Quantitative estimation of barbaloin in *Aloe vera* and its commercial formulations by using HPTLC

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**Abstract:** In Indian Ayurvedic system of medicine the leaf of Ghritkumari (Lat: *Aloe vera*; Fam: Liliaceae ) has been extensively used as a cathartic agent to treat severe stomach constipation. In order to develop reliable quality control marker for quality evaluation and to check the commercial samples of *Aloe vera* for authenticity and purity, its detailed chemical and HPTLC analysis has been done. The cathartic principles viz. Barbaloin was extracted from leaf skin part by using different solvent i.e hexane, ethyl acetate and methanol and was found to present in methanol extract only. Also the, HPTLC studies of Barbaloin compounds revealed that, it is present in the leaf skin but not present even in traces in stem part, leaf gel and root part of the *Aloe vera* plant. It has been found that Barbaloin is present in a few commercial samples which shows that in those samples leaf skin part is used and the samples which lacks barbaloin other part of the *Aloe vera* plant has been used . It gave reproducible results and found linear in the range 1500 – 7500 ng. The method was found to be simple, sensitive, precise, accurate and specific for estimation of barbaloin from *Aloe vera* and its commercial formulations.

**Keywords:** HPTLC; *Aloe vera*; Barbaloin; Cathartic agent.

**Introduction**

In Indian system of medicine a good number of plants have been used as a cathartic agent to treat stomach constipation, of which *Aloe vera* (Fam. Liliaceae), popularly known as “Ghritkumari” is one of the potent plant. It is a succulent herb grows widely in most parts of India. Its leaf which produces latexes is the official part and used as a cathartic agent. It is available in the market, both in pure form and also as one of the ingredients in a number of commercial formulations. Barbaloin (also named aloin, Figure 1), the C-glucoside of aloe emodin anthrone, localizes in the outer rind of the aloe plant, has been reported to constitute up to 30% of the aloe plant dried leaf exudates (Groom and Renolds, 1986) and proposed as a part of the defense mechanisms against herbivores (Gutterman and Chauer-Volfson, 2000). The leaf extracts were found to inhibit the growth of *Mycobacterium tuberculosis*, this activity is attributed to the presence of the bioactive metabolites barbaloin (Gupta et al., 2010). Methanol extract of *Aloe vera* leaf also shows hepatoprotective activity by causing a significant inhibition of rat liver cytosolic alcohol dehydrogenase activity and the active principle for the inhibition of c-ADH and c-ALDH was identified as Barbaloin (Chen et al., 2004).

Orally administered barbaloin is poorly absorbed but is metabolized by intestinal microflora to aloe emodin, which is readily absorbed (Ishii et al., 1994). Because aloe plants containing barbaloin and aloe emodin are widely used for its cathartic properties (Lemli, 1988), the toxicological properties of these compounds have been examined in a number of studies. Barbaloin demonstrates anti-inflammatory and cathartic effects in vivo (Nakagomi et al., 1985). Barbaloin was not directly photocytotoxic, human skin fibroblasts can metabolize barbaloin to aloe emodin (Wamer et al., 2003). These results confirm that *Aloe vera* gel contains toxic low
molecular weight compounds, and every effort must be made to limit the amount of these toxins in the commercially prepared Aloe vera products (Avila et al. 1997). The High performance liquid chromatographic analysis of an Aloe vera “Ghritkumari” leaf latex and Aloes reported to contain Barbaloin as major constituents (Autorhoff et al., 1980; Kawai et al., 1993; Renolds, 1994). Examination of the literature showed lack of methods to analyze commercial products for barbaloin. Most analytical methods were directed toward separation of various anthraquinones from aloes (Reward et al., 1993) or profiling of fresh and aged aloe solutions. Aloenin, however, which is a non-anthraquinone o-glucopyranoside component of Aloe arborescens, has been analyzed in cosmetic products via HPLC (Zonta et al., 1995, Nakano et al. 1985) and GC/MS (Nakamura et al., 1990) methods. Also standardization of Kumari asawa has already been done by HPTLC and HPLC method in which the barbaloin was found to be the major bioactive compound (Elamthuruthy et al., 2005). The use of Aloe vera as ingredients of cosmetics and the concern over the barbaloin content as it shows phototoxic effect in such preparations and also its beneficiary effect in ayurvedic formulation, there is urgency in developing quality control to check quickly the authenticity and purity of Aloe vera and its commercial formulation. Therefore it is absolutely required to develop extraction and HPTLC method to quantify barbaloin in commercial samples. Here we have now developed simple, sensitive, precise, accurate and specific HPTLC method for estimation of barbaloin from Aloe vera and its formulations.

**Material and methods**

**Plant material and commercial formulation**

The Aloe vera plant material was collected from locally in Varanasi (25°18’N, 83°03’E), during January 2006. Its identity was confirmed by taxonomists and voucher specimen (No. 010102) was kept stored in the School of Biochemical engineering, Institute of Technology, Banaras Hindu University, Varanasi, India. The market samples viz., Ghritkumari oil (Surya Pharmaceuticals), Himalaya face wash (The Himalaya Drug Company), Fair & Lovely (Hindustan lever Ltd.), Eve Care (The Himalaya Drug Company), Benzoin tincture (Apex Pharmaceuticals), Rajapravardini vati (Narayana Pharmaceuticals), Kumari Asava (Baidyanath) and Jula Aloe Hydro Gel (Fem Care Pharm Ltd) were procured from local markets.

**Extraction of barbaloin from Aloe vera**

The plant material of Aloe vera has been separated as three parts i.e., leaf, stem and root parts. Leaf part has been further separated into gel and leaf skin. Except leaf gel, all parts were well shade dried and powdered. All these parts were extracted successively with hot n-hexane, ethyl acetate, and methanol. Leaf gel was extracted successively with cold n-hexane, ethyl acetate and methanol. All these extracts were concentrated under vacuum. By the observation of preliminary TLC patterns of all these extracts, only the methanol extracts of leaf skin was found to contain barbaloin. [RF 0.40 (ethyl acetate- methanol-water, 10:2:1)]

**Extraction methods for commercial formulations**

For commercial samples, Rajapravardini Vati (20 g), Ghrit kumari oil (20 ml), Himalaya Face Wash (20 gm), Himalaya Antiseptic Cream (20 gm), Fair & Lovely (20 gm), Eve Care (20 g), Benzoin Tincture (20 g), Kumari Asava (20 g) and Jula Aloe Hydro Gel (20 gm) were dissolved in 100 ml methanol by using magnetic stirrer for 1 hour and transferred into separating funnel respectively. For the removal of oily and fatty material, to that added 100 ml of n-hexane and shook well and set aside for

![Figure 1: Structure of barbaloin.](http://www.openaccessscience.com)
separation of two distinguish layers and collected methanol layer into a conical flask respectively. These concentrated methanol extracts were dissolved in a minimum amount of water and transferred into separating funnel respectively. To that added 100 ml of butanol and shacked well and set aside for separation of two distinct layers. Collect the butanol layer into round bottom flask. Again added 100 ml of butanol to that water layer and collected the butanol layer into the same round bottom flask respectively and concentrated under vacuum. These butanol extracts of Ghrit kumari oil, Himalaya face wash, Himalaya antiseptic cream, Fair & Lovely, Eve Care, Benzoin tincture, Kumari Asava, Jula gel were dissolved in the mini amount of methanol and transferred into 10 ml of volumetric flask and made up the volume with methanol.

**HPTLC Analysis**

**Test Sample Solutions**

Because commercial formulations contain many ingredients, complex extracts were obtained; different sample-preparation procedures were therefore adopted for plant samples and commercial formulations. For pure plant samples, methanol extracts of leaf skin, gel, stem and root were weighed (100 mg) accurately in a sample beaker and dissolved minimum amount of methanol and transferred into a 10 ml volumetric flask and made up the volume with methanol.

**Standard solutions**

The standard solutions of pure isolated barbaloin were prepared by dissolving: a) 1 mg in 1 mL of methanol (for calibration and analysis), b) 1 mg in 10 mL of methanol (for determining limit of detection and spiked standard solutions).

**Sample application**

Different concentrations of standard and test samples were applied in different tracks as bands by Linomat IV applicator in different studies. To determine the Limit of Detection (LOD) a standard solution was applied in volume containing 0.1 – 1.0 g (100-1000 ng). To determine the range of linearity and calibration the standard solution was applied in volume containing 0.9-7.5 g. For quantification of barbaloin in the plant material sample solutions 10 l methanol extract of leaf skin, methanol extract of gel, methanol extract of stem methanol extract of root, commercial sample solutions [ Ghrit kumari oil – 10 l, Himalaya Face wash – 10 l, Himalaya antiseptic cream – 10 l, Fair & Lovely – 10 l, Eve Care – 10 l, Benzoin Tincture – 10 l, Rajapraverdini vati – 10 l, Kumari Asava – 10 l & Jula gel – 10 l] and different concentration of standard solution were applied in volumes containing 0.9 – 7.5 g (900-7500 ng).

**Chromatographic Conditions**

HPTLC was performed on aluminium backed plates precoated with 0.2mm layers of silica gel 60 F254 (E. Merck; cat. No. 1.05554). Samples were applied with a Linomat IV applicator. The mobile phase used for chromatography was ethyl acetate-methanol-water, 10:2:1. Vertical development of the plates was performed to a distance of 8.0 cm in chambers previously saturated for 5 min. Detection of barbaloin was performed in UV light at 340 nm before (gray spot) and after (yellow) treatment of the plate with 5% alcoholic H2SO4 and heating at 105°C for 5min.

Plates were scanned with a Camag TLC scanner – III controlled by Cats4 software (4.05 version).

**Scanning**

After drying, the plates were scanned densitometrically at 360 nm.

**Method development and validation**

**Repeatability**

The plant materials and market samples were extracted by the procedures described above and analysed in triplicate.
Barbaloin in Aloe vera and its commercial formulations

Linearity

The linearity range of barbaloin was 1.5-7.5 μg (1500 –7500ng). The regression equation and co-relation coefficient were obtained with three replicate analyses.

Reproducibility

Methanol extract of leaf skin and Ghrit Kumari oil were taken as blank and spiked separately with different amount (1.5, 3.0, 4.5 μg), standard solutions respectively and three analysis of each spike on the same blank samples.

Stability

The solutions were tested after 24 h and 72 h at room temperature and the results demonstrated that the samples were stable at these conditions. The mobile phase was tested after 1h and no significant change was observed in the RF of barbaloin.

Results

The cathartic principle, Barbaloin was extracted by using methanol from Aloe vera skin. The test sample solutions of the plant materials were prepared as mentioned above. As the commercial formulations showed complicated TLC patterns due to the presence of a number of ingredients in addition to Aloe vera, an efficient procedure was developed for their sample solution preparations. Extensive TLC studies on various extracts reveal that, the solvent systems ethyl acetate: methanol: water – 10: 2:1 for Barbaloin gave well resolved spots with RF 0.40. Although the compound was visualized in UV at 360 nm (Figure 2), different developing reagents have been used as per the following: i) 5% alcoholic – H₂SO₄ spray followed by heating at 105 °C for 5 min – yellow (Barbaloin).

The initial HPTLC finger printing studies were done on the isolated markers and parameters were optimized. The finger printing patterns of the standard Barbaloin is given in Figures 3. Under identical parameters finger printing patterns of the test samples (both plant material and commercial formulations) were recorded. The finger printing pattern of the high barbaloin yielding sample which was collected from leaf skin is given in Figure 4. The HPTLC finger-printing pattern of the butanolic extract of commercial formulations are given in figures 5, 6, 7, 8 and 9. The three dimensional patterns of the standards and test samples reveal the presence of super imposable peaks (RF 0.40 barbaloin). Further, spectrum studies reveal that the peaks corresponding to RF 0.40 of both standard barbaloin and test samples are identical as they showed similar patterns with λ_max at 360 nm. The analytes in solution and during analysis are highly stable as evidenced by their absorption spectra which are consistent throughout the analysis. The correlation coefficients, linearity ranges, regression equations and RSD of slope and intercept are given in Table 1.

Figure 2: UV Spectral graph of Barbaloin λ_max (360 nm).

Table 1: Calibration curve statistical data for barbaloin.

<table>
<thead>
<tr>
<th>Property</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9993</td>
</tr>
<tr>
<td>Linearity range (ng)</td>
<td>1500 - 7500</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 1343.24 + 2505.66. X</td>
</tr>
<tr>
<td>RSD</td>
<td>2.0</td>
</tr>
<tr>
<td>RSD of slope</td>
<td>0.68</td>
</tr>
<tr>
<td>RSD of intercept</td>
<td>1.56</td>
</tr>
<tr>
<td>Number of data points</td>
<td>5</td>
</tr>
</tbody>
</table>

http://www.openaccessscience.com
ijmap@openaccessscience.com
Figure 3: HPTLC fingerprinting of standard ‘Barbaloin’.

Figure 4: HPTLC fingerprinting of standard ‘Barbaloin’ (Rf 0.4) in Aloe vera leaf skin.

Figure 5: HPTLC fingerprinting of standard ‘Barbaloin’ in Eve care.

Figure 6: HPTLC fingerprinting of standard ‘Barbaloin’ (3) in Himalayan ayurvedic cream.

Figure 7: HPTLC fingerprinting of standard ‘Barbaloin’ (4) in Rajopraverdini.
The peak purity test was done by comparing the spectra of the standards and its corresponding peaks in test samples. The correlation coefficients were found to be 0.9990 for barbaloin, which indicates its purity. The percentage of the bio-active marker was determined by calculation mode using peak area parameter and the data is presented in Table 2.

Table 2: Levels (%) of barbaloin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Area of collection /Company name</th>
<th>Barbaloin(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe vera Leaf skin</td>
<td>Bhubaneswar</td>
<td>2.522</td>
</tr>
<tr>
<td>Ghrit Kumari Oil</td>
<td>Surya Pharmaceuticals</td>
<td>0.002</td>
</tr>
<tr>
<td>Himalaya Antiseptic Cream</td>
<td>The Himalaya drug Company</td>
<td>0.0045</td>
</tr>
<tr>
<td>Eve Care Syrup</td>
<td>The Himalaya Drug Company</td>
<td>0.0011</td>
</tr>
<tr>
<td>Benzoin Tincture</td>
<td>Apex Pharmaceuticals</td>
<td>0.0021</td>
</tr>
<tr>
<td>Rajapraverdini Bati</td>
<td>Narayan Pharmaceuticals Pvt Ltd</td>
<td>0.0694</td>
</tr>
<tr>
<td>Kumari Asava</td>
<td>Baidyanath</td>
<td>0.0156</td>
</tr>
<tr>
<td>Fair &amp; lovely</td>
<td>Hindustan lever Ltd</td>
<td>-</td>
</tr>
<tr>
<td>Himalaya Face wash</td>
<td>The Himalaya Drug Company</td>
<td>-</td>
</tr>
<tr>
<td>Jula Aloe Hydro Gel</td>
<td>Fem Care Pharms Ltd</td>
<td>-</td>
</tr>
</tbody>
</table>
The reproducibility studies were done by spiking different concentrations of the standard to their corresponding blank solutions as mentioned above. The percentage of recovery and citation variance for Barbaloin is given in Table 3.

Table 3: Reproducibility, precision and accuracy of the method applied to samples spiked with barbaloin

<table>
<thead>
<tr>
<th>Blank sample</th>
<th>Amount added (ng)</th>
<th>Amount found (ng)</th>
<th>Precision / Reproducibility (C.V.)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf skin</td>
<td>1500</td>
<td>1462.5</td>
<td>1.82</td>
<td>97.5</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>2943.34</td>
<td>0.85</td>
<td>98.11</td>
</tr>
<tr>
<td></td>
<td>4500</td>
<td>4407.76</td>
<td>1.93</td>
<td>97.28</td>
</tr>
<tr>
<td>Ghrit Kumari oil</td>
<td>1500</td>
<td>1452.6</td>
<td>2.20</td>
<td>96.84</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>2926.7</td>
<td>1.78</td>
<td>97.55</td>
</tr>
<tr>
<td></td>
<td>4500</td>
<td>4429.7</td>
<td>1.23</td>
<td>98.43</td>
</tr>
</tbody>
</table>

Discussion

In order to identify the reliable quality control marker of Aloe vera, we have done extensive TLC studies on the cathartic principle viz. barbaloin. These studies revealed that barbaloin accumulates in considerable quantities in both plant samples and a few commercial formulations. Hence HPTLC based quantification studies have been done on barbaloin. In view of the overlapping TLC behavior of commercial formulations due to the presence of a large number of ingredients, different protocols have been adopted to generate sample solutions. In these cases the developed methods showed consistency, linearity, reproducibility and found highly accurate and precise over the linearity range as evident by the data depicted in Table 1. The Coefficient Variance (C.V.) and recovery percentage data indicates that the barbaloin can be estimated within their linearity ranges with accuracy and precision in Table 3. The result shows that barbaloin accumulates several folds more in leaf skin than the commercial formulation. The highest level of barbaloin was found in leaf skin sample. Regarding the commercial samples, barbaloin was found to present in detectable amounts in a few samples in table 2. It is interesting to note that the markers are not present even in traces in the cosmetics samples viz. Jula gel, fair & lovely and Face wash, where Aloe Vera was shown as one of the ingredients. The extraction and HPTLC studies showed that barbaloin was absent in the cosmetics samples. This suggests that in the cosmetics products Aloe gel has been used instead of whole leaf. In case of polyherbal formulation barbaloin was found to be present in all the samples. The present HPTLC method is rapid, simple and accurate for quantitative monitoring barbaloin in of Aloe vera plant and its commercial product.

Conclusions

The potent cathartic compound of Aloe vera viz. Barbaloin was isolated and characterized by chromatographic and spectroscopic methods. In order to identify the reliable marker for quality control, they have been subjected to detailed HPTLC studies. In case of barbaloin, the Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 200 ng and 900 ng respectively and show linearity in the range 1500 – 7500 ng. Further the recovery values for barbaloin were found to be 97 – 98 %, which shows the reliability and suitability of the method. The HPTLC quantification studies reveal that barbaloin accumulates in leaf skin and found to present in a number of commercial formulations. Hence it can be taken as a reliable quality control marker of Aloe vera and its commercial formulations. The present HPTLC method is rapid, simple and accurate for quantitative monitoring of Aloe vera plant and its commercial product with respect to barbaloin.

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