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ISOLATION, PURIFICATION, NMR AND MASS SPECTRAL STUDIES OF TWO NOVEL GOAT MILK OLIGOSACCHARIDES

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ABSTRACT

Milk is a dynamic biological fluid that serves as the gold standard for the nutrition of newborn infants. In addition to its regular constituents, milk contains oligosaccharides; one of the most biologically diverse and significant classes of carbohydrates in living systems. These milk oligosaccharides (MOs) are complex carbohydrates that play crucial roles in numerous molecular processes affecting eukaryotic biology and patho-physiology. They exhibit a wide range of biological activities, including immune-stimulant, hypoglycemic, anti-tumor, antiviral, anticancer, anticoagulant, anti-complementary, immune-modulatory and anti-inflammatory properties. Goat milk, in particular, is a rich source of oligosaccharides with remarkable biological functions such as antibacterial, immunological, anti-inflammatory, hypoallergenic, and therapeutic effects. In traditional medicine, goat milk has been used as a remedy for tuberculosis and is also reported to enhance platelet counts during dengue fever. Given these potential health benefits, goat milk was collected and processed using a modified method of Kobata and Ginsburg. The milk underwent deproteinization, centrifugation, and lyophilization. Subsequently, the oligosaccharides were isolated via gel filtration chromatography and their homogeneity was confirmed by High-Performance Liquid Chromatography (HPLC). The oligosaccharide mixture was then acetylated and further purified using column chromatography. Finally, the acetyl groups were removed to yield oligosaccharides in their native form. The structures of isolated oligosaccharides were elucidated through a combination of chemical transformations, controlled chemical degradation, and comprehensive spectroscopic techniques, including one-dimensional (^{1}H and ^{13}C) and two-dimensional NMR spectroscopy (COSY, TOCSY, HSQC and HMBC), as well as mass spectrometry.

KEYWORDS: Goat milk, oligosaccharides, biological activities, NMR spectroscopy, mass spectrometry.

1. INTRODUCTION

Oligosaccharides, the third most abundant complex compounds found in the milk or colostrum of mammalian species, play a crucial role in various physiological, pathological and biological processes.[1-2] These include functions such as biological recognition, anti-complementary, anticoagulant, anti-inflammatory, antiviral, antibacterial, antitumor, antioxidant, lipid-lowering, immunological, prebiotic and hypoglycemic activities.[3-4] They also contribute significantly to the prevention of inflammatory processes, reduction of diabetes, obesity, and cardiovascular risk factors, modulation of gut microbiota, and enhancement of gastrointestinal function.[5-8] Specifically, goat milk oligosaccharides have demonstrated anti-inflammatory effects in rats with trinitrobenzenesulfonic acid (TNBS)-induced colitis, suggesting their potential in the treatment of inflammatory bowel diseases.[9-10] Furthermore, they play a vital role in intestinal protection and tissue repair in models of dextran sodium sulfate (DSS)-induced colitis, indicating their relevance in managing human intestinal inflammation.[11-12] Additionally, goat milk exhibits antihypertensive and immunomodulatory properties.[13-14] In light of these findings, the present study focuses on the investigation of goat milk for its oligosaccharide constituents and the elucidation of their structural properties.

2. MATERIALS AND METHODS

2.1 General procedures

The presence of sugars was visualized using thin-layer chromatography (TLC) with 50% aqueous sulfuric acid

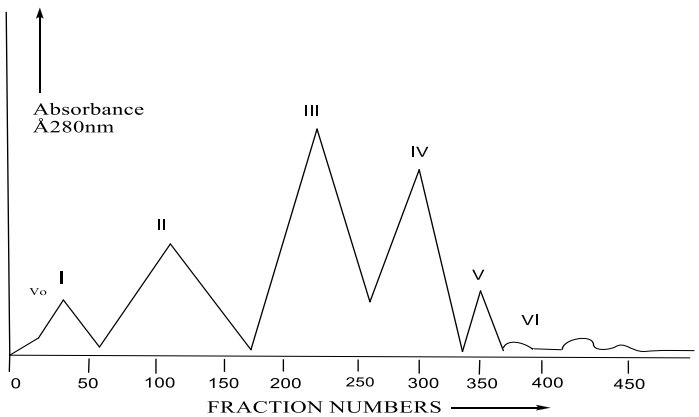
as the detecting reagent and paper chromatography (PC) using acetyl acetone and *p*-dimethylaminobenzaldehyde as chromogenic reagents. For TLC, silica gel G (SRL) was used as the adsorbent, while column chromatography (CC) employed silica gel (SRL, 60–120 mesh). PC was conducted on Whatman No. 1 filter paper with a solvent system comprising toluene, butanol, and water in varied proportions. Gel permeation chromatography was performed using Sephadex G-25 (PHARMACIA). Freeze-drying (lyophilization) of the oligosaccharide mixture was carried out using a CT 60e lyophilizer (HETO), and centrifugation was conducted using a Remi Instruments C-23 cooling centrifuge (JJRCI 763). The homogeneity of the isolated oligosaccharides was confirmed by reverse-phase high-performance liquid chromatography (HPLC), equipped with a Perkin Elmer 250 solvent delivery system, a 235 diode-array detector, and a GP-100 printer plotter. Authentic standards of N-acetylgalactosamine (GalNAc), galactose (Gal), and glucose (Glc) were obtained from Aldrich Chemicals. Optical rotation measurements were performed using an AA-5 series automatic polarimeter with a 1 dm path length cell. ^{1}H and ^{13}C NMR spectra of the oligosaccharides were recorded in D_2O, while spectra of the acetylated derivatives were obtained in $CDCl_3$ at 25°C, using a Bruker AM 300 FT NMR spectrometer. Electrospray ionization mass spectra (ESI-MS) were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. Samples were dissolved in appropriate solvents (methanol/acetonitrile/water) and introduced into the ESI source via a syringe pump at a flow rate of 5 µL/min. The ESI capillary voltage was set at 3.5 kV and cone voltage at 40 V. Spectra were collected in 6-second scans, with final spectra representing an average of 6–8 scans. Elemental analyses for carbon, hydrogen, and nitrogen (C, H, N) were performed using a CARLO-ERBA 1108 elemental analyzer.

2.2 Isolation of goat milk oligosaccharides by modified method of Kobata and Ginsburg[15]

A total of 11 litres of goat milk was collected over a period of 15 days under normal milking conditions from a single domestic goat (Battisi breed) located in Nagla Seth village, Shamsabad, Farrukhabad District, Uttar Pradesh, India. To preserve the milk, an equal volume of ethanol (11 L) was added immediately after collection. The ethanol-fixed milk was transported to the laboratory for further processing. Upon arrival, the mixture was centrifuged at 5000 rpm for 15 minutes at 4 °C. The solidified lipid layer was removed by filtration through a glass wool column under cold atmospheric conditions. Additional ethanol was added to the resulting clear filtrate to achieve a final ethanol concentration of 68%, and the solution was stored overnight at 0 °C. The resulting white precipitate, primarily composed of lactose and proteins, was separated by centrifugation and washed twice with 68% ethanol at 0 °C. The combined supernatants and washings were then filtered through a microfilter and lyophilized, yielding approximately 215 grams of crude oligosaccharide mixture.

2.3 Sephadex G-25 gel filtration of goat milk oligosaccharide mixture

A total of 13.20 g of lyophilized material (containing a mixture of oligosaccharides) derived from goat milk was subjected to gel filtration chromatography using a Sephadex G-25 column for the separation of oligosaccharides from other milk constituents. Glass triple-distilled water (TDW) was used as the eluent at a constant flow rate of 3 mL/min. The oligosaccharide mixture was loaded onto a Sephadex G-25 column (1.6 × 40 cm; void volume = 25 mL), which had been pre-equilibrated with TDW and allowed to stabilize for 10-12 hours. Eluted fractions were collected and analyzed for the presence of neutral sugars using the phenol-sulphuric acid method.[16] Ultraviolet-monitored chromatography revealed seven distinct peaks labeled I to VII. A significant quantity of proteins, glycoproteins, and serum albumins eluted in the void volume, confirmed by positive reactions with both *p*-dimethylaminobenzaldehyde and phenol–sulphuric acid reagents.[16] Fractions corresponding to peaks II, III, and IV tested positive for sugars using the phenol-sulphuric acid assay, indicating the presence of oligosaccharides. These fractions were pooled, lyophilized, and yielded approximately 8.6 g of purified oligosaccharide mixture.



Graph 1: Sephadex G-25 chromatography of goat milk oligosaccharides detected by phenol-sulphuric acid method. Elution was made with TDW.

Table 1: Goat milk oligosaccharide mixture (13.2 g) chromatographed over Sephadex G-25 column chromatography – Details.

Fraction No.	Solvent	Compound (in gm)	Phenol-H_2SO_4 Test for Sugar	Further Investigation
1-60	Glass triple Distilled H_2O	0.73	-ve [I]	-
61-171	”	2.45	+ve [II]	Purified by Column Chromatography
172-275	”	2.95	+++ve [III]	
276-343	”	2.86	+++ve [IV]	
344-374	”	0.96	++ve [V]	-
375-389	”	0.51	-ve [VI]	-

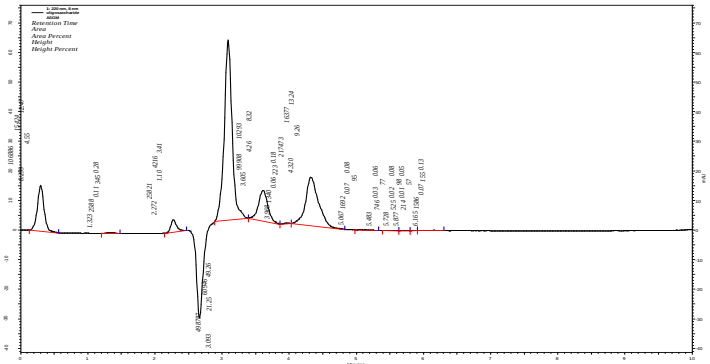
The pooled fractions corresponding to peaks II, III, and IV from the Sephadex G-25 column chromatography yielded 8.6 g of oligosaccharide mixture upon lyophilization. This purification procedure was repeated to ensure consistency and to obtain additional material, resulting in a cumulative yield of 14.35 g of purified oligosaccharide mixture.

2.4 Confirmation of homogeneity of goat milk oligosaccharides by RP- HPLC

The pooled fractions (peaks II, III, and IV) obtained from Sephadex G-25 column chromatography, containing the oligosaccharide mixture, were qualitatively analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC). The analysis was carried out on a Shimadzu CLASS-VP V6.13 HPLC system equipped with a 235-diode array detector and a GP-100 printer plotter. Chromatographic separation was performed on a C18 Purosphere column (25 cm × 0.4 cm, 5 µm particle size; E. Merck). A binary gradient elution system was employed, using a solvent system of acetonitrile and 0.5% trifluoroacetic acid (TFA) in triple-distilled water (TDW). The gradient ranged from acetonitrile:0.5% TFA in water (5:95, v/v) to acetonitrile:0.5% TFA (60:40, v/v) over a period of 25 minutes at a constant flow rate of 1 mL/min. Detection was carried out at 220 nm. Twelve distinct peaks were observed in the chromatogram of the sample (pooled fractions II and III), with retention times ranging from 1.092 minutes to 20.675 minutes. For clarity and reference, the peaks were labeled in ascending order of retention time as follows: [0.299(R_1), 1.323(R_2), 2.272(R_3), 3.093(R_4), 3.605(R_5), 3.968(R_6), 4.320(R_7), 5.067(R_8), 5.483(R_9), 5.728(R_{10}), 5.877(R_{11}) and 6.165(R_{12})].

Table 2: HPLC Table of crude goat milk oligosaccharides.

S. No.	Retention Time (min)	Area%	Area %	Height	Height %
1	0.299	106886	4.55	15424	12.47
2	1.323	2588	0.11	345	0.28
3	2.272	25821	1.10	4216	3.41
4	3.093	498787	21.25	60946	49.26
5	3.605	99908	4.26	10293	8.32
6	3.968	1340	0.06	223	0.18
7	4.320	217473	9.26	16377	13.24
8	5.067	1692	0.07	95	0.08
9	5.483	746	0.03	77	0.06
10	5.728	525	0.02	98	0.08
11	5.877	214	0.01	57	0.05
12	6.165	1586	0.07	155	0.13



Graph 2: Reverse phase HPLC of goat milk oligosaccharides (For the chromatographic conditions, see Tables 1 and 2).

2.5 Acetylation of the oligosaccharide mixture

A total of 11 g of the oligosaccharide mixture, obtained from Sephadex G-25 chromatography, was subjected to acetylation by refluxing with pyridine (11 mL) and acetic anhydride (11 mL) at 60 °C under continuous stirring overnight. To remove excess reagents, the reaction mixture was evaporated under reduced pressure. The resulting viscous residue was dissolved in chloroform ($CHCl_3$, 200 mL) and washed twice with ice-cold water to eliminate any residual pyridine or acetic acid. The organic layer was then evaporated to dryness, affording 12.30 g of acetylated oligosaccharide mixture. The acetylation converted the free hydroxyl groups of the sugars into their corresponding acetyl derivatives, thereby increasing their non-polarity. This modification facilitated better resolution on thin-layer chromatography (TLC), which revealed ten distinct spots, labeled **a** through **j**, indicating the presence of multiple acetylated components. TLC detection was carried out by spraying the chromatoplates with 50% aqueous sulfuric acid followed by gentle heating. Various proportions of chloroform:methanol ($CHCl_3$:MeOH) were used as the mobile phase to achieve optimal separation.

2.6 Purification of acetylated milk oligosaccharides on silica gel column

Purification of the acetylated oligosaccharide mixture (11.0 g) was performed using column chromatography over silica gel (500 g). A gradient elution system was employed, using varying proportions of hexane: chloroform ($CHCl_3$), pure $CHCl_3$, and $CHCl_3$:methanol (MeOH) mixtures as eluents. Fractions of 500 mL each were collected sequentially and analyzed by thin-layer chromatography (TLC). Fractions exhibiting similar TLC profiles (based on R_f values) were pooled, resulting in eight major fractions, designated as: fraction I(1.45gm), II(1.10gm), III(1.75gm), IV(1.12gm), V(1.80gm), VI(1.70gm), VII(1.40gm) and VIII(800mg) respectively. TLC analysis indicated that each of these fractions contained a mixture of three to four compounds. Fractions I to VI were further purified by rechromatography on silica gel, maintaining a compound-to-silica gel ratio of 1:100. Elution was carried out using varied proportions of $CHCl_3$:MeOH, optimized for each fraction. This rechromatographic separation led to the successful isolation of four novel oligosaccharide derivatives, designated as: **compound 'a'** (218 mg), **compound 'b'** (308 mg), **compound 'c'** (142 mg) and **compound 'd'** (154 mg).

2.7 Deacetylation of Isolated Compounds

The acetylated compounds (**a**, **b**, **c**, and **d**) obtained through column chromatography were subjected to deacetylation to yield their natural oligosaccharide forms. Each acetylated compound was dissolved in a mixture of acetone and concentrated ammonia and kept overnight in a stoppered hydrolysis flask at room temperature. After completion of the reaction, ammonia was removed under reduced pressure. The resulting residue was washed with chloroform ($CHCl_3$) to remove any non-polar impurities and finally freeze-dried to obtain the natural oligosaccharides. The deacetylated products were designated with analytical notations as follows: **PGM-1** or **'A'** (from compound *a*), **PGM-2** or **'B'** (from compound *b*), **PGM-3** or **'C'** (from compound *c*) and **PGM-4** or **'D'** (from compound *d*). These purified oligosaccharides (PGM-1 to PGM-4) were then used for further structural and biological characterization.

3. RESULTS & DISCUSSION

In recent years, a variety of oligosaccharides have been isolated from milk of different mammalian origins, exhibiting a wide range of biological activities such as anti-tumor, anticancer, and immune-stimulatory effects. A survey of existing literature reveals that several milk oligosaccharides share common structural core units, which have been described in earlier studies. The structures of these core oligosaccharides were elucidated through a combination of chemical degradation methods and advanced spectroscopic techniques. Previous researchers have assigned the structural identity of these cores-such as lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT)-by comparing the ^{1}H NMR chemical shifts of anomeric protons and ring protons of unknown oligosaccharides with those of known reference compounds and structural reporter groups.[18] While numerous modern chromatographic techniques such as high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and pressure chromatography have been employed for the isolation and purification of oligosaccharides, classical techniques such as thin-layer chromatography (TLC) and column chromatography continue to hold significant value. The integration of classical methods with modern analytical technologies often yields superior results in terms of purity and structural resolution. In the present study, we adopted a combined approach involving high-performance liquid chromatography, gel filtration chromatography, thin-layer chromatography, and column chromatography for the isolation and purification of novel oligosaccharides from goat milk. This hybrid methodology enabled efficient separation and characterization of biologically active oligosaccharide compounds.

The process adopted for the isolation of goat milk oligosaccharides involved multiple sequential stages. In the first stage, crude goat milk oligosaccharides were extracted using a modified version of the Kobata and Ginsburg method. This procedure yielded a complex mixture comprising proteins, glycoproteins, oligosaccharides, and lactose. To isolate the oligosaccharide fraction, this crude mixture (13.20 g) was subjected to gel filtration chromatography using a Sephadex G-25 column, which functions on the principle of molecular sieving. This technique separates components based on their molecular weights: molecules with higher molecular weights elute first, followed by those with lower molecular weights. Accordingly, in the present case, glycoproteins were eluted first, followed by

proteins, oligosaccharides, lactose, and finally monosaccharides. Four distinct fractions (I, II, III, and IV) were collected, corresponding to glycoprotein (1.2 g), protein (7.5 g), oligosaccharides (3.6 g), and lactose (0.72 g), respectively. Fractions II and III were pooled based on positive results with phenol-sulfuric acid and p-dimethylaminobenzaldehyde reagents, confirming the presence of oligosaccharides. These pooled fractions were then analyzed using high-performance liquid chromatography (HPLC) to evaluate the homogeneity and composition of the oligosaccharide mixture. The HPLC chromatogram revealed twelve distinct peaks (R1 to R12), corresponding to twelve different oligosaccharides present in goat milk. Due to their highly polar nature, direct isolation of these oligosaccharides in sufficient quantities using conventional chromatographic methods proved challenging. Therefore, the oligosaccharide mixture was acetylated using acetic anhydride and pyridine at 60°C, converting them into their acetylated forms. This modification reduced their polarity, making them amenable to separation via classical chromatographic techniques such as thin-layer chromatography (TLC) and column chromatography.

The acetylated oligosaccharide mixture obtained after acetylation (12.30 g) was analyzed by thin-layer chromatography (TLC), which revealed ten distinct spots (designated as a to j). These components were effectively resolved using various proportions of chloroform and methanol ($CHCl_3$:CH_3OH) as the mobile phase. The TLC-resolved mixture was then subjected to repeated silica gel column chromatography, as described in the experimental section. This purification process led to the isolation of four novel acetylated goat milk oligosaccharides, designated as compounds a, b, c, and d. These acetylated compounds were subsequently deacetylated by treatment with ammonia in acetone to yield the natural oligosaccharides PGM-1 or A, PGM-2 or B, PGM-3 or C, and PGM-4 or D, respectively. These represent newly identified oligosaccharide components from goat milk. The structures of isolated oligosaccharides were elucidated through a combination of chemical transformations, controlled chemical degradation, and comprehensive spectroscopic techniques, including one-dimensional (^1H and ^{13}C) and two-dimensional NMR spectroscopy (COSY, TOCSY, HSQC, and HMBC), as well as mass spectrometry. The spectroscopic data of isolated compounds **'A'** or **(PGM-1)** and **'B'** or **(PGM-2)** are given as follows:

3.1 Compound A, (PGM-1)

Acetylated compound **'a'** (218 mg) was isolated from silica gel column chromatography. Deacetylation of 50 mg of compound 'a' using ammonia in acetone afforded **compound 'A'** (40 mg), which exhibited an optical rotation of $[\alpha]_D^{25}$ = -22° (*c* 1% H_2O). For analytical purposes, compound 'A' was dried over phosphorus pentoxide (P_2O_5) at 100 °C under 0.1 mm Hg pressure for 8 hours. The compound gave a positive phenol-sulphuric acid test[16], Feigl test[17], and Morgan-Elson test[18], confirming the presence of carbohydrate moieties and N-acetylated sugars.

$C_{26}H_{45}O_{21}N$		%C	%H	%N
	Calculated	44.13	6.36	1.98
	Found	44.12	6.36	1.98

^{1}H NMR of acetylated compound a, in $CDCl_3$ at 300 MHz

6.29 [d, 1H, J=3.0 Hz, *α*-Glc(S-1) H-1], 5.67 [d, 1H, J=8.1 Hz, β-Glc(S-1) H-1], 4.48 [d, 2H, J=8.1 Hz, β-Gal(S-2) H-1 & β-Gal(S-4) H-1], 4.45 [d, 1H, J=7.8 Hz, β-GalNAc(S-3)], 3.84 [m, 1H, β-Glc(S-1) H-4], 3.86 [m, 1H, β-Gal(S-2) H-2] and 3.85 [m, 1H, β-GalNAc(S-3) H-1].

^{13}C NMR of acetylated compound a, in $CDCl_3$ at 300 MHz

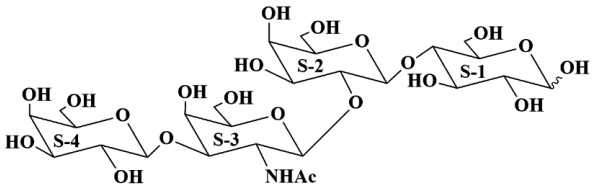
88.95 [1C, *α*-Glc(S-1) C-1], 91.52 [1C, β-Glc(S-1) C-1], 100.92 [2C, β-Gal (S-2) & β-Gal (S-4), C-1] and 101.17 [1C, β-GalNAc(S-3) C-1].

^{1}H NMR of compound A, in D_2O at 300 MHz

5.58 [d, 1H, J=3.3 Hz, *α*-Glc(S-1) H-1], 4.51 [d, 1H, J=7.5 Hz, β-Glc(S-1) H-1], 4.36 [d, 2H, J=7.8 Hz, β-Gal(S-2) H-1 & β-Gal(S-4) H-1], 4.30 [d, 1H, J=7.2 Hz, β-GalNAc(S-3) H-1] and 1.94 [s, 3H, $NHCOCH_3$, β-GalNAc (S-3)].

ES Mass

746[M+K]$^+$, 730[M+Na]$^+$, 707[M$^+$], 669[707-2H_3O^+], 651[669-H_2O], 567[651-2CH_2CO], 545[707-S_4], 487[545-$NHCOCH_3$], 465[545-CH_3OH-HCHO-H_2O], 448[465-OH], 438[487-CH_3OH-OH], 421[438-OH], 406[448-CH_2CO], 342[545-S_3], 304[342-2H_3O^+], 300[342-CH_2CO], 258[304-CHO-OH], 240[300-2HCHO], 222[258-2H_2O] and 180[342-S_2].



Structure of isolated compound 'A'

3.2 Compound B, (PGM-2)

Acetylated compound **'b'** (308 mg) was isolated from silica gel column chromatography. Deacetylation of 50 mg of compound 'b' using ammonia in acetone yielded **compound 'B'** (39 mg), which showed an optical rotation of $[\alpha]_D^{25}$ = -17° (*c* 1% H_2O). For analytical evaluation, compound 'B' was dried over phosphorus pentoxide (P_2O_5) at 100 °C under 0.1 mm Hg pressure for 8 hours. The compound gave a positive phenol-sulphuric acid test[16], Feigl test[17], and Morgan-Elson test[18], indicating the presence of oligosaccharide components and N-acetylated sugar residues.

$C_{28}H_{48}O_{21}N_2$		%C	%H	%N
	Calculated	44.91	6.41	3.74
	Found	44.92	6.41	3.74

^{1}H NMR of acetylated compound b, in $CDCl_3$ at 300 MHz

6.24 [d, 1H, J=3.3 Hz, *α*-Glc(S-1) H-1], 5.68 [d, 1H, J=8.1Hz, β-Glc(S-1) H-1], 4.51 [d, 2H, J=7.5 Hz, β-GalNAc(S-2) H-1 & β-Gal(S-3) H-1], 4.48 [d, 1H, J=8.7 Hz, β-GalNAc(S-4) H-1], 3.86 [m, 1H, β-Glc(S-1) H-4], 3.88 [m, 1H, β-GalNAc(S-2) H-3] and 3.85 [m, 1H, β-Gal(S-3) H-4].

^{13}C NMR of acetylated compound b, in $CDCl_3$ at 300 MHz

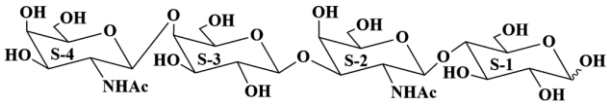
88.83 [1C, *α*-Glc(S-1) C-1], 91.41 [1C, β-Glc(S-1) C-1], 100.77 [2C, β-GalNAc(S-2) C-1 & β-Gal(S-3) C-1] and 101.01 [1C, β-GalNAc(S-4) C-1].

^{1}H NMR of compound B, in D_2O at 300 MHz

5.59 [d, 1H, J= 3.9 Hz, *α*-Glc(S-1) H-1], 4.53 [d, 1H, J= 8.4 Hz, β-Glc(S-1) H-1], 4.38 [d, 2H, J= 7.8 Hz, β-GalNAc(S-2) H-1 & β-Gal(S-3) H-1], 4.31 [d, 1H, J= 7.2 Hz, β-GalNAc(S-4) H-1], 2.08 [s, 3H, $NHCOCH_3$, β-GalNAc(S-4)] and 1.95 [s, 3H, $NHCOCH_3$, β-GalNAc (S-2)].

ES Mass

787[M+K]$^+$, 771[M+Na]$^+$, 748[M$^+$], 690[748-$NHCOCH_3$], 652[690-$2H_3O^+$], 651[652-H$^+$], 545[748-S_4], 503[545-CH_2CO], 481[545-HCHO-2OH], 465[503-$2H_3O^+$], 421[465-CH_3CHO], 406[481-CH_3CHO-CH_2OH], 383[545-S_3], 325[383-$NHCOCH_3$], 319[383-HCHO-2OH], 301[319-H_2O], 259[325-HCHO-$2H_2O$], 223[259-$2H_2O$] and 180[383-S_2].



Structure of isolated compound 'B'

4. CONCLUSION

In the present study, we successfully isolated and characterized two novel oligosaccharides (PGM-1 or 'A' & PGM-2 or 'B') from goat milk using a combination of chromatographic techniques including gel filtration, column chromatography, TLC, and HPLC. The oligosaccharides were initially obtained in their acetylated forms and subsequently deacetylated to yield the natural compounds. These compounds were subjected to standard carbohydrate tests, which confirmed the presence of sugar and N-acetylated moieties. The optical rotation values and consistent purification profiles suggest that these newly isolated goat milk oligosaccharides are structurally unique and may possess significant biological activities. Further, spectroscopic analysis e.g., one-dimensional (^{1}H and ^{13}C) and two-dimensional NMR spectroscopy (COSY, TOCSY, HSQC, and HMBC) as well as mass spectrometry and bioactivity studies are warranted to fully elucidate their structures and potential functional applications in nutrition and therapeutics.

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6. CONFLICTS OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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