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# Anti-angiogenic Rather than Pro-angiogenic and Wound Healing-Promoting Effects of *Lantana camara* L. (*Verbenaceae*) in a Zebra Fish Model of Tissue Regeneration and in Cultured Human Umbilical Vein Endothelial Cells

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author DRAM designed and supervised the study, wrote the protocol, and wrote the first draft of the manuscript. Authors JRT and RB supervised the laboratory studies and performed the statistical analyses. Authors JAH and KO were in charge of the plant collections and extractions. Authors MD, PF and JP carried out and interpreted the cell culture studies. Authors IM and RCS carried out and interpreted the zebra fish studies. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** Plant-derived substances are extensively used for promoting wound healing in various traditional medicinal systems. In this study, extracts from such plants were evaluated for such properties using an animal model of tissue repair and angiogenesis and a cell culture model of

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proliferation, migration, and angiogenesis.
**Place and Duration of the Study:** The study was carried out for sixteen months at the Departments of Pharmacology and Physiology of the Faculty of Medical Sciences, Anton de Kom University, Paramaribo, Suriname.
**Design and Methods:** Aqueous extracts from parts of *Aloe vera*, *Cinnamomum cassia*, *Lantana camara*, *Momordica charantia*, *Psidium guajava*, and *Solanum melongena* were evaluated for their capacity to stimulate the regeneration of the amputated caudal fin of wild-type AB zebra fish embryos; the development of sub-intestinal vessels of Tg(fli1a:EGFP)y1/+ zebra fish embryos; the closure of scratch-wound gaps in human umbilical vein endothelial cell (HUVEC) monolayers; and capillary-like structure formation by these cells in matrigel. The data obtained were compared to those found with untreated controls and considered statistically significantly different when *P* values < .05 (Student's *t* test).
**Results:** None of the plant extracts stimulated fin regeneration and sub-intestinal vessel formation in the fish embryos or HUVEC scratch-wound closure and capillary-like structure formation. However, the *L. camara* extract delayed the regrowth of the amputated fin and the formation of sub-intestinal vessels by approximately 30 (*P* =.004) and 50% (*P* = 0.1), respectively, and inhibited HUVEC scratch-wound closure and capillary-like structure formation by roughly 100 (*P* = .004) and up to about 50% (*P* = .04 and .05), respectively. The remaining plant extracts did not affect either of these phenomena.
**Conclusion:** The *L. camara* extract exerted marked anti-angiogenic characteristics rather than pro-angiogenic and wound healing-promoting properties. This makes it a candidate for evaluation in diseases caused by excessive angiogenesis.

*Keywords: Medicinal plants; wound healing; zebra fish; amputated caudal fin; sub-intestinal vessels; HUVECs; scratch-wound; capillary-like structure formation.*

## 1. INTRODUCTION

A wound can be defined as a loss or the disruption of the cellular, anatomical, and functional continuity of living tissues as a result of trauma [1]. Wound healing occurs through a complex and dynamic, but highly regulated cascade of biochemical and cellular events including inflammation, epithelialization, angiogenesis, the formation of granulation tissue, and the deposition of interstitial matrix [1]. These events involve many cell types, growth factors (including vascular-endothelial growth factor (VEGF) regarded as one of the most important angiogenic growth factors [2]), and other proteins which cooperate in a well-organized manner to restore the normal architecture and physiology of the wounded tissues [1,2]. However, an adequate blood supply to the wound area is generally considered pivotal for restoring the integrity of the wound area [3]. Thus, insufficient revascularization is particularly likely to cause poorly or non-healing wounds [4,5]. For these reasons, many research efforts in the area of wound healing focus on the development of modalities that improve vascularization of the wound area [6].

Human beings have been using plants, herbal preparations, and plant constituents since ancient times for treating a multitude of diseases [7]. The exploration of the traditional medicinal systems from a wide diversity of societies and cultures has led to the isolation of many secondary plant metabolites that have become well-established pharmaceuticals. Examples are the cardiac stimulant digoxin, the antitussive codeine, the antineoplastic drug vincristine, and the oral hypoglycemic agent metformin [7]. Markedly, roughly 25% of the prescription drugs worldwide have a natural origin [8] and nearly 75% of these compounds have been developed following ethnopharmacological leads [9]. This may also hold true for naturally-derived plant-based compounds that stimulate angiogenesis and wound healing.

Notably, extracts, decoctions, and pastes from medicinal plants are used in many parts of the world to promote or accelerate the healing of acute wounds such as lacerations, cuts, burns, bruises, boils, sores, chemicals, heat or cold, and abscesses, as well as chronic wounds caused by infection such as diabetic, vascular, and pressure ulcers [10-12]. This holds particularly true for the populations of many developing countries who have limited health care facilities and have to rely largely on medicinal plants for their primary health care [13]. Table 1 gives a few examples of plants with presumed wound-healing properties [14-16]. So far, however, the scientific evidence to support these claims is conflicting and scant.

As an example, preparations from *A. vera* would promote wound healing [17,18], but systemic reviews and meta-analyses concluded that the evidence to support these claims was insufficient [19-21].

For these reasons, the above-mentioned plants were evaluated for their potential wound-healing properties using an animal model of tissue repair and angiogenesis in conjunction with a cell culture model of proliferation, migration, and angiogenesis of human endothelial cells. Thus, parts of the plants were collected, extracted with distilled water, and assessed for their ability to accelerate the regeneration of the amputated caudal fin of embryos from the zebra fish *Danio rerio*, and to stimulate the formation of sub-intestinal vessels in these fish, the closure of scratch-wound gaps in monolayers of human umbilical vein endothelial cells (HUVECs), and the formation of capillary-like structures by these cells. The results from these studies have been discussed in terms of the potential usefulness of preparations from the plants to promote wound healing.

## 2. MATERIALS AND METHODS

### 2.1 Plants and Preparation of Plant Extracts

Table 1 lists the plants and plant parts investigated in the current study. The samples 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. After authentication by experts from the National Herbarium of Suriname, the collected plant parts were washed with distilled water, dried in open air, washed again with distilled water, and extracted as indicated in Table 1. The extracts were freeze-dried, divided in aliquots of 2 g, and stored at -20ºC until experiments.

### 2.2 Zebra Fish and Maintenance

Adult wild-type (AB) and Tg(fli1a:EGFP)y1/+ zebra fish (Zebra fish International Resource Center, Eugene, OR, USA) 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. The fish were fed three times daily with a combination of dry food and freshly hatched brine shrimp (Ocean Star International, Salt Lake, UT, USA). For experiments, fertilized eggs of both strains of fish were harvested shortly after the light was turned on and kept in Hank's solution (0.137 M NaCl, 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 $NaHCO_3$).

### 2.3 Assessment of Effects of Plant Extracts on the Regeneration of the Amputated Caudal Fin of AB Zebra Fish Embryos

The effects of the plant extracts on tissue regeneration were assessed on the amputated caudal fin of wild-type (AB) zebra fish embryos as described [22]. Thus, at 24 hours post-fertilization (hpf), embryos were collected and removed from the chorion.

**Table 1. Plants and plant parts investigated in the current study, extraction procedures applied, and literature data referring to their presumed wound-healing properties**

| Plant species (Vernacular name) | Plant family | Plant part used and extraction procedure | References on traditional use for wound healing |
|---|---|---|---|
| *Aloe vera* (L.) Burm.f. (True aloe) | Xanthorrhoeaceae | Macerated aerial parts in water, 1 h, 100ºC | [14] |
| *Cinnamomum cassia* (Nees & T. Nees) Farw (Chinese cinnamon) | Lauraceae | Macerated leaves in water, 2 h, 45ºC | [16] |
| *Lantana camara* L. (Wild sage) | Verbenaceae | Macerated aerial parts in water, 2 h, 45ºC | [15] |
| *Momordica charantia* L. (Bitter melon) | Cucurbitaceae | Macerated aerial parts in water, 2 h, 45ºC | [15] |
| *Psidium guajava* L. (Guava) | Myrtaceae | Macerated bark in water, 1 h, 40ºC | [16] |
| *Solanum melongena* L. (Eggplant) | Solanaceae | Macerated roots in water, 1 h, 100ºC | [14] |

Twenty-four hours later, their caudal fin was amputated at the border of the notochord, after which the embryos were exposed to serial dilutions of the plant extracts between 0.1 and 10 µg/mL in Hank's solution containing 0.1% (*v/v*) dimethyl sulfoxide (DMSO; Mediatech, Inc., Manassas, VA, USA). Forty-eight hours later, the surface area of the fin was determined from the notochord on under a stereomicroscope, and expressed relatively to that of controls, *i.e.*, amputated embryos exposed to Hank's solution and DMSO alone.

### 2.4 Assessment of Effects of Plant Extracts on Total Sub-intestinal Vessel Length in Tg(fli1a:EGFP)y1/+ Zebra Fish Embryos

At 8 hpf, eggs from Tg(fli1a:EGFP)y1/+ zebra fish were harvested and exposed to serial dilutions of the plant extracts (0.1 to 10 µg/mL) dissolved in Hank's solution containing 0.1% (*v/v*) DMSO. The fli1 promoter of this transgenic zebra fish line stimulates the expression of enhanced green fluorescent protein (EGFP) in all endothelial cells, enabling visualization of blood vessel development throughout embryogenesis [23]. At 30 hpf, the embryos were removed from the chorion and allowed to swim freely in the plant extract-containing medium. At 96 hpf, 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 controls.

### 2.5 Human Umbilical Vein Endothelial Cells and Maintenance

Human umbilical vein endothelial cells (HUVECs) were 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 37ºC, a minimum relative humidity of 95%, and an atmosphere of 5% $CO_2$ in air. For experiments, exponentially growing cells were detached from the culture flasks using ethylenediaminetetraacetic acid (EDTA)-trypsin (ATCC, Manassas, VA, USA).

### 2.6 Determination of Sub-toxic Concentrations of Plant Extracts

Triplicate cultures of HUVECs between P4 and P10 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 1,000 µg/mL 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 three days and in final volumes of 200 µL per well. Cellular responses at the end of the incubations were assessed with the sulforhodamine B (SRB) assay [24]. 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 Buffer 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 $IC_{20}$ values were derived, *i.e.*, extract concentrations resulting in approximately 20% inhibition of cell proliferation when compared to untreated controls. The studies on wound-gap closure and capillary-like structure formation mentioned hereunder were carried out at these sub-toxic concentrations of each plant extract, because under these conditions the relatively slight inhibitory effects on HUVEC proliferation would not substantially interfere with these phenomena.

### 2.7 Assessment of Effects of Plant Extracts on Wound Gaps in HUVEC Monolayers

Triplicate cultures of HUVEC between P4 and P10 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 80% confluence. Next, the medium was removed and a scratch representing a wound gap was applied in the center of the monolayers using a sterile 200-µL pipette tip [25]. The detached cells were carefully removed, and the cultures were exposed to the plant extracts at the sub-toxic concentrations determined as mentioned in the preceding paragraph 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, after which the samples were photographed. Wound-gap areas were determined using the WimScratch Image Analysis platform (Ibidi GmbH, München, Germany) and expressed relatively to those found for controls, *i.e.*, cell cultures exposed to medium alone.

### 2.8 Assessment of Effects of Plant Extracts on Capillary-Like Structure Forming Activity of HUVECs

Capillary-like structure formation by HUVECs was performed according to a previously described method [26] with minor modifications. Thus, 96-well culture plates were coated with 50 µL of CellMatrix Gel (ATCC, Rockville, MD, USA) which was allowed to solidify at 37 °C for 30 min. Then, triplicate cultures of HUVEC between P7 and P10 were seeded onto the CellMatrix at densities of 6 x $10^3$ cells in 100 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 the sub-toxic concentrations determined as mentioned above in the presence of 100 IU/mL penicillin, 100 µg/mL streptomycin, and 5 µg/mL amphotericin B (Corning, Manassas, VA, USA) and in final volumes of 200 µL per well. Incubations were for 6 h, after which the samples were photographed. 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.

### 2.9 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 Student's *t* test.

## 3. RESULTS

### 3.1 Effects of Plant Extracts on the Regeneration of the Amputated Tail Fin of AB Zebra Fish Embryos

The potential wound healing-accelerating effects of extracts from *A. vera*, *C. cassia*, *L. camara*, *M. charantia*, *P. guajava*, and *S. melongena* were determined by assessing these preparations for their capacity to promote the regeneration of the amputated caudal fin of wild-type AB zebra fish embryos. As shown in Table 2 and Fig. 1, 48 h after amputation, the mean surface area of the caudal fin of embryos exposed to the extract from *L. camara* (10 µg/mL) was approximately 30% reduced when compared to that of untreated, sham-operated controls. This suggests that this plant extract had inhibited the regeneration of the amputated fin rather than exhibiting wound healing-promoting properties. The remaining plant extracts did not affect the growth of the amputated tail fin of the fish embryos statistically significantly.

### 3.2 Effects of Plant Extracts on Sub-intestinal Vessel Growth in Tg(fli1a:EGFP)y1/+ Zebra Fish Embryos

Table 3 gives the total length of the sub-intestinal vessels of Tg(fli1a:EGFP)y1/+ zebra fish embryos exposed to the plant extracts. Total sub-intestinal vessel length of fish treated with the *L. camara* extract at 10 µg/mL was roughly 30% of that found for untreated controls (Table 3; Fig. 2). Thus, this sample inhibited rather than stimulated the formation and proliferation of blood vessels. This suggests that it possessed anti-angiogenic rather than pro-angiogenic properties, substantiating its apparent lack of wound-healing properties mentioned in the previous paragraph. The extracts from *A. vera*, *C. cassia*, *M. charantia*, *P. guajava*, and *S. melongena* had no statistically significant effect on total sub-intestinal vessel length of the fish embryos (Table 3).

**Table 2. Mean surface areas of the amputated caudal fin of AB zebra fish embryos exposed for 48 h to the plant extracts (0.1 to 10 µg/mL) with respect to those found for sham-operated, untreated controls. The latter values were set at 100%. Data are means ± SDs of at least three independent experiments performed in triplicate**

| Plant species | Mean surface areas (± SDs) of the amputated caudal fin of AB zebra fish embryos with respect to that in controls at plant extract concentrations of: | | |
|---|---|---|---|
| | 0.1 µg/mL | 1 µg/mL | 10 µg/mL |
| *A. vera* | 93±18 | 106±16 | 97±25 |
| *C. cassia* | 78±15 | 79±18 | 88±7 |
| *L. camara* | 97±8 | 76±16 | 68±4[1] |
| *M. charantia* | 94±7 | 95±11 | 94±10 |
| *P. guajava* | 93±11 | 86±9 | 89±0 |
| *S. melongena* | 93±11 | 102±7 | 105±7 |

[1]*Statistically significantly different from control (P =.004; Student's t test). The other values did not differ statistically significantly from the control (P > .05; Student's t test)*

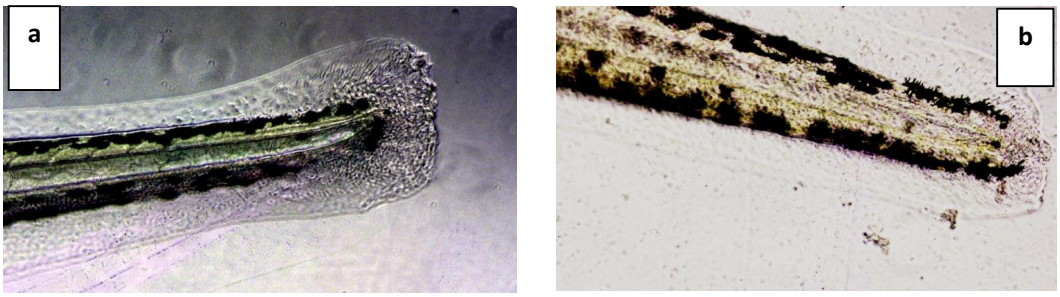


**Fig. 1. Amputated caudal fin of AB zebra fish embryo exposed for 48 h to the *L. camara* extract 10 µg/mL (a) *versus* that in untreated controls (b)**

### 3.3 Effects of Plant Extracts on Scratch-Wound Gap Area in HUVEC Monolayers

Next, the plant extracts were assessed for their effects on the closure of scratch-wound gaps in HUVEC monolayers. These experiments were carried out at the sub-toxic concentrations of the plant extracts (around their $IC_{20}$ values) given in Table 4, which were estimated from dose-response profiles obtained by exposing HUVEC cultures for three days to serial dilutions of the plant extracts and plotting cell densities against extract concentrations. As shown in Table 4 and Fig. 3, the average scratch-wound area in HUVEC monolayers exposed to the *L. camara* extract was roughly twice that of the untreated control. This suggests that the *L. camara* plant extract had considerably inhibited wound gap closure in HUVECs, corroborating its apparent anti-angiogenic properties. None of the other plant extracts exerted a statistically significant effect on HUVEC wound-gap area (Table 4).

**Table 3. Mean total length of sub-intestinal vessels of *Tg(fli1a:EGFP)y1/+* zebra fish embryos exposed for 96 h to the plant extracts (0.1 to 10 µg/mL) 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 | Mean total sub-intestinal vessel length (± SDs) in *Tg(fli1a:EGFP)y1/+* zebra fish embryos with respect to that in controls at plant extract concentrations of: | | |
|---|---|---|---|
| | 0.1 µg/mL | 1 µg/mL | 10 µg/mL |
| *A. vera* | 114±12 | 114±23 | 117±9 |
| *C. cassia* | 85±6 | 82±8 | 86±11 |
| *L. camara* | 103±36 | 106±18 | 48±25[1] |
| *M. charantia* | 113±15 | 105±15 | 101±15 |
| *P. guajava* | 96±15 | 87±24 | 84±13 |
| *S. melongena* | 96±3 | 85±15 | 85±10 |

[1]*Statistically significantly different from control (P =.01; Student's t test). The other values did not differ statistically significantly from the control (P > .05; Student's t test)*

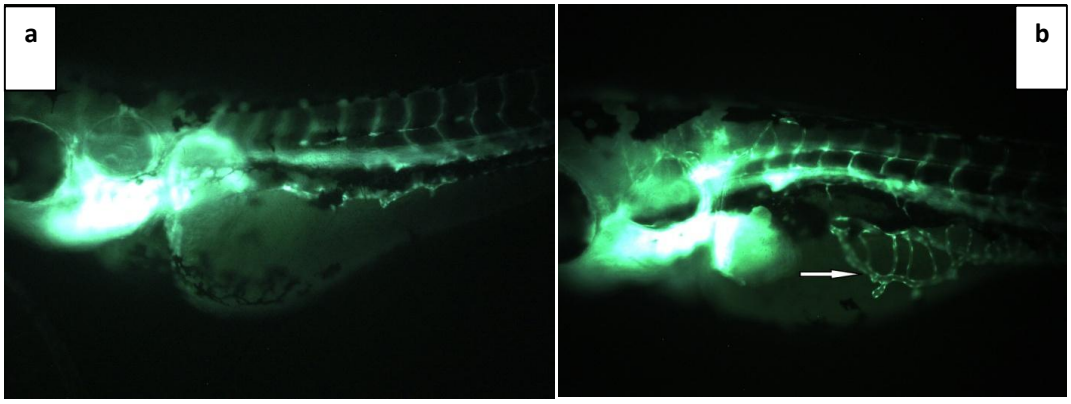


**Fig. 2. Sub-intestinal vessels in *Tg(fli1a:EGFP)y1/+* zebra fish embryos after exposure for 96 h to the *L. camara* extract (10 µg/mL) (a) *versus* those in untreated controls (b; arrow indicates sub-intestinal vessels)**

**Table 4. Mean scratch-wound areas in HUVEC monolayers following exposure for 16 h to the plant extracts at sub-toxic concentrations ($IC_{20}$ values) 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 (Plant extract concentrations used for these experiments) | Mean scratch-wound area (± SDs) in HUVECs with respect to that for controls |
|---|---|
| *A. vera* (10 µg/mL) | 83±22 |
| *C. cassia* (100 µg/mL ) | 101±30 |
| *L. camara* (10 µg/mL) | 226±48[1] |
| *M. charantia* (1 µg/mL) | 104±52 |
| *P. guajava* (0.1 µg/mL) | 144±74 |
| *S. melongena* (0.1 µg/mL) | 102±40 |

[1]*Statistically significantly different from control (P = .004; Student's t test). The other values did not differ statistically significantly from the control (P >.05; Student's t test)*

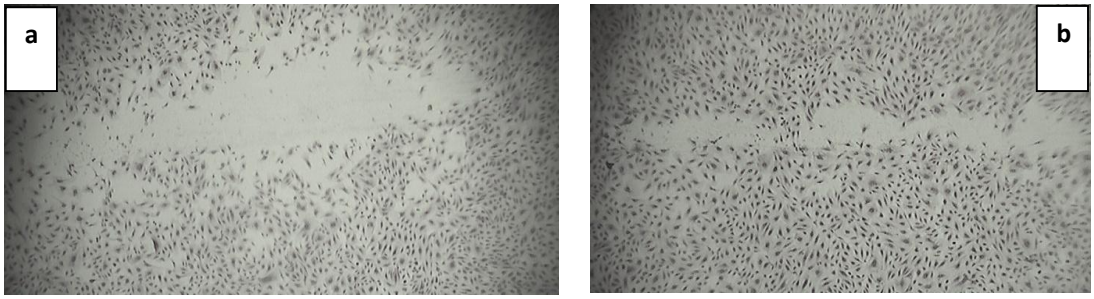


**Fig. 3. Scratch-wound area in HUVEC monolayers following exposure for 16 h to the *L. camara* extract at sub-toxic concentrations ($IC_{20}$ values) (a) *versus* that in untreated control HUVEC monolayers (b)**

### 3.4 Effects of Plant Extracts on the Formation of Capillary-Like Structures by HUVECs

To obtain further support that the *L. camara* extract but not those from the other plants might exhibit anti-angiogenic properties, the plant extracts were evaluated for their effects on the formation of capillary-like structures by HUVECs. These experiments were also carried out at sub-toxic extract concentrations (Table 5). As shown in Table 5, total tube length, the number of branching points, and the number of loops formed by HUVECs treated with the *L. camara* extract at 10 µg/mL was roughly 20, 40, and 50%, respectively, less when compared to those

formed by untreated cells. These observations suggest that this plant extract inhibited capillary-like structure formation by HUVECs, confirming its anti-angiogenic properties. None of the other plant extracts exhibited a statistically significant effect on HUVEC capillary-like structure forming activity (Table 4).

**Table 5. Mean total tube length, number of branching points, and number of loops of capillary-like structures (± SDs) formed by HUVECs following exposure for 6 h to the plant extracts at sub-toxic concentrations (IC20 values) 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 (plant extract concentrations) | Mean total tube length (± SDs) with respect to that for controls | Mean number of branching points (± SDs) with respect to that for controls | Mean number of loops (± SDs)with respect to that for controls |
|---|---|---|---|
| *A. vera* (10 µg/mL ) | 101±8 | 110±21 | 120±63 |
| *C. cassia* (100 µg/mL ) | 97±7 | 107±40 | 127±93 |
| *L. camara* (10 µg/mL) | 81±12[1] | 63±21[2] | 45±31[2] |
| *M. charantia* (10 µg/mL) | 91±7 | 83±14 | 75±32 |
| *P. guajava* (0.1 µg/mL) | 84±22 | 82±41 | 71±59 |
| *S. melongena* (0.1 µg/mL) | 110±17 | 140±58 | 194±178 |

*[1]Statistically significantly different from controls (P =.05; Student's t test). [2]Statistically significantly different from controls (P =.04; Student's t test). The other values did not differ statistically significantly from the control (P > .05; Student's t test).*

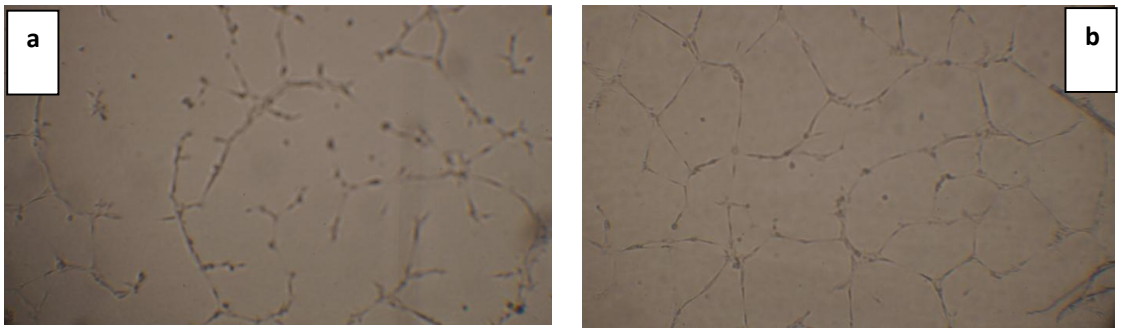


**Fig. 4. Capillary-like structure formation by HUVECs following exposure for 6 h to the *L. camara* extract at sub-toxic concentrations ($IC_{20}$ values) (a) *versus* that in untreated control HUVEC monolayers (b)**

## 4. DISCUSSION

Preparations from *A. vera* (aerial parts), *C. cassia* (leaves), *L. camara* (aerial parts), *M. charantia* (aerial parts), *P. guajava* (bark), and *S. melongena* (roots) are used in various traditional medicinal systems to promote wound healing [14-16]. However, in the current study, none of the aqueous extracts from these plants stimulated the regeneration of the amputated caudal fin of wild-type AB zebra fish embryos and the formation of sub-intestinal vessels of Tg(fli1a:EGFP)y1/+ zebra fish embryos, the closure of scratch-wound gaps in HUVEC monolayers, and capillary-like structure formation by these cells in matrigel. On the contrary, the *L. camara* extract delayed the regrowth of the amputated fin and the formation of sub-intestinal vessels in the fish and inhibited scratch-wound gap closure in, and capillary-like structure formation by HUVECs. Thus, rather than wound healing and pro-angiogenic properties, this preparation exerted marked anti-angiogenic characteristics. The remaining plant extracts did not exhibit either pro- or anti-angiogenic features.

The apparent wound healing-counteracting and anti-angiogenic effects of the *L. camara* extract noted in the current study are consistent with the increased coagulation time and prothrombin time, decreased blood sedimentation rate, and decreased total plasma protein and fibrinogen contents in sheep experimentally poisoned with *L. camara* [27] and with the interference of certain tetracyclic triterpenes in this plant with the blood-clotting cascade [28]. On the other hand, iridoid glycosides such as geniposide in this plant reportedly exerted useful wound healing-

promoting properties [29], and ethanolic extracts from its leaves accelerated the closure of excision wounds, promoted wound contraction and epithelialization, stimulated collagen synthesis, decreased mean wound healing time, and/or reduced the wound area in normal [30-32] as well as alloxan- and streptozotocin-induced diabetic rats [33,34]. The reasons for these discrepancies are not clear. However, the wide cultivation of *L. camara* for over 300 years [35] has led to hundreds of varieties which differ from each other with respect to morphology, physiologically, and genome [36,37]. Therefore, the differences in wound-healing and angiogenic effects between the current study and those cited above may be attributable to the use of *L. camara* varieties with distinct phytochemical compositions and biological activities.

The absence of an effect with the extracts from *A. vera*, *C. cassia*, *M. charantia*, *P. guajava*, and *S. melongena* noted in the current study is neither in agreement with reports on their wound healing-promoting and pro-angiogenic effects, nor with those suggesting anti-angiogenic effects of these plants. In the case of *A. vera*, preparations from this plant stimulated the healing of acute and chronic wounds in rats [17,18], while one of its constituents, the phytosterol β-sitosterol, displayed potent pro-angiogenic activity in the chick chorioallantoic membrane assay, the mouse matrigel plug assay, and an *in vitro* wound-migration assay [38]. On the other hand, anthraquinone emodins in this plant inhibited tubule formation of endothelial cells [39] and suppressed migration and invasion of, and VEGF expression in cultured WiDr colon cancer cells [40], speaking in favor of anti-angiogenic properties of *A. vera* preparations. The differences in outcome between these studies and ours might be tentatively attributed to differences in processing of the plant sample. *A. vera* preparations seem to be sensitive to enzymatic, oxidative, and microbial degradation and must be evaluated when still fresh [41]. However, our experiments were performed with a freeze-dried *A. vera* extract that had been stored at -20 $^{o}$C for several weeks and might have lost part of its biological activities.

That *C. cassia* may promote wound healing and angiogenesis is supported by the stimulatory effects of an ethanol extract from this plant and/or its active compound cinnamic acid on the proliferation, migration, tubule-like structure formation, and VEGF-associated phenomena in HUVECs and bovine aortic endothelial cells (BAECs) *in vitro* [42], and on wound healing and epithelialization in excision wounds in laboratory rats [43,44]. However, similarly to *A. vera*, several studies also support anti-angiogenic properties of *C. cassia*. For instance, aqueous extracts from this plant inhibited platelet aggregation and blood coagulation in laboratory rats [45]; VEGF receptor mRNA expression in cultured HUVECs [46]; VEGF-induced proliferation, migration, invasion, tube formation, associated intracellular signaling events, and matrix metalloproteinase activation in HUVECs and BAECs [47]; VEGF-induced vessel sprouting of rat aorta *ex vivo* [47]; and intersegmental vessel development in Tg(flk1:GFP transgenic zebra fish embryos [46]. The disagreements among these studies might be explained by differences in the plant parts evaluated: the results supporting wound healing and pro-angiogenic effects were obtained with extracts from the whole plant [42-44], those favoring an anti-angiogenic effect with preparations from its bark [45-47], while the current study had evaluated an aqueous extract from the leaves.

Similar considerations may apply to the absence of wound healing-promoting and pro- or anti-angiogenic effects by the preparations from *M. charantia*, *P. guajava*, and *S. melongena* in the current study. Thus, the relatively high blood vessel density and/or the improved wound healing in streptozotocin-induced diabetic Sprague-Dawley rats and normal New Zealand rabbits [48,49] had been accomplished with a preparation from the fruits from *M. charantia*, not with an extract from its aerial parts. The inhibition of VEGF expression in, and migration as well as angiogenesis in cultured DU 145 human prostate cancer cells was achieved with an aqueous extract of *P. guajava* budding leaves [50] instead of a preparation from the bark of the plant. And the suppression of microvessel outgrowth in an *ex vivo* angiogenesis assay using rat aortic rings was observed with the antioxidant anthocyanin nasunin from *S. melongena* peels [51] and not with an extract from the roots of this plant.

## 5. CONCLUSION

Despite folkloristic claims and experimental data suggesting both wound healing-promoting and pro-angiogenic properties and anti-angiogenic characteristics of preparations from *A. vera*, *C. cassia*, *M. charantia*, *P. guajava*, and *S. melongena*, extracts from these plants did not display any of these effects in the current study.

This might be due to the use of other plant parts and/or the application of other processing conditions than those applied in previous studies, but this must be confirmed in future investigations. However, contrary to literature data, the aqueous extracts from *L. camara* exerted substantial anti-angiogenic effects which may render this preparation a candidate for evaluation in diseases caused by excessive angiogenesis such as atherosclerosis, rheumatoid arthritis, diabetes mellitus, psoriasis, endometriosis, adiposity, as well as cancer [52]. For this reason, preclinical experiments to assess *L. camara* preparations for their potential efficacy against various human tumor cell lines as well as studies to characterize the pharmacologically active constituent(s) of this plant are in preparation at our institution.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All experimental procedures described have been approved by the Bioethics Committee of our institution.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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