolia*, *L. acutangula*, *M. charantia*, *P. guajava*, *C. peltata*, and *S. mombin* had no statistically significant effect on total sub-intestinal blood vessel length of the fish embryos (Table 5).

4. Discussion

The formation of new blood vessels is an important aspect of wound healing and occurs during the proliferation phase of this process [17]. In the current study, seven plants that are used for wound care in Suriname [13, 14], were assessed for their potential pro-angiogenic activity *in vitro* and *in vivo*. The plants were *O. bacaba* (stembark), *M. citrifolia* (leaf), *L. acutangula* (fruit juice), *M. charantia* (leaf), *P. guajava* (leaf), *C. peltata* (branch tops), and *S. mombin* (leaf). Aqueous extracts were prepared and evaluated for their ability to stimulate the closure of scratch-scratch-wound gaps in HUVEC monolayers [27] and the formation of capillary-like structures by these cells in Matrigel [28], as well as the development of sub-intestinal vessels in Tg(fli1a:EGFP)y1/+ zebrafish embryos [29]. The results obtained from these experiments indicated that the *O. bacaba* extract promoted scratch-wound wound gap closure in, and capillary-like structure formation by HUVECs, as well as sub-intestinal vessel growth in the zebrafish embryos. The other plant extracts did not display these activities. These observations suggest that the *O. bacaba* sample elicited pro-angiogenic activities which may account, at least partially, for its wound healing-stimulatory activity claimed in Surinamese traditional medicine [13]. The absence of pro-angiogenic activities of the other plants suggests that their alleged beneficial effects in wound healing may be attributable to properties other than those involving angiogenesis.

Table 5 Mean total length of sub-intestinal vessels of Tg(fli1a:EGFP)y1/+ zebrafish embryos exposed for 96 h to the plant extracts at about one-third of their IC_{50} concentrations in HUVECs with respect to those found for untreated controls. The latter values were set at 100%. Data are means ± SDs of at least three independent experiments performed in triplicate

Plant species (concentration used)	Relative length of sub-intestinal vessels in Tg(fli1a:EGFP)y1/+ zebrafish embryos
O. bacaba (25 μg/mL)	136 ± 16[1]
M. citrifolia (400 μg/mL)	99 ± 14
L. acutangula (150 μg/mL)	113 ± 27
M. charantia (150 μg/mL)	105 ± 52
P. guajava (25 μg/mL)	115 ± 22
C. peltata (400 μg/mL)	111 ± 18
S. mombin (100 μg/mL)	96 ± 17

[1]Statistically significantly greater than control (p value < 0.01; ANOVA)

There is some support for the pro-angiogenic activities of *O. bacaba* stembark noted in the current study. For instance, some anthocyanins and phenolic acids identified in parts of *Oenocarpus* species including *O. bacaba* exhibited notable pro-angiogenic activities in several *in vitro* and *in vivo* laboratory models [34, 35]. Some of these polyphenolic compounds stimulated effective wound healing by producing signals for cell survival [36] in addition to protecting tissues against damage and inflammation inflicted by oxidative stress caused by excess reactive oxygen species [37]. Furthermore, these polyphenolic compounds have been identified at relatively high concentrations in fruit, leaf, and root of *O. bacaba* and the closely related *O. bataua* Mart. and displayed appreciable antioxidant activity [34, 38-40] which might be involved in their wound healing-stimulatory activities [39]. However, it must be taken into account that several studies have also associated the polyphenolic compounds with anti-angiogenic instead of pro-angiogenic effects [35]. The contradictory results have tentatively been attributed to, among others, variations in the types, combinations, concentration ranges, and metabolites of the phenolic compounds used; the plant parts they have been derived from; the degree of purity of the samples evaluated; and the laboratory models used [35]. These considerations indicate the need of elaborate studies to establish the precise involvement of angiogenesis in the apparent wound healing-stimulatory effects of *O. bacaba* stembark. However, if validated, this substance may represent an important candidate for managing difficult-to-treat wounds.

The lack of pro-angiogenic activity of the extracts from *M. citrifolia* leaf, *L. acutangula* fruit juice, *M. charantia* leaf, and *P. guajava* leaf noted in the current study is not in accordance with literature data. A methanolic fraction from an ethanolic *M. citrifolia* leaf extract reportedly displayed pro-angiogenic activities, accelerating the closure of excision wounds in mice through its binding to the PDGF and adenosine A_{2A} receptors [41]. As mentioned above, PDGF promotes wound healing by stimulating VEGF production and angiogenesis [20]. And the activated G-protein-coupled adenosine A_{2A} receptor mediates angiogenesis and promotes wound healing in response to tissue injury [42]. In contrast, a fraction of a methanolic *L. acutangula* fruit juice extract, a methanolic *M. charantia* leaf extract, and an aqueous *P. guajava* leaf

extract displayed potent anti-angiogenic rather than pro-angiogenic effects in cultured A-549 and CL1 human lung adenocarcinoma cells as well as cultured DU-145 and PC-3 human prostatic cancer cell lines, inhibiting VEGF expression as well matrix metalloproteinase-2 and matrix metalloproteinase-9 expression, activating tissue inhibitor of metalloproteinase-2 in these cells, and suppressing the migration of the cells *in vitro*, as well as preventing the vascularization of chicken chorioallantoic membranes [43-46].

The reasons that the pro-angiogenic or anti-angiogenic effects of the *M. citrifolia*, *L. acutangula*, *M. charantia*, and *P. guajava* samples did not manifest in the current study might also be attributed to differences in one or more of the experimental conditions applied [40]. Alternatively, the wound healing-stimulatory effects of these plants may be due to mechanisms other than those related to angiogenesis such as the promotion of inflammation and maturation/remodeling. For instance, an *M. citrifolia* ethanolic leaf extract given orally to rats with excision and dead space wounds produced enhanced wound contraction, decreased epithelialization time, and increased hydroxyproline content [47]. A dried and powdered ethanolic *L. acutangula* fruit extract stimulated collagenation, epithelization, and contraction of excision wounds in rats [48], and water and ethanolic fruit extracts displayed anti-inflammatory and analgesic effects in animal models [49, 50]. And the topical administration of an *M. charantia* leaf extract accelerated the rate of wound closure and total protein content in wounds in streptozotocin-induced diabetic Sprague-Dawley rats [51].

Incidentally, plant-derived substances that seemed to promote wound healing through multiple mechanisms have been described before. For instance, the volatile oil from the leaf of the Ngai camphor *Blumea balsamifera* (L.) DC. (Asteraceae) improved wound healing in mice by promoting angiogenesis and perfusion as well as collagen deposition, the formation of organized granulation tissue, re-epithelialization, and wound closure [52]. Extracts from the flower of the common marigold *Calendula officinalis* L. (Asteraceae) stimulated angiogenesis in the chicken chorioallantoic membrane assay and in a cutaneous wound healing model in rats [53] as well as granulation tissue formation by altering the expression of connective tissue growth factor and α-smooth muscle actin in excision wounds in BALB/c mice [54]. And the pentacyclic triterpene madecassoside from the Asian pennywort *Centella asiatica* (L.) Urban (Apiaceae) promoted both angiogenesis and collagen synthesis at the wound site [55], and is now used as a therapeutic agent in wound healing as well as an anti-inflammatory and anti-aging agent [56].

Unlike the samples addressed above, there are no literature data associating the extracts from *C. peltata* (branch tops) and *S. mombin* (leaf) with either pro- or anti-angiogenic activities. These plants may also elicit their alleged wound healing-stimulatory activities through mechanisms other than those involving angiogenesis. Indeed, topically applied and orally administered aqueous and ethanol extracts of *C. peltata* leaf stimulated the closure of excision wounds in laboratory rats as well as collagen turnover in the wounded area as indicated by the increased contents of hydroxyproline and hexosamine [57]. And a methanolic extract from *S. mombin* leaf displayed anti-inflammatory activity in carrageenan-induced paw edema in rats and lipopolysaccharide-treated mice that might occur via suppression of the production of pro-inflammatory mediators and cytokines such as TNF-α and iNO [58]. Thus, these plants may also target the phases of maturation/remodeling and inflammation, respectively, of the wound healing process rather than the angiogenic component of the proliferation phase.

Summarizing, the results from the current study on a possible involvement of angiogenesis in the wound healing-stimulatory activity of *O. bacaba* stembark are partially in line with literature data. These observations support the traditional use of *O. bacaba* stembark preparations as a wound dressing and bleeding stopper [13]. No indications were obtained on the involvement of angiogenesis in the wound healing-stimulatory activity of *M. citrifolia* leaf, *L. acutangula* fruit juice, *M. charantia* leaf, *P. guajava* leaf, *C. peltata* branch tops, and *S. mombin* leaf. Literature data even associated some of these samples with anti-angiogenic rather than pro-angiogenic activities [43-46]. The use of these samples for the management of wounds [13, 14] may involve mechanisms other that those related to angiogenesis such as the stimulation of promotion of inflammation and maturation/remodeling [47-51, 57, 58]. The disagreements with literature data may be due to differences in experimental conditions [35], and must comprehensively be addressed in studies using multiple assays and applying standardized conditions in order to unequivocally establish the therapeutic value of these traditional preparations. At the same time, the value of these preparations for wound care must be assessed with respect to that of currently available therapeutic options. This is particularly important in countries where traditional and conventional forms of treatments are used concurrently such as Suriname.

5. Conclusion

Using several laboratory models, indications were obtained to associate the traditional use of *O. bacaba* stembark for the management of wounds with the stimulation of angiogenesis. The preparations from *C. peltata* branch top, *L. acutangula* fruit juice, *M. charantia* leaf, *M. citrifolia* leaf, *P. guajava* leaf, and *S. mombin* leaf did not display these effects,

suggesting that their traditional use for wound care may be based on distinct mechanisms. These observations emphasize the need of more elaborate assessments of these plants for their presumed wound healing benefits, in order to contribute to the proper and safe use of these and other traditional herbal preparations.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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Statement of ethical approval

The studies described in this paper have been approved by the Ethics Committee of the Faculty of Medical Sciences, Anton de Kom University of Suriname, Paramaribo, Suriname.

Author contributions

DRAM conceived the study, supervised the work, interpreted the data, and wrote the manuscript. MD, JP, and PF collected the plants and carried out the experiments with the human umbilical vein endothelial cells. RS and PM carried out the experiments with the zebrafish.

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