Physico-chemical properties and protein profiling of gum exudates of *Acacia nilotica* collected from different agro-climatic zones in India

Siddiqui Mahtab Zakira[1]*, Thamilarasi K[1]. and Singh Bhoj Raj[2]
1.Processing and Product Development Division, Indian Institute of Natural Resins and Gums, Namkum, Ranchi (Jharkhand), INDIA
2.Centre for Animal Disease Research and Diagnosis, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.), INDIA
*mzs_2009@rediffmail.com

Abstract
Physico-chemical properties and protein profiling of seventeen Acacia nilotica (babool) gum exudates collected from ten Indian States covering five agro-climatic zones of India were studied to find out the intra-specific variations in their moisture level, total color difference (ΔE), yield %, ash content, elemental level, tannin content (mg/g), specific rotation [α], swelling index (%, v/v), viscosity and FT-IR. All the gum exudates showed Newtonian behavior with viscosity being shear rate independent. Gums were thermally characterized using differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA) and differential thermal analysis (DTA). DSC thermogram of gum samples showed presence of a broad endothermic peak at around 90-130 ^{0}C and occurrence of glass transition temperature in the range of 60.82-68.62 ^{0}C.

TGA analysis showed three phases of weight loss indicating that the gum is thermally relatively stable. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of gum samples showed presence of mainly low molecular weight proteins. The observed intra-specific variations in the properties of Acacia nilotica gum exudates collected from different agro-climatic zones of India are presumably due to the effects of difference in their geographical locations, edaphic conditions, climatic factors, botanical sources, season and the age of the tree etc.

Keywords: *Acacia nilotica*, babool, physico-chemical properties, SDS-PAGE, DSC, TGA/DTA.

Introduction

The advantages of natural gums over their synthetic counterparts are their bio-compatibility, low-cost, low toxicity, eco-friendliness and relatively widespread availability[1-3]. The industrial uses/applications of *Acacia* gums, in particular, have been widely reported notably in food, pharmaceutical, adhesive, cosmetic, textile, paint and print industries wherein these are used as food additives, dietary fibres, binders, thickeners, stabilizers, emulsifiers, suspending and surface coating agents, gelling agents etc.[4,5] Physico-chemical properties are the measurable physical and chemical characteristics by which the interaction with other systems takes place and they also collectively determine the quality, suitability, applicability and their end-uses. In gums, these properties are directly influenced by the botanical type, age, location, nature of the soil and the climatic conditions around the resource gum tree. Gums from different species exhibit characteristics that are intrinsically different. Even within the same species, different varieties produce gums with different characteristics. Besides botanical source, the season of collection, harvesting and post-harvest handling also affect the quality. Therefore, physico-chemical characterization of gums is an essential step for establishing their suitability for industrial applications.

Anti-cancer and anti-mutagenic properties of *Acacia nilotica* (Linn.) gum, flower and leaf aqueous extracts on 7, 12- di-methylbenz(a)anthracene (DMBA) induced skin papillomagenesis in Swiss Albino mice have been reported by Meena et al[6] in 2006. Tiwari and Jindal[7] have reported studies on uronic acid materials and structure of *Acacia decurrens* gum polysaccharide in 2010.

Physico-chemical characterization of *Acacia sieberiana* gum has been reported by Oyi et al[8] in 2010. Studies on some physico-chemical properties of the plant gum exudates of *Acacia senegal* (Dakwara), *Acacia sieberiana* (Farar Kaya) and *Acacia nilotica (*Bagaruwa) of Batagarawa, Katsina State, Nigeria have been reported by Yusuf[9] in 2011. The advantages of electrophoresis over gel filtration chromatography for *Acacia* gums analysis have been reported by Safa et al[10] in 2006. *In vivo* and *in vitro* comparative study of protein level in *Acacia nilotica*'s callus, seed, leaf and stem by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been reported by Kshipra and Amla[11] in 2011. They have reported that the protein content in callus was higher than the seed followed by leaf and stem with several protein bands of molecular weight 54.3, 44.1, 42.7, 40.1, 35.6, 31.2, 28.6, 24.7 and 19.5 kDa. The intensity of protein bands was higher in *in vitro* sample compared to *in vivo* samples.

However, out of over 1380 known species of *Acacia* distributed throughout the tropical and sub-tropical regions of

the world, most of the research work on *Acacia* gums has been carried out on *Acacia senegal* and to a lesser extent on *Acacia seyal*, the other *Acacia* gums have received very little attention. The present study reports the intra-specific variation of physico-chemical properties and protein profiling in gum exudates of *Acacia nilotica* (babool) collected from different agro-climatic zones in India.

Material and Methods

Chemicals: All the chemicals, reagents and solvents used for physico-chemical characterization of *Acacia nilotica* (*A. nilotica*) gum exudates were of analytical grade and purchased from Rankem, New Delhi and Merck India Ltd., Mumbai, India. For protein profiling by SDS-PAGE, Lowry's kit (Bangalore Genei), SDS-MES buffer (Sigma), protease inhibitor (Amresco), protein loading dye (HiMedia), protein marker (Fermentas) and silver stain kit for proteins (Sigma) were used during the present study.

The details of seventeen *A. nilotica* gum exudates collected from ten Indian States covering five agro-climatic zones are given in table 1. Planning Commission has identified 15 resource development regions in the country, 14 in the main land and remaining one in the islands of Bay of Bengal and Arabian Sea. The period of collection of all the gum exudates is the same.

Purification of gum exudates: The gum exudates after manual cleaning and sorting were reduced in size using porcelain pestle and mortar and purified adopting the method of Karawya et al[12] with slight modification. Gum (25.0 gm) was dissolved in 50.0 ml distilled water, stirred and left for 24 hrs and then filtered through 25 µm linen cloth. The gum was precipitated from the filtrate by adding 95% ethanol (gum:ethanol 1:6) with stirring and the precipitate was washed several times with 95% ethanol until the gum precipitate crumbled. It was defatted with diethyl ether dried in an oven at 40 ^{0}C for 48 hrs to have a constant weight (yield, 19.7 gm; 78.8%). The yield % of all the gums has been presented in table 2.

Physico-chemical characterization: Physico-chemical characterization of *A. nilotica* gum samples *viz.* solubility, pH, moisture level, total color difference (ΔE), yield %, ash content, elemental (CHN) level, tannin content (mg/g), specific rotation [α], swelling index (%, v/v), viscosity and FT-IR was studied. Gums were thermally characterized using differential scanning calorimetry (DSC), thermo-gravimetric analysis (TGA) and differential thermal analysis (DTA) following standard procedures. Moisture level or loss on drying was determined according to the method described by Association of Official Analytical Chemists[13]. Ash content (total ash) was determined following the method of Ohwoavworhua et al[14] while the method of Bowen et al[15] was adopted for determining swelling index (%, v/v). Elemental analysis of carbon, hydrogen and nitrogen was carried out using a Euro EA Elemental Analyzer. Specific rotation [α] of 0.2 % aqueous gum solution was determined using Rudolph Research Analytical Autopol 1, USA, equipped with a sodium lamp and a cell of 10 cm path length at 589 nm. Tannin (mg/g) in the gum samples was determined using Folin-Ciocalteu method by plotting the standard curve of tannic acid. Digital Brookfield viscometer (Model: LVDV-II+Pro) was used for determination of viscosity. It measures the torque required to rotate an immersed spindle in a fluid.

The instrument features a rotating spindle with multiple speed transmission and interchangeable spindles that measure a variety of viscosity ranges. Viscosity as a function of concentration (10, 20 and 40 %) of *A. nilotica* gum exudates was determined with varying speed of rotations (10, 20, 50 and 100 rpm) to determine its effect on the viscosity values as the drag force is known to alter with changes on the spindle size and rotational speed. For each concentration and at each rotational speed, three measurements were taken. FT-IR spectra of gum samples were recorded in Shimadzu, IR Prestige-21 in the range 4000 to 500 cm^{-1}.

Thermal properties: Thermal properties of gum samples were characterized by using a Q20-TA DSC. Nitrogen at the rate of 50 ml/min was used as purge gas. Five milligram of powdered gum sample was sealed in an aluminium pan and heated up to 300 ^{0}C @10 ^{0}C/min followed by cooling cycle at the same rate. TGA/DTA study was done by Shimadzu, Japan, DTG-60.

Protein profiling: SDS-PAGE with 2-[N-Morpholino] ethane sulfonic acid (MES) buffer following silver staining was employed for the analysis of the proteins in *A. nilotica* gum samples using 12% resolving gel and 5% stacking gel of 1.0 mm thickness. The gum solutions were prepared by dissolving 40.0 mg of gum in 1.0 ml of PBS supplemented with cocktail protease inhibitor. Gum solution was mixed with 5X reducing protein loading dye and denatured at 95 °C for 5 min before loading on the gel.

Gels were loaded with 70.0 µl of a 4% gum solution. Protein marker was also run to estimate the size of the protein bands. The gels were run at 100 V till resolving and then at 120 V constant voltage till the bromophenol blue stain reached the bottom of the gel. The gel was stained with silver stain kit for proteins following manufacturer's instructions. The gel was documented after completion of reaction.

Results

The *A. nilotica* gum exudates numbering 17 collected from ten Indian States covering five agro-climatic zones (Table 1) were light brown to dark brown in color and found to be water soluble at room temperature to form viscous solutions, pH ranged from 4.21-4.70 indicating lack of cross linked polymeric chains. Normally gums having cross linked polymeric chains only swell in water without dissolving[16]. The highest moisture % was found in the exudates collected from Amritsar (Punjab) falling in the Trans Gangetic plain region and the lowest in the exudates collected from Sitapur (Uttar Pradesh) in Upper Gangetic plain region.

The highest total color difference (ΔE) was found in the exudates of IINRG Farm, Ranchi, falling in the Eastern plateau and hills region and the lowest in the case of collection from Amritsar (Punjab). The highest ash % (1.66) was found in collection from Jodhpur (Rajasthan) and the lowest (0.68 %) from Gondia (Maharashtra). Swelling index (%, v/v) of all the gum exudates was determined in distilled water and none of them showed any swelling property.

The quantitative elemental analysis showed that the gum contained low percentage of nitrogen indicating amino acid cross linkage as also the ratio of carbon to hydrogen which was over 8:1 showing polysaccharide composition. The highest tannin content (14.02 mg/g) was found in the gum exudates collected from Bilaspur (Chhattisgarh) and the lowest (0.30 mg/g) in the gum exudates collected from Mayurbhanj (Odisha).

All the gum exudates were found to be dextrorotatory, values ranged from $+44.57^0$ to $+80.98^0$ (Table 2). The observed variations in their specific rotation values may be indicative of the differences in interglycosidic linkages. All the gum exudates showed Newtonian behavior with viscosity being shear rate independent and concentration dependent, as viscosity of 40.0 % solution of gum exudates from Bilaspur (Chhattisgarh) displayed highest cP value (284.9) in comparison to 10.0 % solution (48.8) using spindle number LV-2 at 27.5 ^{0}C (Fig. 1 a and b).

The FT-IR spectra exhibiting strong vibrational mode located at 3000-3600 cm^{-1} are assigned to the stretching vibrations of the O-H bond, the other strong vibrational mode located at 1600 cm^{-1} is assigned to the stretching vibrations of the C=O bond of carboxylate group. The two vibrational modes located at 1076 and 1423 cm^{-1}, with relatively low intensity, are assigned to the stretching vibrations of the C-O bond and the weak vibrational mode located at 2900 cm^{-1} is assigned to the stretching vibrations of the C-H bond. The absorption band located at 2364 cm^{-1}, with relatively low intensity, is usually assigned to the CO_2 vibration[17] (Fig. 2).

Discussion

The ash % i.e. total ash is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. The lowest value of total ash recorded in the gum sample collected from Gondia indicates low levels of carbonates, phosphates, silicates and silica. Almost all the gum samples have ash % within the prescribed limit[18]. Increase in viscosity with concentration may be due to increasing number of high molecular weight polymeric chains of the gums per unit volume and increased interaction between these polymeric chains in aqueous solution, responsible for increase in cohesive density and therefore, greater resistance to flow.

There was also a gradual decrease in viscosity of gum exudates as temperature was increased. Structural and functional group differences in polysaccharide gums influencing the thermal behavior and affecting the transition temperature are generally studied by TGA/DTA and DSC. During the thermal processing, generally dehydration, depolymerization and pyrolitic decomposition are involved in these high temperature stages resulting in the formation of H_2O, CO_2 and CH_4. However, due to the differences in structures and the functional groups, either the degradation routes or the resulting fragments will be different. Most of the polysaccharides comprise of carboxylate or carboxylic acid functional groups. Therefore, the thermal scission of the carboxylate groups and evolution of CO_2 from the corresponding carbohydrate backbone may be a probable mechanism for the thermal transitions[19].

TGA/DTA thermogram of babool gum sample revealed that the gum is relatively stable up to 220 ^{0}C, beyond which degradation starts. In the first zone, the early minor weight loss (18.30%) can be attributed to the desorption of water molecule. In the second zone i.e. the degradation zone at 220-375^{0}C, where a major weight loss (48.87%) was observed, can be attributed to decomposition of gum. In the last zone at 375-670 ^{0}C, the weight loss was observed to be 32.47% indicating that the gum is thermally stable (Fig. 3). DSC thermograms of gum samples indicated presence of a broad endothermic peak at around 90-130 ^{0}C which may be attributed to moisture sorption and occurrence of glass transition (Tg) temperature in the range of 60.82-68.62 ^{0}C. Degradation of gum starts at around 220 ^{0}C which matches with the TGA results of the gum samples. The DSC thermogram of babool gum is shown in fig. 4.

SDS-PAGE, the most reliable and widely used method to estimate the variability in protein fractions at genetic level, was performed in order to find out the banding pattern of the gel using total protein[20]. The highest number of protein bands, ranging from 94 kDa to 19 kDa, were observed in sample 1 and 2 collected from Bareilly and Sitapur (Uttar

Pradesh) falling in Upper Gangetic plain region and sample 6 collected from Amritsar (Punjab) falling in Trans Gangetic plain region. Protein band of molecular weight 37 kDa was found to be present in all the gum samples with slight differences in their intensity and mobility. Protein profile further indicated variability on the basis of presence or absence and intensities of protein bands with banding pattern (Fig. 5). The observed qualitative and quantitative intra-specific variations in the physico-chemical properties and protein profiling of *A. nilotica* gum exudates collected from different agro-climatic zones in India may be attributed to their different geographical locations, edaphic conditions, climatic factors, botanical sources, season and the age of the tree etc.

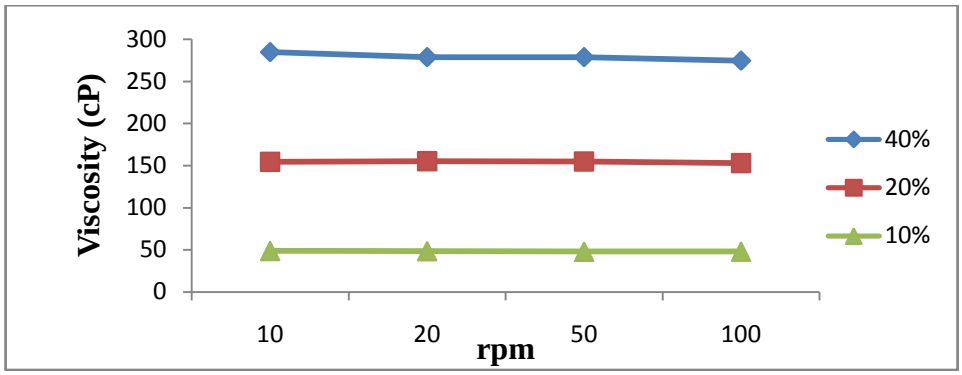

Fig. 1a: Viscosity as a function of concentration of *Acacia nilotica* gum exudates collected from Bilaspur

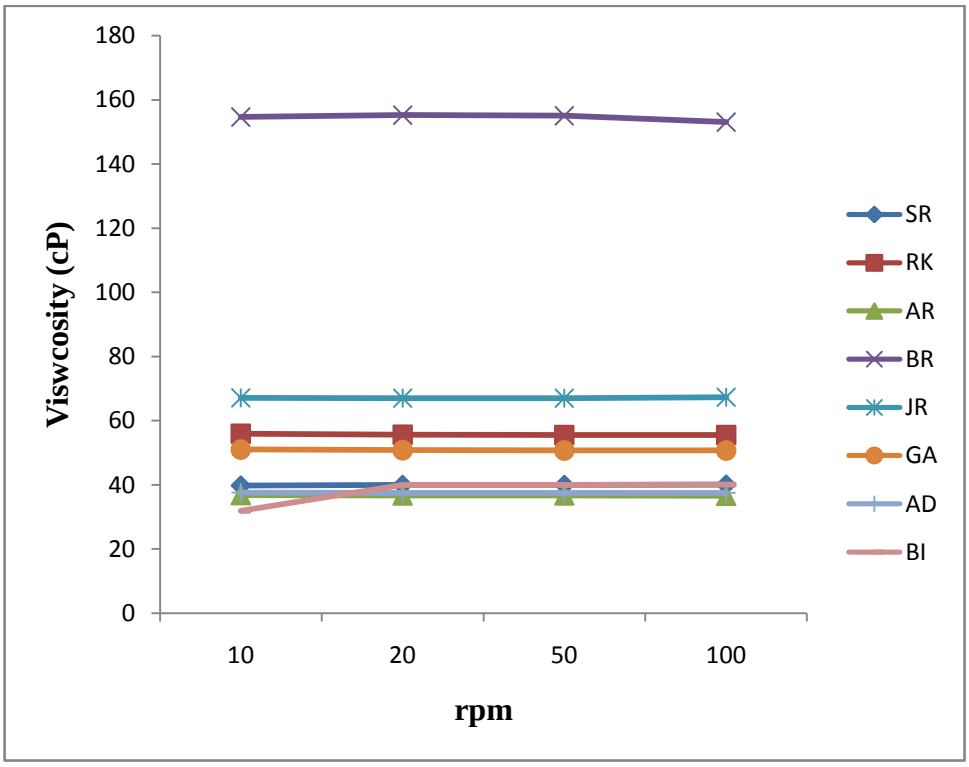

Fig. 1b: Viscosity of 20.0 % solution of *Acacia nilotica* gum exudates collected from different places

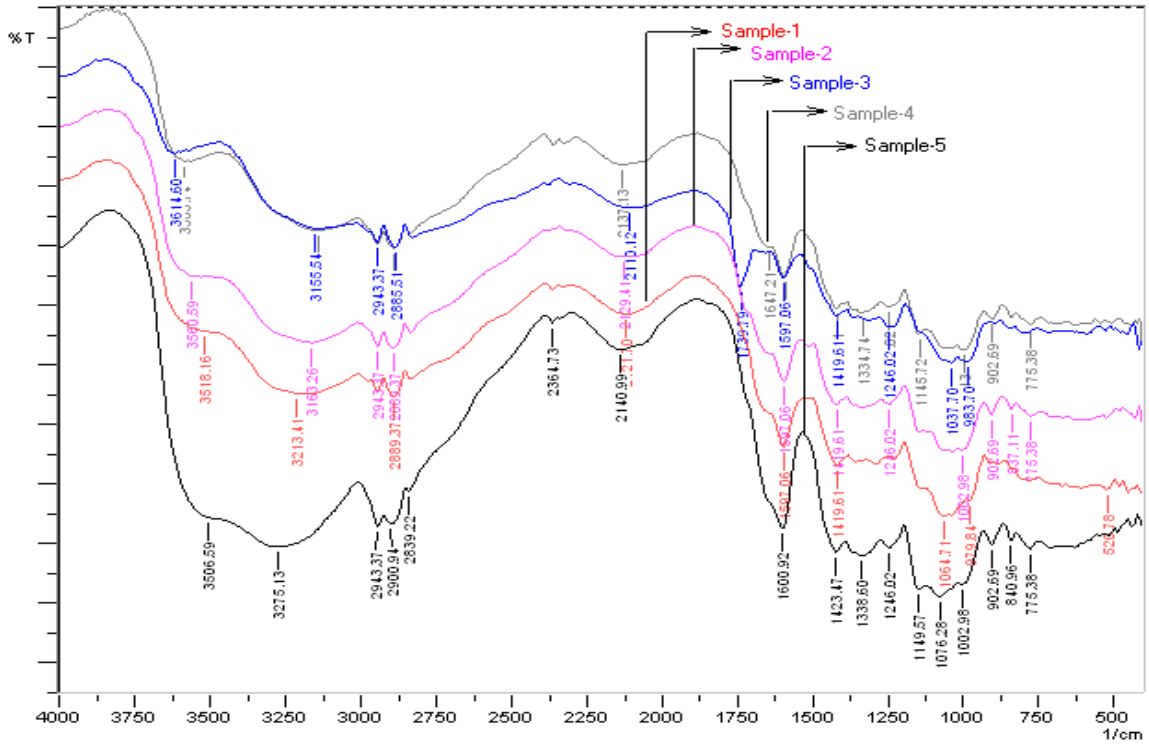

Fig. 2: FT-IR of *Acacia nilotica* gum collected from 1. Jodhpur (Rajasthan), 2. IINRG Farm, Ranchi (Jharkhand), 3. Sitapur (Uttar Pradesh), 4. Bundi (Rajasthan) and 5. Gondia (Maharashtra)

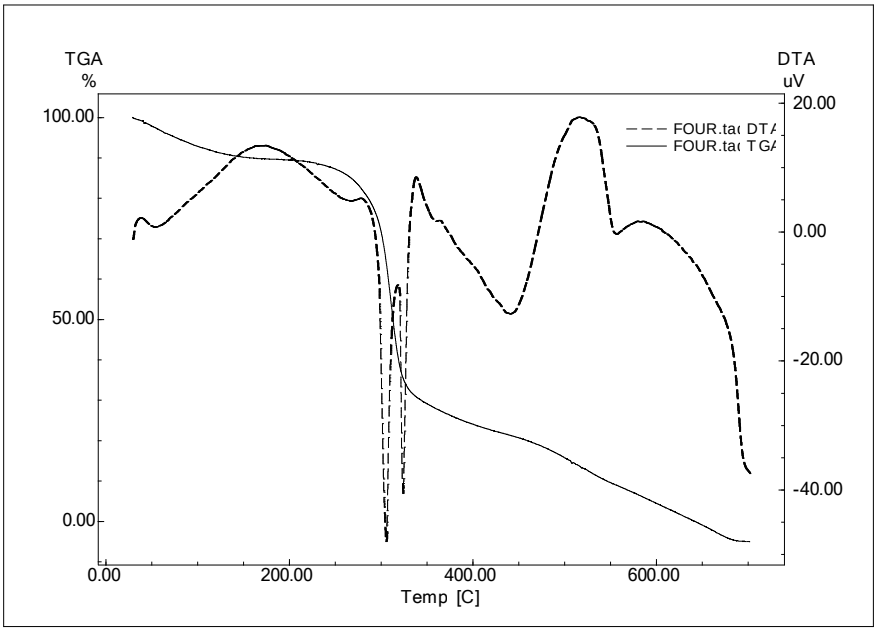

Fig. 3: TGA/DTA thermogram of *Acacia nilotica* gum collected from Bundi (Rajasthan)

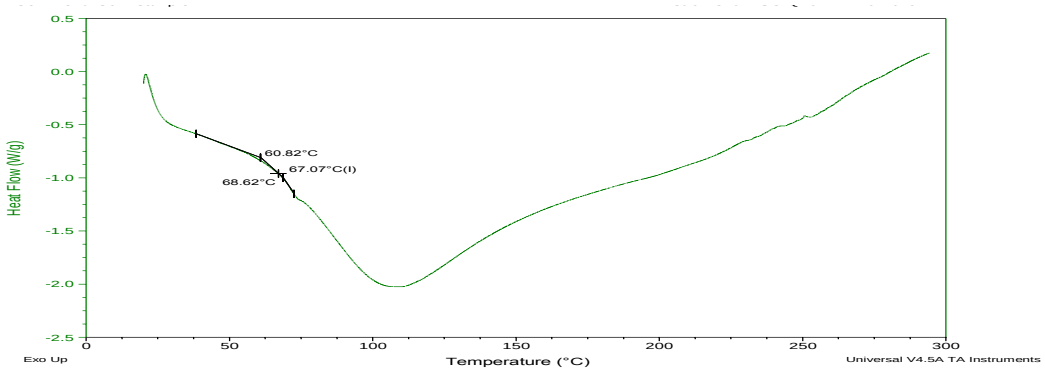

Fig. 4: DSC thermogram of *Acacia nilotica* gum collected from Bundi (Rajasthan)

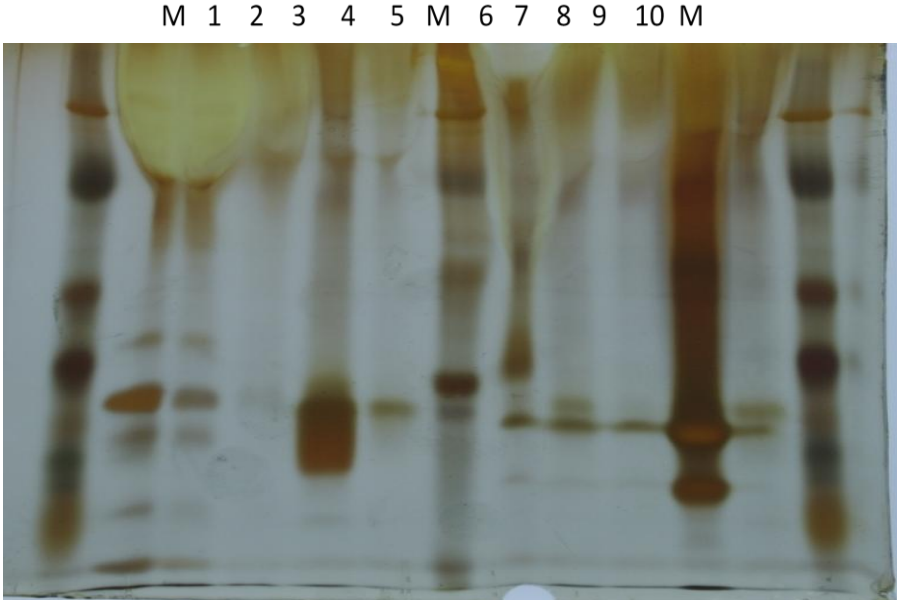

Fig. 5: 12% SDS-PAGE of *Acacia nilotica* gum samples followed by silver staining (In M, from top 1st band-120 kDa, 2-94 kDa, 3-47 kDa, 4-37 kDa, 5-28 kDa and 6-19kDa)

Table 1
***Acacia nilotica* gum exudates collected from different agro-climatic zones**

Agro-climatic zones	Districts/States
Upper Gangetic plain region	Bareilly, Sitapur (Uttar Pradesh).
Trans Gangetic plain region	Hisar, Karnal, Rohtak (Haryana); Amritsar (Punjab).
Eastern plateau and hills region	Bilaspur (Chhattisgarh); Ranchi (Jharkhand); Balaghat, Jabalpur (Madhya Pradesh); Gondia (Maharashtra); Mayurbhanj (Odisha).
Gujarat plains and hills region	Baroda, Anand (Gujarat).
Western dry region	Bundi, Jaipur, Jodhpur (Rajasthan).

Table 2
Physico-chemical characteristics of *Acacia nilotica* gum exudates collected from different agro-climatic zones
BY, Bareilly; SR, Sitapur; HR, Hisar; KL, Karnal; RK, Rohtak; AR, Amritsar; BR, Bilaspur; RI, Ranchi; BT, Balaghat; JR, Jabalpur; GA, Gondia; MJ, Mayurbhanj; AD, Anand; BA, Baroda; BI, Bundi; JR 1, Jaipur and JR 2, Jodhpur

Parameters / Zones	Upper Gangetic plain region		Trans Gangetic plain region				Eastern plateau and hills region						Gujarat plains and hills region		Western dry region		
	BY	**SR**	**HR**	**KL**	**RK**	**AR**	**BR**	**RI**	**BT**	**JR**	**GA**	**MJ**	**AD**	**BA**	**BI**	**JR 1**	**JR 2**
Moisture level (%)	2.44	2.21	2.97	4.84	3.01	6.33	6.27	4.52	3.40	3.17	5.75	6.27	4.60	4.26	3.45	3.31	2.47
ΔE	5.32	6.06	17.93	5.95	7.55	4.99	5.65	29.36	9.01	8.14	8.78	6.97	14.33	16.80	5.28	5.34	4.38
Yield %	78.8	80.3	76.9	76.2	70.5	78.3	80.3	65.0	78.6	80.9	81.0	66.1	66.8	65.3	73.2	69.9	68.2
C % H % N %	44.25 4.02 0.09	41.49 5.00 0.25	48.47 4.70 0.11	44.55 4.11 0.09	34.22 3.76 0.16	43.10 5.49 0.20	42.60 7.73 0.22	42.22 5.72 0.12	44.10 6.08 0.16	32.50 5.26 1.06	38.00 3.61 1.26	44.27 5.25 0.21	43.97 4.30 1.56	40.94 4.61 0.55	32.80 4.94 1.40	30.06 5.24 0.40	38.80 4.91 1.49
Ash content (%)	1.22	1.17	1.06	1.58	1.18	1.61	1.49	1.19	0.69	1.15	0.68	1.22	1.39	1.42	1.38	1.07	1.66
Tannin (mg/g)	0.47	0.38	5.78	6.09	12.54	1.05	14.02	0.95	12.25	10.36	1.16	0.30	0.69	0.81	0.39	0.33	0.35
Specific rotation [α] in H_2O, deg	+ 53.51	+ 56.55	+ 53.13	+ 80.02	+ 80.07	+ 44.57	+ 80.18	+ 68.38	+ 56.40	+ 80.98	+ 79.32	+ 78.28	+ 80.29	+ 80.21	+ 73.77	+ 70.24	+ 78.05

Acknowledgement
The authors are grateful to Dr. R. Ramani, Director, Indian Institute of Natural Resins and Gums, Ranchi (Jharkhand) for constant encouragement and strong support. The technical assistance rendered by Sri D.D. Singh, Ex-Chief Technical Officer, in determining physico-chemical parameters is thankfully acknowledged.

References
1. Ogaji I. and Okafor I.S., Potential of Grewia gum as film coating agent: some physico-chemical properties of coated praziquantel tablets, *Int. J. Pharmaceu. Res.*, **3**, 16 **(2011)**

2. Nep E.I. and Conway B.R., Characterization of Grewia gum, a potential pharmaceutical excipient, *J. Excipients and Food Chem.*, **1**, 30 **(2010)**

3. Emeje M., Nwabunike P., Isimi C., Fortunate J., Mitchell J.W., Byrn S., Kunle O. and Ofoefule O., Isolation, characterization and formulation properties of a new plant gum obtained from *Cissus efescence*, *Int. J. Green Pharmcy*, **3**, 16 **(2009)**

4. Yaseen E.I., Herald T.J., Aramouni F.M. and Alavi S., Rheological properties of selected gum solutions, *Food Res. Int.*, **38**, 111 **(2005)**

5. Kapoor V.P., Farooqi M.I.H., Travel F.R. and Joseleau J.P., Studies on *Acacia nilotica* gum exudates. Structural variation due to different habitats, *Carbohydr. Res.*, **222**, 289 **(1991)**

6. Meena P.D., Kaushik P., Shukla S., Soni A.K., Kumar M. and Kumar A., Anti-cancer and anti-mutagenic properties of *Acacia nilotica* (Linn.) on 7,12-dimethylbenz(a)anthracene- induced skin papillomagenesis in Swiss Albino mice, *Asian Pacific J. Cancer Prevention*, **7**, 627 **(2006)**

7. Tewari A. and Jindal V.K., Studies on uronic acid materials and structure of *Acacia decurrens* gum polysaccharide, *J. Chem. Pharmaceu. Res.*, **2**, 233 **(2010)**

8. Oyi A.R., Shittu A.O. and Mahmud H.S., Physico-chemical characterization of *Acacia sieberiania* gum, *Ind. J. Novel Drug Delivery*, **2**, 99 **(2010)**

9. Yusuf A.K., Studies on some physico-chemical properties of the plant gum exudates of *Acacia senegal* (Dakwara), *Acacia sieberiana* (Farar Kaya) and *Acacia nilotica* (Bagaruwa*), JORIND* **9**,10 **(2011)**

10. Safa M., Patrick R., Karamallah K.A. and Ma Q., The analysis of Acacia gums using electrophoresis, *Food Hydrocolloids*, **20**, 848 **(2006)**

11. Kshipra D. and Amla B., *In vivo* and *in vitro* protein profiling in *Acacia nilotica* (L.), A nitrogen fixing tree, *African J. Microbiol. Res.*, **5**, 2793 **(2011)**

12. Karawya M.S., Balba S.I. and Afofi M.S.A., Investigation of the carbohydrate content of certain mucilaginous plants, *Planta Medica*, **20**,14 **(1971)**

13. Anonymous, Official methods of analysis of the Association of Official Analytical Chemists, 17th ed., Washington DC **(1997)**

14. Ohwoavworhua F.O. and Adelakun T. A., Some physical characteristics of microcrystalline cellulose obtained from raw cotton of *Cochlospermun planchonii*, *Tropical J. Pharmaceu. Res.*, **4**, 1 **(2005)**

15. Bowen F.E. and Vadino W.A., A simple method for differentiating sources, *Drug Deve. Ind. Pharmacy*, **10**, 505 **(1984)**

16. Remington G.R.G., The Science and Practice of Pharmacy, 20th Ed., Lippincott Williams and Wilkins, US **(2000)**

17. Wang Q., Ellis P.R. and Ross-Murphy S.B., Dissolution kinetics of guar gum powders 2, Effects of concentration and molecular weight, *Carbohydr. Poly.*, **53**, 75 **(2003)**

18. FAO, Compendium of food additive specifications addendum 7, Food and nutrition paper No. 52 **(1998)**

19. Vendruscolo C.W. et al, Physico-chemical and mechanical characterization of galactomannan from *Mimosa scabrella*, Effect of drying method, *Carbohydr. Poly.*, **76**, 86 **(2009)**

20. Laemmli U.K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, **227**, 680 **(1970)**.

(Received 15th September 2014, accepted 19th December 2014)