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Original Article

CHARACTERIZATION AND HYPOGLYCEMIC STUDY OF ISOLATED NATURAL POLYMER FROM THE STEM OF *MANILKARA HEXANDRA* (ROXB.) DUBARD

IRUDAYA MONISHA S.*, ROSALINE VIMALA J.

PG and Research Department of Chemistry, Holy Cross College (Autonomous), Trichy, Tamil Nadu, India
Email: irudaya.monisha@gmail.com

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ABSTRACT

Objective: In the present study, the isolated natural polymer from the stem of *Manilkara hexandra* and their physiochemical parameters were investigated. It is further involved in hypoglycemic studies.

Methods: The gum exudates were screened for phytochemicals, physicochemically analyzed for solubility, pH, total ash, moisture content, acid insoluble ash, water soluble ash, sulfated ash, flow property, and it is characterized by Fourier Transform Infrared analysis (FT-IR), Scanning Electron Microscope (SEM), X-ray diffraction analysis (XRD), Nuclear Magnetic Resonance (NMR) studies (^{13}Carbon and ^{1}Proton) and it was examined *in vitro* studies by hypoglycaemic activity.

Results: The isolated gum extracted from the stem of *Manilkara hexandra* (Roxb.) Dubard ash values were found to be low. The gum is found to be hygroscopic in nature due to its high moisture content (0.9131±0.03). Fourier Transform Infrared (FT-IR) spectra show relevant functional groups for gum, which is further confirmed by resonance spectral studies. The X-ray diffraction (XRD) pattern shows that the gum is amorphous as well as crystalline in nature. Scanning Electron Microscope (SEM) image confirms that the gum particles have irregular size and shape. Sugar composition analysis by Thin Layer Chromatography indicated the presence of rhamnose, arabinose. The *in vitro* study of hypoglycemic activity shows the best report compared with the standard. The experimental evidence offers scope to use this natural polymer in the food and pharmaceutical industry.

Conclusion: The isolated natural polymer shows good result in hypoglycemic studies compared with standard.

Keywords: *Manilkara hexandra*, Isolated polymer, Characterization, Hypoglycemic activity

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INTRODUCTION

The position of the earth and the environment conditions makes India the best source of natural products among Asian countries [1]. Natural products are a precious gift of the plants and an assorted lot of pharmaceutical applications such as adhesives, stabilizers, emulsifiers, diluents, binding agents [2], suppository and thickeners [3]. Apart from these applications, the gums and resins produced naturally still add values such as beautifiers, papermaking, and colorants to materials. Such gum-yielding trees occupy a prime place in Non-Wood Forest Produce. Most forest dwellers a viable source of income is from such resin and gum-producing trees.

From very early times natural gums and resins are being used in food, medicine, industries and also in their manufacturing produces. The present-day usage of gums and resins are innumerable. These natural polymers formed by throwing with [4, 5] or without [6] have different properties which depend on their environment [7, 8] and also on their physicochemical characteristics [9]. The gums are hydrophilic or hydrocolloids in nature, which means that they are, water-soluble [10]. A number of gums have been studied to validate their pharmacological usage.

The present study deals with the extraction and characterization of gum from the stem of *Manilkara hexandra* (Roxb.) Dubard (Sapotaceae). It is a huge evergreen tree and broadly present in many regions of India and tropical nations. The stem bark is pleasant, febrifuge, tonic, astringent and medicinal use for helminthiasis, jaundice etc [11]. It is utilized generally to the extensive variety of gastrointestinal manifestations [12]. The seed also contains a medicinal property for piles, ulcers etc [13]. The isolated biopolymer was subjected to micrometric analysis, flow property calculations, characterized by various spectral techniques and evaluated for its hypoglycemic activity.

MATERIALS AND METHODS

Chemicals and reagents

Double distilled water, acetone and other chemicals were purchased from Sigma Aldrich (AR grade). HPLC grade solvents such as, were used in Thin-layer Chromatographyic analysis (Merck). Hydrochloric acid, Sulphuric acid and Nitric acid are used in physicochemical parameters.

Collection of plant

Manilkara hexandra (Roxb.) Dubard (Synonym: *Mimusops hexandra* Roxb.) were collected from Jayakondam at Ariyalur District, Tamil Nadu, India and authenticated by Rapinet Herbarium, St. Joseph's College (Autonomous), Trichy. The sample Voucher number is 00I1. Stems were washed with running water, shade dried, and ground by pestle mortar.

Extraction and isolation of gum

The stem of *Manilkara hexandra* (1 kg) was defatted with petroleum ether and the dried marc was extracted with methanol. During the process, all the saponins were removed [12]. The collected marc was treated with double-distilled water and heated at 60˚C. This filtrate was separated by using muslin cloth (8 fold). The settled precipitate was kept below 6˚C by using airtight containers in the refrigerator. This aqueous volume was reduced (1/3) by applying heat and added acetone to form a gummy precipitate which is settling down in a beaker. After this, a portion was washed with acetone (3 times) and it was separated by using the muslin cloth. The gum was dried till it reaches to constant weight at 35-45˚C in a hot air oven. The dried natural gum was crushed into powder using pestle mortar. Then the isolated polymer powder of *Manilkara hexandra* gum (MHG) yield percentage was calculated.

Phytochemical screening of isolated polymer

The natural polymer was screened using a standard harborne procedure to study the phytochemical properties for the presence of alkaloids, flavonoids, proteins, amino acids, saponin, tannins, carbohydrates, fixed oils and fats, and gums [14]. Hydrolysis test was examined [15].

Physicochemical characterization of natural polymer

The physicochemical was examined using standard procedure [16-18].

Study of flow property parameters

Bulk density

About 10 g of the *Manilkara hexandra* gum was exactly weighed and placed in 100 ml measuring cylinder without disturbing. The bulk volume was measured.

$$\text{Bulk density } (\rho b) = \frac{\text{weight of the sample}}{\text{Bulk volume}}$$

Tapped density

It was obtained by using mechanically tapping a cylinder. The tapped volume was calculated from the tap density tester (Electro lab, Nobel ETD 1020).

$$\text{Tapped density } (\rho t) = \frac{\text{weightofthe sample}}{\text{Trapped volume}}$$

Compressibility index

The Carr' index was calculated from the density of the natural polymer by applying the following formula,

$$\text{Compressibility (C \%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner ratio

The flowability of *Manilkara hexandra* gum (MHG) was calculated by using the Hausner ratio (H). This value was obtained from the following formula,

$$\text{Hausner ratio (H)} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of repose

The stem of *Manilkara hexandra* gum (MHG) powder 10g was introduced into a funnel placed on a horizontal paper surface. Height (H) and radius(R) was measured. The angle of repose (θ) was calculated.

$$\text{Tan}\theta = {}^{H}/_{R}$$

Viscosity

The viscosity of polysaccharide was determined by Oswald's Viscometer [19]. 500µg was taken in a U-shaped long pipette. It consists of two bulbs with a mark in a glass tube. The flow time of the upper and lower mark volume of the bulb was measured between two liquids and calculated by the formula.

$$\frac{\eta 1}{\eta 2} = \frac{d1t1}{d2t2}$$

Where,

η_1 and η_2 = Coefficient of viscosity

d_1 and d_2 = Density

t_1 and t_2 = Time

Fourier transform infrared analysis (FT-IR)

The Fourier Transform Infrared analysis **(**FTIR) spectrum of the gum was studied in the scanning range of 450 to 4500 cm^{-1} with 1 cm^{-1}as the resolution.

Scanning electron microscope (SEM)

The morphological feature (geometry) of the polymer was analyzed at 100µm of voltage 10 kV with different magnification (200 and 213) using the scanning electron microscope (ZEISS).

X-ray diffraction analysis (XRD)

The natural polymer of stem *Manilkara hexandra* gum (MHG) was recorded by X-ray diffractometer (Goniometer). This analysis examined at measurement temperature (25˚ C), voltage (40Kv), and current (30 mA), scan step time (10.16 s), anode material (Cu) and specimen length (10 mm).

Nuclear magnetic resonance (NMR)

The Nuclear Magnetic Resonance **(**NMR) spectra of the gum were recorded in NMR (BRUKER 300 MHz) Ultra shield Magnet with AVANCE II type console to identify its structural features. The H^1 and C^{13} NMR were measured from 0-8 ppm and 50-110 ppm respectively.

Estimation of sugar composition by thin layer chromatography

Mucilages and gums present in plant constituents have pharmaceutical and technical applications which have within them carbohydrates, which are difficult to be analyzed since they are polar and similar structural characteristics. Thin layer chromatography (TLC) was performed to determine the carbohydrates that are available in the gum. The sample was dissolved in double distilled water and spotted in precoated thin layer chromatography (TLC). The Butanol: Acetic Acid: Water (BAW) was highly polar than other systems [20]. So the analysis was done using Butanol: Acetic Acid: Water (4:1:5) solvent system on the aluminium plate (6.5×3.5 cm) with glucose, fructose, sucrose as standard references [21].

***In vitro* of hypoglycemic activity (Non-insulin dependent diabetes mellitus)**

Using phosphate buffer of pH = 6.9 varied concentrations such as 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml of *Manilkara hexandra* gum (MHG) extract were prepared. From each of these concentrations 500 µl of the extract was withdrawn and to that 20 mmol of the buffer and 0.5 mg/ml of α-amylase were added and incubated at 25 ˚C for 10 min. The procedure was repeated by adding 0.5 % starch solution (1000 µl) in 20 mmol phosphate buffer and incubated for 10 min at 25 ˚ C. To that 500 µl of 3, 5-dinitrosalicyclic acid was added in all the five tubes for color development, followed by incubation in a boiling water bath for 5 min. The tubes were cooled to room temperature and their corresponding absorbance was measured at 540 nm. Acarbose was used as the standard with respect to which the inhibitory activity of α-amylase and percentage inhibition was calculated as follows [22].

$$\text{\% of inhibition} = \frac{(\text{Control O. D.} - \text{Test O. D.})}{\text{Control O. D.}} \times 100$$

The experiment was performed in triplicate and its standard deviation value was recorded as percentage inhibition.

RESULTS AND DISCUSSION

Yield percentage

The yield percentage of the isolated natural polymer (fig. 1) is lesser than one (0.88<1).

Fig. 1: Isolated natural polymer from the stem of *Manilkara hexandra*

Phytochemical screening for gum

The results of the phytochemical screening of gum obtained from the stem of *Manilkara hexandra* are shown in table 1, fig. 2a and fig.

2b. The purity of the gum was confirmed from the positive test for carbohydrates and the negative tests for amino acid, alkaloids, protein, oils and fats, flavonoids and tannins which correlates with the report of okra gum [23].

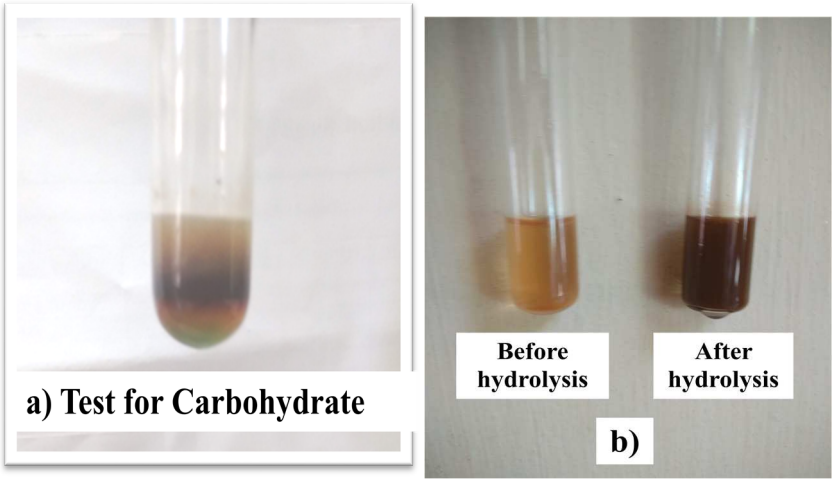

Fig. 2. a.) Molisch test for the stem of *Manilkara hexandra* gum (MHG), b.) Hydrolysis test

Table 1: Qualitative report for stem of *Manilkara hexandra* gum (MHG)

S. No.	Phytocompounds	Observations
1.	Alkaloids	Absent
2.	Amino acid	Absent
3.	Protein	Absent
4.	Flavonoids	Absent
5.	Tannins	Absent
6.	Oils and Fats	Absent
7.	Gum	Present
8.	Sugar	Present

Physicochemical properties

This gum separated from the stem of *Manilkara hexandra* was subject to various physicochemical properties such as solubility shown in table 2. pH, total ash, moisture content, acid insoluble ash, water-soluble ash and sulphated ash as shown in table 3. The gum was soluble in water, slightly soluble in petroleum ether, chloroform, Dimethylformamide (DMF), acetone, and practically insoluble in hexane, ethyl acetate, ethanol, methanol. The pH is 4.98±0.0112 which was moderately acidic which nearly match with the fruit mucilage of *Chrysophyllum lanceolatum* [24]. The low value of total ash (0.0681±0.0094) sulphated ash (0.0601±0.0082), water-soluble ash (0.0341±0.0059), and acid insoluble ash (0.0205±0.0007) indicates that the sample is more pure. Moisture content depends on the stability of the drug [25]. The high moisture content value (0.9131±0.03) generally results in particle agglomeration. It is important for aerosol containing powders. Thus, the gum could be used as protective colloids in suspensions, cosmetics, hot beverages and paint making [26].

Table 2: Solubility analysis of the stem of *Manilkara hexandra* gum (MHG)

S. No.	Solvents	Solubility	Number of parts
1.	Hexane	Nil	More than 10,000 parts
2.	Petroleum ether	Nil	More than 10,000 parts
3.	Chloroform	Nil	More than 10,000 parts
4.	Ethyl acetate	Nil	More than 10,000 parts
5.	Ethanol	Nil	More than 10,000 parts
6.	Methanol	Nil	More than 10,000 parts
7.	Acetone	Sparingly soluble	30-100 parts
8.	DMF	Slightly Soluble	100-1000 parts
9.	Water	Very Soluble	Less than 1 part

Table 3: Study of physicochemical parameters for the stem of *Manilkara hexandra* gum (MHG)

S. No.	Physicochemical parameters	Values*
1.	Total ash	0.068±0.0094
2.	Moisture Content	0.9131±0.030
3.	Sulphated ash	0.1076±0.0022
4.	Water soluble ash	0.0341±0.0059
5.	Acid-insoluble ash	0.0205±0.0007
6.	pH	4.9800±0.0112

*All the experiments were repeated independently three times and the values were represented as an average means±Standard deviation.

Flow property of the gum

The flow properties of the gum are shown in table 4. The results of the flow parameters such as Hausner's ratio and viscosity show that it has low flow property. Bulk density, tapped density and compressibility index values indicate that it is a fine powder. Angle of repose declare the penury glide property which displays stem of *Manilkara hexandra* gum (MHG) was cohesive [27]. The gum of *Manilkara hexandra* gum (MHG) is less viscous due to the complex nature of polysaccharide and monosaccharide derivatives found in it [28].

Table 4: Study of flow property parameters for the stem of *Manilkara hexandra* gum (**MHG)**

S. No.	Flow property parameters	Results* ($\bar{x}\pm\sigma$)
1.	Bulk density (g/ml)	0.7591±0.00001
2.	Tapped density (g/ml)	1.1386±0.00005
3.	Compressibility index (%)	33.56±0.0426
4.	Hausner ratio (%)	1.5±0.01
5.	Angle of repose (˚)	49.01±0.9318˚
6.	Viscosity (cP)	0.93±0.073

*All the experiments were repeated independently three times and the values were represented as an average means±Standard deviation.

Fourier transforms infrared analysis

Fig. 3 shows the characteristic absorption band at 3387 cm^{-1} showing the presence of hydrogen-bonded O-H group. The characteristic peak at 3400 cm^{-1}-3500 cm^{-1} for an amino group would have been masked by the broad O-H band. The presence of alkane C-H stretching, aldehydic C-H stretching and sugar, galactose, arabinose, rhamnose is confirmed by the appearance of an absorption peak at 2927 cm^{-1}. The peak at 1622.13 cm^{-1} is the characteristic band for C=C stretch, N-H amide bend, NO_2 of both aliphatic and aromatic galacto proteins and amino acids of the polymer. The vibrational band at1323.17 cm^{-1} and 1419.61 cm^{-1} are due to C=O sym stretch and O-H bending of glucoronic acid. Band at 1373.32 cm^{-1} are characteristics of CH_3 bend in alkane, aromatic C=C stretch, ketone C-C stretch; amine C-N stretch of polysaccharides and galacto proteins. Alkane bending and alcohol stretching due to sugar backbone is confirmed by the appearance of peak at 1244.09 cm^{-1}. The alkene C-H bends of polysaccharides are found as 1022.27 cm^{-1} [29].

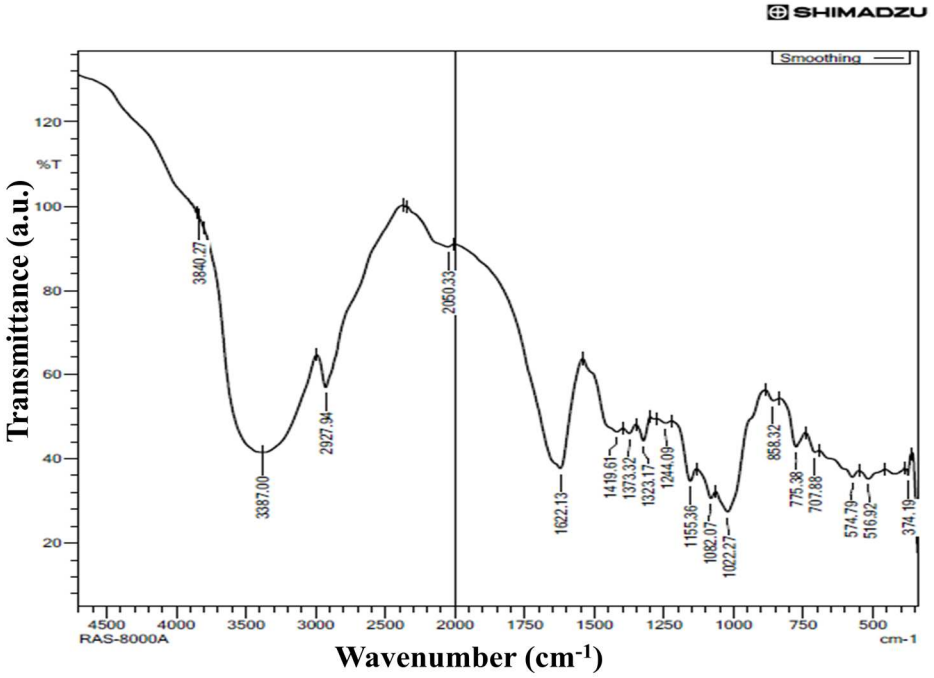

Fig. 3: Fourier transforms infrared spectrum for the stem of *Manilkara hexandra* gum (MHG)

Scanning electron microscope (SEM)

The Scanning Electron Microscope **(**SEM) image of the gum obtained is represented in fig. 4. The microphotographs of the gum shows that the particles are found as aggregates of irregular dimensions, shape devoid of crystallinity, they are fibrous in nature [21]. The *Manilkara hexandra* gum (MHG) non-uniform shape, and it retards from the dosage form due to pore size [30, 31].

X-ray diffraction analysis (XRD)

Physicochemical property of rubbery material depends on the extent of crystallinity of the additives used in polymers. The ease of incorporation and uniformity of dispersion is influenced by the crystalline nature of the polymer matrix, thereby significantly have some role on the physicochemical properties. In view of this X-ray diffraction (XRD) study done for the gummy material is shown in fig. 5. A peak of low intensity at the diffraction angle $2\theta = 24.6^{\circ}$ at the interplanar line spacing of 3.6 Å shows that the gum has a very low degree of crystallinity. Thus, this study supports that it is amorphous as well as crystalline in nature [32]. The obtained 2θ (23˚-25˚) angle is similar to that of *Ocimum basilicum* L. seed mucilage [33].

Nuclear magnetic resonance

The Proton and Carbon Nuclear Magnetic Resonance (NMR) spectrum correlate with the specifications for gum [29]. Using Nuclear Magnetic Resonance (NMR) the structure and property of material (polymer) can be studied. It helps to identify the material, the different functional

group, detect minor compounds and impurity [34]. The signals between 3 and 6 ppm correspond to polysaccharides. Chemical shift at 1.2 ppm is due to the methyl group of rhamnose. An intense peak at 2.1 ppm shows the presence of an acetyl group. Signals between 3.1 to 4 ppm are due to the methoxy group. The presence of non-anomeric protons are seen by the arisement of peaks between 3.63 ppm-4.70 ppm in ^{1}H Nuclear Magnetic Resonance (NMR) and between 60-85 ppm in ^{13}C spectra. An intense peak at 4.80 ppm is due to water molecules shown in fig. 6a and fig. 6b. Typical ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra of the gums are seen by the appearance of peaks in the anomeric region between 4.7-5.9 ppm and 90-110 ppm respectively [35].

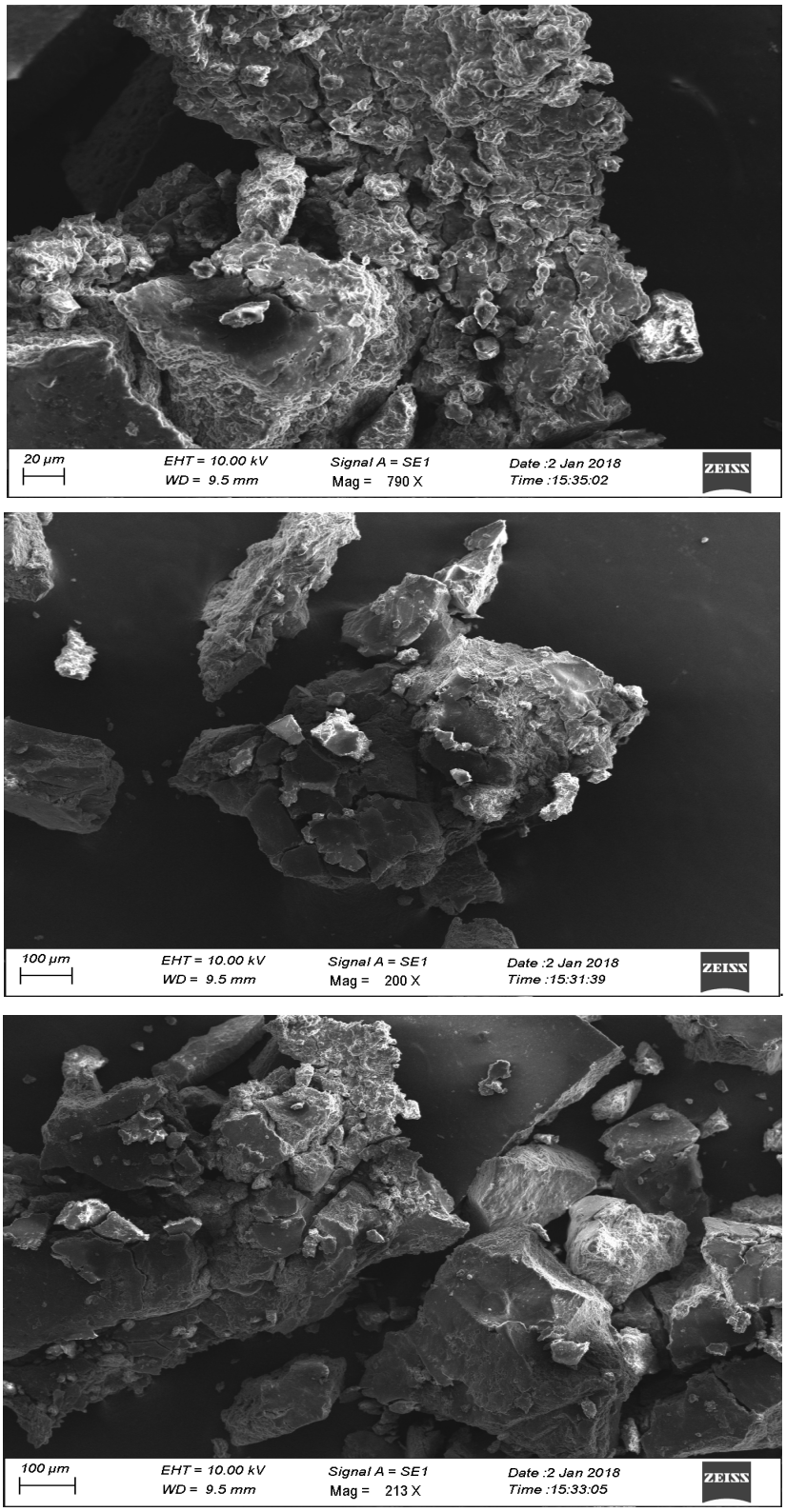

Fig. 4: Scanning electron microscope for the stem of *Manilkara hexandra* gum (MHG) with various magnifications

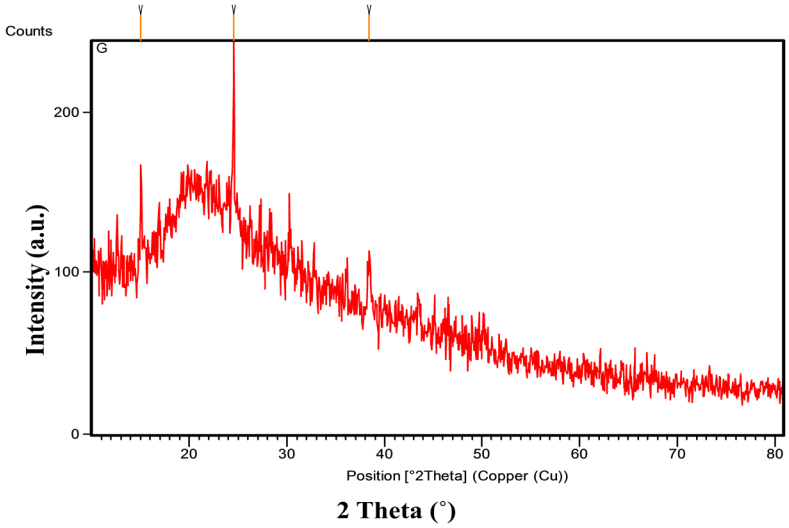

Fig. 5: X-ray diffraction analysis of the stem of *Manilkara hexandra* gum (MHG)

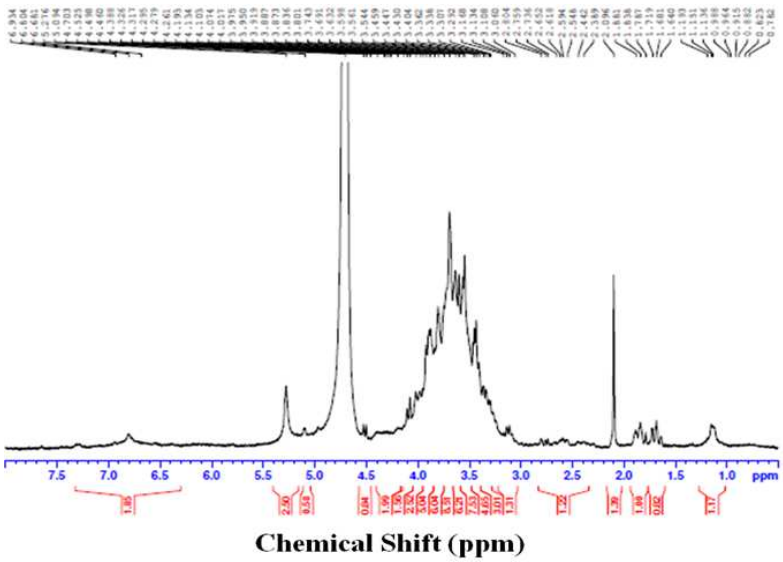

Fig. 6a: ^{1}H nuclear magnetic resonance report for the stem of *Manilkara hexandra* gum

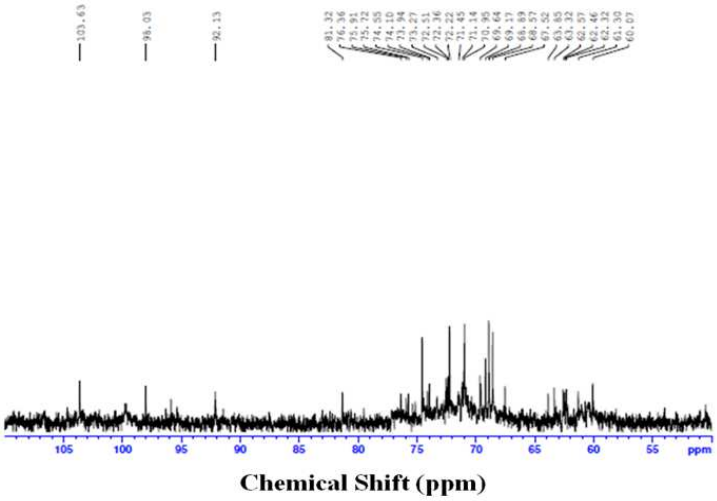

Fig. 6b: ^{13}C nuclear magnetic resonance for the stem of *Manilkara hexandra* gum

Sugar analysis by the thin layer chromatography

In drug industry plant material consisting of carbohydrate play a crucial role. Sugar analysis is very tough to identify, due to the presence of high polar compounds. Thin layer chromatography was done to identify the presence of sugar in the stem of *Manilkara hexandra* gum (MHG). Mucilage is a long chain polysaccharide substance extracted as a viscous or gelatinous dispersion from plant parts (roots, seeds,leaves, fruits etc.) and containing monosaccharides such as L-arabinose, D-galactose, L-rhamnose, D-xylose, and galacturonic acid in various proportions [26]. The R_f value of *Manilkara hexandra* gum MHG was similar to the standards of fructose, maltose, and glucose as shown in the table 5.

Table 5: Comparison of Rf values between standards and the stem of *Manilkara hexandra* gum

S. No.	Standards	Rf value	*Manilkara hexandra* gum (MHG)
1.	Glucose	0.97	0.97
2.	Fructose	0.32	0.34
3.	Maltose	0.93	0.90

Hypoglycemic activity (*in vitro*)

Literature review shows that very few research has been carried out on hypoglycemic activity using plant components [36, 37]. Diabetes Mellitus is classified into two ways (i) Type–I (Insulin dependent) (ii) Type–II (NIDDM-Non Insulin Dependent Diabetes Mellitus). In various approaches contains in the pharmacology field for diabetes, such as inhibition of gluconeogenesis, insulin stimulation, glucose transporters, and decreasing the adsorbed glucose from the intestine [38]. Type–II diabetes control the hyperglycemia through hypoglycemic treatment. In this work successfully discussed by the literature data for hypoglycemic activity. *Manilkara hexandra* gum (MHG) was isolated from aqueous extract since this extract possess active components and it contributes insulin stimulating effect [39] of hypoglycemic activity. The α-amylase enzyme used for digestion of carbohydrates or delay the digestion of carbohydrates increasing rate and it also blunts post pradia [40]. This enzyme acts as a major role to break the diasaccharides to monosaccharide [41] and finally all the monosaccharides to form carbohydrates through the absorption of the small intestine [42]. World Health Organization strongly suggested that this plant possess good research on diabetes mellitus [43]. The percentage inhibition of this sample has a significant difference compared with the standard of acarbose. Finally, this reports project the *Manilkara hexandra* gum may be regulated or control the secretion of insulin level in type-II diabetes mellitus patients. Natural glucose was not alone suitable in diminished blood sugar, regulate metabolism and enhance the insulin activity, but still maintaining the insulin level [44]. The inhibition level was shown in table 6. This reveals that the isolated gum possesses good hypoglycemic activity. The plant efficacy was supported to scientific research and medical evaluation [45].

Table 6: *In vitro* α-amylase inhibition of *Manilkara hexandra* gum

S. No.	Concentrations	*Manilkara hexandra* gum (MHG)	Standard acarbose
		% of inhibition*	
1.	100µg/ml	19.70±1.38	22.45±1.57
2.	200µg/ml	29.57±2.07	36.61±2.56
3.	300µg/ml	38.42±2.69	55.74±3.90
4.	400µg/ml	56.16±3.93	70.31±4.92
5.	500 µg/ml	79.31±5.55	84.84±5.94
6.	IC_{50} (µg/ml)	336.84	274.79

*All the experiments were repeated independently three times and the values were represented as an average means±Standard deviation.

CONCLUSION

Manilkara hexandra gum (MHG) has a various best approach such as Eco-friendly, biodegradable, non-toxic, cheap, no harmful side effects, natural adhesives. The Screening report confirms the presence of carbohydrates and gums. It is soluble in water, slightly acidic, has high moisture content and poor flow property. Ultimately, it is a fluid cohesive powder. Scanning Electron Microscope **(**SEM) and X-ray diffraction **(**XRD) prove that it is crystalline and amorphous in nature. Fourier Transform Infrared analysis (FT-IR), Nuclear Magnetic Resonance (^{1}H and ^{13}C) and the thin layer chromatography confirm the presence of the sugar moiety. Today community faces many obstacles based on the diabetic disorder. From the hypoglycemic activity analyzed it shows that this polymer may be dealt with the crucial issue of obesity owing to the unbalanced secretion of insulin. This research concludes that the isolated gum could be implemented in the food additive, pharmaceutical and medical model system.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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