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Original Research Article

Comparative pharmaceutico-analytical study of Elva prepared with two different methods

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ABSTRACT

Background: Aloe vera has a long antiquity as a medicinal herb with varied therapeutic applications. It has been used as remedies in different form as gel, Elva, juice etc. Among the different forms, Elva is found to be as a constituent in several Ayurvedic formulations but still its standard operating procedure is not mentioned yet. Aim was to develops a standard operating procedure for Elva by preparing it with two different methods.
Methods: The study was carried out by preparing Elva with two different methods, first by water extract (Elva sample I) of aloe vera leaves and secondly by pulp (Elva sample II) of the same.
Results: Elva sample I and II have 4.65% and 2.2% yield respectively. Also, by HPTLC identification, Elva sample I found to be more genuine by taking British Pharmacopoeia as reference standard.
Conclusions: Elva sample I have comparatively better yield and found to be more acceptable by HPTLC.

Keywords: Aloe vera, Elva, Physicochemical, HPTLC

INTRODUCTION

The herb aloe vera has an antiquity dating back to time which, belongs to the family *Liliaceae* is a cactus like perennial plant.[1] This species is inherent to Mediterranean region but is now found in southern parts of North America, Europe and Asia.[2] Aloe vera leaves are formed by a thick epidermis shielded with cuticle surrounding the mesophyll. The parenchyma cells comprises a transparent mucilaginous jelly which is stated as aloe vera gel.[3] The genus Aloe encompass over 400 different species with *Aloe barbadensis Miller*, is considered to be the most biologically active.[4-7] The aloe vera plant has been recognized and used for times for its health, beauty, medicinal and skin care properties.[8] The plant is enrich with many natural health promoting elements. The raw pulp of aloe vera holds approximately 98.5% water, while the mucilage or gel consists of about 99.5% water.[9] The remaining 0.5-1% solid material entails of a range of compounds together with water-soluble and fat-soluble vitamins, minerals, enzymes, mono and polysaccharides, sugar, lignin, phenolic compounds and organic acids.[10-12] Active components in leaf pulp of aloe vera are Vitamins, enzymes, anthraquinones, inorganic compounds, carbohydrates, saccharides, organic compounds, non-essential amino acids.[13] In Ayurvedic Phramacopoeia, *Elva* is defined as dried out juice of leaves of *Aloe barbadensis Mill*.[14] It is also known as *Kumarisaar, Krishnabola, Saartoudbhava and Mussabar*.[15-16] The properties and action of Elva (Table 1).[14,15] Among WHO monographs, Solidified juice in the cells of the pericycle and adjacent leaf parenchyma, made to dry with or without the aid of heat is known as Aloe.[17] It is desc ribed as by the name of *Kanyasaar* in API. In British Pharmacopoeia, three varieties of Aloe namely Curaçao Aloes, Capes Aloes and Standardised Aloes dry extract are described.[18] Beside this, in Dravyagun Vijyanium, 3 varieties of Aloe viz. Curaccao Aloes, Scotorine Aloes and Capes Aloes.[19]

The active principles exist in Aloes are anthraquinones, carbohydrates, saponins, amino acids and lignin.[20]

As aloe is described in various classical text and pharmacopoeias but still a standard operating procedure for Elva preparation yet not to be described in any text. So, in the present study an attempt has been made to set a standard procedure for Elva preparation.

METHODS

In this work a comparative pharmaceutico-analytical analysis has done between the two methods of Elva preparation. The present study was done at Pharmacy of Hans Herbal Pvt Ltd. from 1st April 2015 to 6 April 2015. Elva was prepared by two different methods.

Pharmaceutical study

1st method

Collection of plant materials

Leaves of aloe vera for the present study were obtained from the garden of Prem Nagar Ashram.

Aloe vera authentication had done by Dravyagun Department of Rishikul Campus, Uttarakhand Ayurved University, Haridwar. Method of preparation for *Elva* has been adopted as per reference of *Dravyagun Vijyanium*.[19]

Preparation of Elva

Fresh leaves of aloe vera (7.2 kg) were thoroughly washed with water and cut into small pieces. The pieces were taken in a big steel vessel with sufficient quantity of water and boiled for 2 hrs. After cooling pieces were rubbed to separate pulp from the skin. Extract was then filtered with Markin cloth. The extract was heated on 80-90^0 C till it got boil and after that temp. maintained between 40-50 ^{0} C till it becomes semisolid. The extract was put on tray and allows drying in sunlight. Extract turns Coffey brown in color after drying and collected in air tight jar. Details of vessels, time taken, yield and loss percentage (Table 2).

2nd method

Fresh leaves of aloe vera were collected from Prem Nagar Ashram. The leaves of aloe vera were cleaned and washed with water. The pulp from aloe vera leaves were extracted with the use of knife. The pulp was heated on 50^0 C till it becomes semisolid. The extract was spread on tray and allow to drying in sunlight. After drying it was collected in air tight jar. Details of vessels, time taken, yield and loss percentage are shown in Table 3.

Analytical study

Analytical study was conducted at International Testing Centre, Panchkula, Haryana.

Both the samples of Elva were analyzed on various parameters viz. organoleptic, physico-chemical and HPTLC identification.

Organoleptic parameters

Organoleptic parameters viz. consistency, color, taste, smell and touch of both samples of Elva were shown in Table 1 and 4.

Physico-chemical parameters

pH

pH meter was stabilized for 15-30 min. Now the electrode has been immersed in standard buffer solution of pH 4.0 and stabilized for 1 min. and reading was adjusted at pH 4.0. The electrode was the rinse and immerse in the sample. The reading displayed on the monitor was noted.[21]

Foreign matter

The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below.[2] Representative of sample was taken and spread in a thin layer in a suitable dish or tray Examine in daylight with unaided eye and suspected particles were then transfer to a Petridis, and examine with 10× lens in daylight.

Total ash

The 2-3 gm of accurately weighed ground drug was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450^0 C until free from carbon. It was then cooled and weighed. By adding the filtrate, it was evaporated to dryness, and ignited at a temperature not exceeding 450^0 C. The value of total ash is determined by calculating the percentage of ash with reference to the air-dried drug.[23]

Acid insoluble ash

To the crucible containing total ash, 25ml of dil. HCl was added. The insoluble matter on an ashless filter paper was collected and washes with hot water. Filter paper containing the insoluble matter transferred to the original crucible dry on a hot plate and ignites to constant weight. The residue was allowed to cool in suitable desiccators for 30 minutes and weigh without delay. The content of acid insoluble ash was calculated with reference to the air-dried drug.[24]

Alcohol soluble extractive

Five gm of the air-dried drug coarsely powdered was macerate with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours.

After that it was filtered rapidly, taking precautions against loss of solvent. Now 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and allow drying at 105^0 C to constant weight and weigh. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.[25]

Water soluble extractive

Five gm of the air dried coarsely powdered air-dried drug was macerate with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. After that it was filtered, taking precautions against loss of water. Then 25 ml of filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dry at 105^0 C, to constant weight and weigh. Percentage of water-soluble extractive was calculated with reference to air-dried drug.[26]

Loss on drying

The moisture content is estimated by determining the amount of volatile matter (water drying off from the drug). 10 gm of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 gm) was placed in a tarred evaporating dish and dry at 105^0 C for 5 hours and weigh. The drying was continued and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 percent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in desiccator, show not >0.01 gm difference.[27]

High performance thin-layer chromatography (HPTLC)

Test solution-0.25 g of the powdered drug adds 20 mL of methanol R and heat to boiling in a water-bath. Shake for a few minutes and decant the solution. Store at about 4 °C and use within 24 h.[28] Plate used was TLC silica gel G plate R. Mobile phase used water R, methanol R, ethyl acetate R (13:17:100 V/V/V). Application-10 µL, as bands of 20 mm by maximum 3 mm. Development-Over a path of 10 cm. Drying in air. Detection A was spray with a 100 g/L solution of potassium hydroxide R in methanol R and examine in ultraviolet light at 365 nm.

RESULTS

Analysis of the values of pharmaceutical study revealed that Elva prepared by the water extract of the aloe vera leaves (1^{st} method) have comparatively better yield (4.65%) than the Elva prepared with pulp (2.2%) by 2^{nd} method of the same as shown in Table 2 and 3. Organoleptic characters of Elva prepare by 1^{st} method have coffee brown color with bitter in taste and was solid in consistency whereas Elva prepared by 2^{nd} method was brown in color, astringent in taste and have consistency like flakes as shown in the Table 4. Physico-chemical analysis shows acidic nature of Elva in both samples. HPTLC of both samples of Elva was carried out and of sample I of Elva show 7 peaks at Rf=0.17, 0.41, 0.45, 0.52, 0.57, 0.70, 0.78 and 2 peaks with Rf 0.78 and 0.89 of sample II respectively. Rf=0.78 was found in both samples. Details of HPTLC are presented in the Table 6 and 7 respectively.

Table 1: Rasa panchaka of Elva (Kumarisaar).

Properties	A.P.I.	Dhanvantari Nighantu
Rasa	Katu (pungent)	Tikta (bitter)
Guna	Ushna (hot)	Ushna (hot) Tikshna (sharp)
Viraya	Ushna (hot)	Ushna (hot)
Vipaka	Katu (pungent)	-
Karma	Bhedi (purgative), Pittnirharana (cholagogue purgative), Rajapravartaka (emmenagogue), Jwaranut (anti-pyretic)	Bhedana (purgative), Dipana (appetizer), Aartavajana (emmenagogue), Krimighna (anti-microbial)

Table 2: Details of preparation of Elva.

Parameters	Values
Weight of aloe vera leaves	7.2 kg
Weight of empty steel vessel	2 kg
Circumference of steel vessel	31 cm
Weight of steel vessel with aloe vera extract	6.30 kg
Weight of Elva	200 gm
% loss in whole procedure	95.3
Total time taken	11 hrs
Residue weight	3 kg
% yield	4.65%

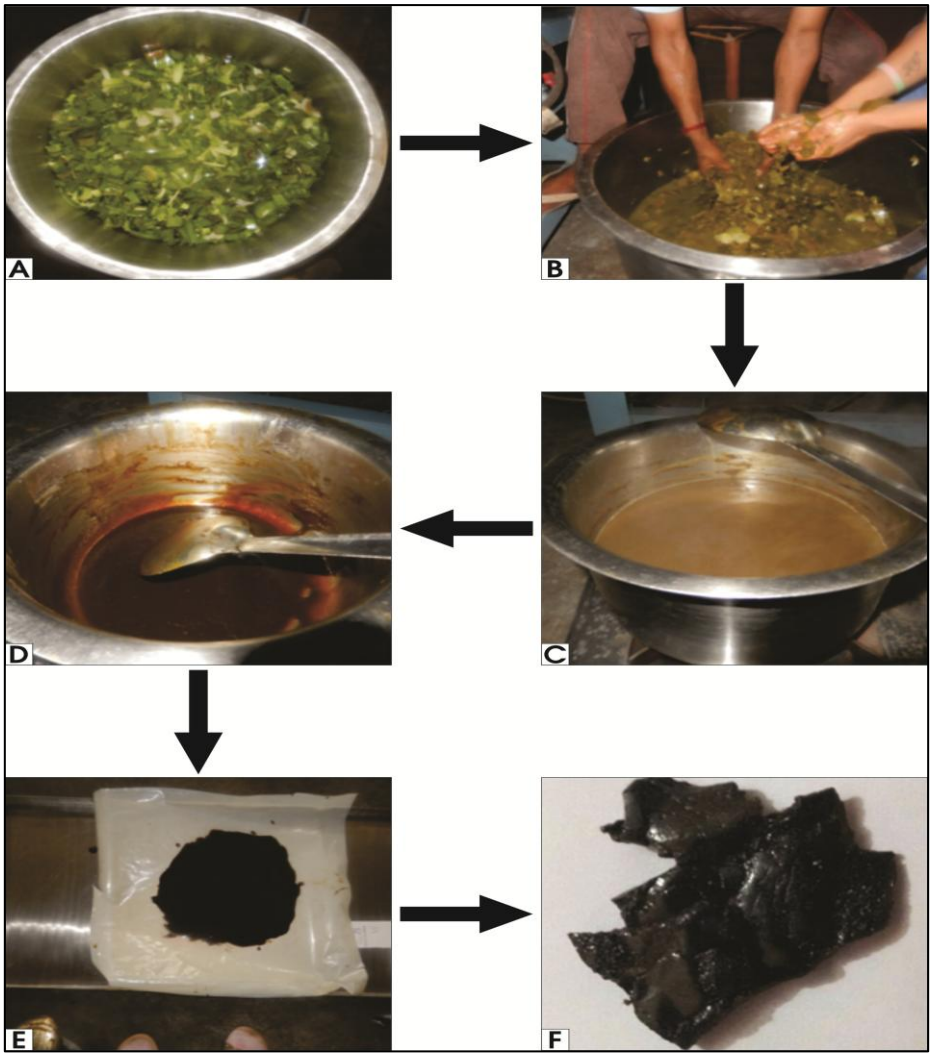

Figure 1 (A-F): First method of Elva preparation.

Table 3: Details of Elva preparation.

Parameters	Values
Weight of aloe vera leaves	7. 5 kg
Weight of empty steel vessel	2 kg
Circumference of steel vessel	31cm
Weight of steel vessel with pulp	5.6 kg
Weight of Elva	80 gm
% loss in weight	97.7
Residue weight	3.5 kg
Total time taken	2½ hrs
% yield	2.2%

Table 4: Organoleptic parameters.

Parameters	Sample I	Sample II
Consistency	Solid	Flakes
Color	Coffey brown	Brown
Taste	bitter	Astringent
Smell	Not clearly distinguished	Not clearly distinguished
Touch	Smooth	Smooth

Table 5: Physico-chemical parameters of Elva samples (as per A.P.I.).

Sample, parameters	Sample I	Sample II
pH	4.07	4.00
Foreign matter (%w/w)	0.01	0.03
Total ash (%w/w)	15.77	14.87
Acid insoluble ash (%w/w)	5.79	5.27
Alcohol soluble extractive (%w/w)	32.83	48.34
Water soluble extractive (%w/w)	76.38	68.98
Loss on drying (%w/w)	13.35	17.29

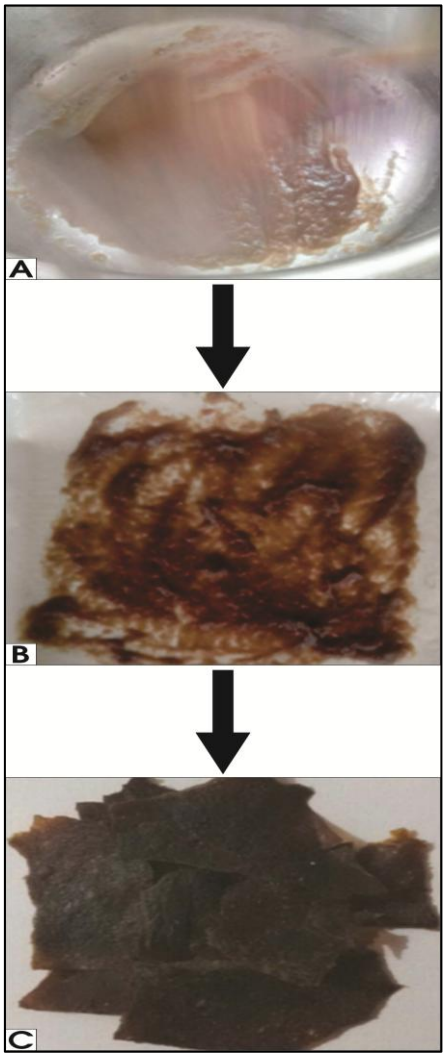

Figure 2 (A-C): Second method of Elva preparation.

Table 6: HPTLC scanning of Elva I sample at 254 nm.

Lane: 1	Type: Sample 1	Name: Elva 1		X-Position: 15.0 mm			
Peak	**Component name**	**y-Pos {mm}**	**Area**	**Area {%}**	**Height**	**Type**	**Rf**
1	:	27.0	398.95	2.7	334.97	f	0.17
2	:	43.7	915.81	6.3	515.50	f	0.41
3	:	46.3	308.62	2.1	210.81	f	0.45
4	:	51.7	1062.86	7.3	470.41	f	0.52
5	:	54.8	566.91	3.9	204.26	f	0.57
6	:	63.8	741.06	5.1	211.11	f	0.70
7	:	69.8	10513.90	72.5	1741.21	f	0.78

Table 7: HPTLC scanning of Elva ii sample at 254 nm.

Lane: 2	Type: Standard	Name: Elva 2	X-Position: 35.0 mm				
Peak	**Component name**	**y-Pos {mm}**	**Area**	**Area {%}**	**Height**	**Type**	**Rf**
1	:	69.4	1855.52	47.5	571.49	F	0.78
2	:	77.6	2049.96	52.5	315.31	F	0.89

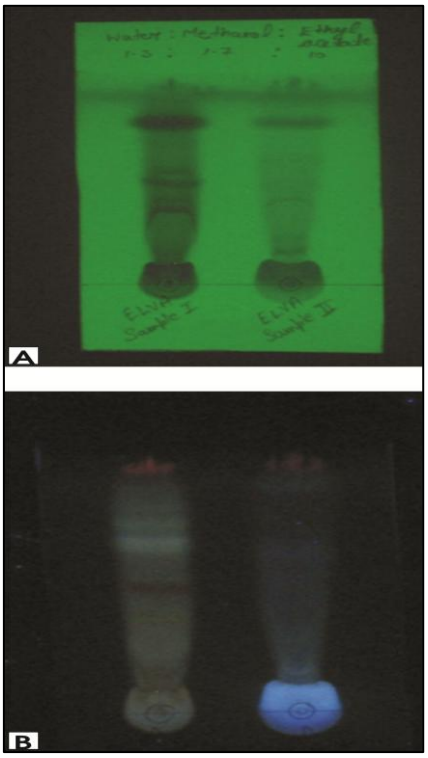

Figure 3 (A and B): HPTLC plates of Elva samples.

DISCUSSION

As per WHO monographs, Aloe is a solidified juice of Aloe vera leaves allowed to dry with or ` Aloe or Elva.[17] In the present study, Elva was prepared by two different methods firstly by ghanpaka (solidified extract) of water extract of Aloe vera leaves and secondly by ghanpaka (solidified extract) of pulp extracted from leaves of Aloe vera.[19] The temperature was maintained between 40^0C-60^0C to prevent the loss of active constituents. In terms of yield, first method comparatively has good yield (4.65%). Also, the color by first method was dark Coffey brown with bitter taste having solid consistency whereas by second have brown color with astringent taste and consistency like flakes.

A.P.I. has been taken as reference standard for all physico-chemical test of Elva.[14] Data concerning to table no. 5 shows total ash values of sample I and II of Elva were 15.77% and 14.87% respectively that are more than permissible limit. But these values could be justified with a study done, who found 12.2% of inorganic content in Aloe vera that was remains preserved even after incineration results in high ash values.[29] Along with this, in a study done by Haque et al the total ash of aloe vera leaves powder after drying found to be 19.50% that also represent the inorganic content in Aloe vera remains conserved even after drying.[8] The values of alcohol soluble extractive in an order for sample I and II of Elva came out to be 32.83% and 48.34% that was less than acceptable limit and comparatively less for sample I that may be because of degradation of constituents during the preparation of Elva as heating was applied for greater time in sample I. Beside this, values of water soluble extractive came out to be 76.38% and 68.98% which are acceptable as per A.P.I. Slightly higher quantity of extract in sample I may be because water extract of leaves of Aloe vera were used to prepare it. The values of loss on drying for sample I and II came out to be 13.35% and 17.29% respectively that are more than acceptable limit. The high values of loss on drying for both samples of Elva may possibly because of its hygroscopic nature. Standard for identification of Elva by TLC was not mentioned in A.P.I. So, reference standard had been taken of Barbados aloes from British Pharmacopoeia Vol. IV (B.P.).[19] After going through the description given in monographs for Barbados Aloes of B.P. details regarding TLC of Elva using mobile phase: water: methanol: ethyl acetate (13:17:100) was found. In the present work HPTLC was performed using the mobile phase water: methanol: ethyl acetate. HPTLC of sample I and II of Elva show 7 peaks at Rf 0.17, 0.41, 0.45, 0.52,

0.57, 0.70, 0.78 and 2 peaks with Rf 0.78 and 0.89 respectively. Rf value 0.78 was found in both samples. The maximum percentage area (72.5%) appears at Rf 0.78 and second at Rf 0.52 (7.3%) whereas in sample II maximum area was found at Rf 0.89 (52.5%) and then at Rf 0.78 (47.5%).After spraying reagent 100 gm/L solution of methanolic KO_4 and examine in UV light 365 nm the chromatogram obtained of sample I shows a yellow fluorescent zone (Rf-0.78) in central part and light blue fluorescent zone (Rf-0.52) in lower part that as per standard were because of barbaloin and aloesine respectively. After heating the plate at 110°C for 5 min, a violet fluorescent zone appears just below the yellow zone similar to reference standard where as in sample II only a blue fluorescent zone (Rf-0.89) was appeared in lower part after spraying the reagent. With these results of HPTLC that sample I was found to be more genuine as per reference standard.

CONCLUSION

With the study done, Elva prepared by water extract have comparatively better yield. Total ash content in both samples of Elva is high because of mineral content present in it. With the HPTLC of Elva samples, the sample prepared by water extract is found to be more genuine as per standard of British Pharmacopoeia.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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