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Extraction, Characterisation And Compatibility Study Of Polysaccharides From Dillenia indica And Abelmoschus esculentus With Metformin Hydrochloride For Development Of Drug Delivery System

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Abstract: The present study was carried out with an aim of studying the ability of polysaccharides extracted from *Dillenia indica* (DI), *Abelmoschus esculentus*(AE) to be used as a polymer in Drug delivery systems. Keeping in view the aim, the natural mucilaginous substances were collected from DI, AE by Acetone precipitation method and were undergone identification tests, quantitative determination of pectin by Carbazole tests and compatibility tests were carried out by FT-IR. The physicochemical tests shows the presence of pectin, quantitative determination by carbazole tests shows the presence of pectin and FT-IR report shows its compatibility with pure pectin as well as Metformin HCl (model drug). The study will provide oppurtunities to researches to use the substance in drug delivery system.
Keywords: *Dillenia indica, Abelmoschus esculentus,* Metformin Hydrochloride,polysachharides , mucilages.

INTRODUCTION

Fruits and Vegetables available in nature contain different types of valuable natural substances. Pectin as such is a polysaccharide and is one of the major constituents of fruits like Apple, Lemon, Bannana, DI, AE[1] .It is a high value functional fruit ingredient available in markets in the form of white to light brown powders and is used as gelling agents in several marketed products in jam, gellies [2] etc. It provides major dietery supplements to our body. Thus if pectin is used as a polymer in Drug delivery systems, it is expected not to have any physiological incompatibility. Pectin is a constituent of DI and AE. Already researches are done using pectous material extracted from DI such as pantoprazole loaded microbeads were prepared using the natural mucoadhesive substances extracted from *Dillenia indica* [3], mucoadhesive nasal gel of felodipine prepared with mucoadhesive substance of *Dillenia indica* [4] . This study was done with an aim to study the compatibility of natural mucilaginous extracts isolated from DI and AE with a model drug. Metformin Hydrochloride was taken as the model drug. The powdered mucilaginous substances were isolated from DI and AE by Acetone precipitation method [5] and identification tests[6] of pectin in the natural mucilaginous extracts were done. The identification tests shows the presence of pectin in the natural mucilaginous substances isolated from DI and AE and the quantitative determination of pectin in the mucilaginous substances of DI and AE was done by Carbazole test[7]. But the physicochemical properties of different chemical constituents present in the powdered mucilaginous substances may create interaction in body. Thus the physical mixtures of model drug (Metformin HCl) in solid form alongwith a series of natural polysaccharide isolated from DI and AE was studied by Fourier Transform infrared spectroscopy (FT-IR). This study was done to investigate that the natural polysaccharides can be used as a polymer in Drug delivery in the coming future.

MATERIALS AND METHODS

Metformin Hydrochloride was received from Ozone Pharmaceutical Ltd., Assam and India. DI and AE were procured from local village in Dibrugarh, Assam.

All the chemicals used were of analytical grade laboratory reagents and were used as such.

1. EXTRACTION OF MUCILAGES [5]

1.1 Aqueous Extraction: The natural mucilages substances was extracted using water as the extracting medium under the following conditions:

Sample and extractant ratio	1:1.5
Temperature of extraction	60 ^{0}C
Time of extraction	5-6 hours
Number of extraction	1
Method of precipitation	Acetone precipitation

1.2 Preparation of the sample

1) The raw materials (DI and AE fruits were weighed).
2) These were thoroughly washed under running tap water for 1 hour to remove the adhering materials and leach out other soluble solids.
3) The raw materials were again rinsed with double distilled water.
4) Fruit covers of DI were chopped into small pieces using knife. In case of AE, both the upper and lower ends of the fruit were cut and the fruit was cut into longitudinal sections. The seeds were removed

1.3 Heating of the sample: Mucilage was isolated in the following steps-

1) The heating mantle was set at 60 ^{0}C and pre-heated for 10 minutes.
2) Two beakers (each 1 L) were taken and filled with chopped pieces of the fruits. Deionised water was added to it in the ratio 1:1.5.
3) The above beakers were then placed on the heating mantle.
4) The above set up was kept for about 5-6 hours for complete recovery of mucilage.
5) The preparation was stirred regularly with glass rod.
6) Temperature was checked at 15 minutes interval to keep it maintained at 60 ^{0}C.
7) After about 6 hours the slurries were strained through a Buchner funnel.
8) The filtrate was kept in refrigerator in a beaker for overnight for sedimentation.
9) The decanted filtrate was taken out of refrigerator and the supernatant was poured into a clean and dry beaker of 1 L size.

1.4 Concentration of the Extract:

The supernatant obtained from the above decantation process is concentrated by evaporating the solution in heating mantle at 50- 60 ^{0}C.The volume of the samples were reduced to 1/5 th of its original volume.

1.5 Precipitation of the Sample

The concentrated samples were washed with three volumes of Acetone. The precipitate was collected and rewashed three times with three volumes of Acetone and the precipitate was collected.

1.6 Drying of the precipitate:

The precipitate was dried at about 50 to 60^0 C in a Hot air oven for 4 hours. On drying, the sample becomes hard and brownish in colour. It was powdered and the powdered samples were passed through sieve nos. 120 & 150.Samples was stored in desiccators under sealed conditions for further study.

2. IDENTIFICATION TEST OF PECTIN [6]

2.1 Stiff gel Test

1g of pectin was heated with 9ml of water on a water bath till a solution was formed, on cooling stiff gel was formed.

2.2 Test with 95% Ethanol

On adding an equal volume of Ethanol (95%) to 1% w/v solution of pectin sample, a translucent, gelatinous precipitate was produced. (Distinction from most gums).

2.3 Test with Potassium Hydroxide (KOH)

To 5ml of a 1% w/v solution of pectin sample, 1ml of a 2% w/v solution of KOH was added and set aside for 15 minutes. A transparent semi-gel was produced When the above gel was acidified with dilute HCl and shaken well, a voluminous, colourless gelatinous ppt. was formed .This upon boiling became white and flocculent.

2.4 Iodine test

To 5ml of recently boiled and cooled 2% w/v solution of sample, 0.15 ml of Iodine solution was added. No blue colour was produced.

2.5 Test for Acidity

An aqueous solution of pectin sample is acidic to blue litmus paper.

3. CHARACTERISATION

Characterisation of the isolated Pectin sample was carried out by spectrophotometric methods described by Elizabeth A. McComb and R.M. McCready [7].

Carbazole test

3.1 Preparation of Reagents

0.150 g of reagent grade carbazole was dissolved in 100 ml of ethyl alcohol. The dissolution of carbazole was slow and required stirring.

3.2 Method

i. 2ml of sample was chilled in 10ml distilled water.
ii. 12 ml of conc. H_2SO_4 was added slowly and carefully through the side of the test tube.
iii. The above preparation was heated for 20 minutes on a water bath and cooled.
iv. 1 ml 0.1% Carbazole in absolute alcohol was added to it and kept for 2 hours.
v. Spectrophotometer reading was taken at 520 nm against a blank prepared by same method without sample.
vi. Calibration curve was plotted at concentration 20µg.ml^{-1}, 40 µg.ml^{-1}, 60 µg.ml^{-1}, 80 µg.ml^{-1} at 520 nm.

4. COMPATIBILITY TEST

The FT-IR spectra of pure pectin, Metformin HCl and both the powdered mucilaginous substances were obtained individually and compared. Physical mixture of drug with powdered mucilaginous substances taking one of them with drug was prepared and FT-IR spectra were obtained. Another FT-IR spectrum was also obtained for the physical mixture of drug with both the mucilaginous substances. The FT-IR spectra were obtained using Bruker, Alpha Infrared spectrophotometer and at mid IR region (wavelength 25 µ to 2.5 µ, wave-number from 200 cm $^{-1}$ to 4000 cm^{-1}), in order to identify the possibility of any interaction between model drug and the powdered mucilaginous substances of DI and AE.

RESULTS AND DISCUSSION

a. Percentage Yield of DI and AE mucilaginous substances

As shown in Table 1, the percentage yield of mucilaginous substances of DI and AE are found to be 0.56 % and 0.24% respectively. The percentage yield is calculated by the following equation:

Percentage yield =
[Amount of mucilaginous substances recovered (g)] x 100
Quantity of fruit (g)

b. Identification Test

After the extraction process, tests were done to ascertain the presence of pectin in the sample. The results of the various identification tests performed are tabulated in Table 2. All the performed tests showed positive results towards the presence of Pectin in the Sample. Further confirmation was done by acidifying the Semi-gel formed in the Test No. 3 in Table 5 with dilute HCl and shaken well voluminous, colorless gelatinous ppt. was formed. This upon boiling becomes white and flocculent. This confirmed the presence of Pectin. The observation of identification of pectin was similar for both the powdered mucilaginous substances.

Table 1: Percentage yield of mucilaginous substances

Plants	Batches	Quantity of Fruit (g)	Weight of mucilageous substances after drying (g)	Percentage Yield (%)
DI	Batch 1	5000	30	0.60
	Batch 2	5000	25	0.50
	Batch 3	5000	32	0.58
AE	Batch 1	7000	15	0.21
	Batch 2	7000	18	0.26
	Batch 3	8000	20	0.25

Table 2: Observation of identification tests of mucilaginous substances

Test No.	Description	Observation
1	Stiff gel Test	A Sticky gel was formed after cooling.
2	Test with 95% Ethanol	Sample formed gelatinous ppt.
3	Test with Potassium Hydroxide(KOH)	A semi-gel or transparent was formed.
4	Iodine Test	No change
5	Test for Acidity	Sample turns blue litmus paper to red.

Table 3: Estimation of the Pectin content powdered mucilaginous substances by Carbazole Test

Powdered Mucilaginous substances.	Absorbance	Concentration (µg/ml) From calibration plot	Weight of recovered powdered mucilaginous substances(gm)	Content of Pectin (calculated)
DI	0.44	48.88	30	29.33
			25	24.44
			32	31.28
AE	0.46	51.11	15	15.33
			18	18.39
			20	20.44

c. Estimation of the amount of pectin by Carbazole test

The amount of pectin in the mucilaginous substances was obtained by Carbazole Test as shown in Table 3. Since the aqueous mucilaginous substances were not purified. Other water soluble substances were also present, due to which, the actual pectin content may vary.

Calibration curve of Pectin

The calibration curve of pure Pectin was obtained by taking absorbance at 520 nm. The regression coefficient was found to be 0.949 as shown in Figure 1.

d. Compatibility test by FT-IR

The FT-IR of pure Pectin, AE powdered mucilaginous substances, DI powdered mucilaginous substances, Metformin HCl, physical mixture of Metformin HCl and AE powdered mucilaginous substances, physical mixture of Metformin HCl and DI powdered mucilaginous substances, physical mixture of Metformin HCl, DI and AE powdered mucilaginous substances are given in Figure 2, Figure 3, Figure 4, Figure 8, Figure 9, Figure 10 respectively. The FT-IR peaks of the powdered mucilaginous substances isolated from DI, AE appeared at the same position as that were found in the pure Pectin. This indicated the presence of pectin in the mucilaginous substances isolated from DI and AE. There was no significant interaction between the Metformin HCl and the powdered mucilaginous substances isolated from DI and AE as the characteristic N-H stretching band of Metformin HCl remained unchanged in the case of physical mixture of Metformin HCl and powdered mucilaginous substances isolated from DI, Physical mixture of Metformin HCl and powdered mucilaginous substances isolated from AE as well as the physical mixture of the Metformin HCl, powdered mucilaginous substances isolated from DI and AE. It indicates that the Metformin HCl and the powdered mucilaginous substances are compatible which shows that Metformin HCl is compatible with mucilaginous substances isolated from DI and AE. Pure Metformin hydrochloride spectra showed sharp characteristic peaks at 3367.34, 3298.05, 3169.04, 2977.89, 1627, 1222, 1064.63, 636.47 $cm^{-1.}$ The peaks appeared at the same wave number of 3367.71, 3172.90,1627.92,937.40, 734.88, 420.48 cm^{-1} in the physical mixture of model drug and AE , Metformin HCl and DI and physical mixture of Metformin HCl, powdered mucilaginous substances of DI and powdered mucilaginous substances of AE. A comparative study of powdered mucilaginous substance of AE with pure Pectin shows a correlation of 94.26% as shown in Figure 5 and powdered mucilaginous substances of DI with pure Pectin shows a correlation of 96.28% as given in Figure 6. Whereas a comparative study of physical mixture of Metformin HCl and powdered mucilaginous substance AE with Metformin HCl shows a correlation of 97.77%, also comparative study of physical mixture of Metformin HCl and powdered mucilaginous substance of DI with Metformin HCl shows a correlation of 93.99% as shown in Figure 11 and Figure 12 respectively. Also the comparative study of physical mixture of Metformin HCl, powdered mucilaginous substances of AE and DI with Metformin HCl shows a correlation of 87.67%. Thus the FT-IR report shows the absence of any interaction of mucilaginous substances isolated from DI, AE with pure Pectin and Metformin HCl are compatible with pure Pectin and Metformin HCl.

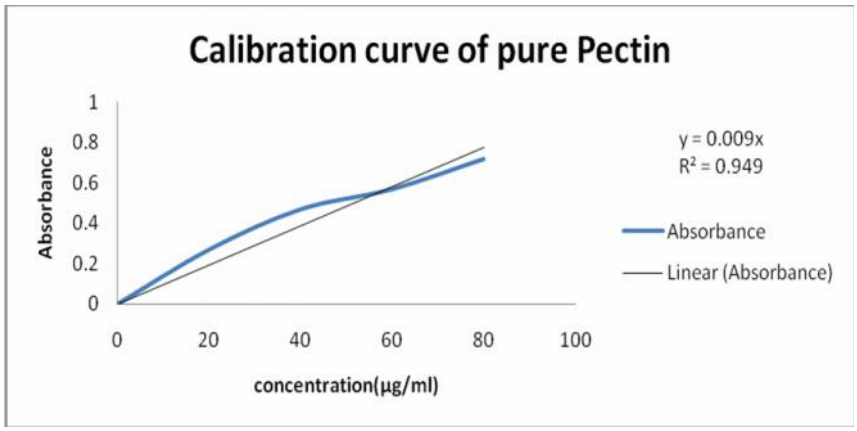

Figure 1: Calibration curve of pure Pectin (λ_{max} = 520 nm).

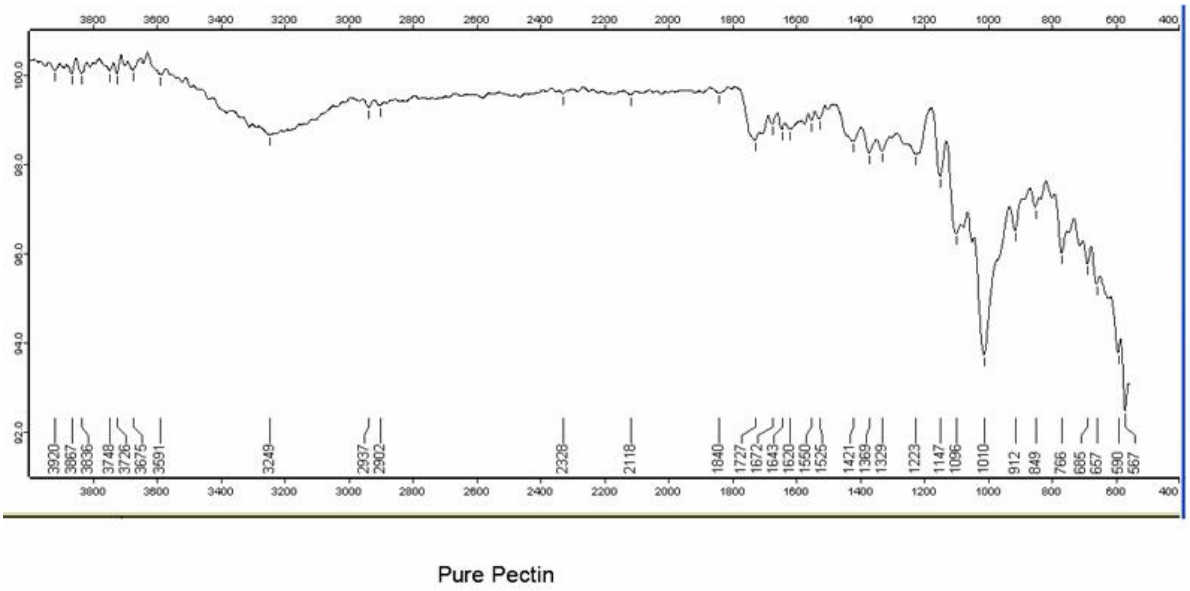

Figure 2: FT-IR of pure Pectin.

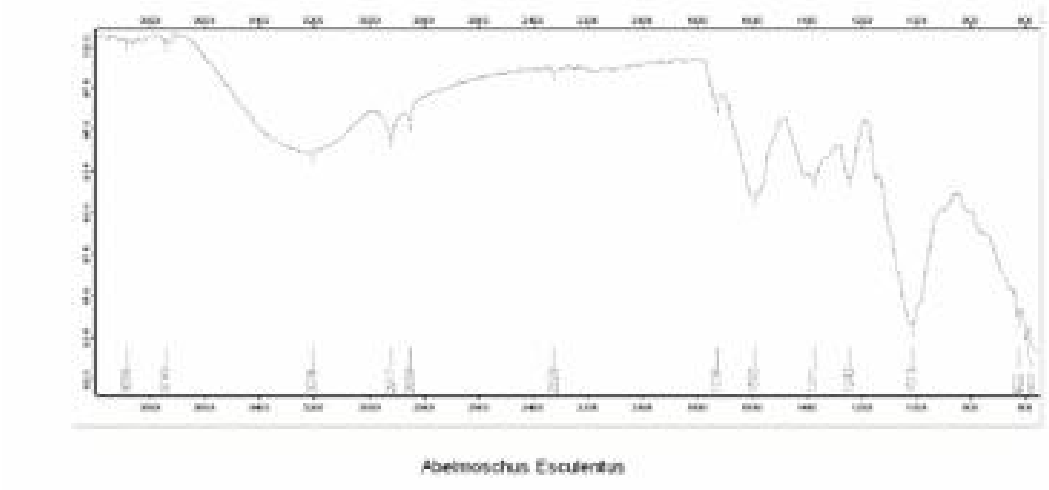

Figure 3: FT-IR of AE powdered mucilaginous substances.

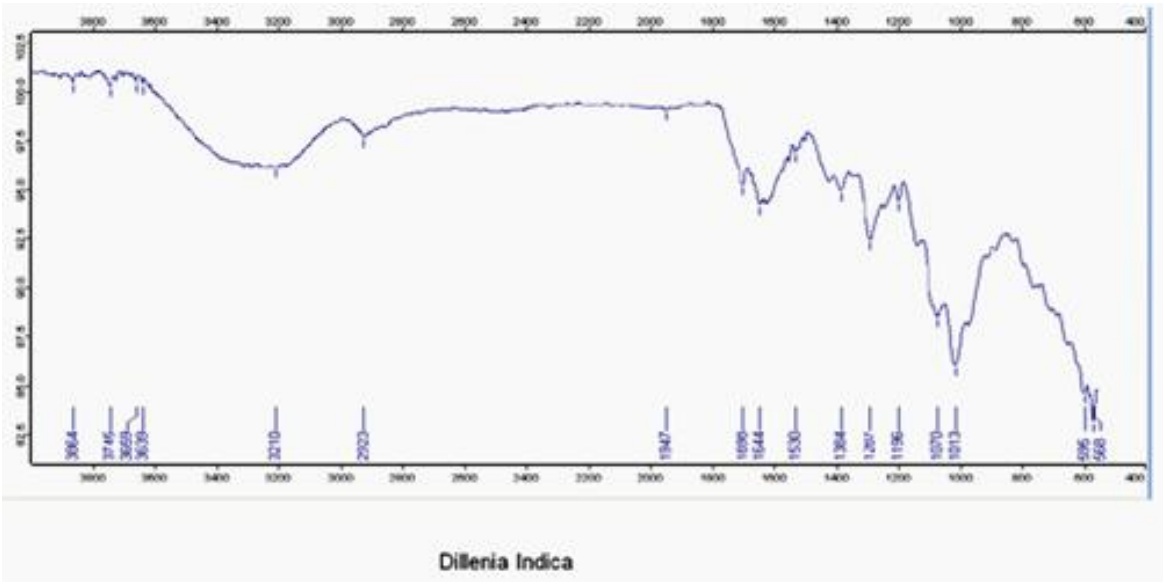

Figure 4: FT-IR of DI powdered mucilaginous substances.

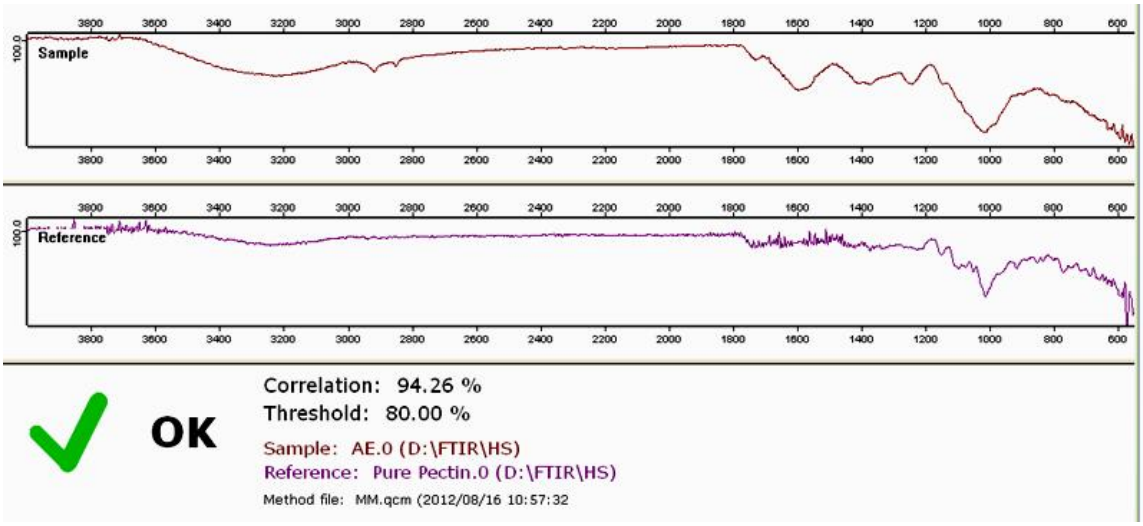

Figure 5: Comparative study of powdered mucilaginous substances of AE with pure Pectin.

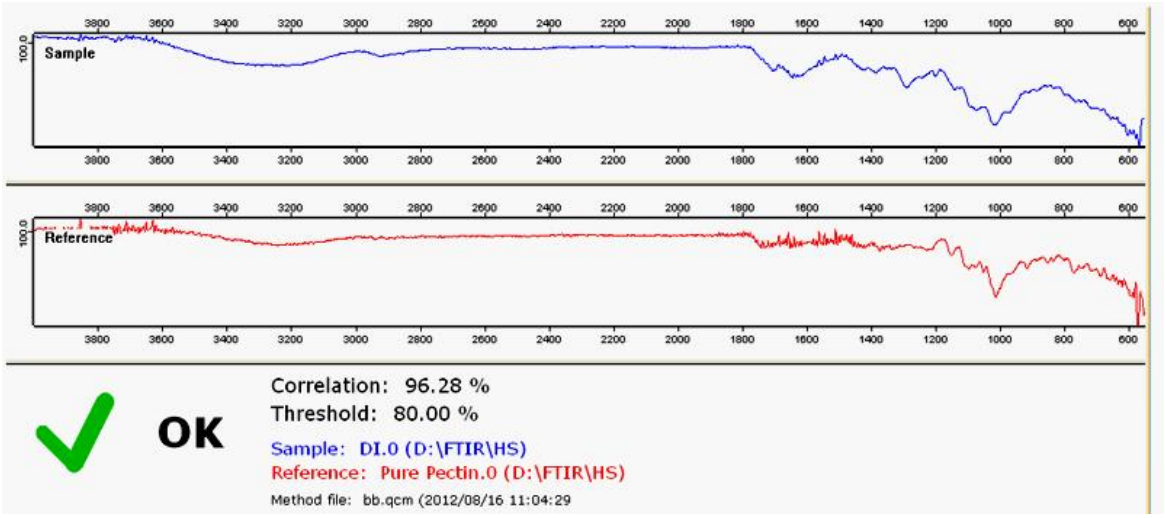

Figure 6: Comparative study of powdered mucilaginous substances of DI with pure Pectin.

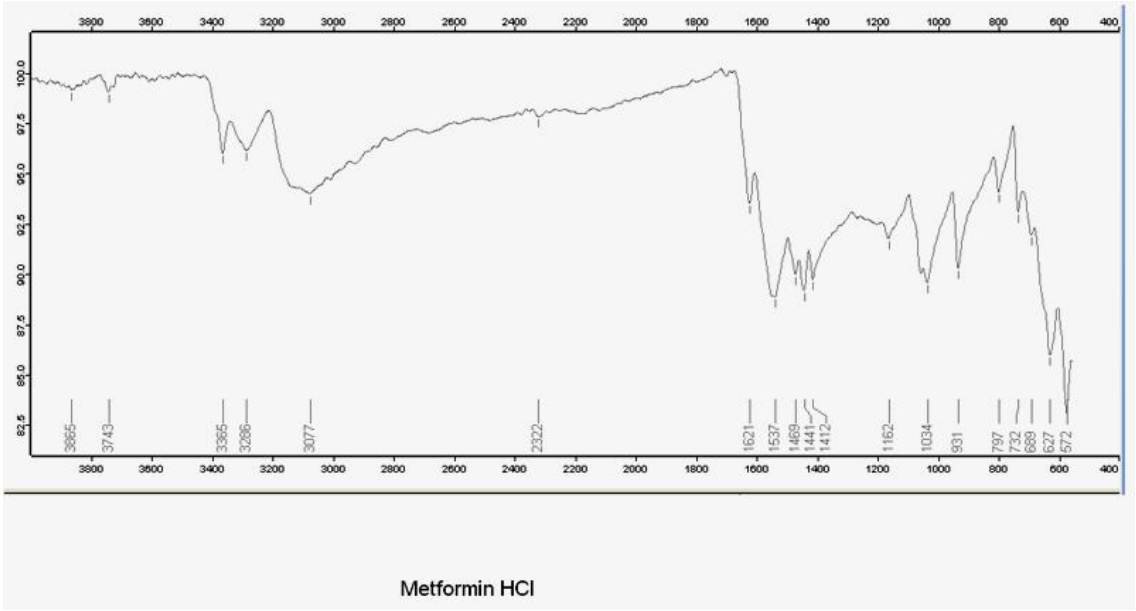

Figure 7: FT-IR of Metformin HCl.

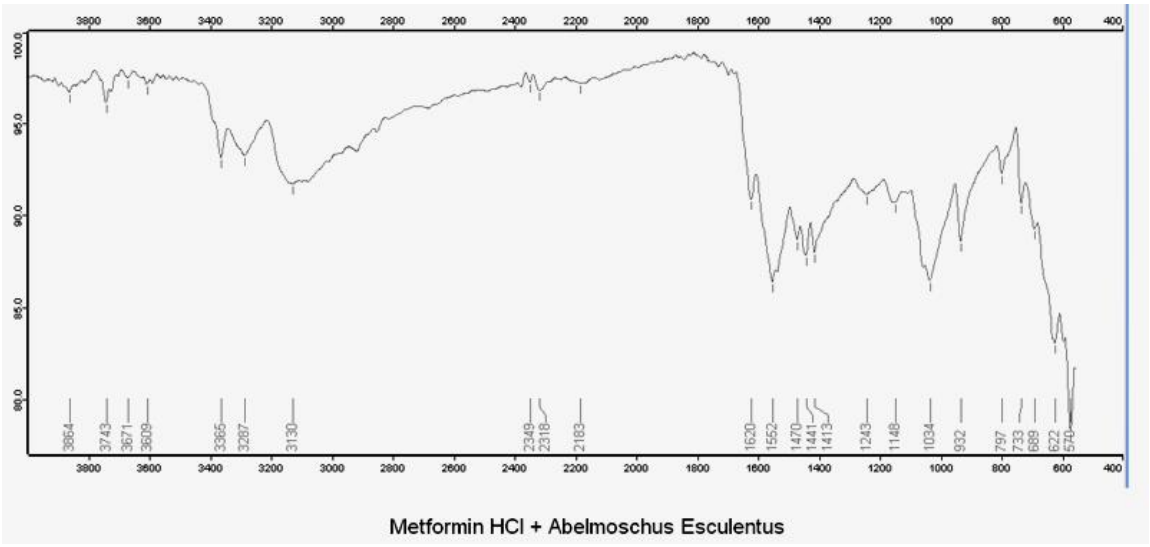

Figure 8: FT-IR of physical mixture of Metformin HCl and AE.

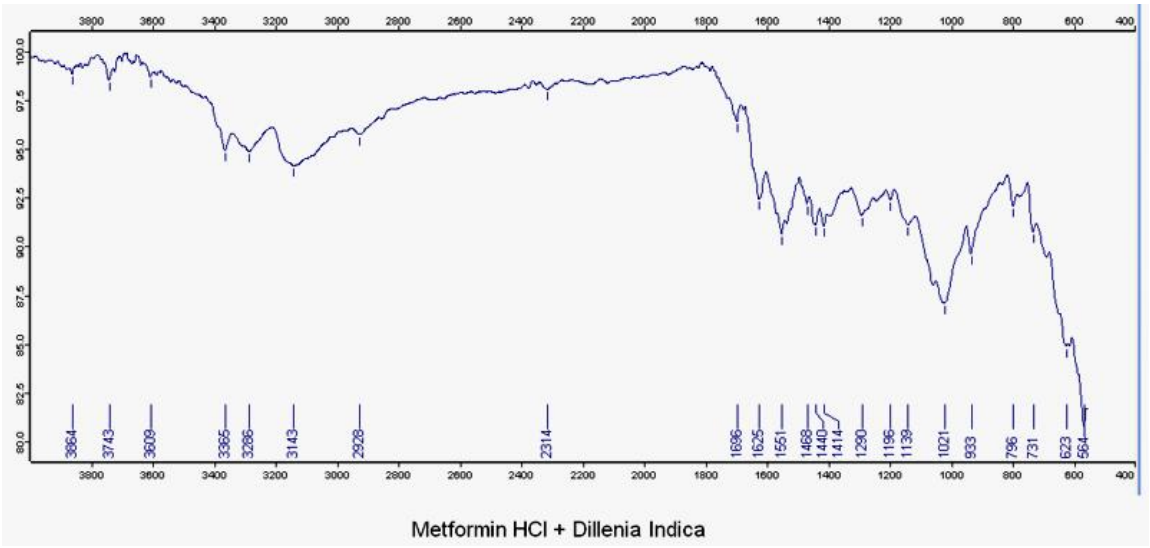

Figure 9: FT-IR of physical mixture of Metformin HCl and DI

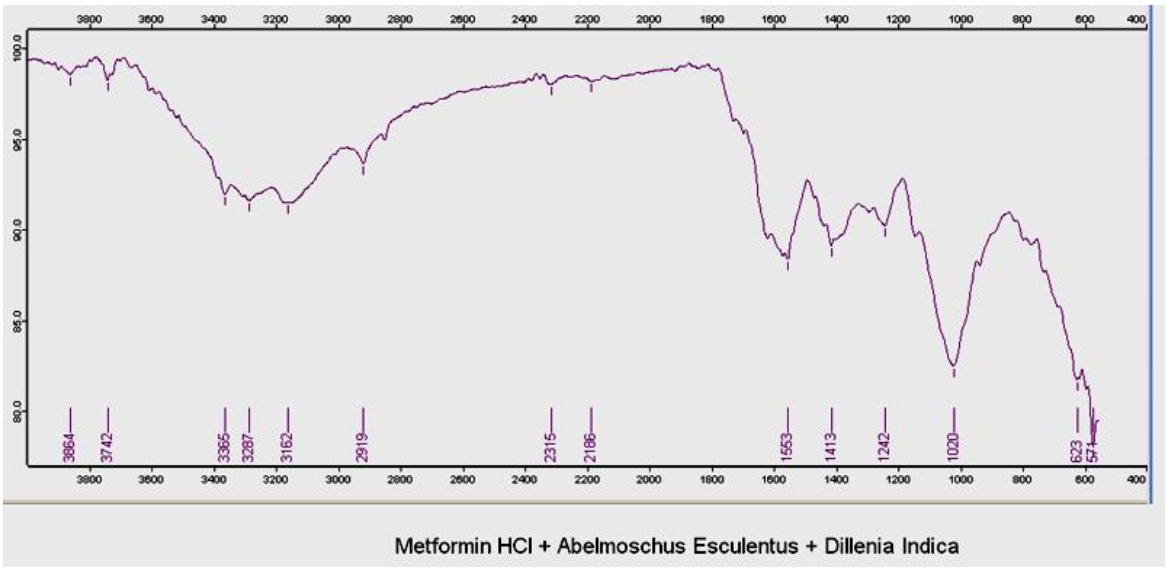

Figure 10: FT-IR of physical mixture of Metformin HCl, AE and DI

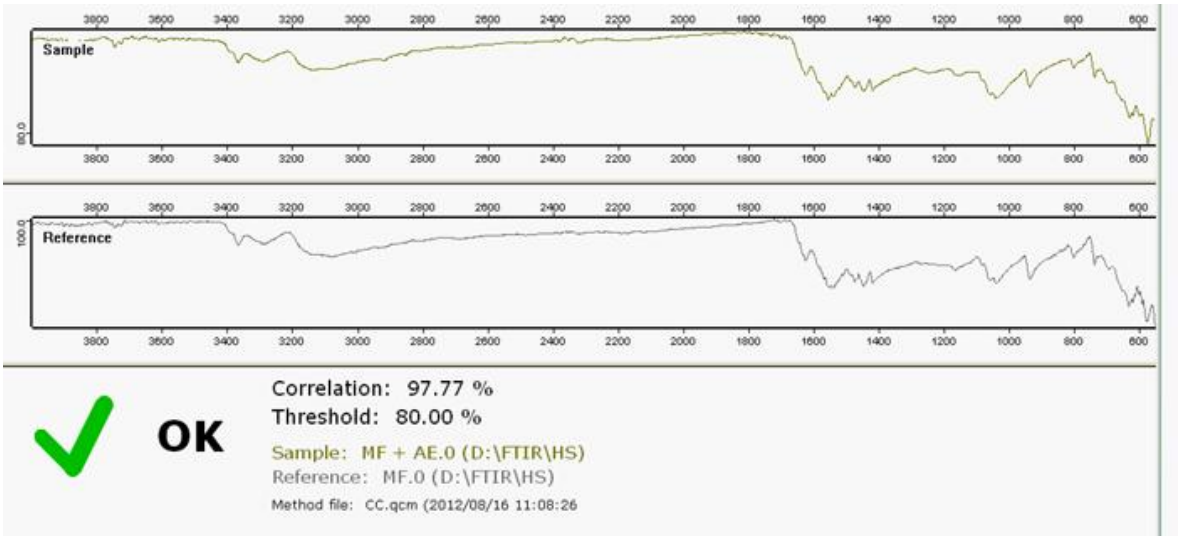

Figure 11: Comparative study of physical mixture of Metformin HCl and AE with Metformin HCl.

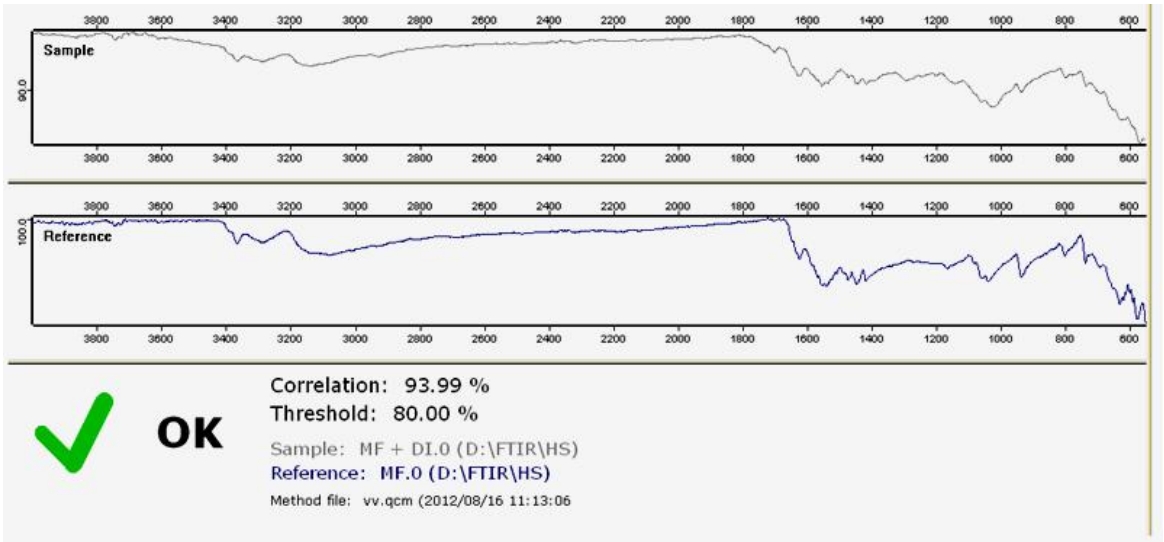

Figure 12: Comparative study of physical mixture of Metformin HCl and DI with Metformin HCl.

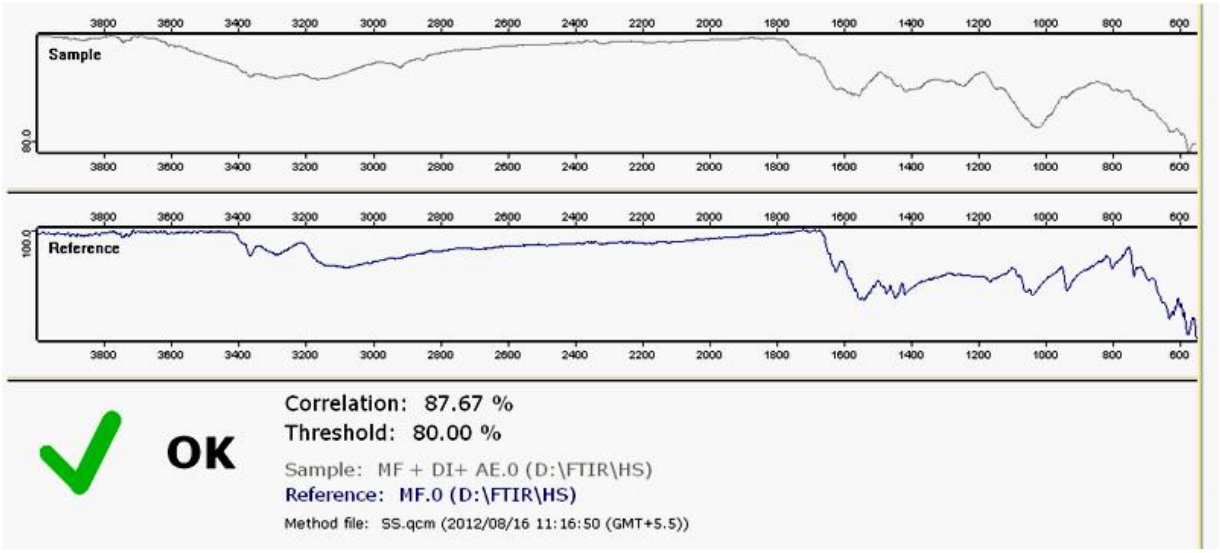

Figure 13: Comparative study of physical mixture of Metformin HCl, DI and AE with Metformin HCl

CONCLUSION

This study shows the presence of pectin in the mucilaginous substances isolated from DI and AE which was confirmed by the identification tests of pectin, and further quantification by Carbazole test. The FT-IR study shows the absence of any interaction between the Metformin HCl and the mucilaginous substances. Thus the mucilaginous substancesis can be a very promising polymer in several Drug delivery systems especially in mucoadhesive drug delivery system.

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