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Research article

Qualitative and quantitative gas chromatography-mass spectroscopy analysis and characterization of naturally isolated mucilage in *Hibiscus cannabinus* L. (Malvaceae)

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Abstract: Deccan hemp is rich in mucilage and of immense value. This study was performed to examine the mucilage of Deccan hemp and its (Gas Chromatography-Mass Spectroscopy) analysis was performed to identify the presence of sugar composition in mucilage for the development of pharmaceutical formulation. Mucilage was found to be 9.54% w/w which was off white in colour, tasteless and with characteristic odour. Physicochemical characterization revealed that mucilage has enough moisture that is 9.34 % w/w and is of neutral pH. It was found to be soluble in hot water and insoluble in organic solvents while in cold water mucilage swelled to form a gel. The GC-MS analysis of mucilage showed the presence of glucose, fructose, sucrose, maltose and xylose that scope to be of scientific relevance particularly plant polymer based excipient and coating material in pharmaceutical products. The present investigation showed that Deccan hemp mucilage has high pharmaceutical significance it can be used as excipient and coating material in pharmaceutical formulation.

Keywords: Pharmaceutical products - Pharmacology - Photochemistry - Organoleptic - Swelling index.

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INTRODUCTION

Hibiscus cannabinus L. commonly known as *Kenef* native of Africa is a member of *Malvaceae* family. It is an erect annual herb, grows up to 2 m in wild and 5 m in cultivars. The stem is cylindrical and slender. The cultivar is unbranched and smooth while the wild is prickly, entirely green, green with red or purple pigmentation or red sometimes lower half green and upper half pigmented. The tap root is well developed measures up to 25cm deep with lateral roots spread horizontally to 1m and the adventitious root on lowest stem section (Shamsuddin & van der Vossen 2003). It is a plant of great therapeutic value with its crude extract exhibiting multidimensional activities which include antidiabetic activity (SundarRajan *et al*. 2011), anti-inflammatory activity (Shaikh *et al*. 2016), fungiotoxic activity (Kobaisy *et al*. 2001), anti-hyperlipidemic activity (Shivali & Pradeep 2010), anti-ulcer activity (Nyam *et al*. 2016) and besides these, it is a good source of mucilage which makes it a drug of pharmaceutical value. Mucilage is a good source of natural polymer with thickening and binding properties in different industrial applications such as food pharmaceutical and cosmetic. Plant material and products are compatible with environmental safety and human health. The plant-based polymer have been effectively applied in various pharmaceutical dosage forms like nanoparticles coating material, film coating agents, matrix controlled system, suspensions, implants, buccal films and microspheres. They have been used as viscosity enhancers, stabilizers, disintegrates, solubilizes, emulsifiers, bio adhesives and binders (Clarke *et al*. 1979, Franz 1989, Zimmermann *et al.* 1994). Mucilage have been extensively used in the field of drug delivery for their easy availability, cost effectiveness, eco friendliness, emollient and non irritant nature, non toxicity, capable of multitude of chemical modifications, bio-degradable and compatible due to natural origin (Durso 1980, Chang & Shukla 2003). Mucilage is intracellular physiological product of

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metabolism (Geetha *et al*. 2009). Which is polysaccharide mixture having high molecular weight *i.e.*, 20000 and more (Narkhede *et al*. 2010); commonly found in various organs of many higher plant species (Hadley 1997) and it is an amorphous polysaccharide, its composition was found to be D-Glucose, D-Fractose, L-Galactouronic acid, D-Galactose, L- Rhamnose, Sucrose, Maltose and Xylose (Baveja *et al*. 1988). Mucilage acts as a membrane thickener and food reserve in the plants (Banker & Anderson 1987), Hence the present study was under taken with the aim to analyze the sugar composition present in mucilage along with physicochemical properties (Fig. 1; Table 1).

Figure 1. Plant of *Hibiscus cannabinus* L. (Deccan Hemp). [Inset: Fruits]

Table 1. Organoleptic and physicochemical characteristic of Deccan Hemp mucilage

S.N.	Properties of Mucilage	Results
1	Swelling Index	9±0.52 % w/w
2	Solubility	Soluble in hot water, Insoluble in organic solvents and in cold water swells to form a gel
3	Loss on drying	9.34 %w/w
4	pH	7.2±0.2
5	Colour	Off white colour
6	Odour	Odour less
7	Taste	Taste less
8	Texture	Irregular
9	Fracture	Rough

MATERIAL AND METHOD

Material

The plant with fruits was collected and authenticated by Dr. A. K. S. Rawat (Principal scientist and Head of the department), Pharmacognosy and Ethenopharmacology divison CSIR-National Botanical Research Institute, Lucknow (voucher field booklet no 254060).

Processing and extraction

100 g of hemp fruit was cut into pieces, soaked in 1 l distilled water for 3–4 hours followed by heating at 70ºC for 5–10 minute and crushed with mechanical blender which was filtered by muslin cloth after which ethanol was added into the filtrate in (1:2) ratio to precipitate mucilage and dried in hot air oven at 40–45ºC. The mucilage powder thus obtained was passed through sieve # 40 and stored in desiccator at room temperature (Kirtikar & Basu 1991).

Organoleptic Evaluation: The isolated mucilage was characterized for organoleptic properties such as colour, taste, odour, texture, and fracture (Baveja *et al*. 1989).

Solubility: 1 g dry mucilage powder was solubilized with polarity gradient solvents and the solubility was determined (Baveja *et al*. 1989).

pH: The mucilage was weighed and dissolved in water separately to obtained 1% w/v solution. The pH of solution was determined using digital pH meter (Baveja *et al*. 1989).

Swelling Index: The swelling index is the volume (in ml) taken up by the swelling of 1g of test material under specified conditions (Baveja *et al*. 1989).

Gas Chromatogrphy Mass Spectroscopy Analysis

Procedure: Preparation of Trimethylsilyl ethers are a convenient way (TMS) to derivative, experimental conditions. Trimethylsilyl ethers derivatives were prepared of several different extracts. Taken 5 mg of the sample was suspended in 40 µl of the solution of methoxylamine hydrochloride in pyridine (20 mg ml^{-1}). The mixture was shaken for an hr at 37ºC before adding 70 µl of the 2,2,2-trifluoro-N-methyl-N-methyl-N-Trimethylsilylacetamide (MSTFA). Shaking was continued for another 30 min. After that resulting derivatized mixture of metabolites were subjected to analysis on Gas Chromatography-Mass Spectrometry (GC-MS) by Thermo Fisher TRACE GC ULTRA coupled with DSQ II Mass Spectrometer instrument using a TR 50 ms column (30 m × 0.25 mm ID × 0.25 × µl film thickness) carriergas, helium temperature programming 5 minutes delay for solvent, at 50ºC temperature hold time 1.0 min, rising at 200ºC min^{-1} to 310ºC and finally held isothermally for 15 min. The injector temperature was 230ºC and carrier flow was constant flow of 1 ml min^{-1} in split mode (1:50) with injector volume 1 µl. The ion source temperature was set at 220ºC, transfer line temperature was 300ºC and the ionization of the sample components were performed in EI mode at an ionization voltage of -70 eV. The mass range was used from m/z 50 to 650 amu. The identification of individual compound was made by comparison of their mass spectra with those of the internal reference mass spectra library (NIST/ Wiley) with authentic compounds. (Pathak *et al*. 2014, Pandey *et al.* 2016).

RESULT AND DISCUSSION

The extracted and isolated mucilage was evaluated for various parameters like percentage yield of mucilage was found to be 9.54% w/w, Organoleptic properties and Physicochemical parameters: solubility, moisture, pH, colour, oduor, taste, fracture, and texture were shown in table 1. The chemical profiling of mucilage of *Hibiscus cannabinus* L. after hydrolysis with the help of GC-MS analysis showed number of peaks out of which five highest characteristic peaks were taken in to consideration. The various peaks in the chromatogram showed various compounds of sugar with a retention time (RT), percentage area of the peaks and it was compared along with the database spectrum of known components stored in the GC-MS library. The retention time (RT) was found to be 37.39 min, 25.42 min, 21.82 min, 41.59 min, 27.56 min indicating the presence of Sucrose, D-glucose, D-fructose, Maltose, D-xylopyronose respectively as shown in table 3 and figure 3. The mucilage sample peaks were also compared to the standard peaks of the sugar shown in tables 2, 3 and figures 2, 3.

Table 2. GC-MS standards of sugar.

S.N.	Retention Time	Peak Area	Peak Height	Peak width	Area %
1	22.90	121630590	264616	0.22	0.02
2	25.59	1528278979	91677442	1.12	14.20
3	26.44	942687997	74970078	0.92	8.76
4	27.46	2693765449	267116185	0.35	25.03
5	27.81	2519904925	247869616	0.51	23.42
6	37.33	89264436	8978875	0.51	2.83
6	38.08	78649343	64592351	0.98	7.11
7	40.79	520419979	31499910	1.20	4.84

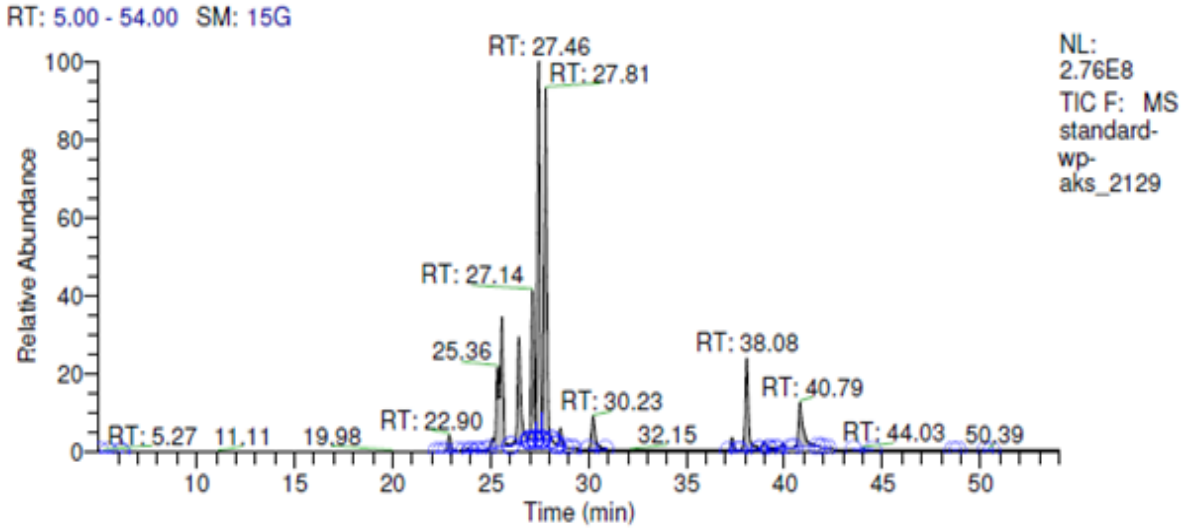

Figure 2. GC-MS chromatogram of standard of sugars showing peaks and retention time indicating the presence of sugar.

Table 3. Sugar present in mucilage of *Hibiscus cannabinus* L.(Deccan Hemp).

S.N.	Retention Time	Peak Area	Peak Height	Peak Width	Area %
1	37.41	12805627845	1277851121	1.08	23.45
2	25.42	14032948788	903750351	0.90	25.70
3	27.56	10973965269	777829934	0.84	20.10
4	24.89	4277049054	436178303	0.43	7.83
5	41.59	3647753787	371474985	0.88	6.68
6	21.82	44313511	2800628	0.47	0.08

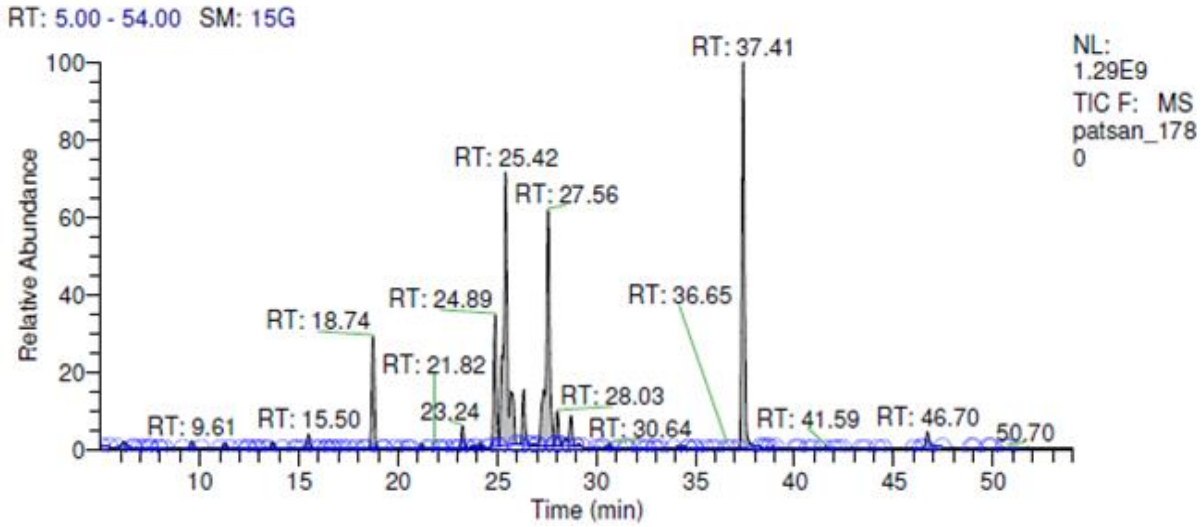

Figure 3. GC-MS chromatogram of *Hibiscus cannabinus* L., mucilage showing peaks and retention time indicating the presence of sugar

CONCLUSION

The obtained mucilage was as efficient as the known ones, so, Deccan hemp was selected for the extraction and isolation of mucilage from natural sources. Inferred Studies that Deccan hemp is an economically important plant with enough mucilage content of high functional value to be used as bio-polymers in pharmaceutical formulations like binding agent super disintegrates and coating material that can be used as in place of currently marketed synthetic polymer and provide a new way to future technologies in Novel Drug Delivery System.

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