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Physicochemical Characterization of *Combretum glutinosum* (Habeil) Gum

M. H. Awad*, Nouri M. A. Soleiman
Al-Mergheb University, College of Arts and Sciences - Kasr Khiar – Libya

***Corresponding Author:**
M. H. Awad
Email: muhash.co1@gmail.com

Abstract: Twenty seven authentic samples of Gum Habeil were collected from bark of *Combretum glutinosum* trees in Blue Nile State - Sudan. Various physicochemical methods, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FT-IR) were applied to characterize this type of gum. Physicochemical analysis exhibited that the average values of Moisture, Ash, pH, Water Holding Capacity, Refractive index, equivalent conductivity, acid equivalent weight , total uronic acid of the gum , Nitrogen and Protein content are 9.88%, 4.32 %, 4.79, 63.03 %, 1.29 , 354.11$sg^{-1}cm^2$, 1291.71, 15.54 %, 0.198 % and 1.316 % respectively. Intrinsic viscosity of freeze dried soluble part ranged from 0.630 to 0.839 dl / g with an average value of 0.691 dl / g, while Intrinsic viscosity of freeze dried deacetylated gum ranged from 1.09 to 1.390 with an average value 1.33 dl / g respectively. Gum Habeil contained a relatively high quantity of calcium, and magnesium, however, unexpected low quantity of sodium ion ($3.53x10^{-3}$ % w/w) was detected. For the rest of the elements Co, Ni, Cr, Zn, Mn, Cu and Fe are of trace values. SEM analysis suggested that the crude gum has irregular particle size, while the soluble part fraction and deacetylated gum were found to be fibrilar. The glass transition temperature of the gum were observed from 30.43 to 46.54 ^oC by DSC. The essential functional groups such as (–OH), (CH_3CO–), (–COO–), and (CH_3–) were recorded at 3410, 1740, 1634, and 1443 cm^{-1} respectively.
Keywords: Combretum glutinosum, Gum Habeil, Morphological and SEM.

INTRODUCTION

Combretum glutinosum, a tree that exudates gum, is widespread all over West Africa and extends to Sudan [1]. *Combretum glutinosum* gum locally known as Gum Habeil [2], which belonged to the *Combretaceae* family [2, 3]. Species from the genus *Combretum* and to a lesser extent *Terminalia* are most widely used for medicinal purposes [4]. Gum Habeil could be fractionated to water soluble part, and gel fraction. Although the gel fraction contains a higher proportion of calcium ion (about 62.23% of total calcium in gum), removal of Calcium ions increases the gum solubility [5]. The objectives of this work to investigate the physicochemical, effect of salt concentration on gum viscosity, minerals composition, structure, and morphological properties of this type of gum, in order to pave the way for its industrial applications. The author 2015 revealed that, the fractionation of Gum Habeil samples exhibited a water soluble part 30 to 40 % w/w, and gel fraction 70 to 60% w/w. Also the author 2015 reported that, By Fedors equation, the intrinsic viscosity values of freeze dried deacetylated gam, and freeze dried soluble part in distilled water equal 2.439, and 2.0741 dl /g respectively. while the salt tolerance parameter (S) of Gum Habeil ranged from 0.083 to 0.101 dl × $M^{1/2}$ / g, and stiffness parameter (B) of gum ranged from 0.0574 to 0.0700 [5]. The author 2013 reported that, the weight average molecular weight of AGP component of gum equal 4.456×10^6, 8.611×10^5, and 1.528×10^6 g / mol., and radius of gyration (Rg) equal 30.03, 72.16, and 81.3 nm of the freeze dried soluble part, freeze dried deacetylated gum , and freeze dried calcium- free gum respectively. Freeze dried soluble part gum shown a highly branched compact chain backbone (low radius of gyration) with higher weight average molecular weight than the modified gum samples "deacetylated and Calcium-free gums" [6].

Sample location and pre-treatment

Gum Habeil samples were collected from forest of the Blue Nile state-Sudan. Gum samples were cleaned from impurities such as bark and sand, then-ground using mechanical blender and sieved using (mesh size: 250µm) [7].

Physicochemical Methods

Standard methods of analysis to determine the physicochemical properties of Gum Habeil were used [8].

Preparation of Deacetylated, and Soluble Part Gum Solutions

Standard methods were used to prepare soluble part, and deacetylated gum solutions [9, 10].

Scanning electron microscopy measurement

SEM photographs of crude, freeze dried soluble part, and freeze dried deacetylated gum were recorded using SEM (JSM-35, No IEP35-3, Jeol Ltd-Tokyo-Japan). Samples were mounted on an aluminum stub with double-sided adhesive tape. The tape was firmly attached to the stub and the sample powder was scattered carefully over its surface. The stub with the specimen was then sputter coated with a thin layer of gold, the processed specimen was subjected to SEM analysis. A thin spread of sample was made on a sheet of A4 paper. An aluminum specimen stub (0.5 inches diameter) was used - double sided, circular adhesive carbon tape (12 mm diameter) was used - as both sides were adhesive, the tape was stuck onto the circular aluminum stub, holding the 'leg' of the aluminum stub, it was pressed against the gum sample on the A4 paper to get a thin, evenly spread layer of sample on it. The aluminum stub was then placed in the vacuum chamber of the sputter coater - after the gauge indicated a suitable vacuum had been achieved (0.1 to 0.2 torr), and the plasma current was 42 mA, gold coating for 140 seconds was commenced - a purple glow was observed during the process. The stub with gold-coated Gum Habeil was then placed in the SEM chamber which was evacuated before the electron beam was turned on. A 10 kV / 2.05A setting was used for the subsequent imaging, the aperture size being fixed at 3.

FT-IR spectroscopy of gum

FT-IR spectra of freeze dried, soluble part, and deacetylated gum were recorded on a FT-IR spectrometer (Spectrum Rα I advanced operation, Perkin Elmer precisely, USA). Five cm^3 of sample in (0.5% w/v) solvent was placed in a weighed pan, and dried on an oven at 60 ^oC for 48 hours to prepare a thin film, and then FT-IR spectra of samples were obtained.

Differential scanning calorimetry of crude gum

DSC was performed using a differential Scanning Calorimeter (SETARAM instrumentation - micro DSC.III, with SET Soft 2000, USA). Accurately weighed (0.5 g / 10 cm^3 deionized water) samples were placed into platinum cell and sealed and compared with the same amount of DI water in reference cell. The temperature range was set 5 to 95 ^oC at a heating rate of 3^oC / min.

Intrinsic viscosity measurement

A Cannon-Fenske routine glass capillary viscometer (75/N94 or 75/ N 104) equipped in a viscometer bath (C.T-500 Series II, USA) was used to determine intrinsic viscosity of gum solution in 1.0M NaCl according to standard method [5,11].

Viscosity and dynamic Rheology measurement

AR-G550 Rheometer, which included a circulating water bath (Julabo F10, Germany with ±0.1 ^oC accuracy), and cone geometry was used to study the influence of ionic strength on gum viscosity.

RESULTS AND DISCUSSION

Tables 1 and 2 show the analytical data of physicochemical characterization and metal composition of gum samples. The average values of pH, total uronic acid, and equivalent weight equals 4.79, 15.54 , and 1291.71 respectively is in good agreement with gum Arabic parameter reported by Karamalla, while the average values of nitrogen , and protein equals 0.198 %, and 1.31% respectively, these results are lower than gum Arabic parameters reported by Karamalla [12]. The highest value of Ash content 4.32% was observed of Gum Habeil may be attributed to its high amount of calcium ions. Fig.1 (A) and (B) show the values of intrinsic viscosity [η] of freeze dried soluble part, and freeze dried deacetylated gum in 1.0 M NaCl equal 0.691 and, 1.33 dl / g respectively. The result shows that viscosity of Gum Habeil is higher than the viscosity of *Acacia Senegal* gum which was 0.11 dl /g . However deacetylated gum fraction have a high viscosity compared to the soluble part fraction.

Table 1: Analytical data of physicochemical characterization of Gum Habeil

Physicochemical parameters	Mean	Range	Standard deviation
Moisture%	9.88	7.75 - 11.59	1.489
Ash%	4.32	2.94 – 5.35	0.956
pH	4.79	4.63 - 4.91	0.1093
Nitrogen%	0.198	0.14- 0.28	0.065
Protein%	1.31	0.924 – 1.84	0.429
Refractive index	1.29	1.20 - 1.40	0.0678
W.H. Capacity	63.02	58.59- 74.75	5.444
Equivalent weight%	1291.71	1323.20 - 1626.20	23.212
Uronic acid%	15.54	12.72 -19.09	3.053
[η] (dl /g) of soluble part	0.691	0.630 – 0.839	0.004
[η] (dl /g) of deacetylated	1.33	1.09- 1.39	0.02
Mw (Da) of soluble part	3.173×10^6	-----	----
Mw (Da) of deacetylated	5.538×10^5	-----	----

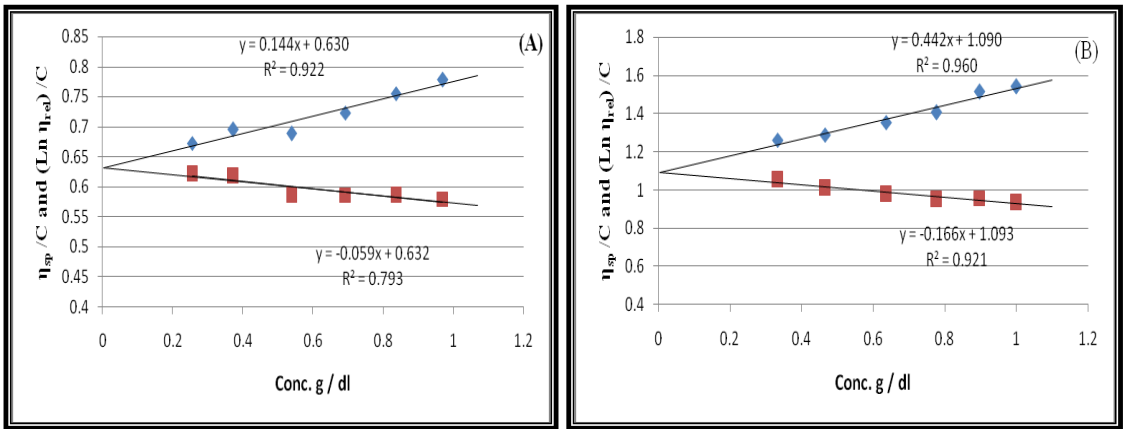

Fig-1: (A) Intrinsic viscosity of soluble part fraction, (B) Intrinsic viscosity of deacetylated gum in 1.0M NaCl at 25 °C

Table 2 shows that the average values of Ca, K, Mg, and Na ions equal 1.29, 0.69, 0.42, and 3.53×10^{-3} % w/w respectively, this indicates that Gum Habeil is salt of calcium, and potassium.

Table 2: Minerals composition of Gum Habeil

Metals % (w/w) In gum samples	Mean	Range	Standard
Ca	1.29	1.23 -1.36	0.044
K	0.69	0.60 - 0.83	0.069
Mg	0.42	0.40 -0.44	0.014
Na $\times10^{-3}$	3.53	3.05 - 3.95	0.321
Co $\times10^{-4}$	4.39	2.00 - 8.50	2.630
Ni$\times 10^{-4}$	2.82	1.05 - 4.90	1.320
Cr $\times10^{-4}$	2.07	1.05 - 4.50	1.000
Zn $\times10^{-4}$	5.41	2.60 - 4.80	1.820
Mn$\times10^{-4}$	3.16	1.00 - 4.35	1.930
Cu $\times10^{-4}$	2.84	1.35 - 3.65	1.160
Fe $\times 10^{-4}$	3.73	1.16 -6.20	1.960

Fig.2 shows the influence of salts on viscosity of dispersion gum solution (0.875% w/v in water and different salts concentration). Viscosity of gum solutions was decreased with the concentration of salt increased, Fig.2 (B) shows that at 2000 mM of calcium chloride (divalent and higher ionic strength) viscosity was increased due to salting out of gum in solution.

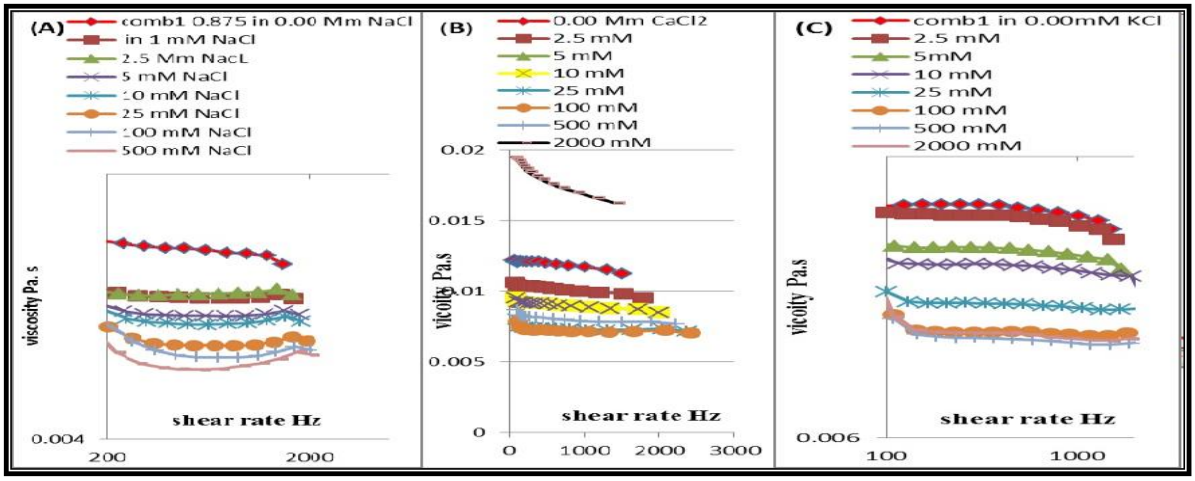

Fig-2: (A, B, & C) Flow curve viscosity vs. shear rate of (0.875 % w/v) gum dispersions at different concentrations of salt (A) NaCl , (B) $CaCl_2$, and (C) KCl at 25°C

Fig.3 shows that the viscosity of the gum solutions at shear rate 100 s^{-1} in specific ionic strength was calculated by using excel interpolation sheet from the viscosity flow curves. The results show that, in salt solutions (μ = 3 x10^{-3} to 11 x10^{-3}), the counter ions of salts shielding the charges on the polymers backbone and disruption of intra-molecular associations, and this decreases the associations between opposite charge on polymer backbone, also that lead to expanding of polymer backbone and increases the viscosity of gum solution with increase ionic strength until equal 11×10^{-3}. In ionic strength ($\mu > 11\times10^{-3}$), viscosity of gum solution was decreased because the access of salts ions eliminated the inter – molecular association between opposite charge in polymer chains, and these actions may lead to a collapsed conformation and decreases the volume of polymer chains and decreases viscosity of gum solution with increase ionic strength . Fig.3A shows that in presence of sodium ions ($\mu > 11\times10^{-3}$) viscosity was decreased by strong slope , while in presence calcium ions ($\mu > 11\times10^{-3}$) viscosity was decreased by sleepy slope Fig.3B, that may be attributed to the sodium ions move higher than calcium ions in aqueous solution because sodium ion have atomic radius and smaller than calcium ion help it to permitted inside the polymer by much number of ions and shielding the charge–charge repulsions and this decreases viscosity.

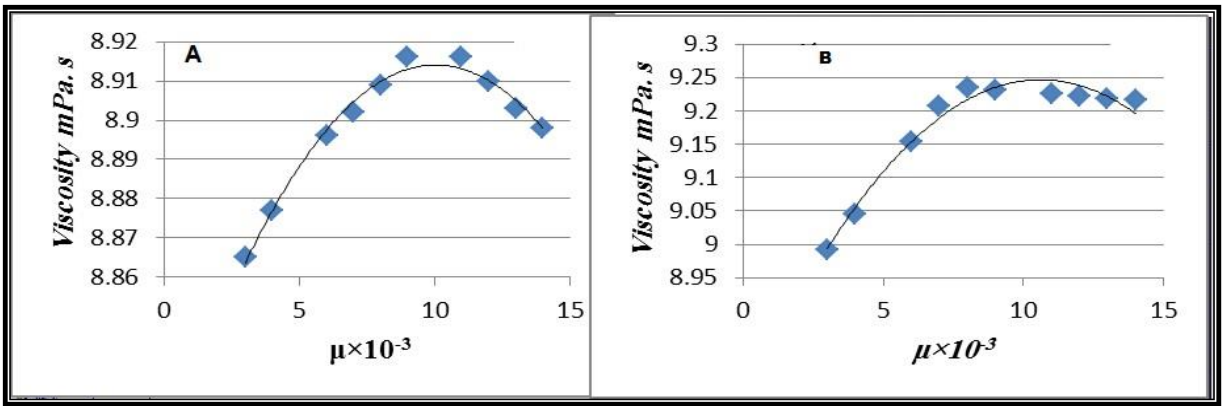

Fig-3: (A) Ionic strength of NaCl, (B) ionic strength of $CaCl_2$ versus gum (0.875 % w/v) viscosity at shear rate 100 Hz and constant temperature 25 ^oC

Scanning electron microphotographs of crude, freeze dried soluble part, and freeze dried deacetylated gum are shown in Fig.4. The SEM Photomicrograph at 120 to 500× magnification and 100 µm scale shows the surface morphology of crude gum has irregular particle size. Deacetylated gum was found to be fibrilar, indicating the loss in particulate morphology that was observed in the soluble part gum, suggesting that acetyl groups are essential in the structural integrity of the gum for particulate appearance in the soluble form. It was reported earlier that particle size and specific surface area influence the hydration behavior of gums, which in turn influence their intrinsic viscosity and molecular mass [13]. The SEM results of crude, freeze dried soluble part, and freeze dried deacetylated gum exhibited that freeze dried, soluble part and deacetylated gum have differences in intrinsic viscosity and in molecular weight value.

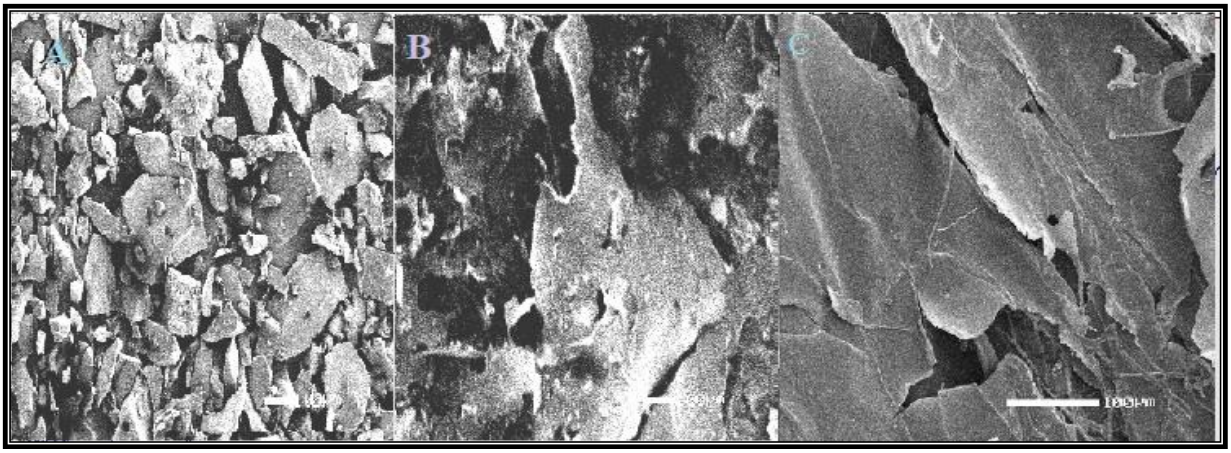

Fig-4: Scanning electron microscopy photomicrograph of, (A) crude gum , (B) freeze dried, soluble part, and (C) freeze dried, deacetylated gum (magnification 500µm, scale bar 50 mm)

Fig.5 and 6 show that the major functional groups present in the FT-IR spectrum of the soluble part fraction (A), and deacetylated gum (B), peak at 1682 cm^{-1} are indicative of acetyl groups. The absorption peaks at 1610 and 1412 cm^{-1} are due to carboxylate groups of uronic acid residues. The absence of the peak at 1740 cm^{-1} in the FT-IR spectrum in Fig.5B showed that deacetylation of the gum with dilute sodium hydroxide solution was completed, while Fig.6 shows that deacetylation by using sodium carbonate doesn't take place.

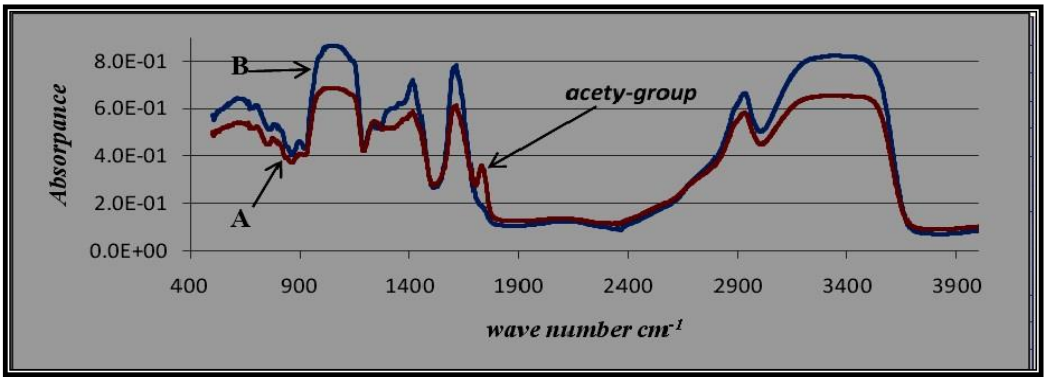

Fig-5: FT-IR spectrum of soluble part fraction (A), and Deacetylated gum (B), indicating the various functional groups

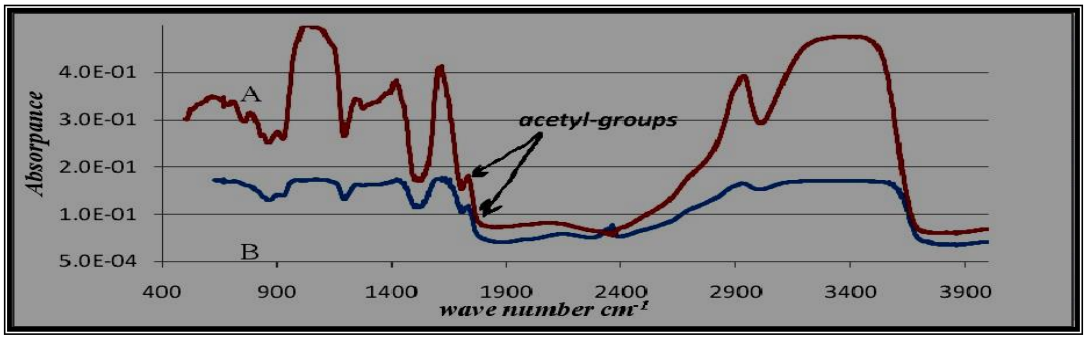

Fig-6: FT-IR spectrum of (A) soluble part fraction, and (B) gum free calcium, indicating the various functional groups

Fig.7 shows heating DSC curves of crude Gum Habeil solutions 5% w/w, at heating rate of 0.5 ^{o}C min^{-1}. The heating (5 – 95^{o}C) curves of gum solutions showed endothermic peak ranged from 30.43 to 46.54 ^{o}C with enthalpies ranged from 0.3332 to 1.393 J/ g respectively, these endothermic peaks is attributed to the helix-coil transition of the gum molecules and the subsequent aggregation of these helices.

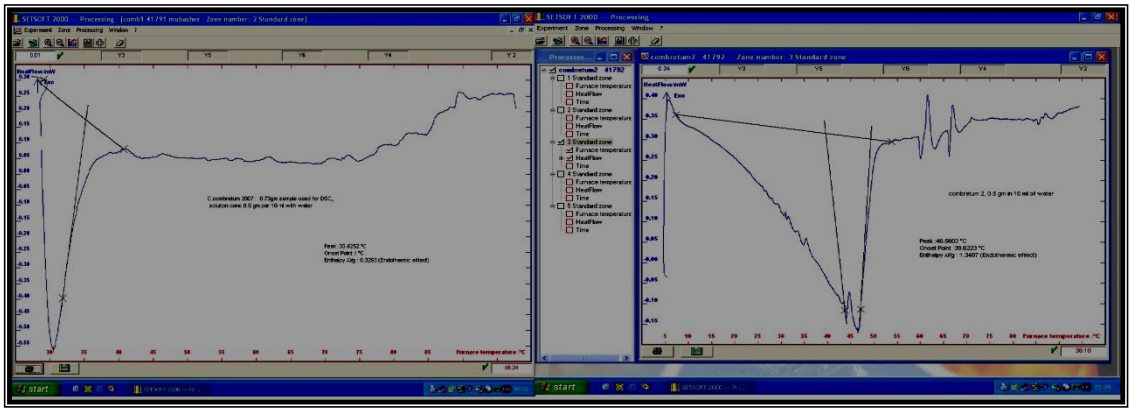

Fig-7: DSC -Thermogram of crude gums in distilled water with heating rate 0.5 ^{o}C / min.

CONCLUSION

Morphological, and physicochemical studies of gum provides further insight into the potentiality of this gum tree among the other tree exudate gums reported in contemporary literature. Morphologically, SEM analysis suggests that the crude gum has irregular particle size, while the soluble part, and deacetylated gum have fibril form. DSC analysis indicated that the thermal transition temperature were ranged from 30.43 to 46.54 ^{o}C with enthalpies / J/ g ranged from 0.3332 to 1.393. Hydroxyl, acetyl and carbonyl were the major functional groups observed in Gum Habeil by FT-IR spectra. Gum Habeil has higher viscosity and lower solubility. Gum Habeil is a complex of partially acetylated polysaccharide obtained as a calcium, potassium and magnesium salt. Based on results of this work it was found that Gum Habeil have good enough properties for industrial applications as an adhesive agent.

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