Revista Brasileira de Farmacognosia 26 (2016) 161–167

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Comparative HPTLC analysis of bioactive marker barbaloin from *in vitro* and naturally grown *Aloe vera*

Devendra Kumar Pandey[a], Sidharth Parida[b], Abhijit Dey[c,*]

[a] *Department of Biotechnology, Lovely Faculty of Technology and Sciences, Lovely Professional University, Phagwara, Punjab, India*
[b] *Department of Biotechnology, MITS School of Biotechnology, Affiliated to Utkal University, Bhubaneswar, Odisa, India*
[c] *Department of Biological Sciences, Presidency University (Erstwhile Presidency College), Kolkata, India*

ARTICLE INFO

Article history:
Received 13 May 2015
Accepted 18 August 2015
Available online 31 December 2015

Keywords:
Aloe vera
Barbaloin
In vitro
HPTLC
Densitometry

ABSTRACT

Aloe vera (L.) Burm. f., Xanthorrhoeaceae, a succulent, produces barbaloin, a bioactive compounds used in various pharmaceutical products. Extracts prepared from the leaves have been widely used as bittering agents, taste modifiers and also as cathartic agent against severe constipation. Barbaloin is reported for its anti-inflammatory, anticancer, antiviral and anticancer activities and these properties are mostly mediated by its antioxidative capacity. Presently, a study has been conducted on the comparative High Performance Thin Layer Chromatography analysis of barbaloin from the dried leaf skin powder of *in vivo* and *in vitro* grown *A. vera*. Shoot tips of *A. vera* were cultured in Murashige and Skoog media supplemented with different combination of 6-benzylaminopurine and 1-naphthaleneacetic acid. [Best multiplication response was noted in benzylaminopurine (2.0 mg/l) + 1-naphthaleneacetic acid (0.1 mg/l) supplemented Murashige and Skoog media]. The quantitative determination of barbaloin was performed on silica gel 60 F_{254} HPTLC plates as stationary phase. The linear ascending development was carried out in a twin trough glass chamber saturated with a mobile phase consisting of ethyl acetate: methanol: water (100:16.5:13.5) at room temperature ($22 \pm 2\,^{\circ}C$). CAMAG Thin Layer Chromatography scanner-3 equipped with CATS software (version: 1.4.4.6337) was used for spectrodensitometric scanning and analysis in the ultraviolet region at $\lambda = 366$ nm. The method was validated for linearity, precision and accuracy. Correlation coefficient, limit of detection, limit of quantification as well as recovery values were found to be satisfactory. Out of the five populations studied, the leaf skin of *A. vera* collected from Jodhpur (Rajasthan, India) and raised *in vitro* was found to contain higher amount of barbaloin (2.78%) when compared to its naturally growing counterparts (2.46%) and other plant populations.

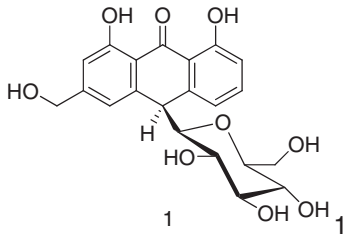
Introduction

Aloe vera (L.) Burm. f., Xanthorrhoeaceae, is a succulent plant indigenous to Northern Africa and Mediterranean countries and has become naturalized almost in all parts of India (Klein and Penneys, 1988). The original commercial use of the plant was meant for the production of a latex substance called barbaloin (**1**) or aloin (molecular formula: $C_{21}H_{22}O_9$) which is yellow-brown in color with and lingering taste and was used as laxative until world war-II (Saeed et al., 2004). *A. vera* has exhibited anticancer, wounds healing, immunomodulatory, antiviral, anti-inflammatory, dental protective, laxative, antiseptic, gastroprotective, moisturizing and anti-aging properties (Surjushe et al., 2008; Park et al., 2011; Yonehara et al., 2015; Hashemi et al., 2015; Mangaiyarkarasi et al., 2015). Oral administration of *A. vera* for the treatment of diabetes mellitus and dyslipidemia has also been investigated (Ngo et al., 2010). Besides barbaloin, the other main ingredient of *A. vera* is called leaf gel, a clear, colorless and tasteless substance, which covers inner portions of the leaves (Reynolds and Dweck, 1999). The gel is used for commercial use in pharmaceuticals, functionals foods and cosmetics (Hamman, 2008). Sterols present in *A. vera* gel stimulated collagen and hyaluronic acid synthesis by human dermal fibroblasts (Tanaka et al., 2015). *A. vera* gel also promoted cesarean wound healing in women (Molazem et al., 2014). Barbaloin (also named aloin), the *C*-glucoside of aloe emodin anthrone, localizes in the outer rind of the plant has been reported to constitute up to 30% of the plant's dried leaf exudates (Groom and Reynolds, 1987) and proposed as a part of the defense mechanisms against herbivores (Gutterman and Chauser-Volfson, 2000; Chang et al., 2006). Barbaloin was reported for its histamine release inhibitory, anti-inflammatory, antiviral, antimicrobial, anticancer, antioxidant and cathartic effects (Patel et al., 2012)

* Corresponding author.
E-mail: abhijit.dbs@presiuniv.ac.in (A. Dey).

http://dx.doi.org/10.1016/j.bjp.2015.08.016
0102-695X/

In another *Aloe* species *A. ferox*, aloesin, aloeresin a and anthraquinone (as barbaloin) was estimated by a reversed-phase high-performance liquid chromatographic (HPLC) method (Zahn et al., 2008). In leaf exudate of *A. secundiflora* the major components were analyzed by HPLC-mass spectroscopy (Rebecca et al., 2003). Barbaloin in aloe capsule was also determined by HPLC methods (Chen et al., 2002). Aloin was selectively determined in different matrices by HPTLC densitometry in fluorescence mode (Coran et al., 2011). Earlier, barbaloin was estimated in *A. vera* and in its commercial products (Pandey et al., 2012). An efficient micropropagation protocol was adopted in *A. vera* in Murashige and Skoog (MS) medium supplemented with growth regulators (Aggarwal and Barna, 2004). Rapid propagation by the shoot generation calli was achieved in polyvinylpyrrolidone (PVP) and growth regulator supplemented MS medium (Roy and Sarkar, 1991). However, no attempt has yet been made to quantify barbaloin (**1**) comparatively in natural or *in vitro* populations of *A. vera*. Since barbaloin is considered as an important biologically active compound in *A. vera*, the present investigation depicts a validated HPTLC protocol for determination of barbaloin from *in situ* and *in vitro* grown populations of *A. vera*.



Materials and methods

Plant material and chemicals

Different populations of *Aloe vera* (L.) Burm. f., Xanthorrhoeaceae, were collected from five districts (from four different states of India) *viz.* Jalandhar (Punjab), Jodhpur (Rajasthan), Varanasi and Lucknow (Uttar Pradesh) and Bhubaneswar (Odisha). Plants with 9–11 leaves and of almost same size and age were harvested in their vegetative stage during the month of June 2012 to September 2012 and maintained at the herbal medicinal garden, Lovely Professional University, Punjab. The materials were identified and authenticated on the basis of morphological characters by a botanist in the Department of Botany, Banaras Hindu University, Varanasi. A voucher specimen (Voucher No. 080912) was deposited at the Department of Biotechnology, Lovely Professional University for future reference. All solvents (HPLC grade) were obtained from E. Merck (Mumbai, India). Standard barbaloin (~97% pure) was purchased from Sigma–Aldrich (USA).

Micropropagation of Aloe vera

MS (Murashige and Skoog) medium supplemented with 6-benzylaminopurine (BAP) (0.5–2.0 mg/l) and 1-naphthaleneacetic acid (NAA) (0.1–1.0 mg/l) and agar (0.8%) was used for shoot proliferation. After 4–5 weeks of culture period, the *in vitro* grown plantlets with newly form shoots were taken out aseptically and the shoots were excised from the parent plant with the help of sterile scalpel blade and forceps and inoculated into new bottles containing solid MS medium with different set of growth regulators (PGR) as mentioned earlier. Newly formed shoots measuring 3–4 cm in length were excised individually from the parent plant and were transferred into the two types of rooting media: MS media supplemented with NAA (0–2.0 mg/l) and indole-3-butyric acid (IBA) (0–2.0 mg/l) individually. All cultures were incubated under 16 h photoperiod (cool, white fluorescent light (30 μM/s)) and temperature of 25 ± 1° C with 50–80% relative humidity. After 30 days of culture on rooting media, the plantlets were shifted to plastic pots for hardening prior to final transfer to natural conditions. For hardening, plants with newly formed roots were taken out from the culture bottles with utmost care to prevent any damage to the roots. The plants were then dipped in warm water to remove any traces of agar following which the plants were dipped in 1% (w/v) solution of Bavistin to prevent any fungal infection in the newly developed plants. The plantlets were then planted in plastic pots containing 1:1 mixture of soil and manure.

Preparation of sample and standard solution

The shed dried leaf skin of *A. vera* were powdered in a mixer grinder (Champ Essentials, Morphy Richards, India). Leaf skin (0.1 g each from *in situ* and *in vitro* grown plants) were separately extracted with methanol (2× 20 ml) for 15 min under reflux on a water bath at 70 °C. The extracts were filtered through Whatman no: 1 filter paper (separately for *in situ* and *in vitro* samples) and were evaporated under vacuum using a rotary evaporator (Eyela, N-1100, China) to furnish a solid mass of extract. The extract was kept in freezing temperature free of methanol because barbaloin (**1**) is converted into aloe emodin and a number of unknown compounds as the glucose part is removed from the compound when kept in methanol (Chang et al., 2006). The extract (0.1 g/ml) obtained from each sample were prepared in HPLC-grade methanol for quantitative analysis. Stock solutions of barbaloin were prepared by dissolving 10 mg of the compound in 10 ml of methanol.

Chromatographic conditions

The HPTLC system was composed of a CAMAG (Muttenz, Switzerland) Linomat-5 automatic sample applicator and CAMAG TLC scanner-3 provided with CATS software (version: 1.4.4.6337). The stationary phase was composed of pre-coated silica gel 60 F_{254} HPTLC plates (20 cm x 10 cm; with 0.25 mm thickness). Samples were administered to the plates as 5 mm wide bands *via* Linomat-5 automatic sample applicator (with nitrogen flow) equipped with a 100 μl Hamilton syringe. Delivery rate from the Hamilton syringe was fixed at 100 nl/s. Linear ascending mode of development up to a distance of 80 mm, with ethyl acetate: methanol: water (100:16.5:13.5) as mobile phase (Wagner and Bladt, 1996; Gutterman and Chauser-Volfson, 2000), was implemented at room temperature (22 ± 2 °C) and 50% relative humidity in a CAMAG twin trough glass chamber (20 cm × 10 cm) saturated earlier with mobile phase vapour for 20 min. After development, the plates were dried at 100 °C for 10 min, derivatized with 100 ml of 10% (v/v) alcoholic KOH solution, following which densitometric scanning was executed at 366 nm (Wagner and Bladt, 1996). The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 100 nm/s. For calibration and to estimate the linearity, marker stocks (0.3, 0.6, 0.9, 1.2, 1.5 μl) were administered to the plate to furnish amounts in the range 300–1800 ng per band. Peak areas were plotted against the corresponding concentrations and regression analysis was executed to generate the calibration equation. To analyze plant samples, 1.0 μl extract of each of the samples was administered to the plate. After development, derivatization, scanning and measurement of peak area the amount of barbaloin (**1**) was determined, assuming the purity of the marker to be 100%. Chromatograms are shown in Figs. 2 and 3.

Method validation

The method was validated *via* calculating linearity, peak purity, limit of detection (LOD), limit of quantification (LOQ),