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By

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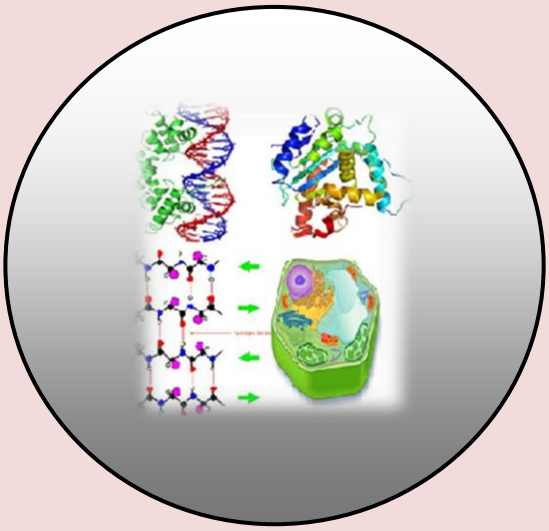

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Buffalo Milk Oligosaccharides, their Structures and Biological Importance: A Review

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ABSTRACT

Buffalo milk is a source of many bioactive components which not only help in meeting the nutritional requirements of the consumers but also play a relevant role in preventing various biological disorders. Milk contains oligosaccharides are reported for the treatment of some human diseases like asthma, gastrointestinal diseases and jaundice. Oligosaccharide mixture of buffalo milk induces significant stimulation of antibody, delayed-type hypersensitivity response to sheep red blood cells in BALB/c mice. It also stimulates non-specific immune response of the animals measured in terms of macrophage migration index. Many novel oligosaccharides have been isolated from the oligosaccharide containing fraction having immunostimulant activity of buffalo milk. These compounds were isolated and purified by a combination of gel filtration chromatography, silica gel column chromatography of derivatised oligosaccharides while the homogeneity was confirmed by high performance liquid chromatography. The oligosaccharides were made up of small monosaccharide units like glucose, galactose, glucosamine, galactosamine. Since NMR is the only non-invasive and physicochemical technique for compound identification. Structure identification of all these oligosaacharides were confirmed by ^{1}H, ^{13}C, 2-D NMR spectroscopy and mass spectrometry. In this review we have presented the structures of various oligosaccharide isolated from Buffalo milk.

Keywords: Buffalo Milk, Oligosaccharides, Hypersensitivity and Chromatography.

INTRODUCTION

In the past complex oligosaccharides were thought of as a food source for a healthy diet and their biological roles were limited to antigenic properties of various blood groups (Wilson et. al, 1997). In recent years various compounds either related to carbohydrates or carbohydrate itself came into the light as important biologically active compounds,

e.g. daunomycin (Cooke et. al, 1980) which is used in the treatment of acute leukaemia, Adriamycin (Cooke et. al, 1980) and bleomycin A2 (Boger et al, 1994) are used in the treatment of solid tumours (Stubbe et. al, 1987). Oligosaccharides play an essential role in many molecular processes impacting eukaryotic biology and diseases and exhibit varied biological activities such as anti-tumour (Chihara et. al, 1969), immunostimulant (Fang et. al 1985), anticancer (Abe et. al, 1985) anticomplementary (Srivasatava et. al 1989), anti-inflammatory (Yamashita et. al, 1982), anticoagulant, hypoglycaemic, antiviral and immunological activities. These oligosaccharides are present in nature as free sugars or in the form of various glycosides. Some of the oligosaccharides isolated from milk have shown structural homology with the carbohydrate carried by glycoproteins and glycolipids on cell surfaces and moreover milk oligosaccharides are currently used in studies on the acceptor specificities of glycosyltransferases and in the biosynthesis of glycoprotein and glycolipid sugar chains and as model compounds for determining the structural requirements of lectins and monoclonal antibodies (Greenwell et.al 1986). During the preliminary screening of buffalo milk in our lab, it was found to be rich in oligosaccharides and haves also shown to have immunostimulant activity. Since buffalo milk is commonly used in northern India, it was of interest to analyse the buffalo milk for its oligosaccharide contents having immunostimulant activity. Keeping in mind the biological activity of buffalo milk oligosaccharide we have isolated 9 milk oligosaccharides from buffalo milk. In this review article we have summarized the structure of milk oligosaccharides isolated from buffalo milk and their biological activity.

Biological Properties of Buffalo Milk Oligosaccharides (Saksena et. al 1999)

Buffalo milk has ability to stimulate non-specific immunological resistance of the host against parasitic infections. Buffalo milk intake influences metabolism, endocrine systems and the nutritional state of neonate. An oligosaccharide mixture of buffalo milk induces significant stimulation of antibody, delayed-type hypersensitivity response to sheep red blood cells in BALB/c mice. This also stimulates non-specific immune response of the animals measured in terms of macrophage migration. Buffalo milk is a rich source of riboflavin, vitamin B12, vitamin A, and thiamine. It contains beneficial compounds that may provide antioxidant protection and improved bone and heart health (Gangwar et. al, 2017).

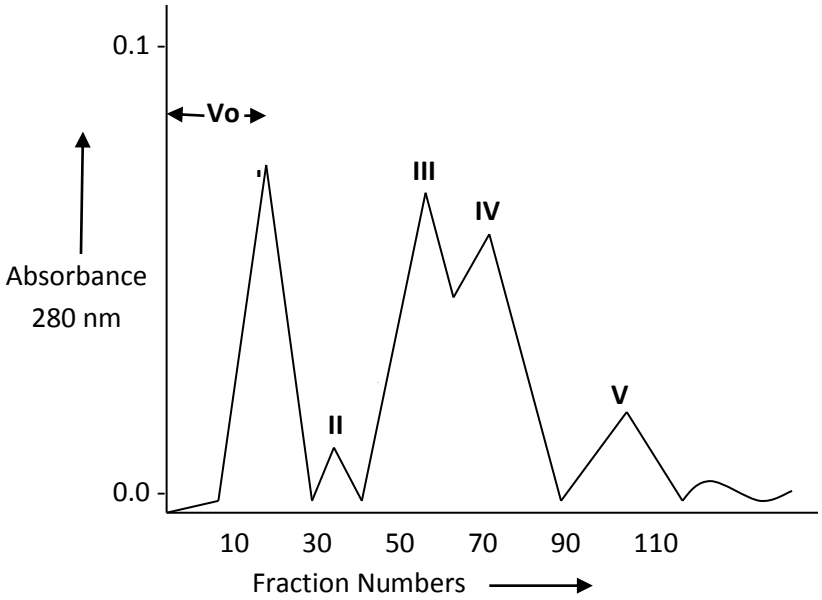
Isolation of milk oligosaccharides by Modified Method of Kobata and Ginsburg (Kumar et al., 2016)

10 liters Buffalo milk (buffalo colostrum) was collected. The milk was fixed by addition of equal amount of ethanol. The preserved milk was taken to laboratory and there, it was centrifuged for 30 min at 5000 rpm at 4^0C. The solidified layer was removed by filtration through glass wool column in cold. More ethanol was added to clear filtrate to a final concentration of 68% and the resulting solution was left overnight at 0^0C. The white precipitate formed, mainly of lactose and protein was removed by centrifugation and washed twice with 68% ethanol at 0^0C. The supernatant and washings were combined and filtered through a micro filter and lyophilized affording crude oligosaccharide mixture.

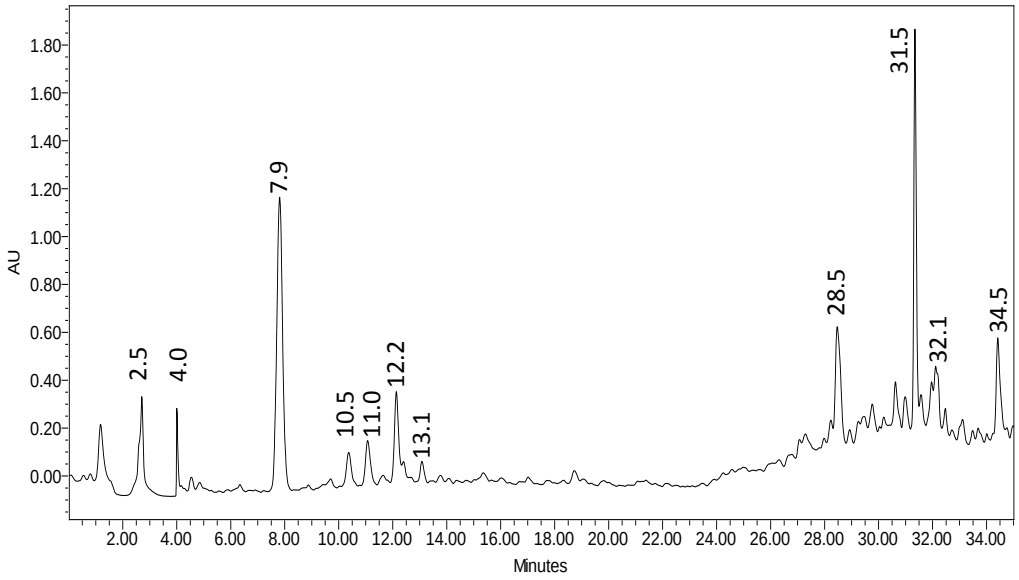
Sephadex G-25 Gel filtration of Buffalo milk Oligosaccharide Mixture (Jamal et. al 2022)

Buffalo milk oligosaccharide mixture obtained from Kobata and Ginsburg was packed in a column (1.6 x 40 cm) (void volume = 25 ml) equilibrated with glass triple distilled water (TDW) and it was left for 10-12 h to settle down.

The material was applied on a Sephadex G-25 column and was eluted for separation of protein and glycoprotein from oligosaccharide (low molecular weight component). In this U.V. monitored Sephadex G-25 chromatography of Buffalo milk oligosaccharide mixture showed five peaks i.e. I, II, III, IV and V. A substantial amount of proteins, glycoproteins and serum albumin were eluted in the void volume which was confirmed by positive coloration with p-dimethylaminobenzaldehyde reagent and phenol-sulphuric acid reagent. Fractions under peaks III and IV gave a positive phenol-sulphuric acid test for sugars which showed the presence of oligosaccharide mixture in Buffalo. These fractions (peak III and IV) were pooled and lyophilized.



Spehadex G-25 Chromatogram of Buffalo Milk Oligosaccharides



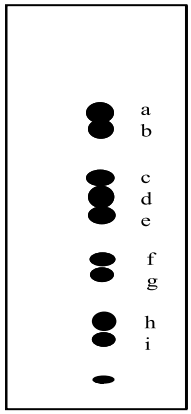
HPLC chromatogram of Buffalo Milk Oligosaccharides

HPLC of oligosaccharide mixture of Buffalo milk (Jamal et. al 2022)

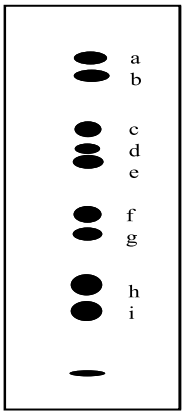
HPLC finger print profile was established. Elution was carried out at a flow rate of 1.5 ml/min with water : phosphoric acid (100:0.3 v/v) as solvent A and acetonitrile : water : phosphoric acid (79.7:20:0.3 v/v) as solvent B using a gradient elution in 0-5 min. with 88-85% A, 5-15 min. with 85-70% of A, 15-20 min. with 70-50% A and 20-25 min. with 50-30% of A and isocratic till 35 min. with 30% of A. Detection was done at 320 nm using 2996 PDA detector.

Acetylation of Buffalo Milk Oligosaccharides

10 gm of crude oligosaccharide mixture for the purpose of isolation of obtained from sephadex chromatography was acetylated by adding pyridine (10 ml) and acetic anhydride (10 ml) at 60^{o} C with constant stirring and was kept overnight. The mixture was evaporated under reduced pressure and the viscous residue was taken in $CHCl_3$ (250 ml) and washed with ice cold wate. The acetylation converted the free sugars into their nonpolar acetyl derivatives which were resolved nicely on TLC, giving eight spots on TLC *i.e.,*A, B, C, D, E, F, G,H and I. The Thin layer Chromatogrphy of acetylated Buffalo milk oligosaccharide is as follows-



$CHCl_3$: MeOH
95:5



$CHCl_3$: MeOH
90: 10

Thin Layer Chromatography of Acetylated Oligosaccharide mixture

PURIFICATION OFACETYLATED OLIGOSACCHARIDE MIXTURE

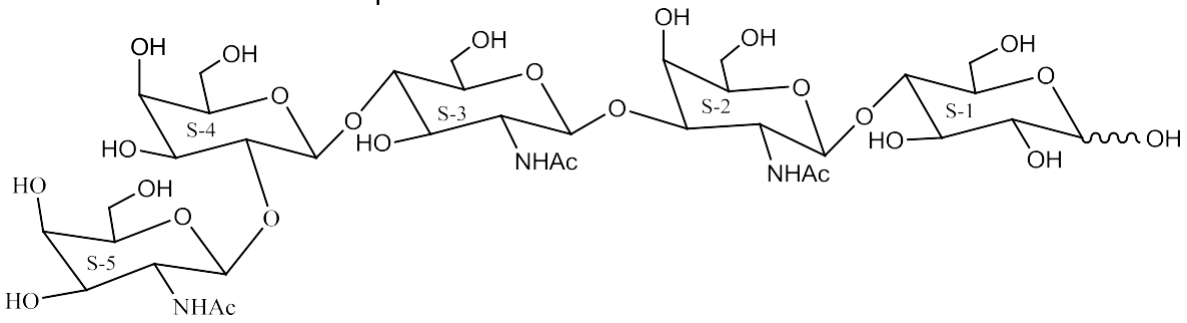
Column Chromatography	Column chromatography plays a very important role in purification of derivatized oligosaccharides
Thin Layer Chromatography	TLC is suitable for analysis of monosaccharide's and oligosaccharides. In TLC plates, the stationary phase is a powdered adsorbent. The resolution of mixture of compounds depends on the suitable solvent system (mobile phase).

By using column chromatography isolation and purification of acetlyalted oligosaccharides were done.After first column we got mixture of acetlated oligosaccharides.These oligosaccharides were further purified by repeated column chromatography at different solvent and at different polarity.

Various Milk Oligosaccharides of Buffalo milk
Compound A Meeniose (Singh et. al 2015)

^{1}H NMR ACETATE (400MHz)	6.19 [d, ^{1}H, J = 3.6 Hz, α-Glc (S-1) H-1], 5.62 [d, 1H, J = 8.0Hz, β-Glc (S-1) H-1], 4.43 [d,1H, J = 8.0Hz, β-GalNAc (S-2) H-1], 5.62 [d, 1H, J = 8.0Hz, β-GlcNAc (S-3) H-1], 5.03[d,1H,J=8.0Hz,β-Gal(S-4)H-1],4.44[d,1H,J=7.6Hz,β-GalNAc(S-5)H-1],3.80[d,1H, β-Glc(S-1)H-4],3.80[d,1H,β-GlcNAc(S-3)H-4],3.74[d,1H,β-GalNAc(S-2)H-3],3.74 [d, 1H, β-Gal (S-4) H-2], 4.07 [d, 1H, β-GalNAc (S-2) H-2], 4.08 [d, 1H, β-GalNAc (S-5) H-2]
^{13}C of Meeniose acetate: : δ in CDCl3 at 400 MHz	88.82[1C, α-Glc (S-1) C-1], 91.40[2C, β-Glc (S-1) & β-GlcNAc (S-3) C-1], 100.79[2C, β-GalNAc (S-5) & β-GalNAc (S-2C-1)], 101.04 [1C, β-Gal (S-4) C-1].
^{1}H NMR of Meeniose: δ in at 300 MHz	5.69 [d, 1H, J = 3.6 Hz, α-Glc (S-1) H-1], 4.50 [d, 1H, J = 8.0Hz, β-Glc (S-1) H-1], 2.10 [s,3H, NHCOCH3, β -GalNAc (S-2)], 1.96 [s, 3H, NHCOCH3, β-GalNAc (S-5)], 1.92s, 3H, NHCOCH3, β -GlcNAc (S-3)].
ES mass	1013[M+Na+K], 990 $[M+K]^{+}$, 951$[M]^{+}$, 933, 893, 862, 748, 657, 729, 700, 657, 586, 550,528, 490, 383, 352, 325, 310, 296, 258, 180

The structure of isolated compound A was as under-



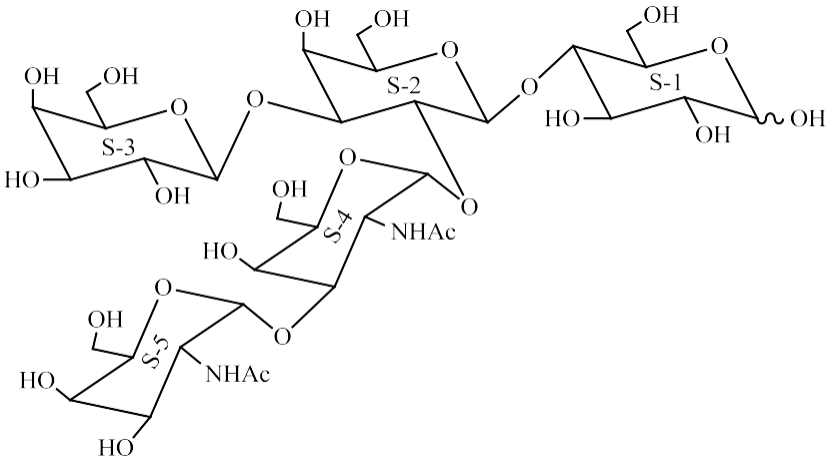
Meeniose

Compound B Murtiose (Singh et. al 2015)

^{1} H NMR of Murtiose: Acetate: δin CDCl3 at 400 MHz	6.08 [d, 1H, J = 4.0 Hz, α-Glc (S-1) H-1], 5.62 [d, 1H, J = 8.0Hz, β-Glc (S-1) H-1], 5.26 [d,1H, J = 1.0 Hz, α-GalNAc (S-5) H-1], 4.70 [d, 1H, J = 2.0 Hz, α-GlcNAc (S-4)H-1],4.45[d,1H,J=8.0Hz,β-Gal(S-3)H-1],4.37[d,1H,J=8.0Hz,β-Gal(S-2)H-1],4.05[d,1H,β-Gal(S-2)H-2],3.74[d,1H,β-Gal(S-2)H-3],3.72[d,1H,β-Glc(S-1)H-4],3.70[d,1H, α-GalNAc (S-4) H-3].

^{13}C of Murtiose acetate: δ in CDCl3 at 400 MHz	89.61[1C,α-GalNAc (S-5) C-1], 90.93[1C, α-Glc (S-1) C-1], 91.29[1C, β-Glc (S-1) C-1], 94.69 [1C,αGalNAc (S-4) C-1], 100.63[1C, β-Gal(S-2)], 100.70[1C, β-Gal (S-3) C-1].
^{1}H NMR MSBC-3 Murtiose: δ in D2O at 300 MHz	δ5.69[d,1H,J=4.0,α-Glc(S-1),H-1],4.63,[d,1H,J=8.0,βGlc(S-1),H-1],5.20[d,1H,J=1.0,α-GalNAc(S-5),H-1],4.49[d,1H,J=8.0,β-Gal(S-3),H-1],4.42,[d,1H,J=8.0,β-Gal(S-2),H-1], 1.96[S, 3H, α-GalNAc (S-4)NHCOCH3], 1.90[S,3H, α-GalNAc (S- 5)NHCOCH3].
Es mass	972 [M+Na+K], 949[M+K] $^{+}$, 910[M] $^{+}$, 875, 851, 820, 785, 748, 671, 717, 545,487, 507,485, 342, 325, 284, 281,180.

The structure of isolated compound B was as under-



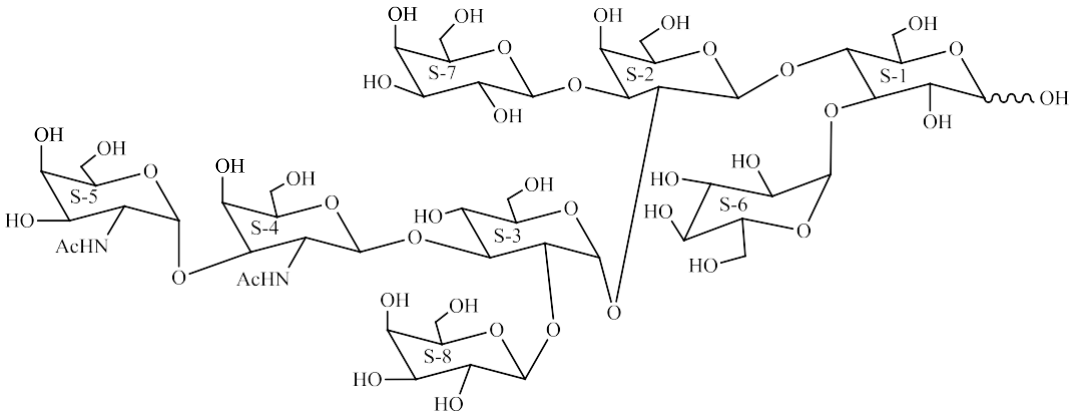
MURITOSE

Compound C Vediose (Chaurasia et. al 2019)

^{1}H NMR of Vediose acetate: δ in CDCl3 at 400 MHz	6.22 [d, 1H, J = 3.2 Hz, α-Glc (S-1) H-1], 5.65 [d, 1H, J = 8.4 Hz, β-Glc (S-1) H-1], 5.57[d,1H,J=2.0Hz,α-Glc(S-3)H-1],5.34[d,1H,J=3.0Hz,α-Glc(S-6)H-1],5.27[d,1H,J=1.6Hz,αGalNAc(S5)H1],4.67[d,1H,J=6.0Hz,β-Gal(S-2)H-1],4.60[d,1H,J=7.2,β-Gal(S-7)H-1],4.54[d,1H,J=8.4, β-Gal(S-8)H-1],4.46[d,1H,J=7.8,β-GalNAc(S-4)H-1],3.52[m,1H,β-Glc(S-1)H-4],3.73[m,2H, β-Glc(S-1)H-3&β-Gal(S-2) H-3], 3.70 [m, 2H, α-Glc (S-3) H-3& β-GalNAc (S-4) H-3], 3.53 [m, 1H, α-Glc (S-3) H-2, 3.40 [m, 1H, β-Gal (S-2)H-2]
^{13}C of Vediose acetate: δ in CDCl3 at 400	89.00[1C,α-Glc (S-1) C-1], 91.08[1C, β -Glc (S-1) C-1], 93.06[1C, -Glc (S-3) C-1], 90.0[1C, -α-Glc (S-6) C-1], 92.01[1C, -GalNAc(S-5) C-1], 96.01[1C, β-Gal (S-2) C-1], 102.02 [3C, β-Gal(S-7, S-8, S-4), C-1]

MHz	
^{1}H NMR Vediose: δ in D2O at 300 MHz	δ 5.20 [d, 1H, J = 3.2, α- Glc(S-1), H-1], 4.62[d, 1H, J = 7.8, β Glc(S-1), H-1], 5.70 [d,1H,J =2.0Hz,αGlc(S-3),H-1],5.54[d,1H,J=3.0, α-Glc(S-6),H1],4.52[d,1H,J=7.4,β-Gal(S-7),H-1],4.51[d,1H,J=7.8,β-Gal(S-8),H-1],4.43[d,1H,J=7.8,β-GalNAc(S-4),H-1], 1.96 [S,3H, β-GalNAc (S-4)NHCOCH3], 1.92 [S,3H, α-GalNAc (S-5)NHCOCH3].
ES mass	1458[M+Na+K], 1435[M+K]$^+$, 1396[M] $^+$, 1297, 1277, 1234 ,1217, 1205, 1072, 1041,1013, 910, 861, 835, 852, 707, 545, 504, 486, 428, 473, 342, 310, 283, 291, 180

The structure of isolated compound C was as under-

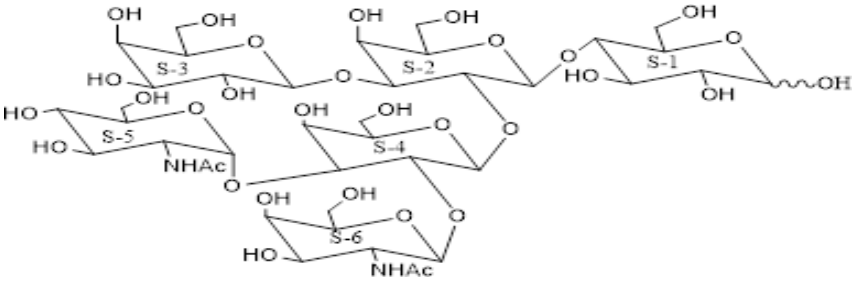


VEDIOSE

Compound D Bebiose (Verma et. al, 2019)

^{1}H NMR of Bebiose: acetate δ in CDCl3 at 400 MHz	6.17 [d, 1H, J = 4.0 Hz, α-Glc (S-1) H-1], 5.69 [d, 1H, J = 5.2Hz, β-Glc (S-1) H-1], 5.40 [d,1H, J = 2.8Hz, α -GlcNAc (S-5) H-1], 4.72 [d, 1H, J = 8.0Hz, βGal (S-4) H-1], 4.59 [d,1H,J=8.0Hz,β-Gal(S-3)H-1],4.52[d,1H,J=8.4,β-Gal(S-2)H-1],4.44[d,1H,J=6.3,β-GalNAc (S-6) H-1], 3.81 [d, 1H, β-Glc(S-1) H-4], 3.80 [d, 2H, β-Gal (S-4) H-2& β- Gal (S- 2) H-3], 3.64 [d, 1H, β-Gal (S-4)H-3]
^{13}C of Bebiose acetate: δ in CDCl3 at 400 MHz	91.00[1C,α-Glc (S-1) C-1], 91.08[1C, β -Glc (S-1) C-1], 90.00 [1C, α-GlcNAc (S-5) C-1],96.00[1C,β-Gal(S-4)C-1],102.00[1C,β-Gal(S-3),C-1],102.00[1C,β-Gal(S-2)C-1],102.00[1C, β-GalNAc (S-6)C-1].
^{1}H NMR Bebiose: δ in D2O at 300 MHz	δ5.69 [d, 1H, J = 4.0, α- Glc(S-1), H-1], 5.20[d, 1H, β Glc(S-1), H-1], 4.52[d,1H, J = 7.8, βGal(S-2), H-1], 4.42 [d, 1H, J = 6.3, β-Gal(S-6), H-1], 1.97 [S, 3H, α-GlcNAc (S- 5)NHCOCH3], 1.96 [S, 3H, β-GalNAc (S-6)NHCOCH3].
ES Mass	1134[M+Na+K], 1095[M+Na], 1072[M$^+$] ,1054, 1013, 869, 833, 811, 666, 648, 606, 504,473, 467, 342, 324, 180.

The structure of isolated compound D was as under-

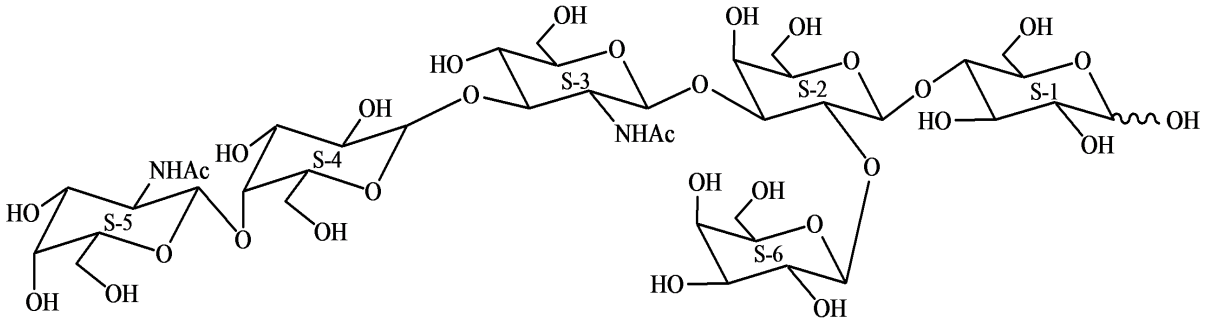


BEBIOSE

Compound E Eutheriose (Gangwar et. al, 2019)

δ in $CDCl_3$: 1H NMR (Acetylated)	δ 6.17 [d, 1H, J=4Hz, α-Glc (S_1)], δ 5.68 [d, 1H, J=8Hz, β-Glc (S_1)], δ 5.40 [d, 1H, J=4Hz, α-Gal (S_4)], δ 5.37 [d, 1H, J=4Hz, α-GalNAc (S_5)], δ 4.74 [d, 1H, J=8Hz, β-GlcNAc (S_3)], δ 4.60 [d, 1H, J=8Hz, β -Gal (S_6)], δ 4.53 [d, 1H, J=8Hz, β -Gal (S_2)], δ 4.12 [m, 1H, J=8Hz, β -GlcNAc (S_3), H-3], δ 3.90 [m, 1H, J=4Hz, α-Gal (S_4), H-4], δ 3.75 [m, 1H, J=8Hz, β -Glc (S_1), H-4], δ 3.69 [m, 1H, J=8Hz, β -Gal (S_2), H-2], δ 3.50 [m, 1H, J=8Hz, β -Gal (S_2), H-3].
δ in $CDCl_3$: 13C NMR (Acetylated)	δ 101.73 [1C, β-Gal (S_6), C-1], δ 100.68 [1C, β-Gal (S_2), C-1], δ 95.00 [1C, β -GlcNAc (S_3), C-1], δ 90.50 [2C, α-Glc (S_1), β -Glc (S_1), C-1], δ 90.0 [2C, α-Gal (S_4), α-GalNAc (S_5), C-1],
δ in D_2O: 1H NMR (Deacetylated)	δ 5.44 [d, 1H, J=4Hz, α-Glc (S_1)], δ 5.21 [d, 1H, J=8Hz, β -Glc (S_1)], δ 5.10 [d, 2H, J=4Hz, α-GalNAc (S_5), α-Gal (S_4)], δ 4.55 [d, 1H, J=8Hz, β -GlcNAc (S_3)], δ 4.40 [d, 1H, J=8Hz, β -Gal (S_6)], δ 4.33 [d, 1H, J=8Hz, β -Gal (S_2)], δ 3.18 [t, 1H, J=8Hz, β -Glc (S_1), H-2], δ 1.88 [s, 3H, α -GalNAc (S_5), $NHCOCH_3$], δ 1.78 [s, 3H, β -GlcNAc (S_3), $NHCOCH_3$].
ES MASS	m/z 1134 [M+Na+K]+, m/z 1095 [M+Na]+, m/z 1072 [M]+, m/z 1041, m/z 1024, m/z 1006, m/z 972, m/z 946, m/z 943, m/z 929, m/z 907, m/z 873, m/z 869, m/z 837, m/z 833, m/z 779, m/z 775, m/z 762, m/z 757, m/z 728, m/z 717, m/z 707, m/z 692, m/z 683, m/z 671, m/z 666, m/z 663, m/z 640, m/z 637, m/z 601, m/z 583, m/z 582, m/z 566, m/z 565, m/z 547, m/z 545, m/z 485, m/z 451, m/z 427, m/z 422, m/z 388, m/z 359, m/z 342, m/z 180

The structure of isolated compound E was as under-

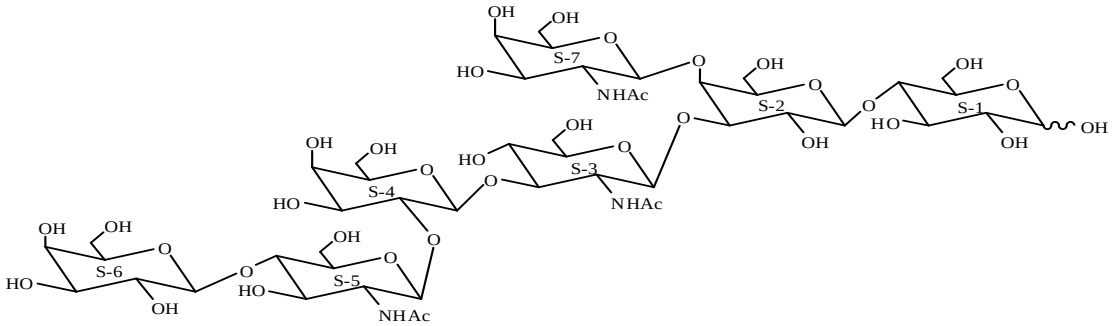


EUTHERIOSE

Compound F Theriose (Jamal et. al, 2019)

δ in $CDCl_3$: ^1H NMR (Acetylated)	δ 103.5 [1C, β-Gal (S_2), C-1], δ 103.0 [1C, β-Gal (S_4), C-1], δ 101.0 [2C, β-Gal (S_6), β-GalNAc (S_7), C-1], δ 95.5 [1C, β-GlcNAc (S_5), C-1], δ 92.5 [1C, β-GlcNAc (S_3), C-1], δ 90.0 [1C, β-Glc (S_1), C-1], δ 90.0 [1C, α-Glc (S_1), C-1].
δ in D_2O: ^1H NMR (Deacetylated)	δ 5.08 [d, 1H, J=4Hz, α- Glc (S_1)], δ 4.52 [d, 1H, J=8Hz, β-Glc (S_1)], δ 4.44 [d, 1H, J=8Hz, β-GlcNAc (S_3)], δ 4.39 [d, 2H, J=8Hz, β-GalNAc (S_7), β-GlcNAc (S_5)], δ4.30 [d,3H, J=8Hz, β-Gal(S_2), β-Gal(S_4) & β-Gal(S_6)], δ3.15 [t,1H, J=8Hz, β-Glc(S_1), H-2], δ 1.88 [s, 3H, β-GalNAc(S_7),NHCOC<u>H</u>$_3$], δ 1.85 [s, 3H, β-GlcNAc(S_5),NHCOC<u>H</u>$_3$] δ 1.75 [s, 3H, β-GlcNAc(S_3),NHCOC<u>H</u>$_3$].
δ in D_2O: 13C NMR (Deacetylated)	δ 104.0 [1C, β-Gal (S_4), C-1], δ 103.0 [3C, β-Gal (S_6), β-Gal (S_2) & β-GalNAc (S_7), C-1], δ 96.0 [2C, β-GlcNAc (S_3), β-GlcNAc (S_5), C-1], δ 92.0 [2C, β-Glc (S_1), α-Glc (S_1), C-1].
ES Mass	m/z 1337 [M+Na+K]+, m/z 1298 [M+Na]+, m/z 1275 [M]+, m/z 1246, m/z 1239, m/z 1205, m/z 1113, m/z 1110, m/z 1079, m/z 1050, m/z 1033, m/z 1002, m/z 910, m/z 876, m/z 847, m/z 830, m/z 748, m/z 712, m/z 678, m/z 545, m/z 511, m/z 509, m/z 482, m/z 465, m/z 342, m/z 325, m/z 291, m/z 180

The structure of isolated compound F was as under-

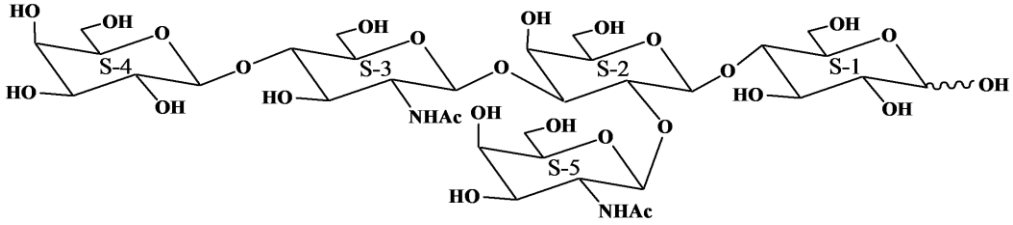


THERIOSE

Compound G Bubaliose (Gangwar et. al, 2017)

δ in $CDCl_3$: 1H NMR (Acetylated)	δ 6.20 [d, 1H, J=4Hz, α- Glc (S_1)], δ 5.62 [d, 2H, J=8Hz, β-Glc (S_1), β-GlcNAc (S_3)], δ 4.46 [d, 1H, J=8Hz, β-GalNAc (S_5)], δ 4.43 [d, 1H, J=8Hz, β-Gal (S_4)], δ 4.42 [d, 1H, J=8Hz, β-Gal (S_2)], δ4.10 [m,1H, J=8Hz, β-Gal(S_2), H-2], δ3.84 [m, 1H, J=8.0 Hz, β-Gal(S_2), H-3], δ 3.78 [m, 1H, J=8.0 Hz, β-Glc (S_1), H-4], δ 3.75[m, 1H, J=8.0 Hz, β-GlcNAc(S_3), H-4].
δ in $CDCl_3$: 13C NMR (Acetylated)	δ 101.05 [1C, β-GalNAc (S_5), C-1], δ 100.79 [2C, β-Gal (S_2), β-Gal (S_4), C-1], δ 91.44 [2C, β-Glc (S_1), β-GlcNAc (S_3), C-1], δ 88.86 [1C, α-Glc (S_1), C-1].
δ in D_2O: 1H NMR (Deacetylated)	δ 103.5 [2C, β-Gal (S_2), β-Gal (S_4), C-1], δ 103.0 [1C, β-GalNAc (S_5), C-1], δ 92.00 [2C, β-Glc (S_1), β-GlcNAc (S_3), C-1], δ 90.0 [1C, α-Glc (S_1), C-1].
ES Mass	m/z 972 [M+Na+K]+, m/z 949 [M+K]+, m/z 910 [M]+, m/z 852, m/z 835, m/z 817, m/z 799, m/z 786, m/z 782, m/z 748, m/z 730, m/z 694, m/z 688, m/z 676, m/z 652, m/z 635, m/z 634, m/z 592, m/z 545, m/z 342, m/z 306, m/z 289, m/z 180.

The structure of isolated compound G was as under-

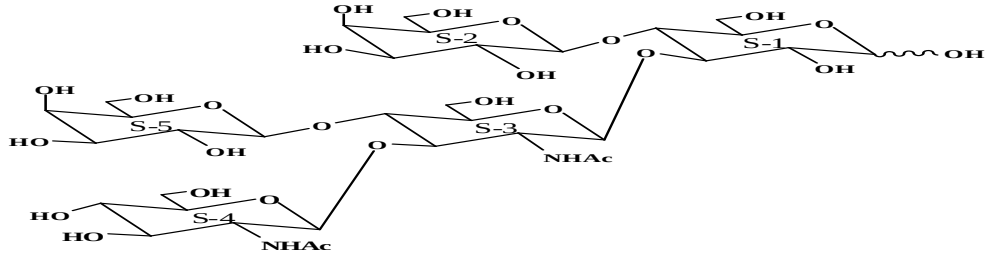


BUBALIOSE

Compound H Orientose (Sharma et. al, 2019)

δ in $CDCl_3$: 1H NMR (Acetylated)	δ 6.22 [d, 1H, J=3.6 Hz, α-Glc (S_1)], δ 5.66 [d, 2H, J=8.4 Hz, β-Glc (S_1), β-GlcNAc (S_3)], δ 4.60 [d, 1H, J=7.5 Hz, β-GalNAc (S_4)], δ 4.57 [d, 2H, J=7.8 Hz, β-Gal (S_5), β-Gal (S_2)], δ4.05 [m,1H, J=8.4 Hz, β-Glc (S_1), H-3], δ 3.82 [m,1H, J=8.4 Hz, β-GlcNAc (S_3), H-3], δ 3.70 [m,1H, J=8.4 Hz, β-Glc (S_1), H-4], δ 3.60 [m,1H, J=8.4 Hz, β-GlcNAc (S_3), H-4].
δ in $CDCl_3$: 13C NMR (Acetylated)	δ 101.83 [2C, β-Gal (S_2), β-Gal (S_5), C-1], δ 101.69 [1C, β-GalNAc (S_4), C-1], δ 91.39 [2C, β-Glc (S_1), β-GlcNAc (S_3), C-1], δ 88.96 [1C, α-Glc (S_1), C-1].
δ in D_2O: 1H NMR (Deacetylated)	δ 5.17 [d, 1H, J=4Hz, α-Glc (S_1)], δ 4.90 [d, 1H, J=8Hz, β-GlcNAc (S_3)], δ 4.59 [d, 1H, J=8Hz, β-Glc (S_1)], δ 4.42 [d, 1H, J=8Hz, β-GalNAc (S_4)], δ 4.38 [d, 1H, J=8Hz, β-Gal (S_5)], δ 4.36 [d,1H, J=8Hz, β-Gal(S_2)], δ 3.18 [t,1H, β-Glc(S_1), H-2], δ 1.92 [s,3H, β-GlcNAc(S_3), NHCOC$\underline{H}_3$], δ 1.91 [s,3H, β-GalNAc(S_4), NHCOC$\underline{H}_3$].
δ in D_2O: 13C NMR (Deacetylated) ES-mass	δ 101.66 [3C, β-Gal (S_5), β-Gal (S_2) & β-GalNAc (S_4), C-1], δ 99.72 [1C, β-GlcNAc (S_3), C-1], δ 94.52 [1C, β-Glc (S_1), C-1], δ 90.56 [1C, α-Glc (S_1), C-1]. m/z 972 [M+Na+K]+, m/z 933 [M+Na]+, m/z 910 [M]+, m/z 748, m/z 730, m/z 712, m/z 699, m/z 681, m/z 664, m/z 663, m/z 545, m/z 527, m/z 511, m/z 498, m/z 482, m/z 467, m/z 465, m/z 342, m/z 325,m/z 307, m/z 278, m/z 260, m/z 180.

The structure of isolated compound H was as under-



ORIENTOSE

Compound I Pentasaccharide sugar (Saksena et. al 1999)

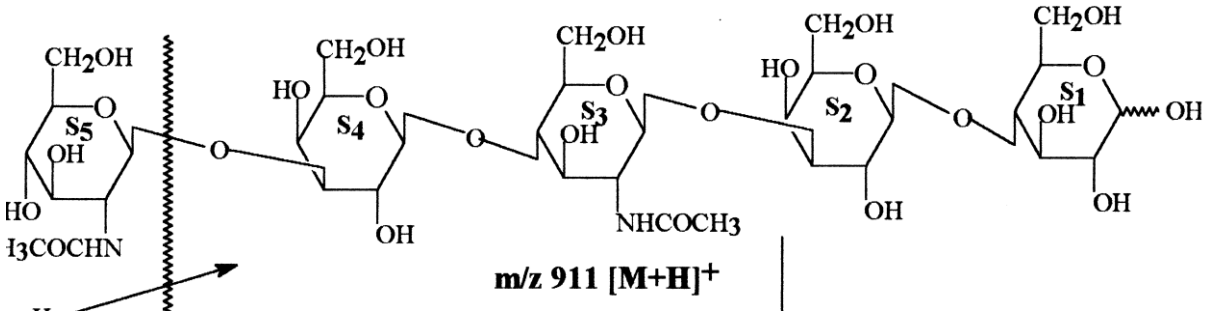
1H NMR of compound P2 (for convenience the five sugar units of compound P2 are designated S1,S2, S3, S4, and S5).N 1.96 (s, 3H, NHCOCH3), 1.99 (s, 3H,NHCOCH3), 3.11

(t, 1H, J=7.5 Hz, L-Glc(S1), H-2), 4.20 (d, 2H, J =3.5 Hz, Gal(S2), H-4 and Gal(S4),H-4), 4.48 (d, 1H, J=7.5 Hz Gal(S2), H-1), 4.52 (d,1H, J=7.5 Hz, Gal(S4), H-1), 5.09 (d, 2H, J =7.8Hz, GlcNAc(S3), H-1 and L-Glc(S1), H-1), 5.22 (d,1H, J=7.8 Hz, GlcNAc(S5), H-1) and 5.49 (d, 1H,J =3.7 Hz, K-Glc(S1), H-1).

Fast atom bombardment mass spectrometry

(FABMS) of P2 : m/z 949 [M+K], 933 [M+Na], 911[M+H], 893 [9113H2O], 880[9113CH_2OH], 857[89332H_2O], 853 [9113$NHCOCH_3$], 849 [8803CH_2OH], 833 [8933CH_2OHCHO], 799 [8573$NHCOCH_3$], 795 [8533$NHCOCH_3$], 791 [8333CH2 =C=O], 775 [9493CH_2OHCHO,3CHOHCHNHCOCH$_3$],758 [7753OH], 749 [7913CH_2 =C=O], 746 [9493S5], 732 [9113S1], 730[9333S5], 716 [7583CH_2 =C=O], 712 [7303H_2O],708 [9113S5], 704 [7463CH2 =C=O], 698 [7163H_2O], 694 [7123H_2O], 693 [74632H_2O,3OH], 690[7083H_2O], 684 [74632CH_2OH], 680 [6983H_2O],676 [6943H2O], 672 [6903H_2O], 657 [6933O],649 [6803CH_2OH], 637 [6723H2O,3OH], 632[6903NHCOCH3], 586 [9113S2,3S1], 579 [6373$NHCOCH_3$], 568 [9333S5,3S4], 567 [9493S5,3S4],566 [6573CH2=C=O,3H2O,3CH2OH], 551[5683OH], 546 [9113S5,3S4], 543 [57932H_2O],529 [5463OH], 528 [5463H_2O], 515 [55132H_2O],511 [5283OH], 487 [5293CH2 =C=O], 468[5283CH_2OHCHO], 409 [4683$NHCOCH_3$], 383[9113S3,3S2,3S1], 365 [3833H_2O], 343 [9113S5,3S4,3S3], 342 [S2,S1], 329 [36532H_2O], 307[3833$NHCOCH_3$,3H_2O], 305 [3653CH_2OHCHO],290 [3073OH], 288 [34233H_2O], 257 [2883CH_2OH], 247 [3073CH_2OHCHO], 226 [2573CH_2OH], 180 [S1].

The structure of isolated compound I (pentasaccharide) was as under-



Pentasaccharide Milk Oligosaccharide

RESULT AND DISCUSSION

The compound Meeniose, Muritose, Vediose and Bebiose were isolated from the buffalo colostrum while Eutheriose, Theriose, Bubaliose and Orientose were isolated from buffalo milk. All these compounds were oligosaccharides which have potent biological activities against cardiac diseases. They promote bone health and reduce risk of osteoporosis. They have different oligosaccharides linkages which are responsible for different physicochemical properties. Buffalo milk oligosaccharides are pentasaccharide, hexasaccharide, septasaccharides and octasaccharide. In all the oligosaccharides, glucose is present on reducing site. ES mass of Milk Oligosaccharides were between 949 to 1337.The structure of isolated compound contained Glucose, Galactose, GalNHAc and GluNHAc as sugar. The linkages were 1-4, 1-3 and 1-2 corresponds for different biological activities.

CONCLUSION

In summary the buffalo milk oligosaccharides are very important compounds in the time of pandemic. By using the biological properties and structure of these compounds chemist synthesized beneficial drugs for humankind.

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