Determination of Sugar Biomarkers in Different Plant Parts of *Lasiurusscindicus*Henrard (Poaceae) through High Performance Thin Layer Chromatography

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ABSTRACT

Thisstudy is a first attempt to present the report of TLC densitometric method, which has been developed and validated for quantification of sugars i.e. Glucose, Fructose, Sucrose and Maltose from hydro-methanolic extracts of leaves (HML), stem (HMS), inflorescence (HMI) and roots (HMR) of desert plant known as *Lasiurusscindicus*Henrard. The best results were obtained by using the mobile phase Propanol: Ethyl acetate: Water (6:3:1 v/v) and the plates are post derivatized with diphenylamine, aniline andorthophosphoric acid, densitometric scanning at 600 nm in absorbance/ reflectance mode. This method gave best resolution of bands at R_f 0.47, 0.48, 0.40 and 0.26, corresponding to glucose, fructose, sucrose and maltose, respectively. The maximum contents of glucose (3.05 w/w), fructose (14.01 w/w), sucrose (7.69 w/w) and maltose (11.42 w/w) are obtained in stem extractions. Sucrose is determined in all parts, whereas, glucose, fructose and maltose are absent in inflorescence and root extracts.

Key words: *Lasiurusscindicus*, economic plant, HPTLC analysis, sugars screening, etc.

INTRODUCTION

Sugars are the primary source of metabolic energy in food. They build up the structure of plant cells and are the main source of energy for plants, animals and human life. The main task of the present research is to analyze and calculate the amount of sugars present in economically most important forage grass of Indian hot desert known as *Lasiurusscindicus*Henrard by High Performance Thin Layer Chromatography.

Plants play an important role in our daily life. These not only provide us nutrition but also have some medicinal value. Poaceae (=Gramineae) are the true grasses which have both

nutritional as well as medicinal importance. Almost all the grasses are used as fodder. Forages are plants or parts of plants eaten by livestock and the variety of plants that are eaten is amazing. Most of the grasses are grown for grains, pasture and lawns (turf), besides ornamental ones.

These include about 10000 to 11000 species belonging to 700 genera from world (Clayton &Renovoize 1989; Watson &Dallwitz 1992). Grasses have been adapted to the conditions in lush rain forests, dry deserts and cold mountain steppes and now a days are most widespread plant types.

Several grass species such as cereals (rice, wheat and maize), millets (jowar, bajra and ragi) and Sugarcane for their food value. Bamboos are used for making building material and paper. Vetiver roots are used for making mats at houses. Some species of grasses are also used for making grooms.Lemon grass is used to make perfumed soaps and flavor curries. Some of the medicinally important grasses are *Coixlacryma-jobi* L., *Cynodondactylon* L. used for leucoderma, bronchitis, piles, asthma, tumors and enlargement of spleen. Traditionally the crushed leaves of *C. dactylon* L. were used to cure wounds and to stop bleeding similar to *Tridaxprocumbens* L. *Acarynthesaspera* L. and *Blumealacera* (Burn.f.) D.C. (Oudhia and Pal 2000).*Vetiveriazizanoides* is used for the ailments of mouth, ulcers, fever, epilepsy, burn, snakebite, scorpion sting, rheumatism, fever, headache, etc. (Jain 1991; Singh &Maheshwarai 1983).

*Lasiurusscindicus*Henrard commonly called Sewangrass, is one of the most important fodder plant. It is the primary grass of extremely arid parts of Jaisalmer, Barmer and Bikaner districts of western Rajasthan in the Indian Thar Desert. It flourishes well under moisture stress on sandy plains, low dunes and hummocks of this region. It is one of the most productive and suitable grass for arid and semi arid zones. Such types of species like Sewan grass are very important in arid environments because they provide forage, which maintains both wild mammals and livestock, and soil cover (Assaeed, 1997). As it is protein rich plant, so that's why it accumulates the higher milk production in dairy animals. Due to the presence of sugars in large amount in leaf and stem, the animals like to eat it.

L. scindicus is a perennial grass. It is bushy, multibranched desert grass with ascending to erect wiry stems. The inflorescence is a silky with long raceme bearing hairy spikelets.

Recently, Ara and Coworkers (2012) analysed the antimicrobial properties by using gas chromatography-mass spectrometric (GC-MS) technique from Saudi Arabia on leaf extracts of seven different plants of difeerent families, one of them is *L. scindicus* of Poaceae family. After that, methanol extracts of some plants native to Saudi Arabia including *L. scindicus*reported antidiarrheal activity and phytochemical constituents. They concluded that antidiarrheal activity is induced due to the presence of their high content of flavonoids, phenolics and tannins. Prasad *et al.* 2014 compiled a review that reveals wide number of phytochemical constituents isolated from the medicinally important plant *Cymbopogonjawarancusa*. It is used for various diseases like skin, vomiting, abdominal tumors, unconsciousness and fever.

There is no scientific literature about the phytochemical analysis of *L. scindicus* from Indian hot desert, Rajasthan. Therefore the objectives of this study is to optimize the quantity of sugars present in all plant parts by High Performance Thin Layer Chromatography techniques..

MATERIALS AND METHODS:

Collection of Plant material:

The material was collected from Bikaner district of Rajasthan, India. The plant is authenticated from Botanical Survey of India, Arid Zone Regional Centre, Jodhpur, Rajasthan. The authenticated plant specimen is submitted to the Herbarium, Department of Botany, Punjabi University, Patiala (PUN 60708).

Standard markers and other Chemicals:

Reference standards (Glucose, Fructose, Sucrose, Maltose, etc) were purchased from Himedia. Chemicals like Propanol, Ethyl acetate, Methanol, Diphenyl amine, Aniline, and Orthophosphoric acid were obtained from SDFCL (S.D. fine chemicals, Mumbai, India). Precoated silica gel 60 F_{254} HPTLC aluminium plates (20×20 cm^2 layer thickness- 0.2 mm, 5-6 µm particle size; E. Merck, Darmstadt, Germany) were obtained from E. Merck Ltd. (Mumbai, India).

Equipment

- Spotting device- CAMAG Linomat V sample applicator (CAMAG, Switzerland)

- Syringe- 100 µl Hamilton syringe
- TLC Chamber- CAMAG twin trough chamber.
- Scanner- CAMAG TLC scanner with D_2 and Hg lamp, Reprostar and winCATS Planar Chromatography manager and CAMAG integration software and TLC viewing cabinet (all from CAMAG, Muttenz, Switzerland).

Preparation of standard stock solution:

Accurately weighed reference standards i.e Glucose Sucrose, Fructose, Mannose, Ribose, Arabinose and acetyl glucosamine (5 mg) were transferred to 10 ml volumetric flask, dissolved in 5 ml 90% methanol (1mg/1ml). These stock solutions were ready to use for HPTLC.

Sample preparation of *L. scindicus*

Fresh plant material is washed under running tap water, air dried and grinded to form fine powder. 5g of dried powder of each part of plant (Leaf, Stem, Inflorescence and Root) sample were extracted with 90% methanolic solutions using soxhlet extractor for 6 hours. The obtained hydromethanolic extracts were then filtered and concentrated using rotaryvacumm evaporator and then Lyophilized with Allied Frost Lyophilizer-FD-3. The obtained lypholized powder of samples (HML, HMS, HMI, HMR) were accurately weighed and then dissolved in methanol (1mg/1ml). These solutions were used as test solutions for HPTLC analysis.

HPTLC analysis

The HPTLC analysis was performed using precoated silica gel 60 F_{254}aluminium plates. Linomat V autosampler was used for spotting of standards (glucose, fructose, sucrose and maltose, 2 µl, 4 µl, 6 µl, 8 µl and 10 µl each) and sample solutions (10 µl each), operated with 6 mm band length, distance between the tracks 8.7 mm, distance from the bottom of the plate, 5 mm. The plates were developed to a distance of 75 mm in a CAMAG twin trough chamber presaturated with mobile phase propanol: ethyl acetate: water (6:3:1 v/v) for 25 minutes. The developed plates were air dried and derivatized because sugars are visualized after derivatization. Derivatization of the plate was done by sprayimg methanolic solution of diphenylamine, aniline and orthophosphoric acid (Wang 2004). The plates were heated at 150˚C for 4-5 minutes. The dried plates were scanned for densitometry measurement, spectra recording and data processing

at absorbance of wavelength 600 nm to record their U.V. spectra and for obtaining wavelength of maximum absorption ($\lambda_{max.}$). The absorbance / reflectance measurement mode was used at a scan speed 100 nm/sec. The plates were photographed in the visible mode after derivation. The peak areas were recorded for all the concentrations. Calibration curve of standards was plotted as peak area versus concentrations of standards applied in triplicates.

Method Validation:

Linearity was studied by applying different concentrations of standard stock solutions (2-10 µl of each reference standard). The calibration curve was developed by plotting peak area versus concentrations with the help of the winCATS software. The areas of peaks were treated by least square linear regression analysis.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the following equation where S.D. is standard deviation and S is the slope of the curve.

LOD = 3.3(S.D. / S)

LOQ = 10(S.D. / S)

The values taken in triplicate were used to determine the accuracy of the study at different levels and different concentrations of standard solutions.

The specificity of the method was ascertained by determining the peak purity of the component by comparing overlay UV spectra of each sugar standard in the sample extract with the absorption spectra of reference standard at the start, middle and end positions of the bands.

Statistical analysis was performed by using Microsoft Excel 2007 which includes computation of linear regression analysis, mean, standard deviation and relative standard deviation.

Results and discussion:

During the present research the quantitative TLC profiling of grass (*L. scindicus*Henrard) has been done along with parallel run of eight sugar standards (glucose, fructose, maltose, mannose, sucrose, ribose, arabinose, cellulobiose and acetylglucosamine) (Table 1). These have been preliminary tried on TLC plates along with plant extracts. Out of these only glucose, fructose,

sucrose and maltose are detected in the different parts of plant extracts. These standards were selected for final results on HPTLC.

Table 1: Preliminary tested sugar markers in *L. scindicus*

Plant	Sugar markers tested								
	Glucose	fructose	Maltose	Mannose	Sucrose	Ribose	Arabinose	Cellulobiose	Acetyl-glucosamine
L. scindicus	+	+	+	-	+	-	-	-	-

* (+) indicates the presence of marker;(-) indicates the absence of the marker

Different proportions of propanol, ethyl acetate and water was used for the separation of different sugars from the hydromethanolic extracts of different parts of *L. scindicus*. Well resolved symmetric bands were obtained by using precoated HPTLC plates with Propanol: Ethyl acetate: Water (6: 3: 1, v/v) as mobile phase . Sugars are visualized after derivatizing with methanolic solution of diphenylamine , aniline and orthophosphoric acid (Wang 2004). Chromatogram was heated after spraying at 150˚C for 4 minutes (Figures. 2-5). These plates were scanned for densitometry measurement, spectra recording and data processing at absorbance of wavelength 600 nm to record their U.V. spectra and for obtaining wavelength of maximum absorption ($\lambda_{max.}$).

During this study, it was found that glucose and fructose shows almost similar retention factor (R_f) i.e. 0.47 and 0.48 respectively. Then the detection of both these compounds is confirmed with overlay absorption spectra of each standard and samples separately in which maximum absorption spectra ($\lambda_{max.}$) of glucose is obtained at 373 nm and fructose at 380 nm. The R_f values of sucrose and maltose are 0.40 and 0.26 respectively. Absorption spectra of sucrose and maltose shows maximum absorption / reflectance at (λ_{max}) 380nm and 376 nm, respectively.

The quantitative analysis of detected sugars in different plant parts is given in the Table 2 along with bar diagram (Figure 1). Hydromethanolic extraction of stem (HMS) shows the presence of maximum amounts of Glucose, Fructose, Sucrose and Maltose i.e. 3.05, 14.01, 7.69

and 11.42 (mg/g of dry wt.), respectively. Sucrose is reported in all the four parts (HML, HMS, HMI, HMR) p0f the plant, whereas, hydromethanolic extracts of root and inflorescence (HMR, HMI) do not show the presence of glucose, fructose and maltose.

Table 2: Results from the quantitative analysis of sugars detected in different plant parts of *L. scindicus*

Different plant extracts	Amount of sugars in different parts of *L. scindicus* (mg/g of dry weight)			
	Glucose	**Fructose**	**Sucrose**	**Maltose**
HML	2.97	3.83	3.12	2.44
HMS	**3.05**	**14.01**	**7.69**	**11.42**
HMI	ND	ND	0.71	ND
HMR	ND	ND	0.14	ND

*HML- leaf extracts; HMS- Stem extracts; HMI- Inflorescence extracts; HMR- root extracts; ND means not detected

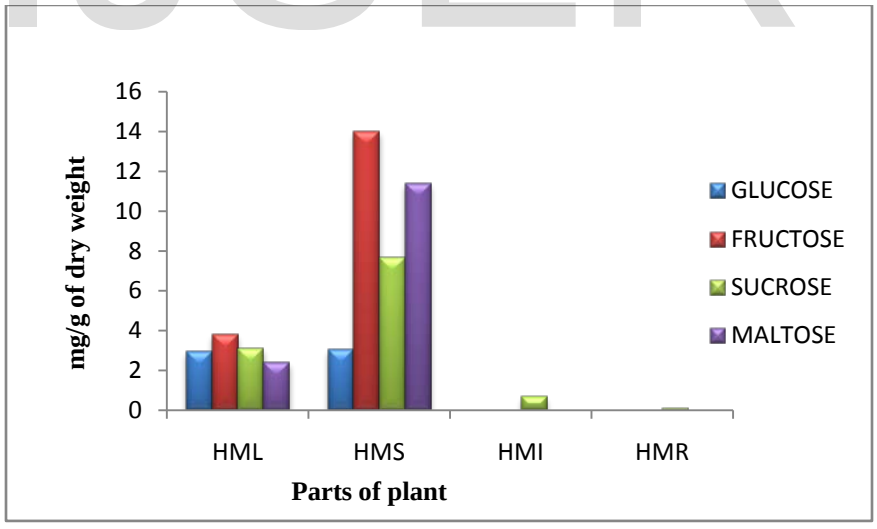

Figure 1: Comparison of amount of sugars calculated in *L. scindicus*

The developed HPTLC method for estimation of referred sugars had been validated using following parameters according to ICH guidelines. Linearity range has been calculated with

acceptable correlation coefficients. It exhibit good linearity between concentration and peak area (Table 3).

Table 3: Method validation parameters for the estimation of sugars by HPTLC densitometry in *L. scindicus*

Parameters	Glucose	Fructose	Sucrose	Maltose
Wavelength (nm)	600	600	600	600
Correlation coefficient (R^2)	0.997	0.997	0.991	0.988
Linearity range	200-1000 ng/spot	200-1000 ng/spot	200-1000 ng/spot	200-1000 ng/spot
Linear regression (y)	2084x	3057x	3441x	911.9x
Slope	2023.08	2926.395	3130.27	855.89
Intercept	449.18	783.85	2403.86	336.05
Specificity	Specific	Specific	Specific	specific

Specificity:

The identity of the bands in the sample extracts were confirmed by comparing the R_f and the overlain UV absorption spectra with those of their respective standard. The correlation coefficient of the standard curves have been obtained from standard curves (Figures 6-9). The absorption spectra of standards and corresponding spots present in extracts matched exactly which indicates no interference by the other plant constituents (Figs. 10-17).

The purity of the bands due to sugars in the sample extract was confirmed by overlaying the absorption spectra recorded at start, middle and end position of the band in the sample tracks (Figs. 10-13).

Conclusion:

In the present study, a very first attempt has been conducted on phytochemical analysis of Sewan grass (*L. scindicus*) from Indian hot desert by High Performance Thin Layer Chromatography. A novel method for HPTLC analysis has been presented along with the results that show the

presence of sugars i.e. glucose, fructose, sucrose, and maltose in the hydromethanolic extracts of plant material. The sugars are beneficiary for husbandry animals as the plant is economically most important for desert animals.

Acknowledgement:

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Conflict of interest

The authors declare that they have no conflict of interest.

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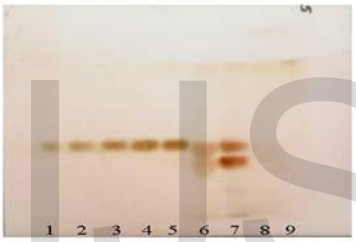

Figure 2: A comparative TLC profile of Glucose along with different plant parts of *L. scindicus* (Track 1-5 Glucose concentrations and tracks 6 (HML), 7 (HMS), 8 (HMI), 9 (HMR) respectively

Figure 3: A comparative TLC profile of Fructose along with different plant parts of *L. scindicus* (Track 1-5 Fructose concentrations and tracks 6 (HML), 7 (HMS), 8 (HMI), 9 (HMR) respectively

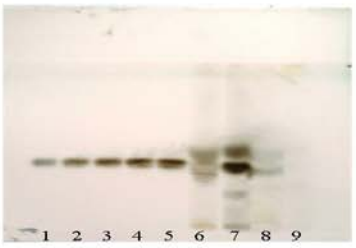

Figure 4: A comparative TLC profile of Sucrose along with different plant parts of *L. scindicus* (Track 1-5 Sucrose concentrations and tracks 6 (HML), 7 (HMS), 8 (HMI), 9 (HMR) respectively

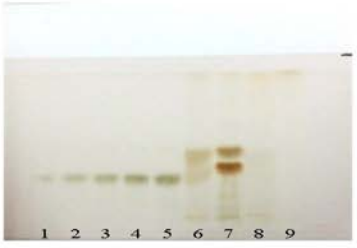

Figure 5: A comparative TLC profile of Maltose along with different plant parts of *L. scindicus* (Track 1-5 Maltose concentrations and tracks 6 (HML), 7 (HMS), 8 (HMI), 9 (HMR) respectively

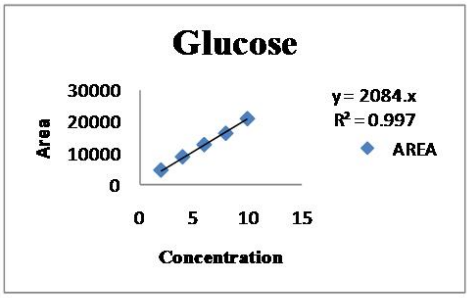

Figure 6 Standard curve of glucose

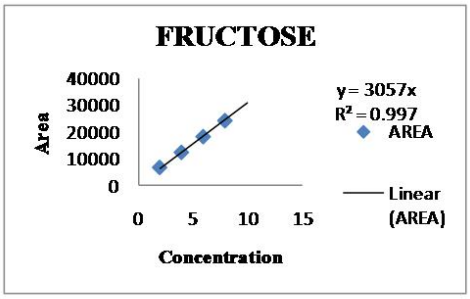

Figure 7. Standard curve of fructose

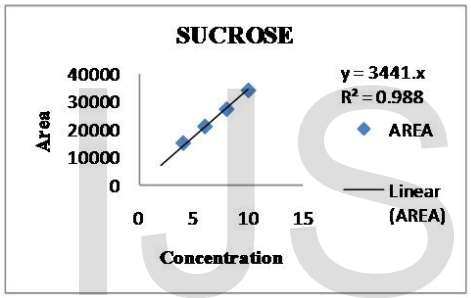

Figure 8. Standard curve of sucrose

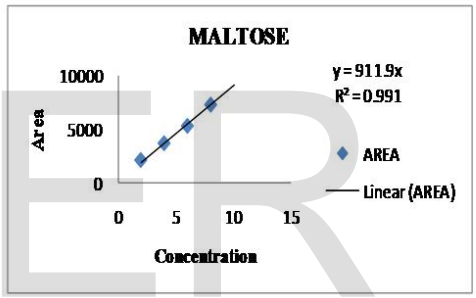

Figure 9. Standard plot of Maltose

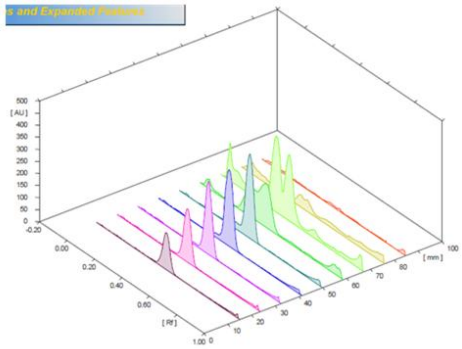

Figure 10. Densitometric scan of standard glucose (a-e) with plant parts (f-i) ($\lambda_{max.}$ 600nm)

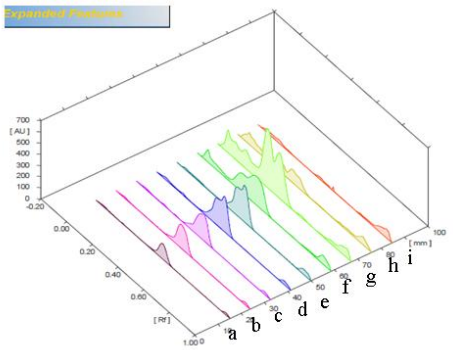

Figure 11. Densitometric scan of standard fructose (a-e) with plant parts (f-i) ($\lambda_{max.}$ 600nm)

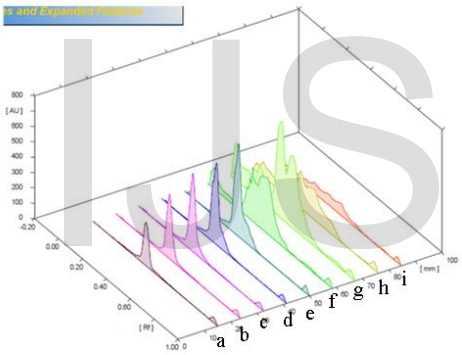

Figure 12. Densitometric scan of standard sucrose (a-e) with plant parts (f-i) ($\lambda_{max.}$ 600nm)

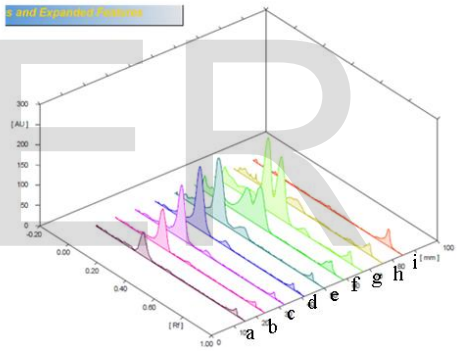

Figure 13. Densitometric scan of standard maltose (a-e) with plant parts (f-i) ($\lambda_{max.}$ 600nm)

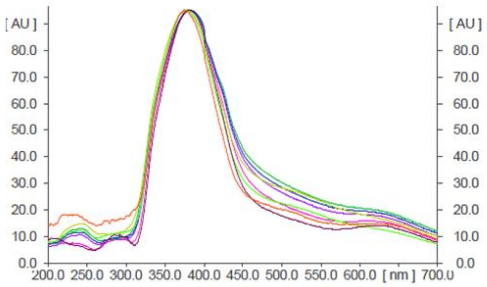

Figure 14: Absorption spectra of Glucose and plant samples

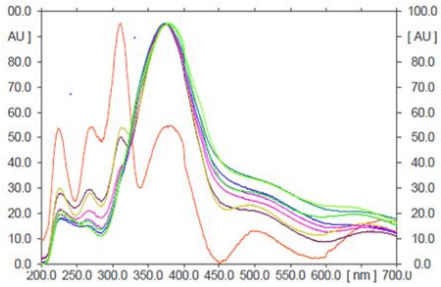

Figure 15: Absorption spectra of fructose and plant samples

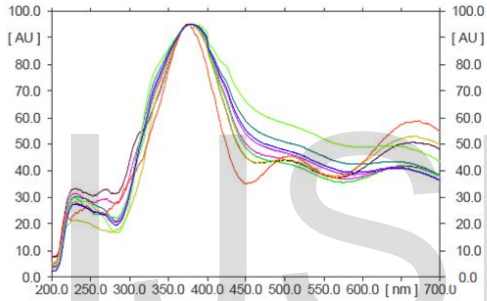

Figure 16: Absorption spectra of sucrose and plant samples

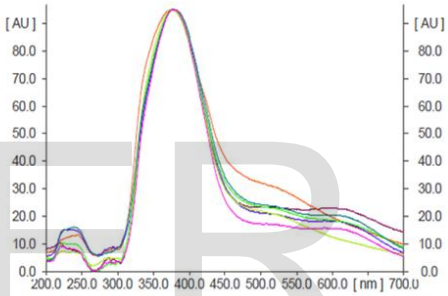

Figure 17: Absorption spectra of maltose and plant samples