

## Aloesin, aloin and aloe-emodin production in *Aloe vera* L. calli

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### Abstract

*Aloe vera* L. (*A. barbadensis* Mill.) belongs to the Aloaceae family and actually is a commercial and medicinal important plant because of the secondary metabolites produced. Aloesin, aloin and aloe-emodin (oxidative product of aloin) are the most important secondary metabolites not only due to the medicinal and cosmetic properties but also due to their physiological role. In this sense, it has been quantified the production of those three metabolites using different *in vitro* explants comparing with fresh leaves of entire *Aloe vera* plants. It has observed that aloesin production was higher in basal calli grown on medium MS1 with 1 mg.l<sup>-1</sup> 2,4-D and 5 mg.l<sup>-1</sup> BA, while aloin content was higher in leaves of entire plants. Aloe-emodin production was greater than the aloin both in basal and fresh leaves calli compared to entire leaves. Probably, this high aloe-emodin production found was due to the aloin oxidation. These results showed that calli cultures represent a promising source for *A. vera* secondary metabolites production.

**Key words:** aloe-emodin, aloesin, *Aloe vera*, aloin, *in vitro* culture, calli.

## Producción de aloesina, aloína y aloe-emodina en callos de *Aloe vera* L.

### Resumen

*Aloe vera* L. (*Aloe barbadensis* Mill.) pertenece a la familia Aloaceae. La importancia de este género radica en los metabolitos secundarios producidos por estas plantas y en sus aplicaciones en la industria cosmética y medicinal. En este trabajo se ha cuantificado la producción de tres de estos metabolitos, aloesina, aloína y aloe-emodina (el producto oxidativo de la aloína), que poseen importantes propiedades medicinales y cosméticas. Esta producción se determinó comparando la cantidad de estos metabolitos encontrada en diferentes explantes con la cantidad hallada en hojas frescas de plantas silvestres. Se observó que la producción de aloesina fue mayor en callos basales cultivados en medio MS1 con 1 mg.l<sup>-1</sup> de 2,4-D más 5 mg.l<sup>-1</sup> de BA, mientras que el contenido de aloína fue más alto en hojas de plantas silvestres. Se encontró que la producción de aloe-emodina fue mayor en callos que en hojas de plantas silvestres. A medida que aumentaba la producción de aloe-emodina, disminuía la de aloína en los diferentes tejidos, lo que explica que probablemente la aloína se oxida para formar aloe-emodina. Estos resultados muestran que los cultivos de callos son una fuente importante para la producción de metabolitos secundarios en *Aloe vera*.

**Palabras claves:** aloe-emodina, aloesina, *Aloe vera*, aloína, cultivo *in vitro*, callos.

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## Introduction

*In vitro* culture is an useful tool for commercial production of secondary metabolites. In fact, *in vitro* plant cell and organ cultures have been used as one of the most important strategies to optimize production of interesting compounds (1, 2). *Aloe vera* L. (*A. barbadensis* Mill.) belongs to Aloaceae family and actually is a commercial and medicinal important plant because of the secondary metabolites produced (phenolic compounds) such as aloesin, aloin and aloe-emodin (oxidative product of aloin) not only due to the medicinal and cosmetic properties but also due to their physiological role. Aloin is the major anthraquinonic component of *Aloe vera* (3). It is a mixture of two diastereoisomers, aloin A and aloin B (4) with numerous functions. It has dermatological applications, it is a potent compound with antimarial (5), antifungal (6), antibacterial and antiviral properties (7). Aloin is an anthraquinone that has been used because of its cathartic and purgative effect and is involved in plant defence mechanism against herbivores (8-10). Aloesin is the second component of *A. vera* and its derivatives have antioxidant and antiinflammatory properties (11). Aloe-emodin is the oxidative product of aloin, and the last investigations have demonstrated that this compound possesses anticancer (12, 13), anti-leukaemic (14), antiviral (15), anti-protozoal (16) and antifungal properties (17).

Considering that *Aloe* species have been mainly studied in order to obtain rapid clonal multiplication for commercial and medicinal aims, and there are few investigations about secondary metabolites production *in vitro*, this work reports the production of aloesin, aloin and aloe-emodin in *Aloe vera* calli.

## Materials and methods

### Plant material

*Aloe vera* plants were collected in Maracaibo (Zulia state, Venezuela) and main-

tained under natural conditions in the gardens of Biology Department of Experimental Faculty of Science of the University of Zulia. Different explants, fresh leaves and apical buds, were obtained from these plants to induce calli formation and also those plants were used for comparison with calli.

### In vitro cultures

#### Basal calli

Many plants, like *A. vera*, form calli in the base of shoots obtained by *in vitro* culture (18). According to this, in order to obtain basal calli, apical buds were isolated from *A. vera* entire plants described above and shoots obtained were cultured using Murashige & Skoog medium (MS) (19) with sucrose 3%, agar 1% and ascorbic acid 0.1 mg.l<sup>-1</sup> supplemented with 2,4-D 1 mg.l<sup>-1</sup> and BA 5 mg.l<sup>-1</sup> (MS1 medium) and 2,4-D 0.5 mg.l<sup>-1</sup> and kinetin 0.1 mg.l<sup>-1</sup> (MS2 medium) combinations. Flasks containing 25 ml of culture medium were placed at 26 ± 1 °C in a culture room at continuous light illuminated with Phillips tubes (F30T12/D/RS). Light intensity was 40 μmol.m<sup>-2</sup>.s<sup>-1</sup>. All the cultures were transferred to fresh medium at 4-week intervals.

After 30 days, *A. vera* calli were isolated from the base of *in vitro* shoots and cultured with the same hormone supplementation that they grown (MS1 and MS2 media), and subcultured every four weeks, under the same environmental conditions as described for shoots. After the 4th subculture, calli were collected for secondary metabolites production analysis.

#### Calli from leaves of entire plants

Fresh leaves (10 cm length) from *A. vera* entire plants were washed, sterilized with sodium hypochloride 10% during 20 min and cut in 15-20 mm<sup>2</sup> pieces under aseptic conditions. Those explants were placed in flasks with 25 ml of MS culture medium with sucrose 3%, agar 1% and ascorbic acid 0.1 mg.l<sup>-1</sup> supplemented with 1 mg.l<sup>-1</sup> 2,4-D and

5 mg.l<sup>-1</sup> BA (MS3) and 1 mg.l<sup>-1</sup> IAA (indoleacetic acid) with 5 mg.l<sup>-1</sup> BA (MS4). They were used 4 explants for each flask for treatment and 10 flasks for every treatment were placed under same conditions for basal calli.

Calli were subcultivated to fresh medium every 4 weeks. After the 4th subculture, calli were collected for secondary metabolites production analysis.

### Extract and HPLC analysis

Ethanolic extracts from leaves of entire plant and *in vitro* material were obtained and quantified by HPLC following the method of Park et al. (20). Leaves of entire plants and calli tissue were removed, frozen at -20 °C for 24 h and placed on a lyophilizer (Labconco Lyo-Lock 4.5) during 48h. Each sample was taken in triplicate.

For each sample, 0.5g of freeze-dried were extracted with 10 ml ethanol (HPLC grade, Merck), filtered (0.45 µm) and chromatographed in HPLC system (AGILENT 1100 Series) using a C<sub>18</sub> reversed-phase column (Phenomenex, 4.6 mm i.d. x 250 mm length, 5 µm). The mobile phase (flow 0.7 ml.min<sup>-1</sup>) was composed of methanol (HPLC grade, Merck) and water.

Phenolic compounds (aloesin, aloin and aloe-emodin) were determined by comparison with spectra previously reported by Nakamura and Okuyama (21), Rauwald and Sigler, (22), Zonta et al. (23), Okamura et al. (24), Yagi (25), Viljoen and Van Wyk (26). Identification was made with diode array detector at UV-visible 293 nm comparing with standards. The standard compounds were aloesin (retention time, r.t. = 10-11 min), donated by Dr. J.H. Park from Seoul National University, aloin (r.t. = 41-42 min) and aloe-emodin (r.t. = 62-63 min), both from Merck.

Quantitative results were calculated transforming area peaks using initial concentration of standard compounds (1 mg.ml<sup>-1</sup>, 20 µL injected). Results are expressed

in µg per gram of dry weight (DW) of plant material.

### Statistical analysis

All data were analysed statistically with the SPSS software. Significance of results was determined using the variance test (ANOVA) and mean differences were analyzed with Duncan test (p≤0.05).

### Results and discussion

Previous reports have demonstrated that aloin is the major constituent of leaves of *A. vera* entire plants (18, 20). On the other hand, it has been observed that the secondary metabolites production can vary depending of tissue type, plant developmental stage and also with weather changes (9, 10).

In this work, secondary metabolites content (aloesin, aloin and aloe-emodin) was determined in leaves of *A. vera* entire plants, calli formed in the base of *in vitro* shoots (MS1 and MS2) and calli obtained from fresh leaves of entire plants (MS3 and MS4) (Fig. 1).

First of all, it has been identified and quantified secondary metabolites from leaves of *A. vera* entire plants, used for comparisons with calli. The HPLC analyses revealed that, as described above, aloin was the major component of leaves followed by aloesin and aloe-emodin (Fig. 2).

Different studies have shown that growth regulators can inhibit or stimulate production of a secondary compound. For example, NAA/BA combination was the most effective in promoting tetrahydroanthracenes production in *A. vera* (11).

In this study, secondary metabolites production in *in vitro* material was variable according to the tissue type and hormonal combinations (Fig. 3). The best medium for aloesin production (498.2 µg.g<sup>-1</sup> D.W.) was MS1 with 1 mg.l<sup>-1</sup> 2,4-D and 5 mg.l<sup>-1</sup> BA. Production was 3 times higher than in leaves of entire plants. As it can see, high BA levels

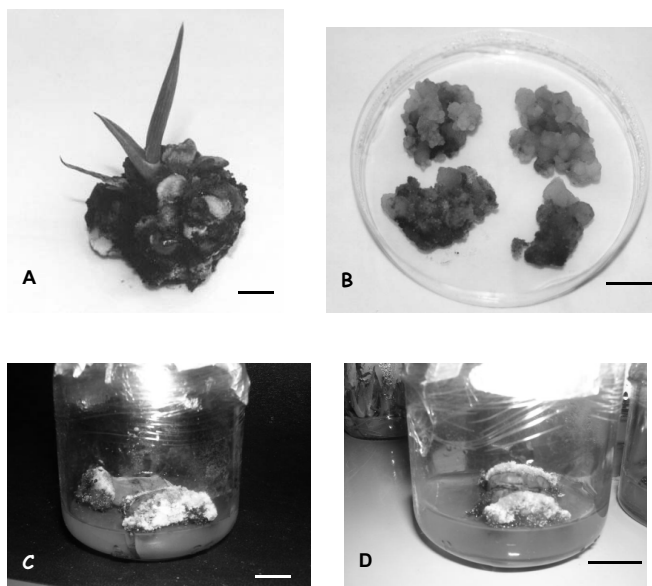


Figure 1. Micropropagated shoots and calli from *Aloe vera*. **A.** Calli formed in the base of shoot grown on MS1 medium. **B.** Basal calli after been isolated. **C.** Calli obtained from leaves of entire plants with MS3. **D.** Calli obtained from leaves of entire plants with MS4. Bar represents 1 cm.

increased aloesin production; similar results have been observed in *Hypericum perforatum* calli cultures where  $4.0\text{-}5.0\text{ mg.l}^{-1}$  induced pseudohypericin production (27). Aloe-emodin production in calli grown in MS1 was too low to be detected, instead, aloe-emodin production in calli grown in MS2 (2,4-D/kinetin) was 2.4 times higher than in leaves of entire plants. Aloin production was low with both media compared to fresh leaves. These results show that better responses in basal calli are obtained with BA instead of kinetine. Other investigations also have described an increase in secondary metabolite production using BA in cultures (28).

Regarding to the calli obtained from fresh leaves of entire plants, it was found that secondary metabolite content was low in these calli, with the exception of the aloe-emodin (Fig. 3). Figures 2C and 2D show that the number of compounds present in chromatograms from these calli is lower compared to basal calli and entire plant leaves. These differences in phenolic composition between *in vivo* plants and *in vitro* cul-

tures have been reported using HPLC method in *Hypericum perforatum* and *H. androsaemum*, probably due to the lack of differentiation in calli cultures which is a limitant for secondary metabolic pathways (27). In fact, secondary metabolism is only one phase of plant differentiation; so, morphologically undifferentiated cultures of many species fail to produce secondary compounds (29).

It is necessary to highlight that aloe-emodin production was higher in calli obtained from fresh leaves (Fig. 3), observing that the quantities in media MS3 ( $14.37\text{ }\mu\text{g.g}^{-1}$  D.W.) and MS4 ( $3.87\text{ }\mu\text{g.g}^{-1}$  D.W.) were 7.2 and 1.9 times higher than those observed in leaves of entire plants ( $2.04\text{ }\mu\text{g.g}^{-1}$  D.W.). This could be related to initial explant type; it is possible that production of calli obtained from another *in vitro* material is lower than those from leaves of entire plants. However, previous work (18) showed that aloe-emodin production was increasing when aloin production was decreasing, probably because of *in vitro* aloin oxidation to give its subproduct aloe-emodin. This could be use-

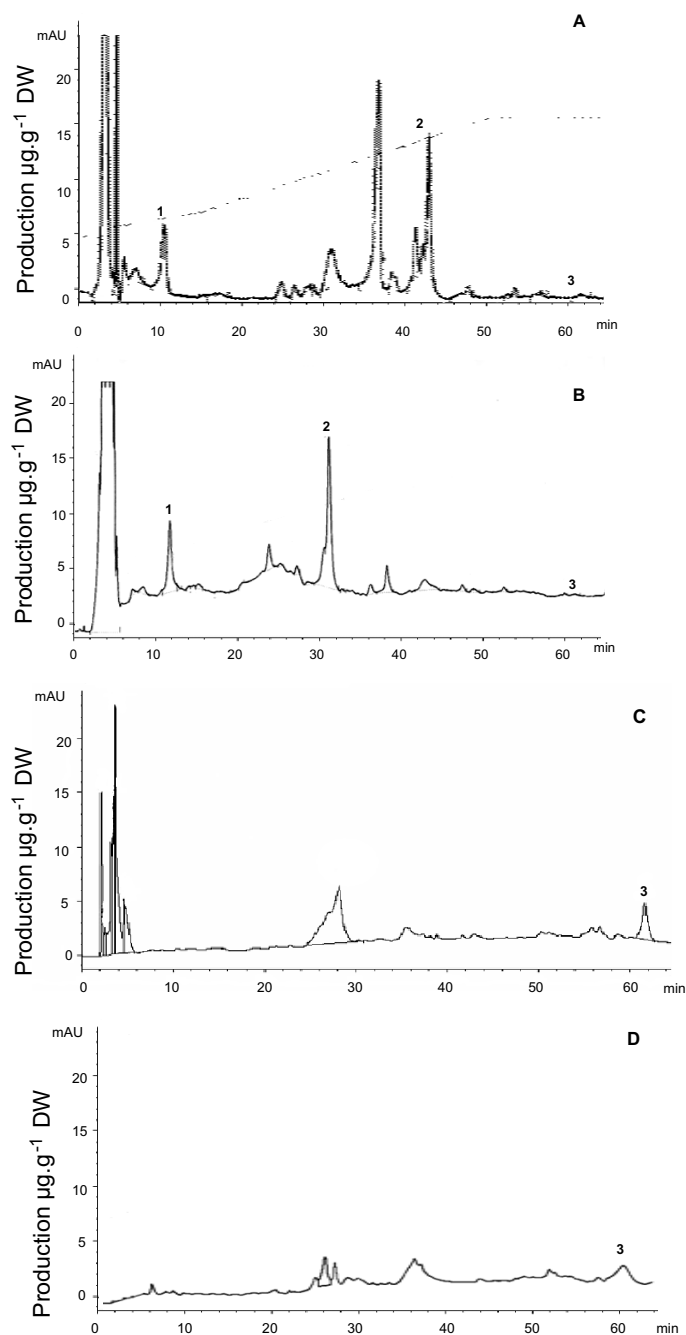


Figure 2. HPLC chromatograms of *Aloe vera* ethanolic extracts. A. Chromatogram of leaves of entire plants. B. Chromatogram of basal calli cultured in medium MS1. C. Chromatogram of calli obtained from leaves of entire plants, grown on medium MS3. D. Chromatogram of calli obtained from leaves of entire plants, grown on medium MS4. Analyses were carried out with a reversed-phase C<sub>18</sub> column (Phenomenex), 293 nm. Retention time (Rt) of aloesin = 11-12 min, aloin Rt = 41-42 min, aloemodin Rt = 61-62 min. 1. Aloesin. 2. Aloin. 3. Aloe-emodin.

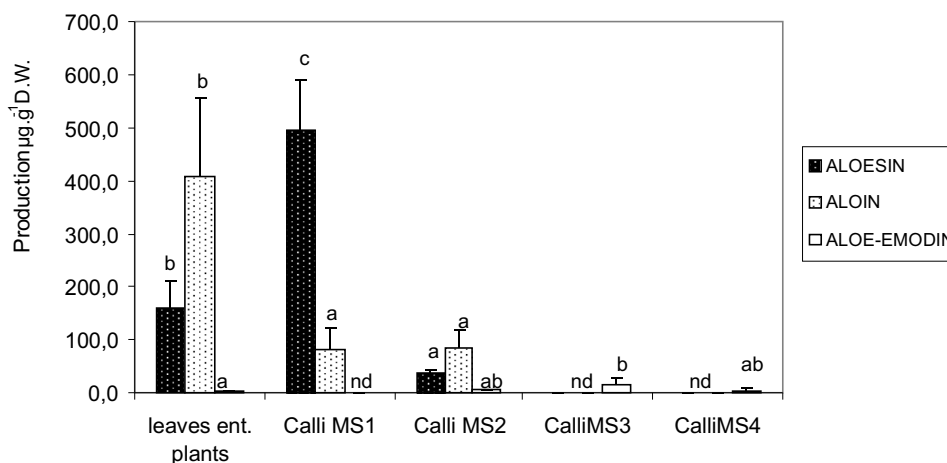


Figure 3. Production of aloesin, aloin and aloe-emodin in leaves of *Aloe vera* entire plants, basal calli (Calli MS1) grown on medium MS1, basal calli (Calli MS2) grown on medium MS2, calli obtained from leaves of entire plants with MS3 medium (Calli MS3) and with MS4 medium (Calli MS4). Each value represents the mean of three independent determinations + S.D. Means are significantly different according to Duncan test ( $p < 0.05$ ). nd: not detected.

ful for future studies oriented to optimisation of phytohormone supplementation and culture conditions in order to increase aloe-emodin production, a compound with many pharmacological properties but in small quantities in leaves of *Aloe vera* entire plants.

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