Physico-chemical evaluation of bitter and non-bitter *Aloe* and their raw juice for human consumption

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Abstract In addition to *Aloe vera* which is bitter in taste, a non-bitter *Aloe* is also found in arid part of Rajasthan. This non-bitter *Aloe* (NBA) is sporadically cultivated as vegetable and for health drink. In spite of its cultivation and various uses, very little information is available about its detailed botanical parameters and chemical characters. This study aims to evaluate the physico-chemical characters of NBA through employing floral morphology, leaf characters and leaf gel and to compare them with those of *A. vera*. Of eleven floral characters studied, eight characters of NBA were significantly different from that of *A. vera*. Most visible difference was observed in their reproductive shoots which are highly branched in NBA (5.21 inflorescence/shoot) as compared to *A. vera* (1.5 inflorescence/shoot). NBA produces less leaf-biomass (−29.32 %) with less leaf-thickness (−31.44 %) but higher leaf length, width, and no. of spine/side by 17.56 %, 21.34 % and 16.11 %, respectively, with significant difference as compared to *A. vera*. But its polysaccharide content (0.259 %) is at par with that of *A. vera*. The raw juice from the leaf of NBA has very low aloin content (4.1 ppm) compared to that from *A. vera* (427.3 ppm) making it a safer health drink compared to the one obtained from *A. vera*. Thus, NBA raw juice emerged as suitable alternative to *A. vera* juice for human consumption.

Keywords Non-bitter *Aloe* · *Aloe vera* · Leaf morphology · Aloin · Floral characters

Introduction

The genus *Aloe* (Liliaceae) is a tropical plant characterized by lance-shaped leaves with jagged edges and sharp points. Of the over 360 *Aloe* species, only few are used for various medical and cosmetic purposes. Many biological activities like antiviral antibacterial, immunostimulative activity, reducing radiation induced skin reaction, accelerated wound healing, antioxidant activity (Grindlay and Reynolds 1986; Rodriguez et al. 2010) have been found in different fractions of *Aloe* species. Some *Aloe* species including *Aloe vera*, also find application in the food industry as a new or functional food (Eshun and He 2004; Rodriguez et al. 2010).

Apart from *Aloe vera* another type of *Aloe*, which apparently looks similar to *A. vera* but non-bitter in taste, is found in India. It is known as *Meetha Guarpatha* (Non-bitter *Aloe*). In some parts of the country this non-bitter *Aloe* (NBA) is cultivated as vegetable and for health drink. On the other hand, the consumption of widely studied *A. vera* is restricted to medicinal uses. In spite of its cultivation and various uses, very little information is available about physico-chemical characters of NBA vis-à-vis *A. vera*. Moreover, previous reports on Indian *Aloes* (Mukerjee and Karkun 1950; Srivastava et al. 1990; Pareek et al. 1999) do not mention existence of NBA. In our preliminary note (Azam et al. 2009), we had reported very few parameters of NBA.

Hence, this study aims to evaluate physico-chemical characters of NBA and to compare them with those of *A. vera*. Suitability of raw juice from both *A. vera* and NBA is also assessed for human consumption.

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Materials and methods

Plant materials

Aloe root suckers (4–6 leaf stage) collected from different locations were established and being maintained in Institute Botanical Garden at Jodhpur, India. After being grown under uniform conditions (regular weeding and irrigation once a month) in sandy loam soil for 3 years, eight landraces each of NBA and *A. vera*, were taken for study in the year 2010. Three plants/landrace were taken and three mature leaves/plant (leaf No.10, 11, and 12 from the top) were studied. Biomass, length, width and thickness of these leaves were recorded (Table 1).

Reagents

Acetonitrile, methanol and water were HPLC grade and supplied by Merck (Darmstadt, Germany). D-mannose and phenol were from Fluka, Buchs, Switzerland. Sulphuric acid was reagent grade supplied by S.D. Fine Chem. limited (India). Aloin A & B, was isolated and identified by reported methods (Haynes et al. 1970; Okamura et al. 1997; Hay and Haynes 1956). Aloe-emodin was prepared by the method of Fairbairn and Simic (1963).

HPLC analysis

Sample preparation

Stock solutions of standard compounds were prepared by dissolving 10 mg of each separately, in 25 ml of HPLC grade methanol. From the stock solution, working solutions of graded concentrations were prepared. For preparation of raw juice, the leaf, after removal of exudates, was washed (with tap water), the green rind was removed, the pulp was homogenized and juice was collected after filtration. For measuring aloin content in raw juice, appropriate quantity (100 ml) of the raw juice so obtained was extracted with ethyl acetate (3×100 ml). Solvent was removed under reduced pressure and the residue was quantitatively dissolved in 100 ml methanol. After microfiltration, the solution was subjected to HPLC analysis.

Instrumentation and chromatographic conditions

The chromatographic system consisted of two pumps in isocratic mode (Shimadzu, LC-10AD model), a Rheodine 6161 injector with 20 µl loop, and a variable wavelength UV–VIS detector (SPD –10A). Separations were obtained on a Shim pack CLC- ODS stainless steel column (150×4.6 ID with 5 µm particle size) kept in a HPLC column oven. The mobile phase was composed of a water/acetonitrile (80/

20) mixture. The flow rate was 1.5 ml/min. The detector wave length was set at 354 nm with sensitivity, 0.05 AUFS and column temperature, 45 °C.

Calibration curve

Analyte standard solutions (prepared daily) at seven different concentrations, were injected in triplet into the HPLC system. The analyte peak areas obtained were plotted against the corresponding concentrations of the analyte (expressed as mg/ml) and the calibration curves were set up by means of the least-square method.

Test sample analysis

Five microlitres of each standard and test samples after filtration through 0.45 µm membrane filters, were accurately injected in triplicate into the HPLC column. Identification of aloin A & B was carried out by comparing their retention times with those of authentic standards. Quantitative determination was carried using calibration curves of the standards and expressed as parts per million (ppm).

Aloe polysaccharide estimation

Sample preparation

After harvesting, leaves were washed with distilled water to remove dirt. The spines, placed along their margins, were removed. The epidermis was carefully separated from the parenchyma using knife. The fillets were extensively washed with distilled water to remove exudates from the surface. The fillets were crushed and juice was separated by filtration. Test samples were prepared by dissolving 1 ml of the freshly prepared juice in 250 ml of 1.75 % sodium chloride solution.

Sample analysis

Aloe polysaccharide (APS) in test samples in triplicate was estimated by Leyes Method (Leyes et al. 2000). D- mannose was used as standard. A test sample of 400 µl was mixed with 200 µl of 6 % phenol solution and 1 ml concentrated sulfuric acid. The mixture was reacted for 20 min at room temperature. The absorbance of the reaction mixture was measured at 490 nm.

Floral characters

Initiation of flowering in NBA starts in the last week of July. Its buds and flowers both have mauve color (Sr. No. 45) with greenish top. In *A. vera* flowering takes place in the

Table 1 Physicochemical characteristics of fresh leaf of non- bitter *Aloe* and *Aloe vera*

Origin of sample (sample number)	Fresh leaf weight (g/leaf)	Leaf length (cm)	Leaf width (cm)	Leaf thickness (mm)	No. of spines (each side)	Gel content (g/leaf)	GC/FLW ^a	Polysaccharide content (%)	Aloin content in raw juice (ppm)
<i>Non-bitter Aloe</i>									
Shiv Bari (1)	314.8	69.6	10.2	13.4	67.0	95.4	0.322	0.239	4.0
Sagar (2)	323.5	74.7	13.3	13.4	75.6	98.7	0.324	0.299	4.2
Udasar (3)	329.0	72.6	11.8	14.6	70.8	98.5	0.295	0.263	4.4
Laxamangarh (4)	348.9	75.3	10.4	14.6	74.8	112.6	0.319	0.197	4.3
Jaisalmer (5)	348.3	74.3	12.8	14.6	75.5	119.5	0.322	0.287	4.1
Barmer (6)	320.1	71.3	11.0	14.4	74.1	101.1	0.315	0.237	3.7
Jodhpur (7)	326.5	72.3	10.2	14.2	71.2	97.9	0.312	0.267	3.9
Gangasahar (8)	342.6	71.4	11.8	13.9	70.7	102.1	0.306	0.283	4.1
Grand Mean	331.7	72.7	11.4	14.1	72.4	103.2	0.320	0.259	4.1
SEm±	7.1	1.2	0.4	0.3	1.8	5.0	—	0.017	14.8
CD ($p=0.05$)	20.7	3.6	1.3	NS ^c	5.0	14.4	—	0.049	NS ^c
<i>Aloe vera</i>									
Bhinasar (9)	438.6	56.8	7.2	19.8	61.2	161.2	0.367	0.255	393.5
Churu (10)	428.7	60.5	9.9	18.6	61.8	155.4	0.362	0.300	455.6
Sagar (11)	409.8	62.4	9.4	19.5	68.5	148.8	0.363	0.221	468.3
Badusar (12)	437.3	62.9	9.9	18.3	59.0	163.7	0.363	0.248	449.1
Laxamangarh (13)	412.0	60.0	9.9	18.0	56.7	147.8	0.374	0.311	417.8
Jaisalmer (14)	427.8	61.4	9.7	17.6	57.4	164.4	0.372	0.276	437.3
Jodhpur (15)	453.3	54.8	7.9	18.8	61.1	171.4	0.369	0.251	377.3
Gangasahar (16)	424.6	60.5	8.0	17.9	60.6	154.9	0.364	0.216	420.0
Grand Mean	429.0	59.9	9.0	18.6	60.8	158.5	0.367	0.260	427.3
SEm±	7.1	1.2	0.4	0.3	1.8	5.0	—	0.017	14.8
CD ($p=0.05$)	20.7	3.6	1.3	1.1	5.3	14.4	—	0.049	42.9
SEm ± between non-bitter <i>Aloe</i> and <i>A. vera</i>	3.5	0.6	0.2	0.2	0.9	2.5	—	0.008	7.4
CD ($p=0.05$) between non-bitter <i>Aloe</i> and <i>A. vera</i>	10.3 ^b	1.8 ^b	0.6 ^b	0.5 ^b	2.6 ^b	7.2 ^b	—	NS ^c	21.4 ^b

^a Ratio of gel content (GC) and fresh leaf weight (FLW)^b significant at $p=0.01$ level also^c NS- Non significant

first week of November. Its buds and flowers look pink (Sr. No.30) with greenish top, and coral pink with yellowish top, respectively (Graf 1973). Investigated floral characters of NBA and *A. vera* were, number of inflorescence per reproductive shoot, number of flowers per inflorescence, pedicel length (cm), bract length(cm), tapel length(cm), filament length(cm), anther length(mm), anther width (mm), style length(cm), ovary length(mm), and ovary diameter(mm).

Statistical analysis

From each land race data were collected in triplicate and analyzed using CRD, for comparison of two groups of *Aloe*

(NBA & *A. vera*). It compared *Aloe* land races within the group and between these two groups.

Results and discussion

Leaf morphology

Leaf morphological characters were recorded in Table 1. The fresh leaf biomass among the land races of NBA significantly ($p=0.05$) varied from 314.83 to 348.97 g/leaf (mean, 331.76 g/leaf). Other parameters except leaf thickness also varied significantly ($p=0.05$). The range of variation of leaf length, leaf width, no. of spines/side were 69.60

to 75.38 cm (mean, 72.72 cm), 10.23 to 13.36 cm (mean, 11.48 cm), and 67.07 to 75.60 (mean, 72.49), respectively. The mean leaf thickness of NBA was 14.15 mm.

Similarly, among the land races of *Aloe vera*, fresh leaf biomass, leaf length, leaf width, leaf thickness, no. of spine/side varied significantly at $p=0.05$ which were 409.80 to 453.33 g (mean 429.05 g), 56.80 to 62.93 cm (mean 59.95 cm), 7.26 to 9.96 cm (9.03 cm), 17.67 to 19.83 mm (mean 18.60 mm), and 56.70 to 68.53 (mean 60.81), respectively. Similar variation in leaf characters of *A. vera* has also been reported by previous workers (Srivastava et al. 1990).

Statistical analysis of the data showed that all leaf characters of NBA were significantly different from that of *A. vera* at $p=0.01$. The leaf of NBA is longer (17.56 %) and wider (21.34 %) at base. On the other hand, *A. vera* leaf has higher mean biomass/leaf and more thickness. When percent increase in the values of these leaf characters of NBA over those of *A. vera* was calculated, leaf thickness was most prominent (−31.44 %), followed by leaf biomass (−29.32 %), leaf width (21.34 %), leaf length (17.56 %), and no. of spines/side (16.11 %).

Floral characters

The results of the investigation done on 11 floral characters in NBA and *A. vera* (Table 2) showed that there were highly significant difference (at $p=0.01$) between the two aloes for the characters- number of inflorescence per reproductive shoot, number of flowers per inflorescence, pedicel length, tapel length, anther length, anther width, ovary length and ovary diameter. Bract length and style length of NBA were also significantly different from that of *A. vera* at $p=0.05$. Also, Multi- branched (Mean, 5.21 inflorescence/shoot) NBA looks visibly different from *A. vera* (Mean, 1.50 inflorescence/shoot).

Floral characters, according to Heywood and Moore (1984) are known to provide the most valuable indicators of taxonomic affinity. Akinyele (2006), on the basis of difference in floral characters like ovary length, pedicel length, bract length, tapel length, and anther length, distinguished four *Aloe* species of Nigerian origin from one another. Thus floral characters of NBA are pointing towards its distinct separation from *A. vera*.

Our previous work on RAPD analysis of the landraces of NBA and *A. vera*, had also differentiated NBA from *A. vera* (Azam et al. 2009) by showing two distinct clusters.

Leaf gel and chemical characterization

The gel content which is dependent on fresh leaf biomass varied significantly ($p=0.05$) from 95.40 to 119.53 g/leaf (mean, 103.25 g/leaf) among NBA whereas in *A. vera*, it ranged from 147.80 to 171.47 g/leaf (mean, 158.50 g/leaf)

(Table 1). Also, leaf gel content of NBA was significantly different from that of *A. vera* at $p=0.01$. The ratio of 'gel content' to 'leaf biomass' of NBA (0.320) and *A. vera* (0.367) were also different. When quantified, NBA was found to produce 53.51 % less gel than *A. vera*.

Polysaccharide content

Polysaccharide content in the gel of the land races of NBA and *A. vera* varied significantly (at $p=0.05$) from 0.197 to 0.299 % (mean, 0.259 %) and 0.216 to 0.311 % (mean, 0.260 %), respectively. But, statistically, polysaccharide content of NBA is at par with that of *A. vera* (Table 1).

Aloin content

Aloin content in the raw juice of NBA and *A. vera* varied from 3.76 to 4.43 ppm (mean, 4.12 ppm) and from 377.3 to 468.3 ppm (mean, 427.36 ppm), respectively (Table 1). Aloe juice for human consumption, should not contain aloin, a suspected DNA damaging and carcinogenic agent (Lachenmeier et al. 2005), beyond the maximum permitted limit of 0.1 ppm set by European Council (Anon 1988) and 10 ppm as per International Aloe Science Council (Anon 2009). Aloin content in raw juice of NBA is within the safe limit set by IASC. Hence, NBA raw juice may be considered safe for human consumption. Moreover, polysaccharide content in NBA is at par with that of *A. vera*. It is the polysaccharide of the gel to which many of the health benefits have been attributed (Hamman 2008). On the other hand, in *A. vera* raw juice, aloin content (mean 427.36 ppm) is beyond the safe limit. Further processing of raw juice of *A. vera* is required for reducing its aloin content to safe limit. In contrast, raw juice of NBA having aloin content much lower than the prescribed safe limit can be consumed as such and without any further processing. NBA is therefore, a safe and viable alternative source of raw juice as compared to *A. vera*. Botanical characters described in this paper also make it a distinct type that can be promoted for large scale growing and putting up its processing units which will benefit growers, manufactures and consumers.

Conclusion

The study showed that leaf parameters of non-bitter *Aloe* are significantly different ($p=0.01$) from that of *Aloe vera*. Floral characters and flowering time also distinguished non-bitter *Aloe* from *A. vera*. Most visible difference was observed in their reproductive shoots which are highly branched in NBA (5.21) as compared to that of *A. vera* (1.5). Though, NBA produces less gel per leaf but the quality of its gel in terms of bioactive polysaccharides is at

Table 2 Floral characters of Non-bitter Aloe (NBA) and *Aloe vera*^a

Origin of sample (sample number)	No. of inflorescence	No. of flowers	Pedicle length (cm)	Bract length (cm)	Tapel length (cm)	Filament length (cm)	Anther length (mm)	Anther width (mm)	Style length (cm)	Ovary length (mm)	Ovary diameter (mm)
Non-bitter Aloe (NBA)											
Shiv Bari (1)	4.67	33.67	1.17	0.67	3.23	3.17	2.27	1	2.30	7.16	3.37
Sagar (2)	5.33	35.33	1.23	0.63	3.23	3.15	2.09	1	2.23	7.66	3.10
Udasar (3)	5.67	38.00	1.07	0.57	3.27	3.10	2.43	1.01	2.03	7.00	3.43
Laxamangarh (4)	5.33	41.33	1.10	0.73	3.17	3.17	2.21	1.03	2.20	7.16	3.16
Jaisalmer (5)	5.00	42.00	1.17	0.67	3.23	3.00	2.48	0.96	2.21	6.83	3.30
Barmer (6)	5.67	37.67	1.07	0.63	3.17	3.16	2.42	1.07	2.23	7.33	3.43
Jodhpur (7)	5.67	38.33	1.17	0.67	3.27	3.11	2.34	1.03	2.24	7.00	3.26
Gangasahar (8)	4.33	43.67	1.13	0.73	3.10	3.14	2.38	1.03	2.30	6.84	3.40
Grand Mean	5.21	38.75	1.14	0.66	3.21	3.13	2.33	1.02	2.22	7.12	3.31
Sem(±)	0.341	2.936	0.041	0.0363	0.0399	0.0539	0.1113	0.1003	0.087	0.234	0.082
CD ($p=0.05$) ^a	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<i>Aloe vera</i>											
Bhinasar (9)	1.33	124.67	0.73	0.67	3.37	3.26	3.32	1.30	2.30	5.68	2.26
Churu (10)	1.33	124.33	0.73	0.73	3.43	3.29	3.55	1.30	2.53	5.00	2.33
Sagar (11)	1.00	124.33	0.77	0.63	3.40	3.09	3.52	1.49	2.20	4.82	2.37
Badasar (12)	2.00	118.00	0.73	0.73	3.30	3.12	3.33	1.33	2.30	4.66	2.42
Laxamangarh (13)	1.33	123.00	0.77	0.73	3.33	3.12	3.66	1.43	2.37	5.16	2.30
Jaisalmer (14)	1.67	116.00	0.70	0.67	3.37	3.16	3.21	1.47	2.23	4.80	2.50
Jodhpur (15)	1.33	114.00	0.77	0.77	3.37	3.10	3.33	1.43	2.24	5.00	2.30
Gangasahar (16)	2.00	119.67	0.73	0.70	3.33	3.07	3.50	1.40	2.23	5.33	2.33
Grand Mean	1.50	120.50	0.74	0.70	3.36	3.15	3.43	1.40	2.30	5.06	2.35
Sem(±)	0.341	2.94	0.411	0.0363	0.0399	0.054	0.111	0.1003	0.087	0.234	0.08
CD ($p=0.05$) ^a	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEm ± between non-bitter Aloe and <i>A. vera</i>	0.17	1.47	0.021	0.018	0.02	0.027	0.06	0.05	0.043	0.12	0.047
CD ($p=0.05$) ^a between non-bitter Aloe and <i>A. vera</i>	0.493 ^b	4.24 ^b	0.059 ^b	0.052	0.06 ^b	NS	0.16 ^b	0.15 ^b	0.13	0.34 ^b	0.12 ^b

^a Initiation of flowering: Non-bitter *Aloe* –last week of July; *A. vera* – First week of November^b NS non- significant^c Significantly different at $p=0.01$ also

par with that of *A. vera*. In view of distinct botanical identity and safer raw juice, NBA can be a profitable alternative to the existing source of raw juice i.e. *A. vera*.

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