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# Standardization of marketed *Kumariasava*—an Ayurvedic *Aloe vera* product

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**Abstract**

*Kumariasava* is a marketed ayurvedic formulation containing *Aloe vera* as one of the main ingredients. Present study aims to standardize *Kumariasava* based upon chromatographic and spectral studies. Various extracts of *Kumariasava* have been prepared and evaluated. Chloroform extract indicated presence of three well-resolved fluorescent components. Spectral data of these three fractions (III–V) have been reported as a valuable analytical tool for routine standardization of *Kumariasava*. Fraction V indicated presence of anthraquinones, which is reported as the main constituent of aloe, namely aloin. Hence, isolation and evaluation of aloin has been undertaken. Aloin can be used as possible marker compound for standardization of *Kumariasava*.


*Keywords:* Standardization; *Kumariasava*; *Aloe vera*; Marker

## 1. Introduction

Ayurveda is the indigenous system of medicine. It has been practiced in India since ancient times. This plant-based system of medicine has already gained worldwide attention due to its safety and efficacy. The Ayurvedic system tauted as an 'alternative system of medicine' has become specially significant in the post-GATT era [1]. With the growing need for safer drugs, attention has been drawn to their quality, efficacy and standards of the Ayurvedic formulations [2].

Ayurvedic pharmacy advocates the use of quality control tests to make sure that the prepared medicines adhere to the standards mentioned in Ayurveda. Most of the tests described in ancient literature appear to be based on observation and seem subjective without valid scientific backing. Hence standardization and development of reliable quality protocols for Ayurvedic formulations using modern techniques of analysis is extremely important [3]. Ayurveda is a plant-based system of medicine and consist of various Ayurvedic formulations such as solid dosage forms (pills, powders), liquid dosage forms (asavas, aristhas) and semisolid dosage forms (ghritas, avlehas).

Asavas are medicinal preparations made by soaking the drugs (powder or decoction) in a solution of sugar or jaggery for a specified period of time during which it undergoes a fermentation process generating alcohol, thus facilitating the extraction of active principles contained in the drug(s). The alcohol generated, also serves as a preservative [4].

*Kumariasava* is a marketed Ayurvedic herbal formulation included in Ayurvedic formulary containing *Aloe vera* as the main ingredient. It is a self-fermented galenical containing about 40–50 crude drugs [5].

It is one of the most widely used over the counter products indicated as general tonic for children and adults and used in liver sluggishness, weak digestion, urinary disorders and cough [6].

## 2. Aims and objectives

Pharmacopoeial standards for Ayurvedic formulations published by the Central Council for Research in Ayurveda

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and Siddha give certain physical parameters as standards for *Kumariasava*. However, these are not based on modern analytical methods. It is therefore essential, that there are definite and accurate analytical tools to ascertain consistency and quality of Ayurvedic formulations.

Thus, the present study aims to develop standardization method for *Kumariasava* and to identify analytical marker/s in the formulation.

## 3. Experimental

All the solvents purchased from E. Merck and S.D. Fine Chemicals, Mumbai. All solvents used for extraction, TLC, and HPTLC studies were distilled before use. Solvents used for UV and IR studies were of spectroscopy grade. Solvents used for HPLC analysis were of HPLC grade. Precoated silica gel GF-254 plates procured from E. Merck, Mumbai were used for TLC and HPTLC studies. The UV spectra were recorded on a JASCO V 530 spectrophotometer. The fluorescence analysis was performed on Shimadzu RF-5000 fluorimeter. The FT IR spectra were recorded on JASCO FT IR 410. HPTLC studies were carried out using CAMAG LINOMAT IV applicator and CAMAG SCANNER III. The HPLC analyses were done on a TOSOH–CCPM system.

All the results are obtained by repetition of the each experiment at least three times.

### 3.1. Procurement of drug

Commercially available brand (M/S Sandu Brothers) of *Kumariasava* was procured from local market.

### 3.2. Standardization using physicochemical parameters

The sample of *Kumariasava* was analysed for various parameters such as pH, specific gravity, total solids content, alcohol content, amount of reducing and non-reducing sugars [7].

### 3.3. Preparation of extracts

*Kumariasava*, 100 ml, was evaporated on a steam bath to about 50 ml, cooled and then diluted to 100 ml with water. This was extracted successively with solvents such as petroleum ether (60–80 °C), benzene, chloroform, ethyl acetate and ethyl ether. All the extracts were dried under reduced pressure at room temperature.

### 3.4. Qualitative chemical examination

All the extracts were qualitatively evaluated by chemical tests and TLC studies for the presence of various phytoconstituents like alkaloids, carbohydrates, saponins, phenolic compounds and tannins, phytosterols and anthraquinone glycosides [8].

#### 3.4.1. TLC studies

TLC studies of all the extracts were carried out using Silica gel GF-254 as stationary phase and chloroform: ethyl acetate (75:25) as mobile phase. Spots were observed under UV and visible light. Chloroform extract was found to contain well-resolved fluorescent components. The $R_f$ values of the components were 0.360, 0.540, 0.718, 0.809, 0.850. Out of the five components, three components were separated in appreciable amount by preparative TLC using same mobile phase. The fractions III–V were thus obtained with $R_f$ values of 0.718, 0.809, 0.850, respectively.

#### 3.4.2. HPTLC studies

HPTLC fingerprint of chloroform extract was recorded at 254 and 366 nm.

Fractions III–V were subjected to HPTLC studies to develop fingerprints using same conditions as used for TLC.

#### 3.4.3. Spectral studies

UV, IR and fluorescence spectra were recorded for fractions III–V.

UV spectra were recorded in methanol and ethanol.

IR spectra were recorded of neat sample.

#### 3.4.4. HPLC studies

Fraction V indicated presence of anthraquinones which is reported to be a major component of *Aloe vera*. Fraction V was further analysed by HPLC using following conditions:

Column: $C_{18}$ (25 cm × 4.6 mm, i.d.), 10 μm
Mobile phase: methanol:water (60:40)
Detection: at 254 nm
Flow rate: 1 ml/min

#### 3.4.5. Isolation of aloin

Aloin was extracted from dried juice of *Aloe vera* by dissolving it in water. It was filtered and the filtrate was acidified with sulfuric acid to precipitate resin. The precipitated resin was filtered and the filtrate was neutralized with ammonia. Crude aloin thus obtained was purified by recrystalization in 50% ethanol [9–11].

TLC, HPTLC and HPLC studies of aloin were carried out under same conditions as mentioned for fraction V.

## 4. Results and discussion

Standardization of *Kumariasava* as per pharmacopoeia was carried out based on the physicochemical parameters [7]. The marketed sample of *Kumariasava* was found to pass all the pharmacopoeial tests (Table 1).

Aqueous solution of *Kumariasava* was successively extracted with the various solvents, i.e. petroleum ether, benzene, chloroform, ethyl acetate and ethyl ether and TLC studies of all the extracts were carried out. Petroleum ether, benzene and chloroform extracts showed one, three and five

Table 1
Standardization of *Kumariasava*—physicochemical parameters

| Serial number | Physicochemical tests | Standard values | Observed values |
|---|---|---|---|
| 1 | Description | Fragrant clear liquid with bitter taste | Dark brown colored fragrant liquid with bitter taste and with some sediment |
| 2 | Total solids | 10–25% (w/v) | 24.408% (w/v) |
| 3 | Specific gravity | 1.03–1.09 | 1.036 |
| 4 | Sugars—reducing | 3–7% (w/v) | 5.55% (w/v) |
| | Non-reducing | Not more than 1% (w/v) | 0.91% (w/v) |
| 5 | pH | 3.5–5 | 4.38 |
| 6 | Alcohol content | 5–10% (v/v) | 7.90% (v/v) |

spots, respectively, while no spots were seen for ethyl acetate, ethyl ether and water extracts. A summary of the qualitative analysis is given in Table 2.

After preliminary chemical testing of all the extracts, it was decided to focus attention on the chloroform extract since this extract showed presence of maximum number of spots. TLC profile and HPTLC fingerprints were developed for chloroform extract. The TLC profile of chloroform extract was found to contain five fluorescent components that were well resolved. The $R_f$ values of the components were 0.360, 0.540, 0.718, 0.809, and 0.850.

Out of the five components, three components were separated in appreciable amounts by preparative TLC using same mobile phase. The fractions with $R_f$ values of 0.718, 0.809 and 0.850 were separated as fractions III–V, respectively.

Fractions III–V were evaluated by chemical and spectral methods to study the nature of the components. The color characteristics in UV and visible light revealed presence of flavones and/or flavonol in fraction III and isoflavones in fraction IV. Fraction V was found to contain anthraquinones (Table 3). Spectral studies confirmed the results of TLC studies. UV and IR spectra of fraction V gave characteristic peaks indicating presence of quinones. The UV, IR, fluorescence spectra and HPTLC fingerprints of fractions III–V can be used for routine standardization of *Kumariasava* (Table 4) (Figs. 1–3).

Table 4
Spectral data of isolated fractions

| Fraction | UV (methanol) | IR ($cm^{-1}$) | Fluorescence | |
|---|---|---|---|---|
| | | | Excitation maximum (nm) | Emission maximum (nm) |
| III | 218, 272 | 2928 | 297 | 336 |
| IV | 213, 273 | 2845 | 340 | 393 |
| V | 212, 256, 427.5 | 1698, 1585 | 395 | 490 |

Fraction V of chloroform extract was found to be crystalline solid with orange red color and slightly tacky in nature. It gave Modified Borntrager's test positive. On spraying the TLC plates with 10% methanolic KOH solution, original yellow color of the spot with $R_f$ 0.850 changed to purple. It's ethanolic solution gave absorbance maximum at 431 nm in visible region and two absorbance maxima at 214 and 257 nm in the UV region.

As reported in literature, anthraquinones were detected by their yellow-brown appearance in visible and UV radiation. Spraying with 10% methanolic KOH, the original yellow and yellow-brown colors changed to red, violet, green or purple. The chemical tests, color reactions and the spectral

Table 2
Qualitative chemical evaluation of extracts

| Serial number | Phytoconstituents | Extracts | | | | | |
|---|---|---|---|---|---|---|---|
| | | Pet ether | Benzene | Chloroform | Ethyl acetate | Ethyl ether | Water |
| 1 | Alkaloids | – | – | – | – | – | – |
| 2 | Carbohydrates | – | – | – | + | – | + |
| 3 | Saponins | – | – | – | + | – | + |
| 4 | Phenolic compounds and tannins | – | – | – | + | + | + |
| 5 | Phytosterols | + | + | + | – | – | – |
| 6 | Anthraquinone glycosides | – | + | + | + | – | + |
| 7 | TLC studies: number of spots | 1 | 3 | 5 | – | – | – |

(+) Indicates presence, (–) indicates absence. The chemical evaluation reported here is for the entire extract and not for the individual spot.

Table 3
TLC studies of isolated fractions of chloroform extract

| Isolated fraction | $R_f$ | Color characteristics | | | Indication |
|---|---|---|---|---|---|
| | | Visible | Fluorescence in UV light | | |
| | | | Alone | With $NH_3$ | |
| III | 0.718 | Colorless | Dark purple | Faint purple | Flavones and/or flavonol |
| IV | 0.809 | Colorless | Faint blue | Intense blue | Isoflavones |
| V | 0.850 | Yellow | Reddish orange | No change | Anthraquinones |

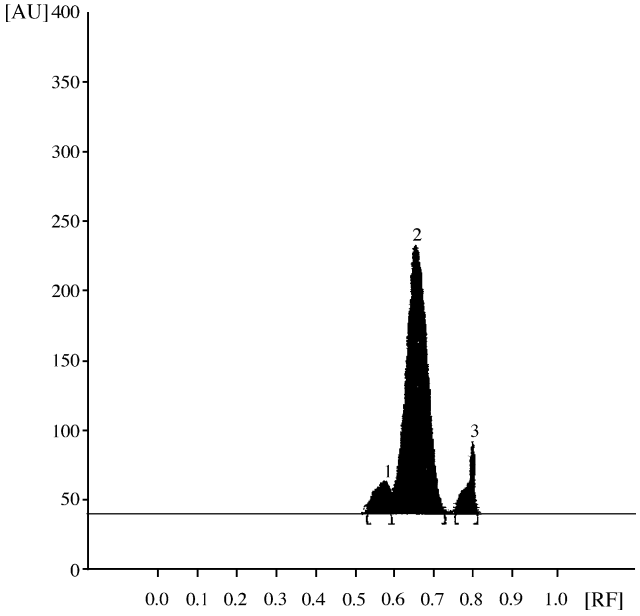


Fig. 1. HPTLC fingerprint of fraction III.

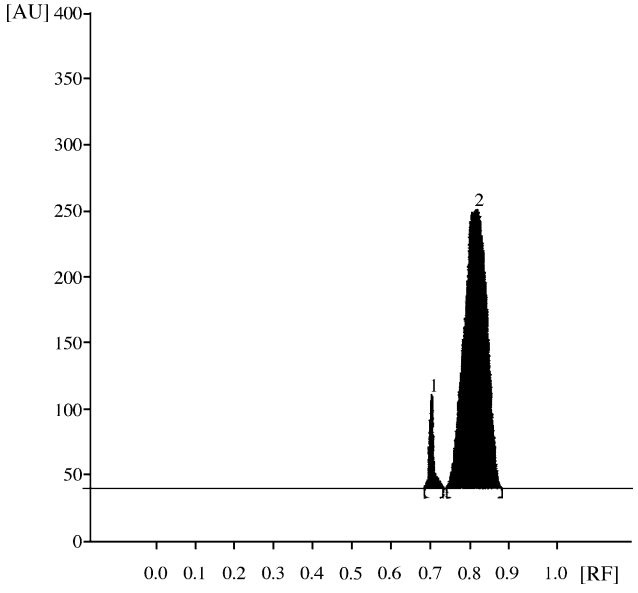


Fig. 3. HPTLC fingerprint of fraction V.

data strongly indicated that fraction V was an anthraquinone moiety (Table 5) and hence further analysis of the same was undertaken.

### 4.1. Characterization of fraction V

The HPLC analysis of this fraction gave two peaks with retention times of 3.506 and 11.243 min, respectively, (Fig. 4) using same conditions as mentioned earlier.

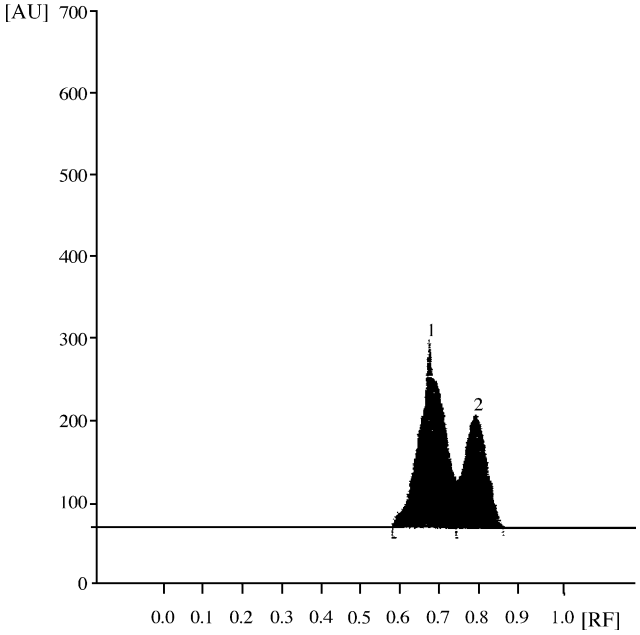


Fig. 2. HPTLC fingerprint of fraction IV.

Since aloin is one of the major constituents of Aloes, it was isolated from dried juice of *Aloe vera*. Isolated aloin gave modified Borntrager's test positive for anthaquinone C-glycosides. TLC analysis of aloin on silica gel plate gave single reddish orange colored spot with $R_f$ 0.847 at 366 nm. HPLC analysis of aloin under same conditions as mentioned for fraction V gave a single peak with RT 3.532 min (Fig. 5). Fraction V of chloroform extract of *Kumariasava* and aloin were compared by TLC, HPTLC and HPLC analysis.

Fraction V and aloin showed same color characteristics before and after derivatization at 366 nm. Both gave positive modified Borntrager's test indicating presence of anthraquinone C-glycosides. One of the components of fraction V showed same $R_f$ value by TLC and HPTLC as that of aloin (0.847 and 0.81, respectively) and same retention time in HPLC profiles (3.5 min) (Table 6). Thus, if can be said that fraction V may contain aloin. It can be the possible analytical

Table 5
Chemical and spectral studies of fraction V of $CHCl_3$ extract

| Tests | Observation | |
|---|---|---|
| Nature | Orange red crystals, slightly tacky | |
| Modified Borntrager's test | Development red color | |
| Spraying of with 10% methanolic KOH | Original yellow color changed to purple | |
| | Methanol (nm) | Ethanol (nm) |
| Spectral data | | |
| Visible spectrum | 427.5 | 431 |
| Ultraviolet spectrum | 211.5, 256 | 214, 257 |
| Infra red spectrum | 1696 $cm^{-1}$ indicating presence of quinones | |

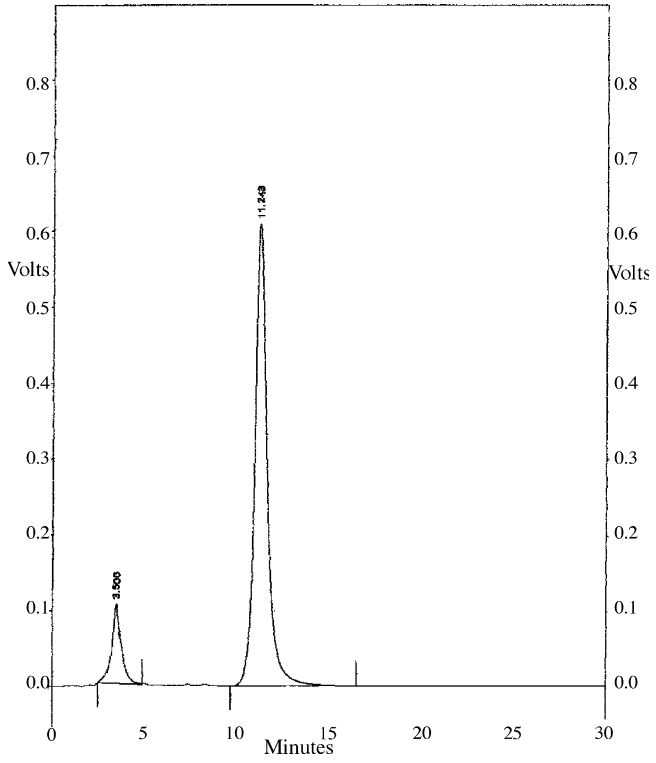


Fig. 4. HPLC profile of fraction V.

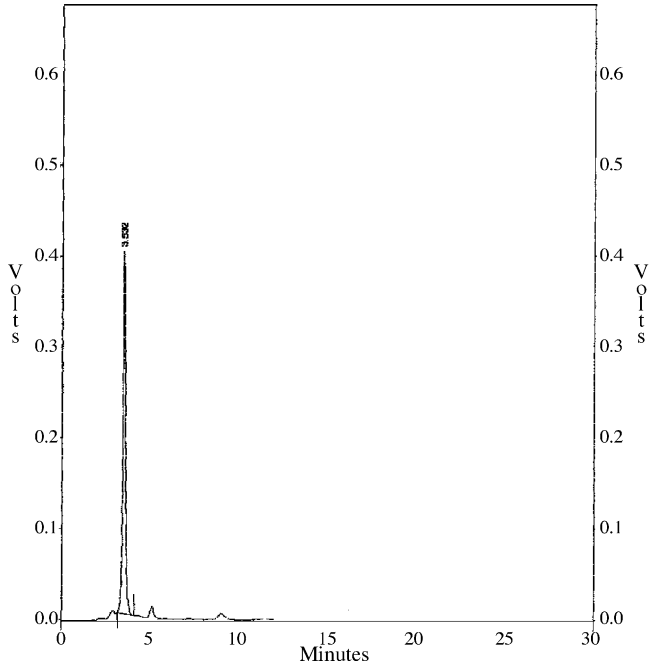


Fig. 5. HPLC profile of aloin.

Table 6
Comparision of aloin and fraction V

| Tests | Aloin | Fraction V of $CHCl_3$ extract of *Kumariasava* |
|---|---|---|
| Modified Borntrager's test | Positive | Positive |
| TLC: number of spots and $R_f$ | 1 (0.847) | 2 (0.791, 0.847) |
| Color at 366 nm | Reddish orange | Reddish orange |
| Color after spraying with 10% methanolic KOH | Purple | Purple |
| HPLC: number of peaks and RT | 1 (3.536 min) | 2 (3.502 and 11.243 min) |
| HPTLC: number of spots and $R_f$ | 1 (0.81) | 2 (0.69, 0.81) |

marker for standardization of *Kumariasava*. After quantitative estimation of aloin, specifications can be stated. This should then serve as a simple, accurate and routine method of analysis for *Kumariasavas*.

## 5. Conclusion

The spectral data and HPTLC fingerprint of chloroform extract of *Kumariasava* could be used as a valuable analytical tool in the routine standardization of *Kumariasava* to check the batch to bath variation.

Aloin can be used as one of the appropriate analytical markers for standardization of *Kumariasava*.

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