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Research Article
Angiogenesis-Interfering Potential of Wound Healing Plants in Subintestinal Blood Vessels of Tg(fli1a:EGFP)y1/+Zebrafish Embryos

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Abstract

Background and Objective: Plants are often traditionally used for managing wounds. Angiogenesis is an important event in wound healing. In this study, six traditionally used wound-healing plants from Suriname (South America) have been evaluated for their capacity to stimulate Subintestinal Vessel (SIV) formation in Tg(fli1a: EGFP)y1/+zebrafish embryos. **Materials and Methods:** Extracts were prepared from *Carapa guianensis*, *Copaifera guyanensis* (stembark), *Punica granatum* (fruit) and *Piper betle*, *Stachytarpheta jamaicensis* as well as *Uncaria guianensis* (leaves). Zebrafish embryos were exposed to the plant extracts (10^{-5} - 100 μg mL^{-1}) in Hank's solution containing dimethyl sulfoxide 0.1% (v/v) from 8 hrs post-fertilization (hpf) *in ovo* until 96 hpf *ex ovo*. Total SIV lengths were quantified using the Axiovision 4.8.1 Image Acquisition and Management Software. The numbers of surviving embryos were also recorded. Data were compared to those found with untreated controls (ANOVA, $p<0.05$). **Results:** None of the plant extracts produced greater SIV lengths than controls. However, the *C. guianensis* extract at 0.01 μg mL^{-1} produced a decrease of about 40% in SIV length and left about 70% of the embryos unharmed. The *P. betle* and *S. jamaicensis* preparations at 100.0 μg mL^{-1} also produced a decrease in SIV lengths of around 50% but killed more than half of the embryos. **Conclusion:** The traditional use of the plants for wound healing may not involve proangiogenic events. However, the *C. guianensis* stem bark extract may possess antiangiogenic properties. This may impede wound healing but may be useful against conditions associated with excessive angiogenesis.

Key words: Suriname, traditional medicine, medicinal plants, wound healing, Tg(fli1a: EGFP)y1/+zebrafish, subintestinal vessels, angiogenesis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A wound is the disruption of the protective function of the skin due to loss of the continuity of the epithelium with or without damage to the underlying connective tissue, following an incision, laceration, abrasion, puncture, avulsion or amputation[1]. The body responds to a wound by initiating a complex and dynamic cascade of four precisely timed and partially overlapping phases, namely hemostasis, inflammation, proliferation as well as maturation and remodeling[2,3]. These events involve, among others, the formation of a fibrin clot by the aggregation of thrombocytes, the removal of bacteria and cell debris by white blood cells, the rebuilding of the wounded area with new granulation tissue that is vascularized by the ingrowth of blood vessels and the increase of tensile strength to the wound by newly formed collagen[2,3]. For a wound to heal successfully, all these events must occur in the proper sequence and time frame[2,3].

Minor or acute open wounds such as superficial scratches, needle pricks and shallow cuts may not require medical treatment and sanitization of the wound and removal of any debris to prevent infection generally suffice[4]. If necessary, topical antibiotics such as polymyxin B, bacitracin, and/or neomycin can be used to fight microbial infections[5]. On the other hand, severe open wounds with substantial bleeding usually require immediate medical attention involving, among others, stopping the bleeding, cleaning the wound, preventing infection using oral antibiotics and closing and dressing the wound[3,6]. Non-steroidal anti-inflammatory drugs such as naproxen, ibuprofen and diclofenac can be taken during the healing process, although their therapeutic benefit has been disputed[7].

In extreme cases, when one or more phases in the healing process fail(s) to proceed correctly, chronic wounds occur[8]. Chronic wounds are considered wounds that do not heal spontaneously within three months[9] and may be caused by, among others, increased formation of toxic free radicals, delayed granulation tissue formation reduced angiogenesis and decreased collagen reorganization[9]. Examples of such lesions are diabetic, vascular and pressure ulcers and they represent a major burden to patients, their families, health care professionals and health care systems[10,11]. Treatment of these types of wounds is more complicated and may involve the use of artificial skin substitutes in combination with collagen, protease-modulating matrices such as Promogran®, growth factors such as vascular endothelial growth factor and basic fibroblast growth factor or acellular collagen-based matrices that mimic the extracellular matrix[12].

Apart from these allopathic therapies, a variety of plant-based formulae and procedures are traditionally used for managing wounds[13,14]. The clove basil *Ocimum gratissimum* L. (Lamiaceae) may promote blood coagulation by shortening the activated partial thromboplastin time[15]. The sappanwood *Biancaea sappan* (L. 1753) Tod. 1875 (Fabaceae) exhibits broad antibacterial activity[16] and may stimulate proliferation and migration of as well as collagen synthesis by dermal fibroblast[17]. The frankincense *Boswellia sacra* Flueck (Burseraceae) may help diminish inflammation, stimulate the growth of granulation tissue[18] and reduce the time of wound closure via direct effects on neovascularization[19]. And the Mongolian milkvetch *Astragalus propinquus* Schischkin (Fabaceae) may also promote neovascularization[20,21] and help remove reactive oxygen species[22].

The Republic of Suriname (South America) has an extensive ethnopharmacological tradition[23] that has its roots in various traditional forms of medicine originating in parts of the Americas, Africa, Asia and Europe[24]. As a result, a considerable number of traditional plant-derived preparations is used for managing wounds[25-30]. The data of Table 1 gives six such plants[25-30] as well as the references for the pharmacological support for their presumed wound healing properties. Unfortunately, there is insufficient information on the mechanisms of action of the plants. Considering the importance of neoangiogenesis in the wound healing process[2,3], it was decided to assess these plants for their potential to stimulate the formation of new blood vessels.
For this purpose, extracts from the plants have been assessed for their ability to accelerate the formation of Subintestinal Vessels (SIVs) in developing embryos of the transgenic fluorescent Tg(fli1a:EGFP)y1/+zebrafish (*Danio rerio*).

MATERIALS AND METHODS

Location and duration of the study: This study on the proangiogenic potential of plants that are used in Suriname for managing wounds has been carried out at the Department of Pharmacology of the Faculty of Medical Sciences, Anton de Kom University of Suriname, Paramaribo, Suriname, in the period between May, 2019 and February, 2020.

Plant material: The plants investigated in the current study are mentioned in Table 1. The plants have been selected from literature data on their traditional use for managing wounds[25-30] and the pharmacological support for these applications[31-44] (Table 1). They have been collected in rural areas of Suriname (Table 2) that had been free from herbicidal

Table 1: Plants investigated in the current study, their most common traditional medical uses in Suriname, as well as the pharmacological support for managing wounds

Plant species (vernacular name)	Family	Most common traditional medical uses in Suriname (references)	Pharmacological support for wound healing stimulating activity
Carapa guianensis Aubl. (crabwood)	Meliaceae	Various types of wounds[25], Disinfection of wounds[25,26]	Stimulation healing of incision wounds in alloxan-induced diabetic Wistar rats[31]
			Beneficial effects on healing of excision, incision, and dead space wounds in rats[32,33]
Punica granatum L. (pomegranate)	Lythraceae	Gingival bleeding[27], Sores[27]	Stimulation healing of tooth extraction wounds in guinea pigs[34]
			Stimulation healing of deep second-degree burn skin wounds in rats[35]
Piper betle L. (betel leaf)	Piperaceae	Nose bleeding[26,28], Sores and pustules[28], Disinfection of wounds[28]	Stimulation healing of burn and excision wounds in Swiss mice[36]
Stachytarpheta jamaicensis (L.) Vahl.(Jamaica vervain)	Verbenaceae	Sores[25,29], Open wounds[29]	Stimulation healing of excision and dead space wounds in diabetic rats[37,38] and normal rats[39]
Uncaria guianensis (Aubl.) J. F. Gmel.(cat's claw)	Rubiaceae	Disinfection of wounds[25,30], Gingival bleeding[30]	Efficacious in osteoarthritis of the knee[40], Protection of rats from induced gastritis[41]
Copaifera guyanensis Lindl. (hoepelhout)	Fabaceae	Infected wounds[25,26], Superficial and deep cuts[25,26]	Stimulation wound healing by genus *Copaifera* in rats[42]
			Beneficial effects on healing of wounds in rabbit's ears[43] and dorsum of rats[44]

or pesticide use for at least the preceding 6 months. The collections were done in close collaboration with the National Herbarium of Suriname (BBS) 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. None of the collected plants was on the International Union for Conservation of Nature's Red List of endangered or threatened species[45]. When necessary, free, prior and informed consent had been sought from the indigenous and tribal communities on whose territory the plants were collected[46]. The collection sites have been determined using a GPSmap® 60CSx receiver (Garmin Ltd., Miami Beach, FL, USA) and have been recorded (Table 2). From all collected plant species, voucher specimens have been prepared which have been assigned a reference number (Table 2) and have been stored at the BBS for future reference.

Drugs and chemicals: Brine shrimp was from Ocean Star International (Salt Lake, UT, USA), Dimethyl Sulfoxide (DMSO) from Mediatech, Inc. (Manassas, VA, USA) and tricaine from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were from our laboratory stock and were of the highest grade available.

Preparation of plant extracts: The collected plant parts (Table 1 and 2) were first thoroughly washed with tap water, then with distilled water, dried in the open air and extracted as indicated in Table 2. The extracts were filtered, freeze-dried and divided into aliquots of 10 mg that were stored at -20°C. The methods for preparing the plant extracts are approximations of the ways they are made by Surinamese traditional healers.

Zebrafish and maintenance: Adult Tg(fli1a:EGFP)y1/+ zebrafish were from Zebrafish International Resource Center (Eugene, OR, USA) and were maintained under standard laboratory conditions using a light schedule of 14 hrs on and 10 hrs off and at a temperature of 28°C[47]. The fli1 promoter of this transparent and transgenic zebrafish line stimulates the expression of Enhanced Green Fluorescent Protein (EGFP) in the endothelial cells, enabling visualization of blood vessel development throughout embryogenesis[48]. The fish were fed three times daily with a combination of dry food and freshly hatched brine shrimp[47]. For experiments, fertilized eggs were harvested shortly after the light was turned on and kept in Hank's solution (0.137 M NaCl,

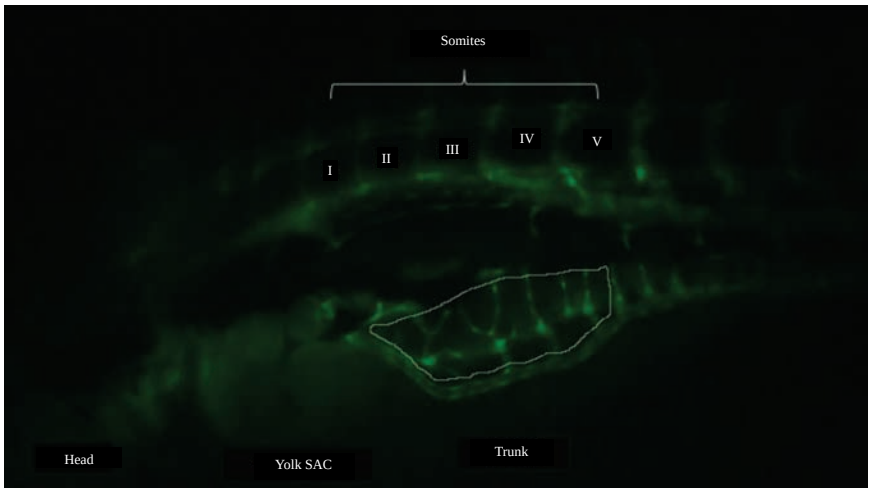

Fig. 1: Fluorescence microscopic visualization of blood vessels of Tg (fli1a:EGFP)y1/+zebrafish embryo at 96 hpf

For all embryos, the length of the subintestinal blood vessels was measured inside the delimited area, underneath the five indicated somites, total subintestinal blood vessel length was expressed in µm

Table 2: Collection sites, herbarium voucher numbers, parts used and methods of processing of the plants investigated in the current study

Plant species	Collection site	Herbarium voucher number	Plant part used	Method of processing
C. guianensis	Brokopondo district (21N 0712666, 0582200)	UVS 18.499	Stembark	Maceration and extraction for 2 hrs with petroleum ether
P. granatum	Nickerie district (21N 0507685, 0652131)	UVS 18.494	Fruit	Maceration and filtration
P. betle	Wanica district (21N 0674859, 0648865)	UVS 18.495	Leaf	Maceration and extraction for 1 h with distilled water at 100°C
S. jamaicensis	Paramaribo district (21N 0680139, 0673271)	UVS 18.496	Leaf	Maceration and extraction for 1 h with distilled water at 45°C
U. guianensis	(Para district (21N 0689695, 0635638)	UVS 18.497	Leaf	Maceration and extraction for 2 hrs with distilled water at 100°C
C. guyanensis	Brokopondo district (21N 0712913, 0581341)	UVS 18.498	Stembark	Maceration and extraction for 2 hrs with petroleum ether

All reference vouchers have been stored at the National Herbarium of Suriname (BBS) at the Anton de Kom University of Suriname, Paramaribo, Suriname (UvS: Universiteit van Suriname)

5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 4.2 mM $NaHCO_3$).

Assessment of effects of plant extracts on total subintestinal vessel length in and survival of Tg(fli1a:EGFP)y1/+zebrafish embryos: At 8 hrs post-fertilization (hpf), eggs from the Tg(fli1a:EGFP)y1/+ zebrafish were harvested and exposed to serial dilutions of the plant extracts between 10^{-5} and 100 µg mL^{-1} dissolved in Hank's solution containing DMSO 0.1% (v/v). Eggs exposed to medium alone served as controls. The incubations took place at a temperature of 28.2°C and a minimum relative humidity of 95%[47]. At 30 hpf, the hatched embryos[49] were carefully removed from the chorion under a Stemi 2000-C stereomicroscope (Carl Zeiss AG, Oberkochen, Germany) and using pincers and allowed to swim freely in fresh plant extract-containing medium or fresh medium alone. The media were refreshed at 48 and 72 hpf. The experiments were terminated at 96 hpf, because SIVs partially degenerate after this period[49,50].

At the end of the experiments, the embryos were anaesthetized by transferring them to a medium containing tricaine 150 mg L^{-1} [49], after which their SIVs were visualized under an Axiovert 40 CFL fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany) and photographed. All photographs were from the five upper somites and have been taken from embryos placed in the same position[51] (Fig. 1). Total subintestinal vessel lengths were determined with the Axiovision 4.8.1 Image Acquisition and Management Software for Light Microscopy (Carl Zeiss AG, Oberkochen, Germany) and were in µm[48]. The numbers of embryos surviving under the various conditions were also recorded.

Data processing and statistics: Total SIV lengths in zebrafish embryos that had been exposed to a plant extract were expressed relative to those found for untreated controls. Similarly, numbers of surviving embryos at 96 hpf after exposure to a plant extract were expressed to that of untreated controls surviving at that time point. All experiments have been carried out at least three times in

Table 3: Mean subintestinal blood vessel length (±SDs) in Tg(fli1a:EGFP)y1/+zebrafish embryos at 96 hpf after treatment with the plant extracts relative to that found for untreated controls

	Mean relative subintestinal blood vessel lengths (expressed in % of control values) of zebrafish embryos at 96 hpf at plant extract concentrations							
	Concentration (μg mL^{-1})							
Plant varieties	0.00001	0.0001	0.001	0.01	0.1	1.0	10.0	100.0
C. guianensis	110±52	91±40	86±53	58±35*	N.d.	N.d.	N.d.	N.d.
P. granatum	N.d.	N.d.	N.d.	N.d.	98±41	86±42	91±51	79±52
P. betle	N.d.	N.d.	N.d.	N.d.	100±36	93±48	76±42	40±33
S. *jamaicensis*	N.d.	N.d.	N.d.	N.d.	86±51	89±44	77±52	50±38
U. guianensis	N.d.	N.d.	N.d.	N.d.	97±39	87±43	79±45	67±55
C. guyanensis	98±36	102±31	118±34	104±55	N.d.	N.d.	N.d.	N.d.

*Statistically significantly different from untreated controls (p = 0.001, one-way ANOVA), Data are in percentages and have been expressed to that of the controls which were set at 100%, N.d.: Not done

Table 4: Mean percentage of Tg(fli1a:EGFP)y1/+ zebrafish embryos surviving at 96 hpf after treatment with the plant extracts relative to that found for untreated controls

	Mean percentage of zebrafish embryos surviving at 96 hpf (expressed in % of control values) at plant extract concentrations							
	Concentration (μg mL^{-1})							
Plant varieties	0.00001	0.0001	0.001	0.01	0.1	1.0	10.0	100.0
C. guianensis	97±12	89±9	81±21	71±29	N.d.	N.d.	N.d.	N.d.
P. granatum	N.d.	N.d.	N.d.	N.d.	92±13	92±16	80±32	62±22
P. betle	N.d.	N.d.	N.d.	N.d.	81±14	78±23	65±25	33±23
S. *jamaicensis*	N.d.	N.d.	N.d.	N.d.	90±14	81±12	73±18	41±17
U. guianensis	N.d.	N.d.	N.d.	N.d.	85±21	84±16	68±34	55±20
C. guyanensis	84±17	85±17	85±11	81±17	N.d.	N.d.	N.d.	N.d.

Latter value was set at 100%, N.d.: Not done

triplicate. Results are Means±SDs and are given in Table 3 and 4. The p-values ≤0.05 were taken to indicate statistically significant differences according to one-way ANOVA.

RESULTS

Effects of plant extracts on subintestinal vessel length of zebrafish embryos: Six extracts from plant species that are popularly used for treating wounds have been evaluated for their capacity to stimulate the formation of SIVs in developing Tg(fli1a:EGFP)y1/+ zebrafish embryos at 96 hpf. The rationale for this study was based on the importance of new blood vessel formation to the process of wound healing.

Table 3 gives the total SIV length of Tg(fli1a:EGFP)y1/+ zebrafish embryos at 96 hpf after exposure to the plant extracts. The total SIV length of the untreated controls at that time point was on average about 1,400 μm. None of the six plant extracts produced at any of the concentrations tested a statistically significantly greater total SIV length when compared to that of the untreated controls. This suggests that none of the plant extracts stimulated blood vessel formation in the zebrafish embryos and that none of them elicited proangiogenic activities under the experimental conditions applied in the current study.

However, total SIV length in embryos treated with the *C. guianensis* extract at 0.01 μg mL^{-1} was 58±35%, which was statistically significantly different from control values that were set at 100% (p = 0.001, one-way ANOVA) (Table 3). This difference is illustrated in Fig. 2 which clearly shows the difference in SIV length in arbitrarily selected control fish (Fig. 2a, about 1,568 μm) and that in fish treated with the *C. guianensis* extract 0.01 μg mL^{-1} (Fig. 2b, about 476 μm). Total SIV lengths in embryos exposed to the *P. betle* or *S. jamaicensis* extract at 100.0 μg mL^{-1} were also less (about 60 and 50%, respectively) when compared to those of the controls.

Effects of plant extracts on the survival of zebrafish embryos: Assessment of the numbers of zebrafish embryos surviving at 96 hpf after exposure to the plant extracts indicated that the *C. guianensis* extract had relatively little effect on the viability of the zebrafish embryos, leaving 71±29% of them unharmed at the concentration of 0.01 μg mL^{-1} (Table 4). This suggests that this preparation may have exerted antiangiogenic effects in the current study. However, the use of the *P. betle* or the *S. jamaicensis* extract at 100.0 μg mL^{-1} led to only 33±23 and 41±17%, respectively, of the zebrafish embryos surviving when compared to the controls (Table 4). This strongly suggests that

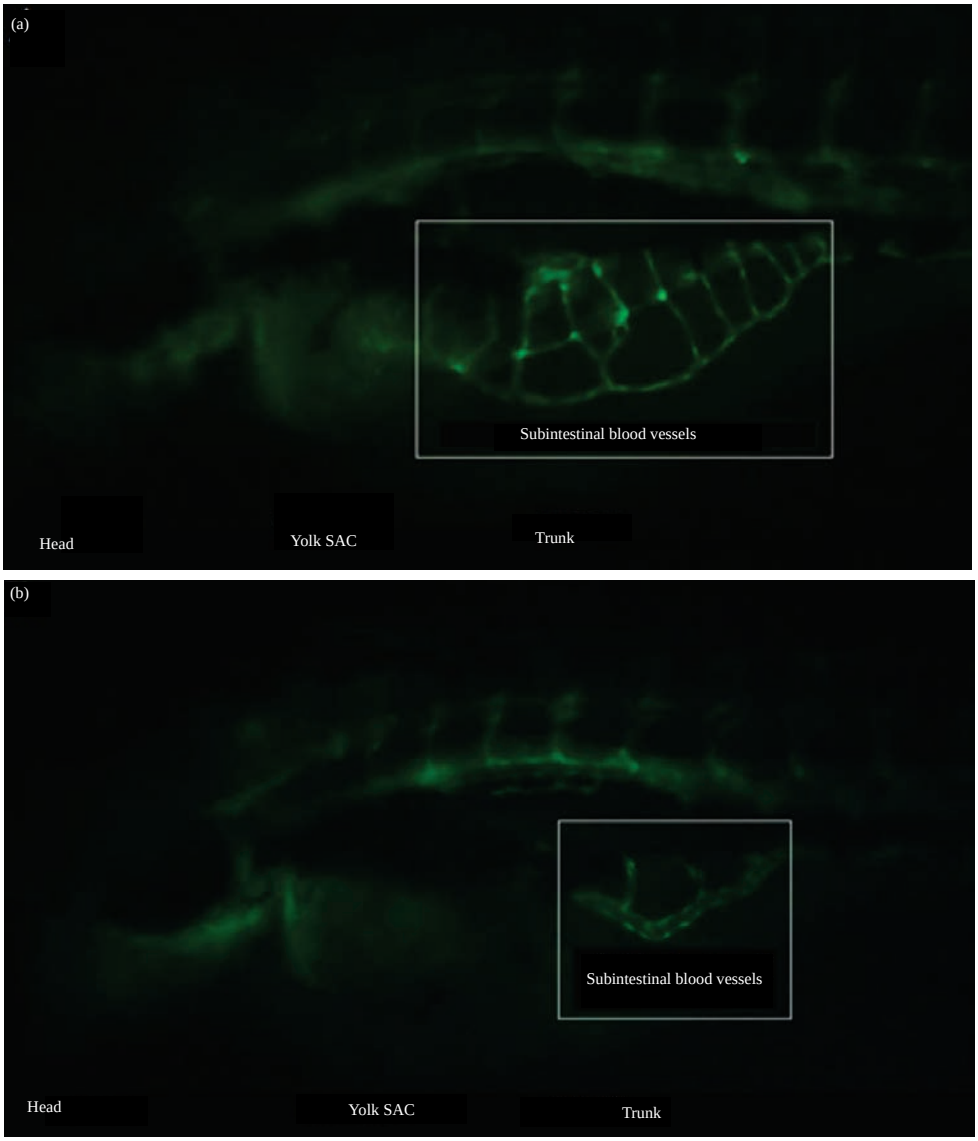

Fig. 2a-b: Fluorescence microscopic visualization of blood vessels of Tg(fli1a:EGFP)y1/+ zebrafish embryo at 96 hpf in control fish (a) and in fish treated with the *C. guianensis* extract 0.01 μg mL^{-1} (b)

Total subintestinal blood vessel lengths in these arbitrarily selected control and *C. guianensis* extract-treated embryos were about 1,568 μm and 476 μm, respectively

the effects of these plant extracts on SIV lengths were attributable to general toxicity rather than to an antiangiogenic effect as inferred for the *C. guianensis* extract.

DISCUSSION

Neoangiogenesis is an important phase in the process of wound healing[2,3]. In this study, preparations from six medicinal plants that are traditionally used in Suriname for managing wounds[25-30], have been evaluated for their potential to stimulate angiogenesis. To this end, the plant extracts have been assessed for their stimulatory effects on total SIV length in developing Tg(fli1a:EGFP)y1/+ zebrafish embryos. The plants and plant parts investigated were the stem bark from *C. guianensis* and *C. guyanensis*, the fruit from *P. granatum* as well as the leaves from *P. betle*, *S. jamaicensis* and *U. guianensis*. Preparations from *C. guianensis* seed oil and leaf stimulated wound healing in rodent models[31-33] as did the oleoresin from the bark of various *Copaifera* members[42-44]. The same held for the *P. granatum* fruit juice[34,35] as well as the leaf extracts from *P. betle*[36] and *S. jamaicensis*[37-39], while *U. guianensis* leaf preparations were efficacious in

osteoarthritis of the knee[40] and protected rats from induced gastritis[41]. Together, these data suggest that these plants possess wound healing stimulating activities that may be associated with angiogenesis. However, the use of none of the preparations from the plant parts led to a greater SIV length in the zebrafish embryos, suggesting that none of them exhibited proangiogenic properties under the experimental conditions applied. However, exposure of the fish to the *C. guianensis* extract led to a lower total SIV length when compared to untreated controls, suggesting that this preparation possessed antiangiogenic properties.

The apparent antiangiogenic effect of the *C. guianensis* stem bark extract is at variance with the previously reported proangiogenic activities of andiroba oil prepared from the seed of the plant. Given by oral gavage, the oil stimulated angiogenesis along with fibroblast proliferation and other parameters of healing in open wounds in the cecum of Wistar rats[52]. The stimulation of wound healing by topically applied andiroba oil in alloxan-induced diabetic rats was accompanied by the promotion of neovascularization[31]. The use of a topical commercial emulsion containing andiroba oil (called Tegum®) led to the improvement of the healing of and upregulation of transforming growth factor β_3 levels as well as an increase in the number of capillaries reactive to factor VIII-related antigen in full-thickness cutaneous wounds in Wistar rats[53]. Notably, in addition to the seed oil[31], extracts from the leaves[32] but also those from the stembark[33] stimulated wound healing in laboratory rats.

These data support that *C. guianensis* possesses proangiogenic properties and make it difficult to explain the clear antiangiogenic effect observed in the current study. As explained in critical analyses of the antiangiogenic properties of plant-derived substances[54] and commonly used angiogenesis assays[55], differences in extraction procedure and laboratory model may produce substantially different outcomes. This may even involve opposite effects on the degree of blood vessel formation although the test compounds may elicit comparable wound healing-stimulating effects[54,55]. In the current study, *C. guianensis* stem bark was extracted with petroleum ether and the highly lipophilic fraction obtained was given to zebrafish embryos for absorption through the skin. On the other hand, in one of the previous studies[33], the stembark was extracted with water and the typically hydrophilic fraction obtained was orally administered to rats. Thus, the discrepancy between the current results and that described in the literature[33] may be attributed, at least in part, to differences in extraction conditions, chemical nature of the test compounds, administration route and/or laboratory model. This supposition must be verified in future studies. This is particularly important since the use of an antiangiogenic compound can impede proper wound healing, although such a compound may have merit in diseases associated with excessive blood vessel formation.

Comparable considerations may account for the discrepancies between the current results with the *P. granatum*, *P. betle* and *S. jamaicensis* preparations on the one hand and data reported in the literature on the other hand. Incidentally, the literature data were also not consistent with each other. Thus, *P. granatum* juice did not affect total zebrafish embryo SIV length in the current study but stimulated healing as well as Vascular Endothelial Growth Factor (VEGF) and Platelet-derived Growth Factor (PDGF) expression in tooth extraction wounds in guinea pigs[34], suggesting that it possessed proangiogenic properties. On the other hand, *P. granatum* fruit juice, fruit extract, a polyphenol fraction from the fermented fruit juice or punicalagin, an antioxidant ellagitannin in pomegranate juice, exerted antiangiogenic effects in several human carcinoma cell lines as well as human umbilical vein endothelial cells[56,57], chick chorioallantoic membrane assays[56,58,59] and tumour xenograft-mouse models[60-62].

A methanol extract from *P. betle* leaf stimulated the proliferation of fibroblasts in a scratch-wound healing assay[36], hinting that it possessed proangiogenic properties. In contrast, the phenolic compound eugenol that is abundantly present in *P. betle* leaf exhibited chemopreventive[63] and antiangiogenic activities in Wistar rats with N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric cancer[64]. Importantly, the antiangiogenic activity of eugenol might be mediated by interference with VEGF levels and VEGF-receptor-1 expression[64]. And *S. jamaicensis* leaf preparations stimulated wound healing in diabetic and normal laboratory rats[37-39] but elicited an antiangiogenic effect in a chick chorioallantoic membrane assay[65]. The disagreements between these observations on the one hand and the lack of an effect of the *P. betle* and the *S. jamaicensis* extracts in the current study, on the other hand, may also be attributable to differences in experimental conditions[54,55]. This also must be confirmed in additional studies.

To our knowledge, there are no studies on the effects of *U. guianensis* leaf and *C. guyanensis* stembark preparations on blood vessel formation. However, a hydroalcoholic extract from the stembark of *Uncaria tomentosa* (Willd. ex Schult.) DC. increased the expression of cytokines such as IFN-γ that downregulated angiogenesis in endothelial cells[66] and reduced staining for Factor VIII in subcutaneously injected B16-BL6 murine melanoma cells in C57BL/6 mice[67]. On the

other hand, an ointment containing 10% copaiba oil from the stembark of *Copaifera langsdorffii* Desf. Kuntze stimulated angiogenesis and accelerated the viability of random skin flaps in laboratory rats[68], while creams prepared from either the oleoresin from *C. langsdorffii* stembark or a hydroalcoholic extract from the leaf of this plant promoted angiogenesis as well as reepithelialization, wound retraction and remodelling in skin wounds in Wistar rats[69]. These observations suggest that *Uncaria* preparations may possess antiangiogenic properties and that those from *Copaifera* preparations may have proangiogenic characteristics. These dissimilarities with the current study where no effects on blood vessel formation were observed, may also tentatively be explained by differences in experimental conditions[54,55] but this also remains to be determined.

Summarizing, the results from this study suggest that preparations from *C. guianensis*, *C. guyanensis*, *P. granatum*, *P. betle*, *S. jamaicensis* and *U. guianensis* do not possess proangiogenic properties. This suggests that the traditional claims of wound healing activities of these plants may not be associated with proangiogenic events. The apparent antiangiogenic properties of the *C. guianensis* extract may even contraindicate its use for wound healing but may make it useful against conditions associated with excessive angiogenesis. However, these conclusions must be regarded with some caution. The developing zebrafish embryos used in the current study have mainly absorbed the plant extracts through their skin instead, which might have led to relatively high and potentially toxic concentrations in their entire body. This might well have perturbed organogenesis including the development of the circulatory system[70]. This may be of particular relevance to zebrafish embryos which are reportedly much more susceptible to potentially toxic compounds when compared to adult zebrafish and other *in vivo* models of angiogenesis[71]. These considerations underscore the need for multiple model systems to evaluate compounds for their potential effect on angiogenesis. Until these additional studies have been carried out, the traditional use of the plants for managing wounds should be discouraged.

CONCLUSION

The results from this study suggest that the traditional use of preparations from *C. guianensis* and *C. guyanensis* stembark, *P. granatum* fruit as well as *P. betle*, *S. jamaicensis* and *U. guianensis* leaf for wound healing cannot be explained by proangiogenic effects in the wound area. The extract of *C. guianensis* stembark may even possess antiangiogenic properties, suggesting that it may have merit in conditions associated with excessive angiogenesis. The presumed wound healing-stimulating activities of the plants may be attributable to (a) mechanism(s) other than the promotion of blood vessel formation. These possibilities should be investigated in comprehensive studies using various *in vitro* and *in vivo* models.

SIGNIFICANCE STATEMENT

Many conditions are treated with plant-derived traditional medicines, often without sufficient evidence for clinical efficacy. This may lead to the use of inefficacious or even unsafe medications. Nevertheless, further evaluation of these is warranted, not only to establish their medicinal usefulness but also to explore unforeseen applications.

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