Original Article

Shodhana (processing) of *Rakta-Snuhi* (*Euphorbia caducifolia Haines.*) latex with *Chincha* (*Tamarindus indica* L.) leaf juice: A pharmaceutical analysis

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Abstract

Background: Latex of *Euphorbia caducifolia* Haines. (Euphorbiaceae), botanical source of *Rakta Snuhi*, a caustic irritant, is being used in different Ayurvedic therapeutics, after proper processing (*Shodhana*) in some specific media. *Shodhana* of *Snuhi* latex with *Chincha-Patra Swarasa* (juice of tamarind leaves) using *Raudra Yantra* (instrument/pot kept under sunlight for drying) has been recommended in Ayurveda texts. *Snuhi* is one of the *Upavisha* (sub toxic group of herbal drugs) and a well-known plant in the Indian system of medicine. It is used in pharmaceutical procedures like preparation and processing of drugs. **Aim:** The aim of the study was to carry out *Shodhana* of *E. caducifolia* latex with *Chincha-Patra Swarasa*. **Material and methods:** Fresh latex of *Snuhi* was collected from the Sapada area of Jamnagar, Gujarat and fresh leaves of *Tamarindus indica* L. were collected from the herbal garden of the university and *Swarasa* was extracted by traditional expression technique. *Shodhana* of *Snuhi Kshira* was done under sunlight dried and shade dried method by mixing it with leaf juice of *Tamarindus indica* L. in a glass bowl in different ratio i.e. *Kshira*: leaf juice was 8:4, 8:2, 8:1 respectively. **Results:** This study reveals certain changes in physico-chemical parameters (pH) and organoleptic characters of processed *E. caducifolia* latex by *Tamarind* leaf juice both in shade-dried and sunlight-dried samples. Under HPTLC, *Shodhana* of *E. caducifolia* latex with *Tamarind* leaf juice alters the number of spots both sunlight-dried and shade-dried samples. In sun-dried sample, the number of spots increased when the concentration of *Tamarind* leaf juice is decreased in HPTLC study. The study reveals that in case of shade-dried *Shodhita* (processed) *Snuhi* latex sample, the concentration of lupeol increases with the increasing quantity of *Tamarind* leaf juice. **Conclusion:** *Shodhana* with *Tamarind* leaf juice changes both qualitative and quantitative property of *Snuhi* latex.

Keywords: *Euphorbia caducifolia* Haines, latex, purification, *Rakta Snuhi*, *Shodhana*, *Tamarindus indica* L.

Introduction

Rakta-Snuhi (E. caducifolia), botanically identified as *Euphorbia caducifolia* Haines., is a member of *Upavisha* group (sub-toxic herbal group)[1] and is being used extensively in different formulations of therapeutic significance, being advocated, in diseases such as *Gulma* (abdominal lump), *Arsha* (piles), *Grahani* (sprue), *Pandu* (anemia), *Kushtha* (skin diseases) and *Shotha* (edema).[2] It's latex is used as *Bhavana Dravya* (lavigation) in the preparation of *Vati* (tablets), *Varti* (suppositories) and herbo-mineral compound formulation.[3] The latex is used in the management of *Arsha* (piles) and *Bhagandara* (fistula) and in the preparation of *Ksharasutra* (alkaline thread).[4] *Euphorbia caducifolia* latex contains number of chemical constituents such as flavonoids, terpenoids, reducing sugar, carbohydrates and amino acids, among which terpenoids is considered as the main responsible constituent for the poisonous effect.[5] The latex or sap of many Euphorbia plants is toxic and may cause inflammation of the skin[6] and eye[7,8] on contact. *Euphorbia caducifolia* has also been reported to cause ocular injury.[9] The latex of

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E. caducifolia is acrid and liable to cause dermatitis.[10] To minimize or to prevent the poisonous effect of *Rakta-Snuhi* latex and to enhance the potency and efficacy of the drug, *Shodhana* is recommended.[11] According to Ayurvedic literature, *Snuhi* latex should be mixed with leaf juice of *Tamarindus indica* and then the mixture should be kept under sunlight, in *Raudra Yantra* (drying in sunlight keeping the contents in a saucer), till it is dried properly.[12,13]

Lupeol is a pentacyclic triterpene which has a wide therapeutic usage, has been reported in euphorbia species, and possesses anti-inflammatory and antioxidant activity[14] and thus was chosen as a marker in this study. Impact of *Shodhana* on *E. caducifolia* latex has not been reported till date. Hence, the present study was carried out to evaluate the impact of purification of *E. caducifolia* latex by tamarind leaf juice and to develop physicochemical and high-performance thin-layer chromatographic (HPTLC) profile.

Materials and methods

Collection and selection of drug

The plant *Rakta-Snuhi* was identified by a local plant taxonomist and the botanical name, i.e., *Euphorbia caducifolia* Haines, was confirmed by studying the morphological characters, as described in Flora of Orissa and Trease and Evans, Pharmacognosy.[15,16] Sample specimen was authenticated by an expert of Pharmacognosy laboratory of IPGT and RA, Gujarat Ayurved University, Jamnagar, and herbarium of the sample has been deposited to Institute's pharmacognosy museum which consisted of *Euphorbia caducifolia* Haines (Phm/6213) and its latex that was collected by the scholar from its natural habitat of surrounding place of Jamnagar, during February 2016, early in the morning (7 am). The latex has been stored in a glass jar. *Chincha (T. indica)* leaves were collected from the campus of Gujarat Ayurved University, Jamnagar.

Procedure

Method of purification of *Rakta-Snuhi* latex was carried out in two groups. In one group drying latex was mixed with *T. indica* leaves juice, in a *Raudra Yantra* and was kept mixed in the sunlight in the ratio of 8:4, 8:2, and 8:1 in a glass bowl. In the other group each of these content were kept in shade (in the absence of direct sunlight). Each procedure was repeated three times to validate the pharmaceutical procedure.[12] Final samples were stored in a airtight glass container and labeled as samples 1 (8:4 shade processed sample), sample 2 (8:2 shade processed sample), sample 3 (8:1 shade processed sample), sample 4 (8:4 sunlight processed sample), sample 5 (8:2 sunlight processed sample) and sample 6 (8:1 sunlight processed sample) [Table 1].

Tamarind leaf juice was prepared by adding 20 ml of water to 100 g of, *T. indica* leaves and this mixture was titrated and processed in an electronic mixer. Later, tamarind leaves juice was extracted using a muslin cloth.

Organoleptic character and pH

pH and organoleptic characters of these samples were evaluated following standard procedures recommended by Ayurvedic Pharmacopoeia of India.[17]

High-performance thin-layer chromatographic study

Chemicals percolated silica gel 60 F254 thin-layer chromatography (TLC) aluminum plates (10 cm × 10 cm, 0.2 mm thick), analar reagent (AR) grade toluene, ethyl acetate, 5% alcoholic potassium hydroxide, chloroform and vanillin-H2SO4 reagent were obtained from M/S Merck Ltd. Mumbai, India. Lupeol (purity 95% by gas chromatography), the reference standard, was procured from Natural Remedies Pvt. Limited, Bangalore, India.[18]

Samples for high-performance thin-layer chromatography (HPTLC)

The extracts of all samples for HPTLC were made in the same process as mentioned below:

1. Methanolic extract – 2.5 g of sample was macerated with 50 ml of methanol for 24 h and filtered. The filtrate was concentrated to 30 mg and used for spotting
2. One ml of the sample of latex was mixed in an equal quantity of 5% alcoholic potassium hydroxide and subjected to heat and then an unsaponified layer was obtained by adding the mixture with 3 ml of chloroform. The filtrate was concentrated to 30 mg and used for spotting.

The samples were titled as Track-1 to 11 and the details are as below:
Track-1: 2.5 µg of standard lupeol
Track-2: 5 µg of standard lupeol (taken as reference standard)
Track-3: 7.5 µg of standard lupeol

Table 1: pH of different processed samples of *Rakta Snuhi* (in shade dried and sunlight dried)

Sample number	Ratio for *Shodhana* (*Snuhi Ksheera* and *Chincha Patra Swarasa*)	Parameter	0 h	12 h	24 h	36 h
	Shade dried					
1	8:4	pH	3.54	3.46	3.34	3.27
2	8:2	pH	3.75	3.58	3.49	3.42
3	8:1	pH	3.98	3.85	3.69	3.58
	Sunlight dried					
4	8:4	pH	3.54	-	-	-
5	8:2	pH	3.75	-	-	-
6	8:1	pH	3.98	-	-	-

Track-4: 10 µg of raw latex
Track-5: 10 µg of raw *Chincha* leaf juice
Track-6: 10 µg of 8:1 shade processed sample
Track-7: 10 µg of 8:2 shade processed sample
Track-8: 10 µg of 8:4 shade processed sample
Track-9: 10 µg of 8:1 sunlight processed sample
Track-10: 10 µg of 8:2 sunlight processed sample
Track-11: 10 µg of 8:4 sunlight processed sample

Mobile phase: toluene: ethyl acetate (14:6) v/v

Detection: spray with vanillin-H_2SO4.

Chromatographic conditions

A Camag Linomat V HPTLC system equipped with an automatic TLC sampler, TLC scanner III and integrated software Win CATS was used for the analysis. Precoated silica gel GF_{254} plates were used for the study. Development was carried out in Camag Twin Trough Chamber where chamber saturation was 30 min, development time was 30 min, and development distance was 10 cm.

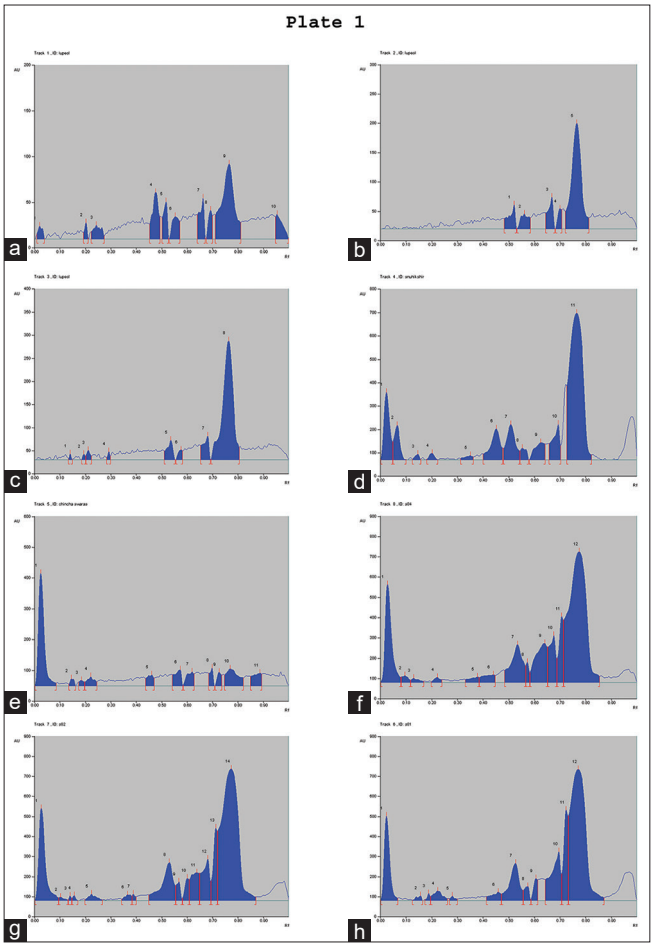

Figure 1: (a) Linearity (two-dimensional densitograms) graph of lupeol (2.5 µl). (b) Linearity (two-dimensional densitograms) graph of lupeol (5 µl). (c) Linearity (two-dimensional densitograms) graph of lupeol (7.5 µl). (d) Densitogram showing lupeol in latex of *E. caducifolia*. (e) Densitogram showing lupeol in leaf juice of *T. indica*. (f) Densitogram showing lupeol in 8:4 Latex of *E. caducifolia*: Leaf juice of *T. indica*, shade processed sample. (g) Densitogram showing lupeol in 8:2 latex of *E. caducifolia*: Leaf juice of *T. indica*, shade processed sample. (h) Densitogram showing lupeol in 8:1 latex of *E. caducifolia*: Leaf juice of *T. indica*, shade processed sample

Results

pH

pH of raw *Euphorbia caducifolia* was 4.35 and *T. indica* leaf juice was 2.70 and pH of various processed samples are presented in Table 1. It took 11 and 7 days for complete drying of samples under shade and in sunlight, respectively.

Odor

The odor of samples was noted at a regular interval of 12 h. Sunlight-dried processed samples became odorless after 12 h, while shade-dried processed samples became odorless after 48 h.

Color

The colors of all the processed samples were noted after 24 h. The color of all shade-dried samples changed from milky

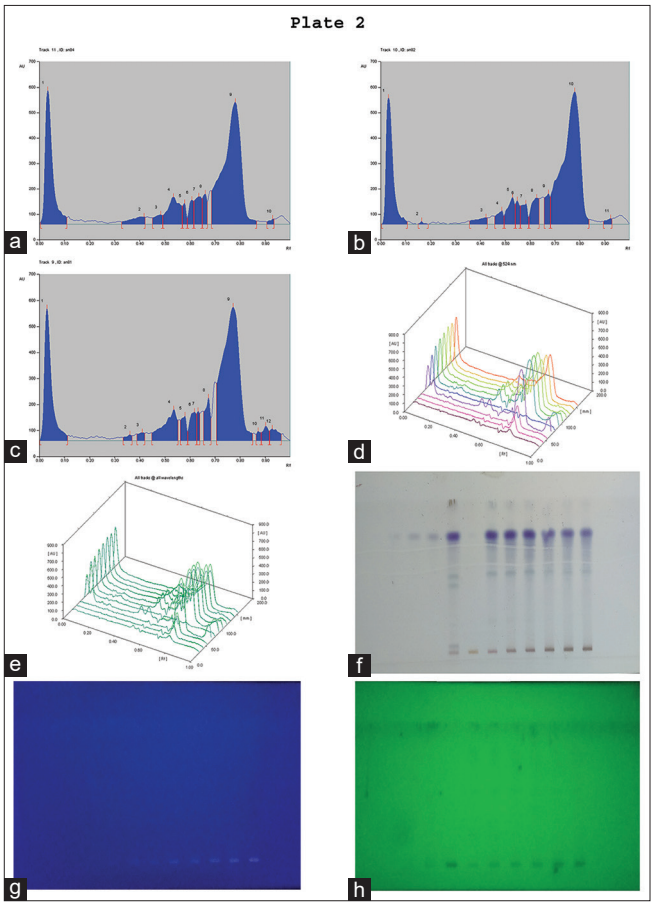

Figure 2: (a) Densitogram showing lupeol in 8:4 latex of *E. caducifolia*: Leaf juice of *T. indica*, sunlight processed sample. (b) Densitogram showing lupeol in 8:2 latex of *E. caducifolia*: Leaf juice of *T. indica*, sunlight processed sample. (c) Densitogram showing lupeol in 8:1 latex of *E. caducifolia*: Leaf juice of *T. indica*, sunlight processed sample. (d) Densitogram showing three-dimensional wavelength graph. (e) Densitogram showing multi-wavelength graph. (f) Thin-layer chromatography plate in vanillin-H2SO4. (g) Thin-layer chromatography plate at 254 nm. (h) Thin-layer chromatography plate at 366 nm

white to creamish white on the 3rd day and then changed to light brown after 7th day. While in sun light-dried samples, the milky white color changed to creamish white color on the 2nd day and then creamish light brown on the 4th day.

Estimation of lupeol

The lupeol quantities in different tracks were noted and details are presented in Table 2.

Discussion

Tamarind leaf juice is highly acidic (2.70) in nature. There was a significant reduction in pH as the amount of tamarind leaf juice proportion increased, i.e., 4.35–3.27 which differs in different processed samples due to differences in the ratio of *E. caducifolia* latex and *T. indica* leaf juice. Tamarind leaf juice neutralizes the poisonous effect of *E. caducifolia* latex by its chemical composition and strong acid nature.

Sun light processed samples became odorless in 12 h and dried in 7 days, while shade dried samples became odorless in 48 h and dried in 11 days. This may be due to the direct effect of heat.

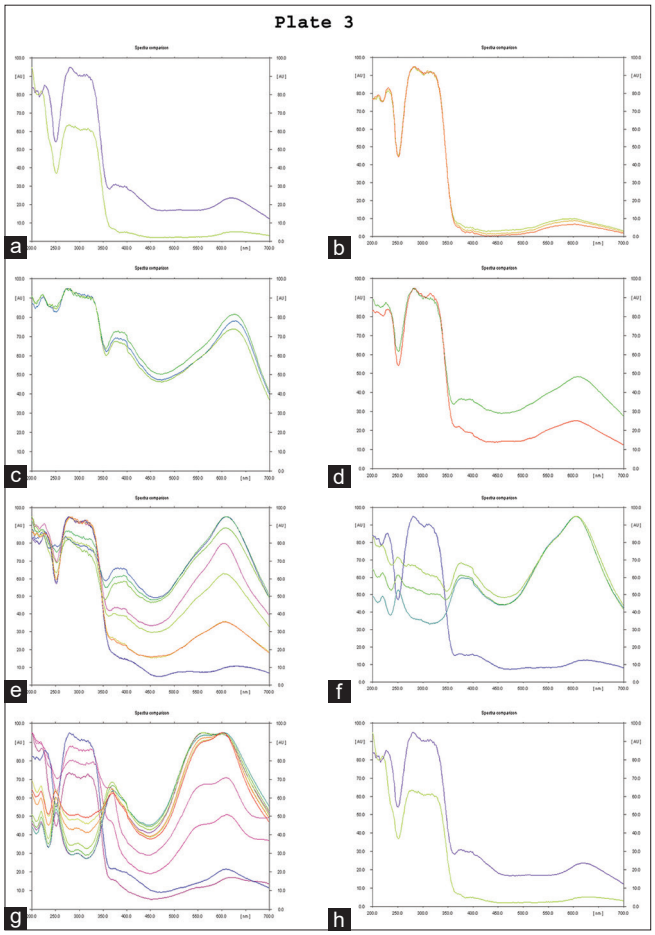

Figure 3: (a) Spectral comparison at 0.45 R_f. (b) Spectral comparison at 0.41 R_f. (c) Spectral comparison at 0.53 R_f. (d) Spectral comparison at 0.60 R_f. (e) Spectral comparison at 0.68 R_f. (f) Spectral comparison at 0.73 R_f. (g) Spectral comparison at 0.77 R_f. (h) Spectral comparison at 0.89 R_f

High-performance thin-layer chromatographic findings

The concentration of lupeol in *E. caducifolia* latex was found to be increased in shade-dried processed sample when the concentration of *T. indica* leaf juice was increased.

On the other hand, the concentration of lupeol gets decreased when the sample is exposed to sunlight. The concentration of lupeol in ample quantity in *T. indica* leaf juice provides potency to *E. caducifolia* latex. It has been reported that the concentration of lupeol depends on temperature and duration;[19] thus, lupeol was in higher concentration in shade sample than sun-dried sample.

R_f value

HPTLC study at 524 nm showed 11 spots in raw latex sample, 11 spots in tamarind leaf juice, 12 spots in 8:4 shade-dried sample, 14 spots in 8:2 shade-dried sample, 12 spots in 8:1 shade-dried sample, 10 spots in 8:4 sun-dried sample, 11 spots in 8:2 sun-dried sample and 12 number of spots in 8:1 sun-dried sample. 2D densitograms shows variation of lupeol during purification methods of *E. caducifolia* latex (Figure 1a-h, Figure 2a-h).

Details of common R_f value having similar spectra in different processed samples are given in Table 3.

After purification process, there is a change in concentration at similar R_f, but no new moiety is formed at a similar R_f.

Table 2: Lupeol quantity in different samples of processed latex of *Euphorbia caducifolia*

Samples	Maximum R_f	Area	Concentration (μg)
latex of *Euphorbia caducifolia* (EC)	0.76	26586.9	3.025
Leaf juice of *Tamarindus indica*	0.89	2199.3	0.250
E. caducifolia shade 8:4	0.77	34919.3	3.973
E. caducifolia shade 8:2	0.77	34623.8	3.939
E. caducifolia shade 8:1	0.77	31023.5	3.530
E. caducifolia sun 8:4	0.93	26865.7	3.057
E. caducifolia sun 8:2	0.92	28623.1	3.257
E. caducifolia sun 8:1	0.93	28134.6	3.201

E. caducifolia: *Euphorbia caducifolia*

Table 3: Common R_f values in different processed samples of *Rakta Snuhi Ksheera*

R_f	Processed sample
0.45	Latex of *E. caducifolia* sample and 8:4 shade dry sample
0.41	8:4, 8:2, and 8:1 sun-dried sample
0.53	8:4, 8:2, and 8:1 sun-dried sample
0.60	8:2 shade-dried sample and 8:4 sun-dried sample
0.68	Latex of *E. caducifolia* sample; Leaf juice of *T. indica*; 8:4, 8:2, and 8:1 shade-dried sample; 8:4, 8:2, and 8:1 sun-dried sample
0.73	Leaf juice of *T. indica*; 8:4, 8:2, and 8:1 shade-dried sample
0.77	Latex of *E. caducifolia* sample; Leaf juice of *T. indica*; 8:4, 8:2, and 8:1 shade-dried sample; 8:4, 8:2, and 8:1 sun-dried sample
0.89	Leaf juice of *T. indica* and 8:1 sun-dried sample

At 0.77 R_f, similar spectra were observed both in lupeol and Tamarind leaf juice, whereas dissimilar spectra were observed in *E. caducifolia* latex and in all processed samples at 0.77 R_f. Spectral comparison graph of different samples during purification methods of *E. caducifolia* (Figure 3a-h).

Conclusion

Shodhana (purification process) of *Euphorbia caducifolia* latex with tamarind leaf juice changes both its qualitative and quantitative properties of *Rakta Snuhi* latex. *Shodhana* alters the pH, color, and odor of *Euphorbia caducifolia* latex. It also alters the number of spots both in sun and shade-dried samples. The study reveals that in case of shade-dried processed latex sample, the concentration of lupeol increases with the increasing quantity of *Tamarind indica* leaves juice.

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Conflicts of interest

There are no conflicts of interest.

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