Acta Chimica & Pharmaceutica Indica

*Acta Chim. Pharm. Indica: 2(1), 2012, 60-66*
*ISSN 2277-288X*

# CHEMICAL ANALYSIS OF *AGAVE SISALANA* JUICE FOR ITS POSSIBLE UTILIZATION

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(*Received : 16.10.2011; Revised : 29.10.2011; Accepted : 01.11.2011*)

## ABSTRACT

*Agave sisalana* Perrine (family: Agavaceae), popularly known as Sisal is a commercially used fibre yielding plant. This herbaceous plant occupies sixth place among fibre plants, representing 2% of the world's production of plant fibre. Approximately 240.7 tonnes of sisal is globally produced. Sisal industry in India is largely unorganised. Sisal is mainly grown in arid and semi-arid regions across the various states in the country. Processing of the sisal leaves for fibre production generates vast quantity of juice which remains underutilized and thus can have negative environmental impact from its disposal. Research programmes driven by societal demand, aiming at evaluation of the agricultural / forestry byproducts in possible value-added applications, have increased significantly during the last decade. The chemical analysis of the juice was undertaken to find its possible utilization. The contents of water, ash, crude protein, total soluble sugars and inulin were determined. The potential of the juice for production of inulin was revealed. Inulin is widely used in functional foods for its health promoting properties. Storage of the juice at 4º C showed a considerable reduction in the inulin content and therefore, the use of fresh juice was suggested.

**Key words**: *Agave sisalana*, Juice, Inulin.

## INTRODUCTION

*Agave sisalana* Perrine (Agavaceae), popularly known as sisal in is a monocotyledonous plant from Mexico. Sisal derives its name from a small port in the Yucatan peninsula of Mexico through which the earliest supplies of Agave fibers were exported and it became known to commerce as Sisal or 'sisal hemp'. The documented use of agaves in the Peninsula of Yucatan is as a source of fiber. Global production of sisal fibre in 2007 amounted to 240 thousand tonnes of which Brazil, the largest producing country, produced 113,000 tonnes, Tanzania produced approximately 37,000 tonnes, Kenya produced 27,600 tonnes, Venezuela 10,500 tonnes and 9,000 tonnes were produced in Madagascar. China contributed 40,000 tons with smaller amounts coming from South Africa, Mozambique, Haiti and Cuba. Though Indian climatic conditions suit Agave plants, yield in India is extremely low compared to the other countries (54.5 to 9.1 kg/ha). Average annual production of sisal fibre in India is far less than the total demand for the Agave fibre. India imports annually Agaves from Tanzani, Kenya and other countries.

*Agave sisalana* is chiefly cultivated for its fibre which is eminently suited for cordage of all kinds and trials have shown that for marine cordage the fibre compares favourably in durability with the Manila hemp obtained from *Musa textiles* Nees. The leaves are cut for fibre between the third and fourth year. The

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lowest leaves are cut close to the trunk. Each plant yield 250-300 leaves during its lifetime of 7-8 years. For extraction of the fibre, two methods are employed viz. the retting process and the mechanical process. The fibre yield in retting process is about 5.5% and in the mechanical process is about 3-4% based on the weight of green leaves.

Sisal fibre being strong and more resistant to dampness than other fibre is used for binder twines,ship cordage, webbing and sacking, and as a substitute for manila hemp which is used for heavier twines, ropes, marine cordage,fish, nets, etc. It is used as a substitute of jute for mats, rugs, sacks for coffee, wagon cover and floor covering. Sisal occupies 6th place among fiber plants, representing 2% of the world's production of plant fibers (plant fibers provide 65% of the world's fibers).

The leaves contain several steroidal sapogenins: Hecogenin, tigogenin, neotigogenin, neotigogenone, Δ9(11)-dehydrohecogenin, sisalagenin, rockogenin, 5-α-pregnan-3β, 20β-diol, 12-epirockogenin and chlorogenin. They contain β-sitosterol and two trihydroxysapogenins viz. hainangenin and hongguanggenin. Hecogenin occurs in the plant in the form of its glycoside, heconin. Sisal waste and the juice expressed from the leaf pulp by decortication of fresh leaves are the commercial source of hecogenin.

Preliminary pharmacological investigation shows that the juice of the leaves lowers the blood pressure in dogs and stimulates their intestinal movements. It possesses ebolic properties and may be used as an abortifacient as it activates the uterine motility. The juice may be administered with Pitocin to intensify the ebolic action[1]. It is a uterine stimulant, emmenagogue, laxative and hypotensive drug.

Only 5% of the decortications of the leaves of sisal (*A. sisalana*) produce a hard fiber that is used for various purposes; the remaining 95% consists of solid waste (mucilage) and waste liquid (juice of the sisal) that are normaly discarded by sisal farms[2]. Thus, sisal waste principal y contains plant tissue (lignin and cellulose), primary and secondary metabolites, and water, amongst others. In an attempt to utilize this waste, some small local producers have systematicaly applied this residue to crops in an attempt to obtain improved production, or in the feeding of animals[3]. In this way, researchers have studied this practice and published the use of sisal waste against larvae of mosquitoes, which transmit tropical diseases[4]. The leaf extract at 5000 ppm concentration possesses molluscicidal activity. It also destroys surrounding aquatic fauna and flora[5]. The objective of study was to carryout chemical analysis of *Agave sisalana* juice with respect to the parameters such as water content, ash content, pH value crude protein content, inulin content and total soluble content to explore the possibility of its utilization

## EXPERIMENTAL

### Plant material

*Agave sisalana* leaves were obtained from Girish Grih Udhyog Avam Resha Udpadan Samiti, Kimsaar, Kotdawar.

### Chemicals and reagents

All chemicals and solvents used were either analytical or HPLC grade (E. Merk Ltd., Mumbai, India)

### Extraction of juice from *Agave sisalana* leaves

The chopped leaves were processed through compressor cum dewatering unit and fresh juice was collected from the outlet. The juice was then followed by centrifugation at 2000 rpm for 15 min. Clear supernatant liquid (1600 mL) was collected and to it 16 mL of toulene was added. The juice was stored at $4^{o}$ C until analyzed.

**Determination of water content**

Water content was determined using the standard method[6]. For this juice (10 mL) was taken in the petri dish and evapourated on the water bath till it became dry. Dishes were further heated in hot air oven at 100° C for specified period of time till constant weight was obtained.

**Determination of ash content**

Ash content was determined using the standard method[7]. The juice (10 mL) was taken in silica crucible and heated in muffle furnace at dull red heat (600° C) till it was completely converted to white ash. The crucible was kept in desiccator to cool and then the weight of the ash was obtained.

**Determination of pH value**

The pH value of juice was determined using pH meter.

**Determination of crude protein by Kjeldahl's method**

0.5 g of finely powdered sample was taken in a long necked digestion tube and 10 mL of conc. $H_2SO_4$ and 10 g of catalyst mixture was added. The sample was digested on Kjeldahl's heating unit until the solution became light green in color. The sample was cooled and transferred to 50 mL volumetric flask. In the kjeltic machine, the digestion tube were rinsed several times with small amount of distill water and the washings were poured in the volumetric flask. Finally the volume was made to 50 mL with distilled water. Now 25 mL of boric acid solution was taken in 250 mL conical flask. The receiving flask was placed in such a way that the outlet of the condenser of kjeltic machine apparatus dipped into the boric acid solution. 5 mL of acid digestion sample was transferred to the steam chamber along with 5-8 mL of 40% NaOH to the aliquot of digestion sample followed by passing of the steam through the steam chamber to distill ammonia till boric acid containing flask changed its color from red to greenish-blue. Now the receiving flask was removed and the condenser outlet was rinsed into the receiving flask with distill water. The contents of receiving flask were titrated against 0.01 N $H_2SO_4$ till the greenish-blue color changed to electric grey. A blank preparation was run which was identically prepared except that it did not contain the sample[8].

The amount of Nitrogen is calculated from the formula:

$$N_2 = 14.01 \text{ x } \frac{(\text{Reading of burette} - \text{Reading of blank})}{\text{Weight of sample x } 10} \text{ x } 0.13754$$

Crude Protein = 6.25 X $N_2$

**Determination of Inulin content**

**(a) Chemical test for presence of Inulin in *Agave sisalana* juice**

The presence of inulin in *Agave sisalana* juice was detected using method reported by Blaydes[9].

The juice was diluted with distill water and a drop of thymol was added followed by addition of a drop of Con. $H_2SO_4$. Appearance of bright red color at once and its disappearance soon indicated the presence of inulin.

**(b) Determination of Inulin content**

Preparation of Standard solution: A stock standard solution of 200 ppm of inulin was prepared by dissolving accurately weight 20 mg pure inulin in 100 mL of distill water. This stock solution was used to

prepare serial dilutions containing 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 ppm inulin solution. 3 mL of each solution was used employing the above procedure to construct a calibration graphs (shown in Fig. 1) and concentration of inulin in the sample was determined using the calibration graph. The assay figure obtained from calibration graph was multiplied by dilution factor (= 50) to give the inulin concentration as ppm followed by dividing the ppm values by 10,000 to convert to the percentage values. The method reported in literature by Sadasivam and Manickam[10] was employed for estimation of inulin content.

To 1 mL of juice was diluted 50 times using distill water. To 3 mL of sample, were added equal amount of Conc. HCl and 0.3 mL resorcinol reagent. The mixture was warmed on digital waterbath at 80$^{o}$ C for 10 min. and the absorbance was observed at 520 nm in a UV-Spectrometer (shown in Fig. 2).

**Determination of total soluble sugar content**

The total soluble sugar was determined using ferricyanide method (volumetric method).

To obtain aliquot, 1-2 mL of saturated neutral lead acetate was added to the collected clear supernatant (juice). After properly mixing it for 15 min, it was filtered through Whatman No.1 filter paper and the volume was made to 250 mL with distilled water. Excess of lead acetate was then precipitated out with solid sodium oxalate. 5 mL of potassium ferricyanide and 5 mL of aliquot of the sample extract (as obtained above) was taken in a test tube, heated for 15 min in boiling water bath and then cooled. 5 mL of iodine-solution followed by 3 mL of 5% glacial acetic acid was added to the above solution. The excess iodine was titrated against 0.01 N $Na_2S_2O_3$ till the color of solution turned pale yellow. Now starch indicator solution was added, upon which the color changed to blue. The titration was completed till disappearance of blue color. A blank taking water instead of sugar solution or sample aliquot was run and proceeded in the same manner. Volume of $Na_2S_2O_3$ used for the sample was deducted from that consumed for the blank[8].

The amount of total soluble sugar was calculated from the following relationship:

mg of total soluble sugar in 5 mL of sample extract = $\mu$ (x + 0.05)

where: ($\mu$ = 0.338 and x = Vol. of 0.01 N $Na_2S_2O_3$ used for sample, i.e. Vol. of $Na_2S_2O_3$ used in blank– Vol. used in sample).

## RESULTS AND DISCUSSION

The data revealed that *Agave sisalana* juice was acidic (pH = 5.42) in nature. The juice contained good amount of water (= 93.73%) along with 1.11% of total soluble sugar. Protein and ash content were found to be 11.56% and 1.48%, respectively.

Now a days, a great interest exists in the obtaining of nondigestible oligosaccharides (soluble fibre) for its use in nutraceutical drinks. The agave species have been reported to be a source of nondigestible oligosaccharides derived from inulin, but scarce studies about its extraction exists. Inulin is a term applied to a heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates. It has been used in many countries to replace fat or sugar and reduce the calories of foods such as ice cream, dairy products, confections and baked goods. It has lower caloric values than typical carbohydrates and is nondigestible by human intestinal enzymes. Inulin is widely used in functional foods through out the world for their health-promoting and technological properties. It is ingredients of the future that meet the needs of the food industry today, and is on the leading edge of the emerging trend toward functional foods[11].

The chemical composition of *Agave sisalana* juice* can be summarized below:

**Table 1: Chemical composition of *Agave sisalana* Juice**

| S. No. | Parameter | Value |
|---|---|---|
| **1** | Water content (%) | 93.73 |
| **2** | Ash content (%) | 1.48 |
| **3** | pH value at 28.9$^{o}$ C | 5.42 |
| **4** | Crude protein (%) | 11.56 |
| **5** | Inulin content (mg/g) | 8.7$^{\$}$, 20.87$^{\#}$ |
| **6** | Total soluble sugar content (%) | 1.12 |

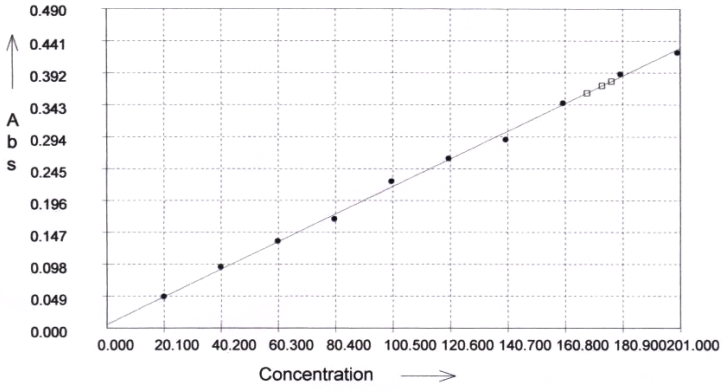
$^{\$}$Inulin content of stored juice, # Inulin content of fresh juice

The values corresponded to the average of the three replicas.

**UV-Vis Double Beam Spectrophotometer 6.77**

**Standard Curve - Quantitative Mode**

| | | | |
|---|---|---|---|
| Method : | One Wavelength Method | Signed By: | User |
| Instrument Sr. No. : | 2747/0307 | Date : | 11-May-2010 |
| Sample Name : | Quantitative Test | Time : | 4:14:54 PM |
| Analysed By : | PKukreti | Dated : | 10 - May - 2010 |
| Abs -> K1 x (Conc) + K0 | | Wavelength : | 520nm. |
| K1 -> 0.0021, K0 -> 0.0056 | | Calibration mode : | Linear Mode |




**Standard values**

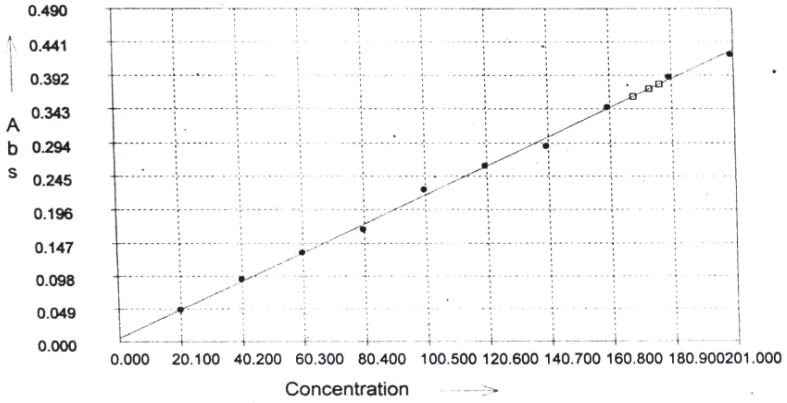
| Stds. | Abs | Conc. |
|---|---|---|
| Std #1 | 0.049 | 20.000 |
| Std #2 | 0.094 | 40.000 |
| Std #3 | 0.134 | 60.000 |
| Std #4 | 0.168 | 80.000 |
| Std #5 | 0.226 | 100.000 |
| Std #6 | 0.261 | 120.000 |
| Std #7 | 0.290 | 140.000 |
| Std #8 | 0.346 | 160.000 |
| Std #9 | 0.390 | 180.000 |
| Std #10 | 0.423 | 200.000 |

**Fig. 1: Calibration curve of standard inulin solution**

**UV-Vis Double Beam Spectrophotometer 6.77**

**Standard Curve with samples - Quantitative Mode**

| | | | |
|---|---|---|---|
| Method : | One Wavelength Method | Signed By: | User |
| Instrument Sr. No. : | 2747/0307 | Date : | 11-May-2010 |
| Sample Name : | Quantitative Test | Time : | 4:13:37 PM |
| Analysed By : | PKukreti | Dated : | 10 - May - 2010 |
| Abs -> K1 x (Conc) + K0 | | Wavelength : | 520nm. |
| K1 -> 0.0021, K0 -> 0.0056 | | Calibration mode : | Linear Mode |




**Sample values**

| Samples | Abs | Conc. |
|---|---|---|
| shipra#1 | 0.372 | 173.673 |
| shipra#2 | 0.361 | 168.460 |
| shipra#3 | 0.379 | 176.991 |
| shipra#4 | 0.372 | 173.673 |
| shipra#5 | 0.882 | 415.379 |
| shipra#6 | 0.883 | 415.853 |
| shipra#7 | 0.894 | 421.066 |
| shipra#8 | 0.900 | 423.910 |

**Fig. 2: Calibration curve of sample solution**

High inulin content has been found in *Agave americana* and hence it is used for the production of alcoholic drinks through conversionof inulin into fermentable fructose and galactose.

In *Agave tequilana* inulin content (22.00 mg/g) has been reported[12]. However *Agave sisalana* has not been studied for its inulin content. It was therefore thought worthwhile to evaluate *Agave sisalana* juice for inulin content.

The presence of inulin in the juice was ascertained through chemical test and it was found to be positive. The concentration of inulin in the fresh juice was determined and was found to be 20.87 mg/g. A comparison of inulin concentration in fresh and juice stored for thirty two days was also made using the same method. A considerable reduction in inulin content in the stored juice (8.7 mg/g) was observed.

This is the first report on chemical composition of *Agave sisalana*, an industrial waste with respect to water content (93.73%), ash content (1.4%), pH value (5.42), crude protein content (11.56%), inulin content (8.7 mg/g), total soluble sugar (1.11%).

The knowledge generated in the work may form a basis for the possible utilization of the juice however the hecogenin (0.1%) reported to be present in the juice needs to be removed before making it usable for good purpose.

## ACKNOWLEDGEMENT

We are thankful to Director, Forest Research Institute (FRI), Dehradun and Head, Chemistry Division, FRI, Dehradun for providing necessary facilities and encouragement.

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