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Characterisation of Gallstones Using Fourier Transform Infrared Spectroscopy

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ABSTRACT: *Gallstone formation is the primary underlying disease that results in gall bladder illness. Gallstone formation in the gall bladder is a common disease and constitutes a major health problem in the world wide. Cholesterol gallstones, pigment gallstones and mixed gallstones are formed in gallbladder. The aim of the study is to determine the constituents and their compositions of gallstones using Fourier transform infrared spectroscopy. The study reveals that cholesterol stones and mixed stones type of gallstones were predominant whereas pigment stones were less frequent in the selected region.*
Keywords: *Gallstone, Gall bladder, Fourier transform infrared spectroscopy.*

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I. Introduction

Gallstones are small, hard deposits that can form in the gallbladder, a sac-like organ that lies under the liver on the right side of the abdomen. Most people with gallstones don't even know they have them. But in some cases a stone may cause the gallbladder to become inflamed, resulting in pain, infection, or other serious complications. The formation of gallstone is a complex process that starts with bile, a fluid composed mostly of water, bile salts, lecithin (a fat known as phospholipids) and cholesterol. The process of gallstone formation is referred to as cholelithiasis. It is generally a slow process, and usually causes no pain or other symptoms. The majority of gallstones are either cholesterol or mixed type. Gallstones can range in size from a few millimeters to several centimeters in diameter. Most gallstones are formed from cholesterol. Pigment stones are also very common, they are formed from a brown – coloured substance called calcium bilirubinate. Patients can have a mixture of the two gallstone types. The occurrence of gallstone disease is 2-3 times more common in women than in men [1]. 20-30% of western and around 10% of non-western populations has been affected by gallstones [2]. Cholesterol stones contain more than 70% of cholesterol whereas pigment stones contain mainly of various bilirubinate salts with less than 20% cholesterol by weight. Pigment stones are predominant in India [3]. Pigment stones are further subdivided into laminated brown stones and amorphous black stones [4]. Brown, black stones are chemically, morphologically and clinically distinct. Black stones are formed in the gallbladder and are associated with hemolysis, cirrhosis and old age [5, 6]. Classification of pigment stones was made on the basis of the proceeding of the first National Institute of Health-International Workshop on pigment gallstone diseases [7]. Chemical composition of gallstones is essential for actiopathogensis of gallstones diseases [8]. Non spectroscopic techniques such as enzymatic and calorimetric methods have been used to determine chemical compositions of gallstones. The lack of specificity, sensitivity and inaccurate values are common in these methods. Spectroscopy method is the most widely used method because of its advantages over chemical analysis. This technique requires minimal sample volume, specificity of all components and also provides quantitative results with greater reproducibility [9]. The present study was carried out to analyze the chemical composition of human gallstones using Fourier Transform Infrared Spectroscopy (FTIR).

II. Material And Methods

Gallstones used in the present study are collected from Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER) Pondicherry, India. The age of the patients range is lies between 35-70 for both men and women. The stones are collected from September 2014 to December 2015. All the stones are washed in running water then with saline water and air dried for several days. During analysis the stones were cut into two halves by using a razor blade and one quarter of the collected gall stones were powdered using clean pestle and agate mortar. The powered samples are then pelletised by applying 15 - 16 KPa pressure. FTIR studies were carried out using NICOLET 6700 FTIR SYSTEM at IIT Chennai, India. During the FTIR studies the frequency range 4000–400 cm^{-1} at 4 cm^{-1} resolutions is used. To obtain a high signal/noise ratio 100 scans were accumulated for each sample.

III. Result And Discussion

FTIR spectrometer (NICOLET 6700 FT - IR) is used to identify the functional groups and measurements are carried out in the mid - infrared range (4000 - 400 cm^{-1}) at 4 cm^{-1} resolution rate in the transmittance mode. FTIR spectra of 15 samples are shown in figures 3.1 – 3.15. The chemical components and its corresponding IR transmittance bands of gallstones are given under the FTIR spectra. From the FTIR spectra, the collected gallstones were grouped into cholesterol (9), mixed (4) and pigment (2) type gallstones. This analysis showed that cholesterol crystal is the predominant composition in cholesterol and mixed gallstones.

3.1. CHOLESTEROL STONES

Presence of cholesterol in the gallstone samples GS 2, GS 3, GS 4, GS 8, GS 9, GS 10, GS 12, GS 13 and GS 14 is characterized by large O – H stretching absorption bands at 3394.0, 3398.3 cm^{-1}, C - H stretching vibration band occurring from 2935.4 to 2941.3 cm^{-1}, C – H deformation bands obtained at 1463.8, 1461.9 cm^{-1} in the FTIR spectrum of these samples. A sharp absorption peak is observed at 1054.9, 1053.09 cm^{-1} due to the ring deformation of cholesterol [2, 10, 11]. Band occurring from 1373.3 to 1378.2 cm^{-1} is due to CH_2 and CH_3 bending vibration of cholesterol gallstones [10, 12-14]. A very weak intensity band at 1666.3 cm^{-1} due to bilirubin salts. Aragonite form of $CaCo_3$ shows weak intensity band at from 1085 and 699 cm^{-1} [15]. The presence of calcium is identified by the C – O stretching bands occurring between1461.9 - 1463.9 cm^{-1}.

3.2. MIXED STONES

In GS 1, GS 6, GS 11 and GS 15 cholesterol is abundant as described in cholesterol stones from GS 2, 3, 4, 8, 9, 10, 12, 13, 14. Bilirubinate salts have characteristic bands at 1645.8 cm^{-1} and a band at 3442.6 cm^{-1} due to N –H stretching vibration of pyrrole of bilirubin, the 1647.1 cm^{-1} band also comes from bilirubinate salt. Generally, calcium palmitate is the most abundant component of gallstone. Identification of calcium palmitate could be based on the presence of specific peaks at 1461 and 668.8 cm^{-1}.

3.3. PIGMENT STONES

In FTIR GS 5 and GS 7 are identified to have as calcium bilirubinate salts. This must be the reason for the amorphous nature of these gallstones. Calcium bilirubinate have characteristic band at 1615.6, 1622,1246.2, 1666.4, 1453.5, 1571.3 cm^{-1} which are assigned to (C = C, C – N, C = O) stretching vibration of lactam, C = O stretching of COOH, (C = O ,C – N, C = C) stretching, asymmetric stretching γ as (COO) and (C – O) stretching or C – N stretching coupled with NH deformation γ (C –N) + δ (NH), respectively [16-17, 5, 14]. Calcium palmitate could be identified by the presence of specific peaks at 612.5, 856.2, 2921.9 and 2923.8 cm^{-1}. Weak O – H stretching absorption band at 3398.3, 3352.0 cm^{-1} and sharp absorption peak at 1041.4, 1043.4 cm^{-1} can be attributed to the ring deformation of cholesterol. FTIR analysis of gallstones shows that cholesterol to be the most abundant component, bilirubin is the next abundant component. Calcium carbonate ($CaCo_3$) in the form of two polymorphs namely calcite and aragonite obtained by FTIR studies.

IV. Conclusion

Fifteen stones were analysed by Fourier Transform Infrared Spectroscopy (FTIR). From these studies it is found that cholesterol was the main component in GS 1, GS 2, GS 3, GS 4, GS 6, GS 8, GS 9, GS 10, GS 11, GS 12, GS 13, GS 14 and GS 15, remaining two stones (GS 5, GS 7) is found to be pigment calcium stones by FTIR studies. It has been identified that in mixed stones (GS 1, GS 6, GS 11 and GS 15) cholesterol also is found to be abundant. Next to cholesterol derivatives bilirubin occurrence is more, this is due to the presence of bilirubin in cholesterol stones and calcium bilirubinate in pigment calcium gallstones. These results are confirmed by FTIR studies. From the FTIR studies finally concluded that the majority of patients are affected by cholesterol and mixed gallstones compared to pigment gallstones. These cholesterol stones formations may be due to the intake of food, age factor and liver diseases in and around the region of Pondicherry peoples.

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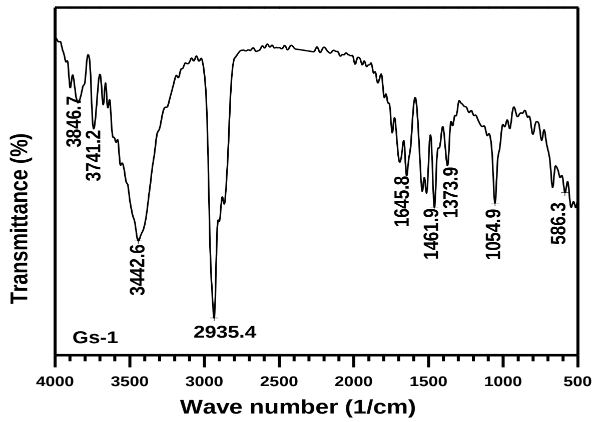

Figure – 3.1: FTIR Spectrum of GS 1

Wave number cm^{-1}	Compound
3846.7	**Aragonite**
3442.6	**Aragonite**
2935.4	**Cholesterol**
1645.8	**Calcium palmitate**
1461.9	**Cholesterol**
1373.9	**Cholesterol**
1054.9	**Bilirubin**
586.3	**Calcium phosphate**

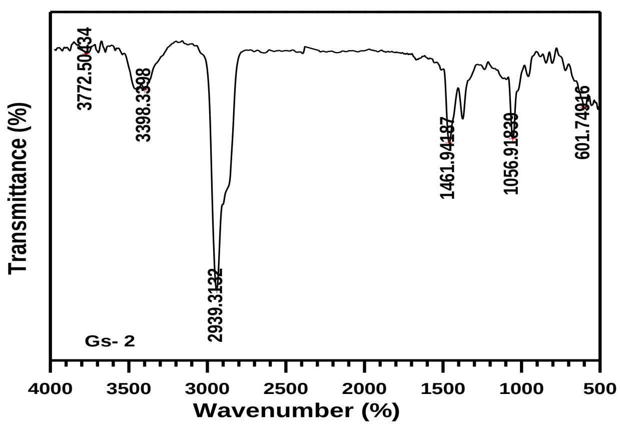

Figure – 3.2: FTIR Spectrum of GS 2

Wave number cm^{-1}	Compound
3398.3	Cholesterol
2939.3	Calcium palmitate / Cholesterol
1461.9	Cholesterol
1056.9	Cholesterol / Bilirubin
601.7	Cholesterol

Figure – 3.3: FTIR Spectrum of GS 3

Wave number cm^{-1}	Compound
3398.3	Cholesterol
2939.3	Calcium palmitate / Cholesterol
1461.9	Cholesterol
1373.2	Cholesterol
1053.0	Cholesterol / Bilirubin
594.0	Cholesterol

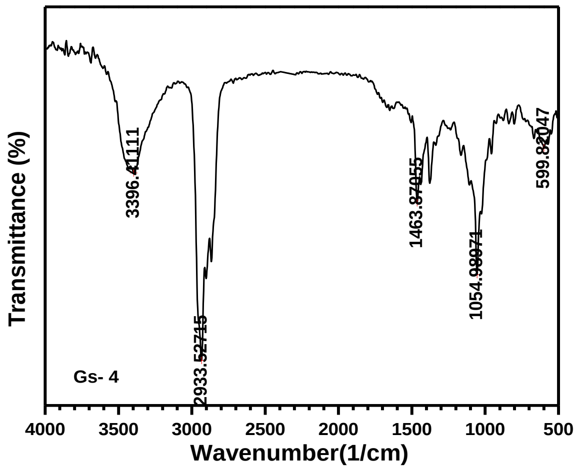

Figure – 3.4: FTIR Spectrum of GS 4

Wave number cm^{-1}	Compound
3396.4	Bilirubin/ Cholesterol
2933.5	Cholesterol
1463.9	Cholesterol / calcium palmitate
1054.9	Cholesterol / Bilirubin
599.8	Cholesterol

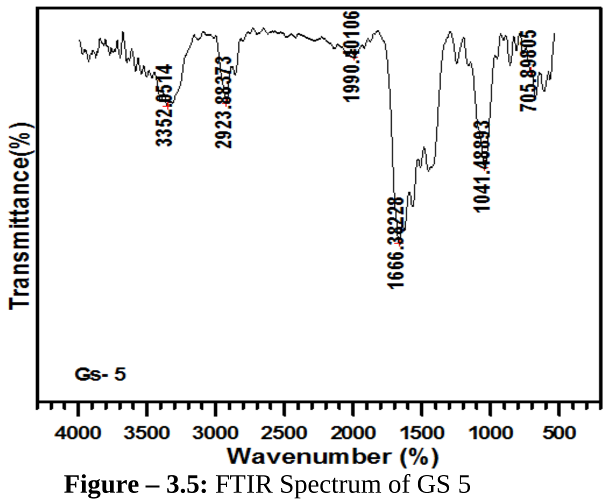

Figure – 3.5: FTIR Spectrum of GS 5

Wave number cm^{-1}	Compound
3352.0	**Bilirubin**
2923.8	**Cholesterol / Calcium palmitate**
1666.3	**Calcium palmitate**
1041.5	**Bilirubin /Cholesterol**
705.9	**Calcium carbonate/Araganite**

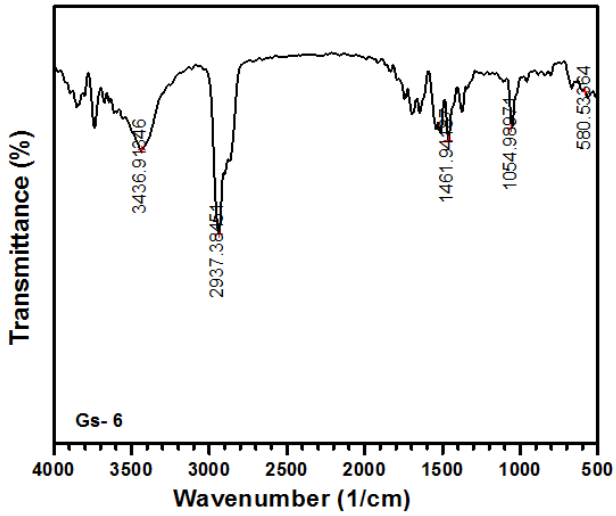

Figure – 3.6: FTIR Spectrum of GS 6

Wave number cm^{-1}	Compound
3436.9	**Aragonite**
2937.3	**Cholesterol / Calcium palmitate**
1461.9	**Aragonite**
1054.9	**Bilirubin / Cholesterol**
580.5	**Calcium palmitate / Calcium phosphate**

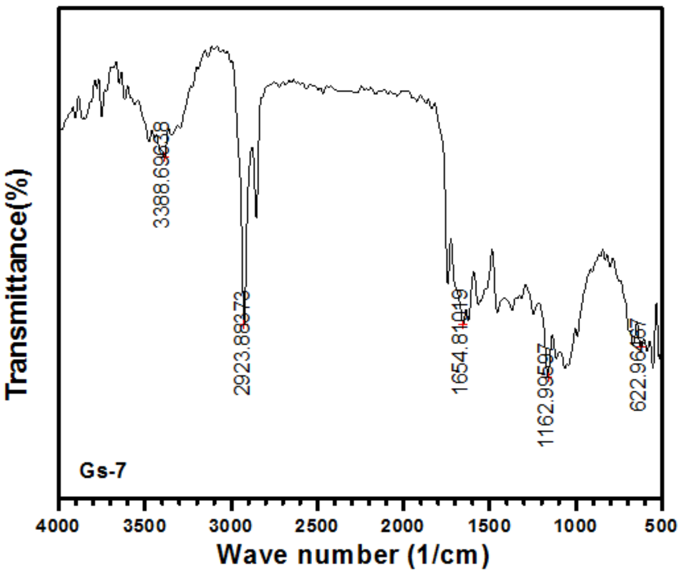

Figure – 3.7: FTIR Spectrum of GS 7

Wave number cm^{-1}	Compound
3388.7	**Bilirubin**
2923.8	**Calcium palmitate/ Cholesterol**
1654.8	**Calcium palmitate / Sodium chlorate**
1162.9	**Bilirubin**
622.9	**Sodium chlorate**

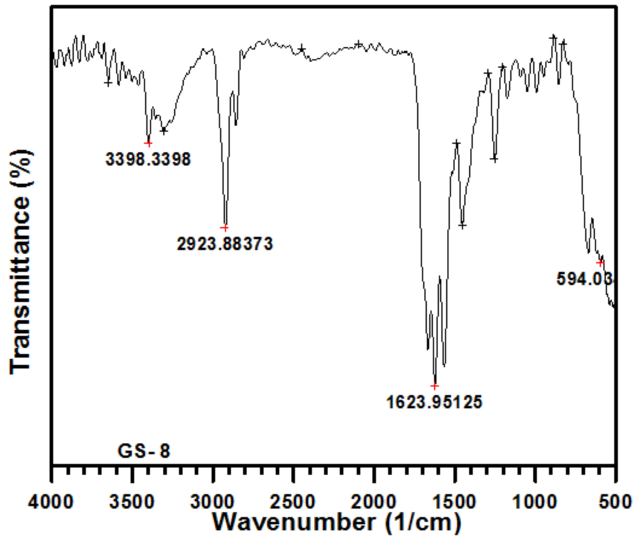

Figure – 3.8: FTIR Spectrum of GS 8

Wave number cm^{-1}	Compound
3398.3	**Cholesterol**
2923.8	**Calcium palmitate/ Bilirubin**
1623.9	**Sodium chlorate/unconjugated bilirubin**
594.0	**Cholesterol**

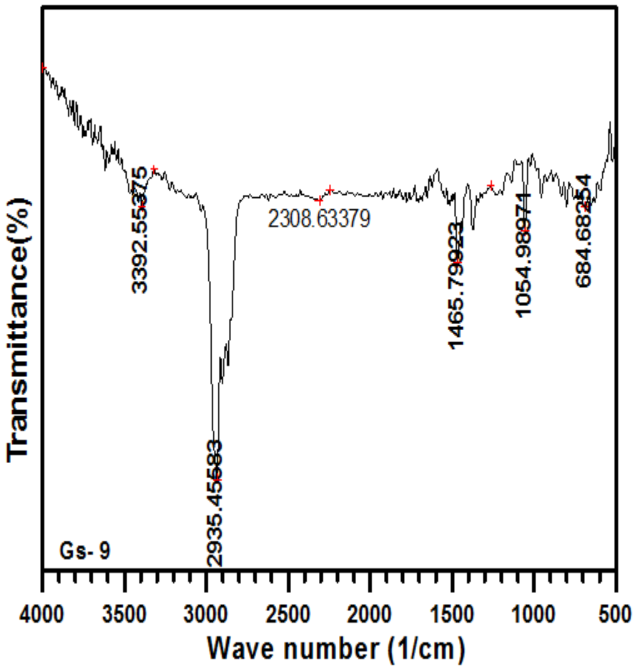

Figure – 3.9: FTIR Spectrum of GS 9

Wave number cm^{-1}	Compound
3392.5	**Bilirubin/ Cholesterol**
2935.5	**Cholesterol**
1465.8	**Cholesterol / Calcium palmitate**
1054.9	**Cholesterol**
684.7	**Araganite**

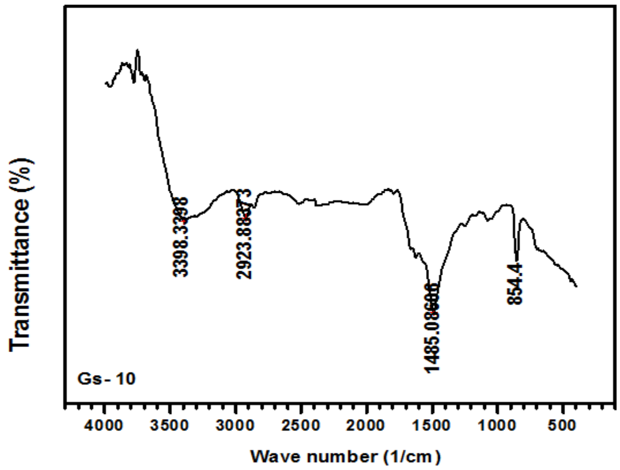

Figure – 3.10: FTIR Spectrum of GS 10

Wave number cm^{-1}	Compound
3398.7	Chlosterol
2923.8	Chlosterol
1485.0	Aragonite
854.4	Aragonite

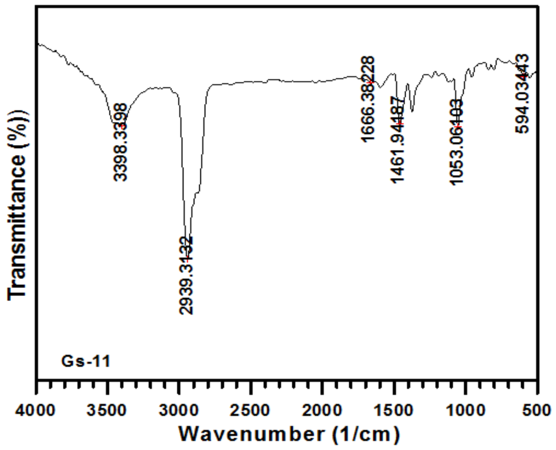

Figure – 3.11: FTIR Spectrum of GS 11

Wave number cm^{-1}	Compound
3398.3	Chlosterol
2939.3	Cholesterol
1666.3	Calcium palmitate
1461.9	Chlosterol / Calcium palmitate
1053.0	Cholesterol / Bilirubin
594.0	Cholesterol

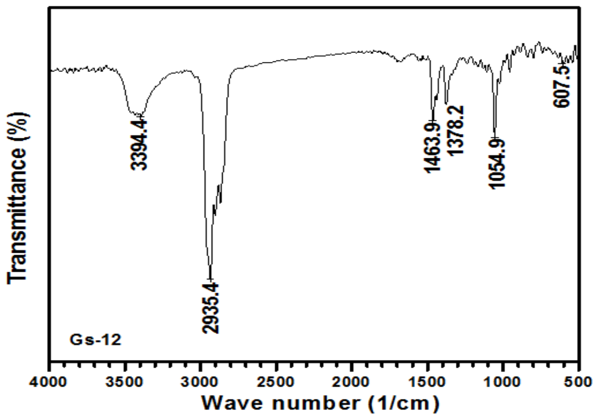

Figure – 3.12: FTIR Spectrum of GS 12

Wave number cm^{-1}	Compound
3394.4	Bilirubin/ Cholesterol
2935.4	Cholesterol
1463.9	Chlosterol / Calcium palmitate
1378.2	Chlosterol
1054.9	Cholesterol / Bilirubin
622.9	Chlosterol

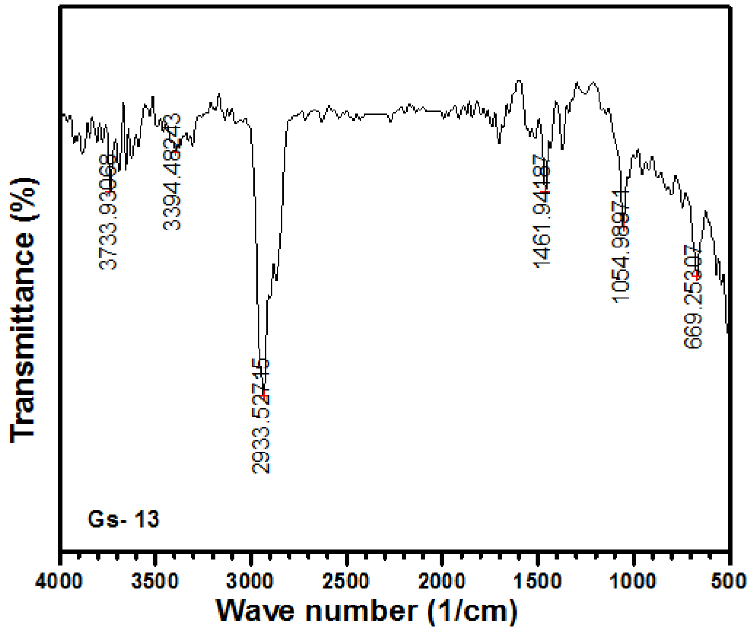

Figure – 3.13: FTIR Spectrum of GS 13

Wave number cm^{-1}	Compound
3394.5	Bilirubin/ Cholesterol
2933.5	Chlosterol
1461.9	Calcium palmitate
1054.9	Chlosterol / Bilirubin
699.2	Calcium palmitate

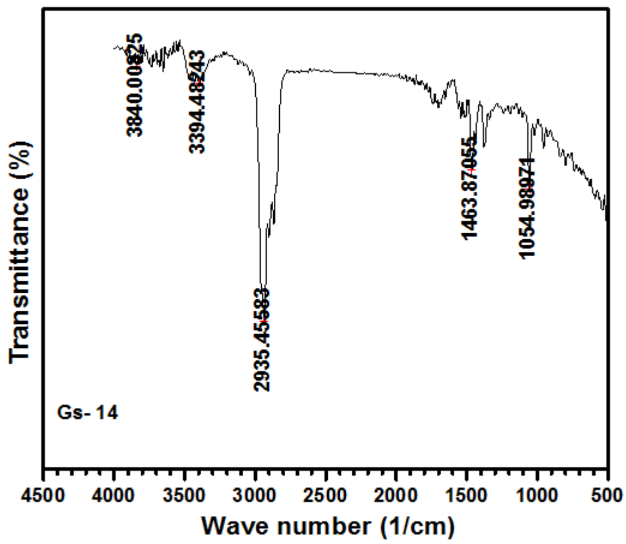

Figure – 3.14: FTIR Spectrum of GS 14

Wave number cm^{-1}	Compound
3394.4	Bilirubin/ Cholesterol
2935.4	Calcium palmitate/ Cholesterol
1463.8	Chlosterol / Calcium palmitate
1054.9	Chlosterol / Bilirubin

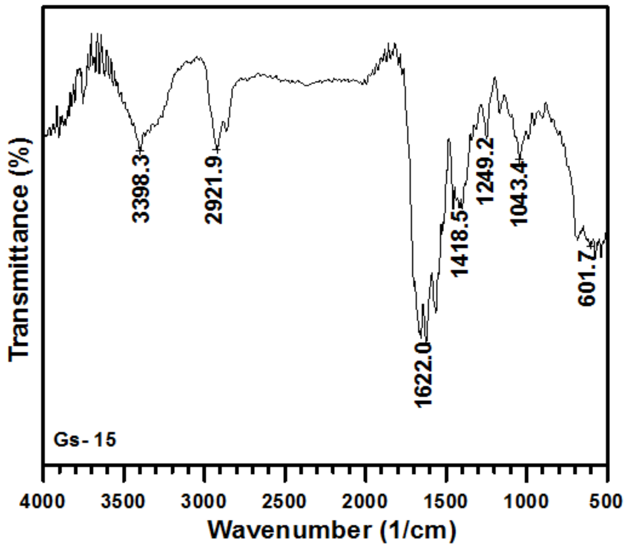

Figure – 3.15: FTIR Spectrum of GS 15

Wave number cm^{-1}	Compound
3398.3	Chlosterol
2921.9	Chlosterol / Calcium palmitate
1622.0	Sodium chlorate
1418.5	Calcite
1249.2	Chlosterol/Calcium bilirubinate
1043.4	Bilirubin
601.7	Chlosterol

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