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Microvascular protective activity of anthocyanosides-rich extract from *Acalypha langiana*

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ABSTRACT

A mixture of anthocyanosides from the leaves of *Acalypha langiana* consisting of cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-galactoside, petunidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, malvidin-5,5-diglucoside and the anthocyanidin pelargonidin, were characterised by means of HPLC and UV-VIS. Phenolic components were extracted with pure methanol. The samples were diluted in the mixture of methanol and formic acid just before the injection on the column and separated on Hypersil PEP 300 C18 chromatographic column using gradient solvent system consisting of formic acid, water and methanol. DAD detector was employed at 520 nm for anthocyanins. An *Acalypha langiana* anthocyanosides preparation (equivalent to 30% of anthocyanidins) demonstrated significant vasoprotective in rabbits, the skin capillary permeability increase, due to chloroform, was reduced after i.p. (25-100 mg/kg) anthocyanosides. Comparison was made between the action of the methanol extract and a known protective microvascular drug as troxerutin (25 mg/kg). The mixed of anthocyanosides was more active that the troxerutin.

KEY WORDS: *Acalypha langiana* , anthocyanidins, vasoprotective activity.

INTRODUCTION

Flavonoids tend to decrease capillary permeability and increase capillary resistance. Several mechanisms have been proposed such as inhibition of ascorbic acid oxidation, blood cell aggregation and stimulation of pituitary-adrenal axis (1). Studies show anthocyanins positive influences on a variety of health conditions (2). Possess ability to protect both large and small blood vessels from oxidative damage derives from a range of effects, including mitigating microvessel damage from high blood-sugar levels that cause complications in diabetics (3). By the same token, diabetic retinopathy, which damages eyesight, is caused by leaking, damaged capillaries (4). Anthocyanin pigments and associated flavonoids have demonstrated ability to protect a myriad of human diseases. Anthocyanin-rich mixtures of bioflavonoids may provide protection from DNA cleavage, estrogenic activity (altering development of hormone-dependent disease symptoms), enzyme inhibition, boosting production of cytokines (thus regulating immune responses), anti-inflammatory activity, lipid peroxidation, decreasing capillary permeability and fragility, and membrane strengthening (5).

Anthocyanins help maintain microcapillary integrity by stabilizing capillary walls. Blocked or reduced oxygen followed by reestablishment of normal supplies is called ischemia-reperfusion. Ischemia-reperfusion creates oxidants that result in white blood cell adhesion to microcapillary walls, increases capillary wall permeability, reduces blood flow, and often causes permanent capillary damage (6). Chemical structure (position, number, and types of substitutions) of the individual anthocyanin molecule also has a bearing on the degree to which anthocyanins exert their bioactive properties (7) and the structure/function relationships also influence the intracellular localization of the pigments (8).

Acalypha langinia Muell. (Euphorbiaceae) is commonly known as “arlomo”. It is a common herb that grows wild and abundantly in the fields of Mexico. A water extract of the leaves has long been used for Guerrero indigenous for the treatment of diabetes and wounds and the use in Aguascalientes of powdered leaves to treat ulcers was reported a long time ago. In Guerrero a water extract of the leaves has long been used for indigenous to clean wounds and when abundant hemorrhages are present during postpartum, it is applied as a vaginal wash (9). Based on our primary

ethnobotanical field research with healers in, various uses were attributed to the plant related to antidiabetic and anti-inflammatory effects, like hepato-protective and wound-healing . Decoction of the roots is drunk for the treatment of hepatitis. Decoction of the root or the leaves is also used to wash wounds and a powder of the same plant parts is applied topically as a wound healer. The genus *Acalypha* with about 450 species is the fourth largest genus of Euphorbiaceae are used in Central America as folk medicine (10). Here we report the vascular protective action of anthocyanosides rich extracts from *Acalypha langinia*. Activity was compared to that of troxerutin.

MATERIALS AND METHODS

Plant material.

Acalypha langiana stems were collected in Colima State, Mexico. The material was identified by Edith Lopez Villafranca of the Department of Botany of ENEP-Iztacala UNAM, and a voucher specimen of the plant (7859) is deposited in the herbarium of this Department for reference.

General Experimental Procedure.

The HPLC apparatus was a Hewlett Packard 1100 liquid chromatograph with DAD detector (190-600 nm).

Preparation of Standards.

Various suppliers provided 15 anthocyanidins standards with purity of 95-99%. Cyanidin-3-rutinoside, cyanidin-3-rutoside, and delphinidin were purchased from Extrasynthese (Genay, France), peonidin-3-galactoside, petunidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, from Polyphenol co. (Finland), while malvidin-5,5-diglucoside, cyanidin, petunidin, pelargonidin, peonidin, malvidin and pelargonidin-3-glucoside and pelargonidin-3-rutinoside were purchased from Sarsyntex (Merignac, France). Methanol (HPLC gradient grade) was purchased from J. T. Baker and formic acid (98-100%) from Sigma. Initially, solutions containing flavonoids were used to obtain retention time and spectral information for the identification of the anthocyanidins present in the *A. langiana* extract. These solutions were prepared by dissolving standard compound in methanol (1-2 mg/mL of each compound). After removing an aliquot for HPLC analysis, the standard solution was mixed with 5 mL of Reagent A ($HCOOH/H_2O$), and 10 mL de methanol. This reacted solution was also analyzed by HPLC. Two series of standard solutions (each series containing different glycones found in the *A. langiana* were analyzed by HPLC to determine the quantity of compound versus chromatograph peak area information. The most concentrated solution in each series was precisely prepared from four solid standards. Specifically, 300-500 µg of each of the glycones was weighed to the nearest 0.1 µg. The amounts were combined, then dissolved and diluted to 5.00 mL with Reagent A. Next, the concentrate was diluted 1.00 mL to 10.00 mL and 0.100 mL to 10.00 mL with Reagent A to produce two other solutions for the series. The standard solutions were analyzed by HPLC using 2, 10, and 20 µL injections.

Extraction and preparation of sample for HPLC and spectrophotometric analysis.

50 g of *A. langiana* were homogenised in 80 mL of methanol (99.9 %). After 25 min of extraction at room temperature (while agitating) the homogenate was centrifuged (3000 min^{-1}, 15 min). The supernatant was removed and then evaporated under reduced pressure at 30 °C to yield a syrup-like residue. The residue was in turn diluted in 25 mL of methanol and stored in freezer prior to analysis.

HPLC analysis of phenolic components in A. langiana.

The LC system employed is a Hewlett-Packard (HP) 1050 Series modular system equipped with an 1100 Series diode array detector and a HP Kayak computer with HP HPLC 3D ChemStation software for instrument operation and data analysis. The LC method uses a HP Zorbax Eclipse XDB-C 18 column (250 x 4.6 mm) preceded by a guard column of similar stationary phase. The column Hypersil PEP 300 C18, 250 x 4.6 mm, 5 m particle size is maintained at room temperature. Guard column Alltech, 10 x 4.1 mm, Injection volume 20 L and Row rate 1.0 mL/min. The mobile phase is run mobile phase A = $HCOOH/H_2O$, pH = 2.1, B = CH_3OH (HPLC grade), gradient 8 % B (0 min), 12 % B (11 min), 30 % B (17 min), 33 % B (28 min), 100 % B (30-35 mill), 8 % B (36 min). Detection was carried out at 520 nm for anthocyanins. A 10-minute re-equilibration period was used between individual runs. The chromatograms were recorded at room temperature (20 °C).

Characterisation and quantification of phenolic compounds by HPLC.

Compounds were characterized on the basis of retention times and UV-VIS spectra of previously described standards and by using data of already published UV-VIS spectra (11-14). The ratio of the average absorbance in the 400-440 nm range versus the absorbance maximum in the visible range of spectrum (A400-440/ Avis max) was calculated from the recorded UV-VIS spectra and used as an indicator of 3-

glycoside structure of anthocyanins (12).

Experiment on microvascular permeability

Animals used.

Adult, healthy, New Zealand white rabbit weighing between 2.0-2.5 kg were used in these experiments. The animals were kept in an air-conditioned animal room at temperature of 25 °C in the Biotery of the University. The animals were given a commercial feed prepared by Purina and allowed tap water *ad libitum*.

Microvascular permeability.

The male rabbits (2 kg mean weight, 12 animals in each experimental group) were treated intraperitoneally with either NaCl (0.9%) (control group) or the extract (25, 50, 100 mg/kg). After 30 min three zones of depilated skin were irritiated with chloroform applied by means of a cotton tipped glass tube pressed lightly on the skin for 30 s. After a further 60 min the histamine was administered intradermally, 0.8 μg to each of the three zones (i.e. 2.4 μg/rabbit}, followed by immediate intravenous application of Evans blue (25 mg/kg, as 10% aqueous solution). The animals were killed 30 min after histamine administration and the skin zones (each 3 x 3cm) were accurately cut off and extracted with 4 mL formamide for 72 h at 45°C. The absorption of the decanted supernatant was measured at 620 nm and the Evans blue content read from the calibration curve (15).

RESULTS

The retention times for the 15 anthocyanins were initially investigated. Of the listed therein, eight flavonoids were found in the leaves of *A. langinia* extract including pelargonidin, cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-galactoside, petunidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, and malvidin-5,5-diglucoside. Calibration of these eight compounds yielded straight lines with coefficients of 0.998 or greater. In the standards, the limits of detection (LODs) ranged from 42ng to 176 ng.

Based on well-known spectral characteristics (ratio $A_{400\text{-}440}/A_{vis\ max}$) of anthocyanin-3-glycosides as well as on comparison between UV-VIS spectra (16) of eluted standards, we assigned eight compounds detected at 520 nm and identified the peaks as follows: peak 5 matched the retention time and spectral characteristics of cyanidin-3-glucoside, while peak 4 of cyanidin-3-rutinoside. Similary, peak 1, 2 and 3 were identified as pelargonidin, malvidin-3-glucoside, malvidin-5,5-diglucoside and elution order of peaks 6,7 and 8 indicated that they were most probably peonidin-3-galactoside, petunidin-3-glucoside and delphinidin-3-glucoside. The others peaks could not be identified against available standards.

Extract from *A. langinia* significantly inhibited capillary permeability in rabbits and showed a concentration-dependent. The protective microvascular activity was measured as a counteracting effect on the leakage of Evans blue introduced intravenously. Troxerutin (25 mg/kg) a protective microvascular was used as reference drug in this study. The results are shown in Table 1. The extract of *A. langinia* 120 min after peritoneal treatment at a dose of 25 and 50 mg/kg produced a inhibitory effect on the capillary permeability in rabbit skin increased by chloroform and histamine of 48.1 and 78.3% respectively. This effect was more pronounced at 100 mg/kg (91.2% reduction).

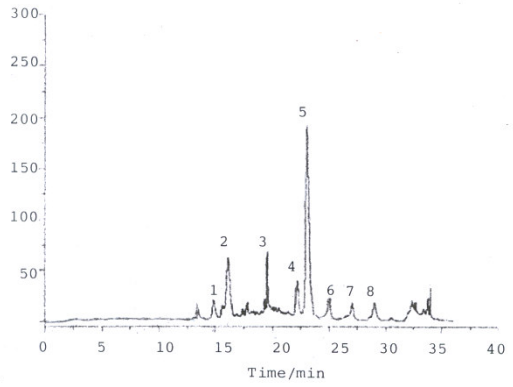

Fig 1. HPLC separation of anthocyanosides in Acalypha langiana monitored at 520 nm. Peaks: Pelargonidin 1, malvidin-3-glucoside 2, malvidin-5,5-diglu- coside 3, cyanidin-3-rutinoside 4, cyanidin-3-glucoside 5, peonidin-3-galactoside 6, petunidin-3-glucoside 7, delphinidin-3-glucoside 8.

Table 1. Effect of the anthocyanosides-rich methanol extract of Acalypha langinia on increased microvascular permeability induced by both chloroform and histamine in rabbits.

Treatment mg/kg	Inhibition (%)
0.9% NaCl	0
25	48.1
50	78.3
100	91.2
Troxerutin 25	46.9

DISCUSSION

Methanol extract of *A. langinia* have an composition of cyanidin-3-glucoside and malvidin-3-glucoside as the major anthocianins, and the pelargonidin as minor anthocianin. The results showed that the anthocyanosides-rich methanol extract from *Acalypha langiana* produced a significant inhibitory effect on the capillary permeability in rabbit skin.

CONCLUSION

The effect of methanol extract of *Acalypha langiana* stems on the capillary permeability in rabbit skin is presented. Also compared the effects of methanol extract with those of troxerutin. It is, commonly used as protective microvascular exhibited less activity that the extract. All the concentrations of extract assayed decreased the capillary permeability.

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