Original Article

Bioactivity and Characterization of Karaya Gum

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Abstract

Natural Biopolymers has much applications and do possess various bioactivity. In this study, karaya gum was collected and used for bioactivity studies. Gum was subjected for phytochemical screening and characterized using thin layer chromatography (TLC), UV-Vis Spectrophotometer, FTIR. It was also subjected for bioactivity studies like antibacterial against *E.coli* and *S.aureus* and antioxidant activity. Karaya gum showed a good antioxidant and antibacterial activity against *E.coli* and *S.aureus*. UV-VIS spectroscopy analysis showed the presence of sugars like sucrose, glucose, xylose etc. FTIR analysis showed the presence of functional groups like alcohol, phenols, aldehydes, ketones.

Keywords: Karaya gum, bioactivity

INTRODUCTION

Plant gums have been used widely in several medical applications, as it is the cheapest and most available raw material for polysaccharide production[1]. These gums are usually formed after a wound in a superior plant as a result of their protection mechanisms. The ability of these materials to be bio-safe and biodegradable, makes it perfect to create a drug delivery system to enhance drug-delivery matrix due to their elevated water-produced swelling, dispersible in tablets, availability, low cost, and thickening characteristics in oral-administered liquids[2-5]. But, the chemical composition of the gum is important since it can affect the extraction technique and also can define the uses of the gum[6].

The karaya gum is an exudate from a big bushy tree known as *Sterculia urens*, this tree is originated from the family Sterculiaceae, that can be found in a dry forest located at the central and northern part of India. Another source for karaya gum is from *S.setigera* in Senegal and Mali, and minorsup- pliesform *S. villosa* in Sudan, India and Pakistan[7-8]. The production of these gums is so critical, the exudation will only begin after tapping the trunks by a manpower and then it continues for several days, the large exudes is dried in hot and dry weather, broken, cleaned to take out the unwanted materials and the bark, Then it will be categorized based on the quality and stored. The Gums harvested during the hot climate (April, May and June) are the one characterized with the highest quality and they are exported internationally as grade one. Grade one gum is usually found as a powder or granules and used in pharmaceutical and food industries since they have food solubility, high viscosity, moisture retention, and transparent color. World production is around 3000 tons a year, half of it originated from India and the rest originated from NorthAfrica[7]. It has an acetic flavor and odor; it can create a soft film when it's plastified with glycols. The chemical structure has been found to contain D-glucuronic acid, D-galacturonic acid, D-galactose and L-

rhamnose, but in different ratios depending on the type, source, and the quality of the gum[8-10]. The viscosity of Karaya gum is inversely proportional to the shear rate, where the viscosity in 0.5% dispersions has a value near the 120-400 centipoise[9]. Usually the viscosity develop linearly in low-concentration solutions, until reaching 0.5% of karaya gum concentration. Viscosity reduces as the gum ages[11]. While regarding the pH stability, Karaya gum dispersion is resistant to acidic environments since it has a high uronic acid concentration. The dispersion stability can be only maintained at an acidic range [12]. The FDA categorized Karaya gum as safe after several teratogenic, toxicological tests and mutagenic studies[13], thus being used in drug delivering [14].

MATERIALS AND METHODS

Materials used

Ethanol, Trichloroacetic acid,Acetone, Sulphuric acid , Phenol,Sodium Tri Meta Phosphate,Iodine, Potassium Iodide, Picric Acid, Chloroform, Ferric Chloride, Sodium Hydroxide, Ninhydrin reagent, Iodine Crystals, Nutrient agar, Nutrient broth, DPPH reagent, Methanol, Hydrochloric acid, *E.coli*, S.aureus. Centrifuge, Weighing balance, UV-Vis spectrophotometer, and FTIR.

Procurement of *Sterculia urens* gum exudate

Karaya gum was obtained from *Sterculia urens* trees found in the highlands of central and northern India. The gum granules were completely dried and powdered using pestle and mortar.

Defatting of Karaya gum

First, the Karaya gum granules were crushed into a powder by using pestle and mortar, then the powder was soaked in 100% ethanol overnight and dried using the hot air oven at 55–60 °C to obtain a defatted gum. 6g of the defatted gum powder was dissolved in 300 ml of water and stirred continuously for 2 hours using the magnetic stirrer, later the viscous solution was filtered by passing it through a cotton gauze to remove all the mucilage.

Characterisation of crude karaya gum:

Karaya gum powder was tested using various techniques to detect the characteristics of the polysaccharide. The crude gum was subjected to UV-Vis Spectrophotometer for characterization which was taken at 200-800nm. Additionally the extract was then analyzed using FT-IR analysis at the frequent range of 450-4000 cm -1

Solubility tests

1 gram of the crude Karaya gum was weighed and dissolved in 20 ml of six various solvents which are water, Ethanol, Methanol, Acetone, Hydrochloric acid, and DMSO to check the solubility rate of the crude gum.

Moisture Content

An empty dish plate was first weighed on an Avery laboratory balance, and the weight was recorded, one gram of the crude gum was added to the dish and the weight was taken and recorded again. The sample was placed in an oven with a temperature of 50-55 °C. Later every 10 minutes the gum sample was taken out of the oven and weighed again. This procedure was repeated until the gum weight remained constant. The percentage of the gum moisture content was then calculated using the following formula:

Moisture Content (%) = (amount of the moisture loss / Weight of bone dried sample) x 100

Determination of Swelling index

The swelling capacity of the crude Karaya gum was measured using Meka et al[15]. First, one Gum granule was taken and weighted using an Avery laboratory balance and then transferred into a 100ml beaker. 50ml of distilled water was added and the beaker was shaken thoroughly and kept at room temperature for 6 hours. The weight of the granules was taken every 30 min and recorded. The swelling index was determined using the following equation

Swelling Index = (Final Volume - Initial Volume / Final Volume) x 100.

Phytochemical analysis

Several phytochemical analysis was conducted to detect the components of the gum extract and search for bioactive agents that can be applied in the synthesis of useful drugs and drug carriers , the first one was the detection of alkaloids by applying

Wagner's test and Hager's Test. The gum extract was first dissolved in dilute Hydrochloric acid and filtered, later for Wagner's test the filtrate was mixed with a few drops of Wagner's reagent that is made of iodine in potassium iodide. While for Hager's test the filtrate was mixed with 1 or 2 drops of Hager's solution which is picric acid then the color was observed for both tests. Second test was Salkowski's test for the detection of Phytosterols, where the gum extract was mixed with 2ml of Chloroform and filtered and then the filtrate was mixed with 3 ml of concentrated sulphuric acid, later the mixture was allowed to set for a few minutes and the color change was observed. Third test was ferric chloride for detection of Phenols, where a few drops of ferric chloride solution was mixed with 2ml of the gum extract, and the color change was observed and recorded. Ninhydrin test to detect proteins and amino acids was conducted after, the gum extract was mixed with 0.25% w/v Ninhydrin reagent and boiled for 5 minutes till the color change was observed. Alkaline Reagent test was also done to detect flavonoids, 3-5 drops of Sodium Hydroxide solution was added to the gum extract and the yellow color observation was done. To add more, a Foam test to detect Saponins was carried on by mixing 2ml of water with 0.5ml of gum extract, the mixture was shaken for 2min and the foam persisting was observed for 10 minutes. Lastly, Benedict's test was conducted to test the sugar reduction; 1ml of the gum extract was placed into a clean test tube and 2ml of Benedict's reagent was added to the sample, the sample is then placed in a boiling water bath for few minutes, and the color change of the solution was detected. For all the tests, distilled water was used as the negative control[16].

Thin Layer Chromatography

Thin layer chromatography was performed on the Karaya gum extract to detect the number of components, and to determine the monosaccharide composition found in the Karaya gum extract. The procedure was placed using the solvent systems ethanol: water (1:1), methanol: water (1:1) and acetone:water (1:1). The TLC film was prepared first, and a room to load the sample was made by gently scratching the surface of the plate; by using a pipette a less than 1mm diameter sample was placed on the spot. Later the TLC films were placed at an angle of 60° in three different beakers (with an aluminum foil lid) which have the same volume but different types of solvents, and the films were allowed to sit in the beaker till the solvent and the sample almost reached the top of the film. The observation of the sample was done by first visualizing the films under the short length wavelength UV light, and the observed spots were outlined with a pencil, followed by placing the films in a jar containing iodine crystals for a few minutes. Lastly, the observed spots was also outlined and the R_f value was calculated.

Antibacterial activity

The Antibacterial activity of the gum extract was tested by using agar well diffusion technique. For this method, the Nutrient agar plates were prepared and the mother cultures of the *E.coli* and S.aureus were cultured for 24h in the nutrient broth. Later, six 10mm diameter wells were made in each plate by using a punching kit and needle. The broth culture of each bacteria was inoculated on each agar plate using cotton swab, then the gum extract were added on each well in different concentration (100%, 75%,50%, 25% and 0%), where in the last well an Ampicillin antibiotic disc were added as the positive control. Lasly, the *E.coli* and the S.aureus plates were incubated at 37° C for 18-24h. If the gum extract was able to inhibit the bacterial growth, it would form a clear zone around the well known as inhibition zone. The diameter of these zones were measured to determine the effectiveness of the gum extract against the bacteria. The larger the zone, indicates a strong antibiotic possessing.

Antioxidant activity

Determination of the antioxidant activity of the Karaya gum extract was tested by performing DPPH assay (Radical Scavenging assay) and Phosphomolybdenum assay. DPPH test is a cost effective and simple procedure to detect the antioxidant capacity of the extract, this test is done by the interaction of the hydrogen donors with the stable 2 2-diphenyl-1-picrylhydrazyl that leads to the decolorization of the purble DPPH. For this test, 0.1mM of DPPH solution was prepared in methanol. Later five different test tubes were prepared with different concentrations of the gum extract each containing 3ml; 1ml of the prepared DPPH solution was added to each tube and mixed well and allowed to sit in the dark at room temperature for 30min. The absorbance was recorded at 517 nm using a UV-spectrophotometer. Ascorbic acid was prepared as well in 5 different concentrations and used as the standard. Lastly the Scavenging activity was calculated based on the following formula[16]

"A0 - A1"/ A0 x 100, where A0 is the control absorbance and A1 is the sample absorbance

Phosphomolybdenum Assay was conducted to determine the presence of antioxidant components in the gum extract based on reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V). The reagent was prepared by mixing 0.04g of ammonium molybdate, 0.03g of sodium phosphate, 0.6ml of concentrated sulfuric Acid, and then the volume was made up to 5ml by adding distilled water. Later five tubes were prepared with the respective volume 100-500µl of the gum extract. 1ml of the reagent was added to each tube and then they were allowed to a set in a water bath at 95 °C for 90 minutes. Lastly, the absorbance at 625 nm was then taken and recorded.

RESULT and DISCUSSION

Solubility Testing

The crude Karaya gum showed a low solubility, even by using different types of solvents (Table 1). The presence of insoluble matter, large and high weight molecules and impurities affect the solubility of gum, which reduces the solubility capacity of the crude Karaya gum. One gram of the crude karaya gum was able to dissolve partially in water, due to the acetyl group found in the gum structure, therefore it leads to the formation of viscous colloidal dispersion after the gum absorbs the water rapidly[17]. While for Hydrochloric acid, the crude Karaya gum was able to dissolve completely. On the other hand, Ethanol, Methanol, Acetone and DMSO were not able to dissolve the crude Karaya gum; and that because the physical characteristics of alcohols are based on the hydrogen bonding ability of the -OH group. When the gum was added to the ethanol,methanol or acetone solvents , which are less polar than water, it decreases the solubility and potential hydrogen bonds of the starch , since the water is more attracted to the alcohol than the starch, the starch is dehydrated by the alcohol and precipitates.

Table 1. Solubility of karaya gum in various solvents

Solvents	Solubility
Water	Partially Dissolvable
HCL	Dissolvable
DMSO	Non Dissolvable
Ethanol	Non Dissolvable
Acetone	Non Dissolvable
Methanol	Non Dissolvable

Determination of Swelling Index

The initial weight of the gum granule was 2.64g, then the granule weight was taken each 30 min for 6 hours. The weight was 5.28g, 6.20, 8.01 and 9.61g respectively. The swelling index then was calculated to be 50%, 57%, 67% 72.5% (Figure 1). This shows that the gum can be used as disintegrant which on swelling may explode and produce enough energy to free up drug contents; therefore absorb enough moisture that allows it to swell and lead to the disintegrating of the tablets disintegrated. This test allows to discover the flow properties of the karaya gum, packaging and arrangement of particles and hence the compatibility of the granulation.

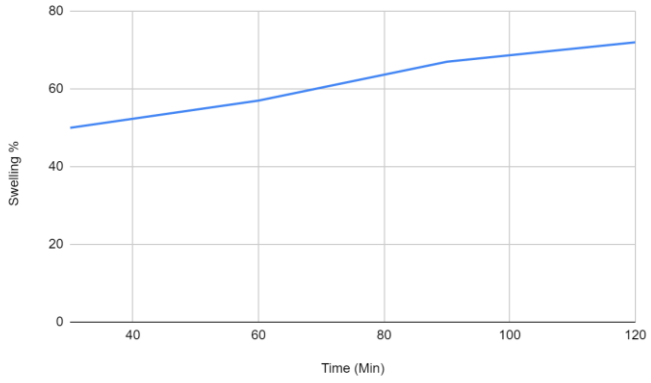

Figure 1. The swelling index percentage as the time increases

Moisture Content

The moisture content of the karaya gum versus drying time at 55 °C. It shows that the initial moisture content of 16% was reduced to 4%, 2%, and 1% every 10 minutes for 70 minutes till the moisture content became stable. More energy is required for it to be dried more, which means higher temperature can be used as well to reduce the moisture content further. Hence the drying time needed to decrease the moisture content to any certain level is based on the temperature (Figure not shown here).

Phytochemical Screening

The gum extract, shown to contain alkaloids after conduction Wagner's and Hager's Test. Positive results were observed for Wanger's test by detecting a brownish-reddish precipitate , while for Hager's test a yellow precipitate formed indicating the presence of alkaloids. To add more, the Ninhydrin test showed positive results, after the observation of the blue color, which indicates the presence of protein and amino acid in the gum extract. Phytosterols were detected in the gum extract after conducting Salkowski's test, a yellow golden color was observed indicating the presence of triterpenes. On the other hand, Negative results were observed for Phenols, Flavonoids, saponins and reducing sugar, where no color change were observed.

Thin Layer Chromatography

TLC was conducted for the extract Karaya gum; the separated bands showed the presence of monosaccharides. (R_f -0.75) indicates the presence of Rhamnose, (R_f -0.46) indicates the presence of glucose, Lastly (R_f -0.67) indicates the presence of Xylose and Ribose (figure not shown here). The difference in the R_f values was detected because of the polar nature of the mobile phase towards the monosaccharides present in the Karaya gum.

UV-Vis Spectrophotometer

The aqueous Karaya gum extract was characterized using UV-Visible Spectrophotometer, absorbance maximum was found between the range of 270 – 280 nm which indicated the presence of sucrose and glucose (Figure 2). According to Sarazin et al.[18] the absorbance of sucrose is at 260 nm to 270 nm while glucose is from 260-270nm. Additionally, a peak at 210 nm and around 255 nm might indicate the presence of xylose. Similarly, Samrot et al.[19] have reported that the sharp peak at 254 nm indicates the presence of xylose in *Mangifera indica L* Gum.

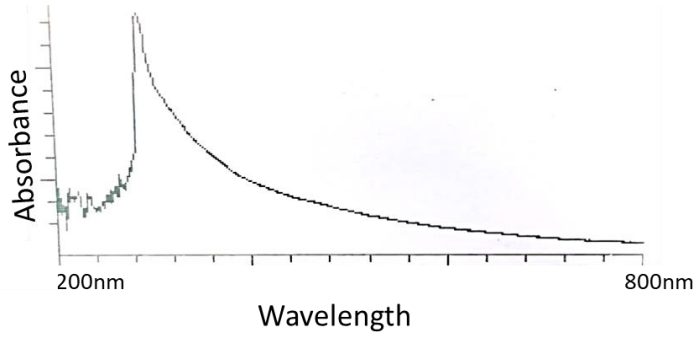

Figure 2. UV-Vis analysis of the Karaya gum extract

Fourier transform infra-red spectroscopic (FTIR)

FTIR analysis showed a peak at 3266 cm-1 indicates the hydrogen crosslinking of water molecules and shows O-H stretching, with compound type Alcohol and Phenols (Figure 3). 1721 cm-1 indicates the -C- stretching, with compound type Aldehydes and Ketones. 1601 cm-1 shows the C=C stretching, with compound type Alkenes. 1372 cm-1 represent C-H bending, with compound type Alkane. The peak at 1241 cm-1 indicates the stretching of the C-N, with compound type Amines. Lastly the peak 1036 cm-1 represents C-O stretching with compound type primary alcohol. Samrot et al.[20] have depicted that the *Terminalia Catappa L.* gum had a large peak at 3417 cm-1 for O-H stretching, 2940 cm-1, 1300-1450 cm-1 for alkenes and 1725 cm-1 for the aldehyde group, respectively.

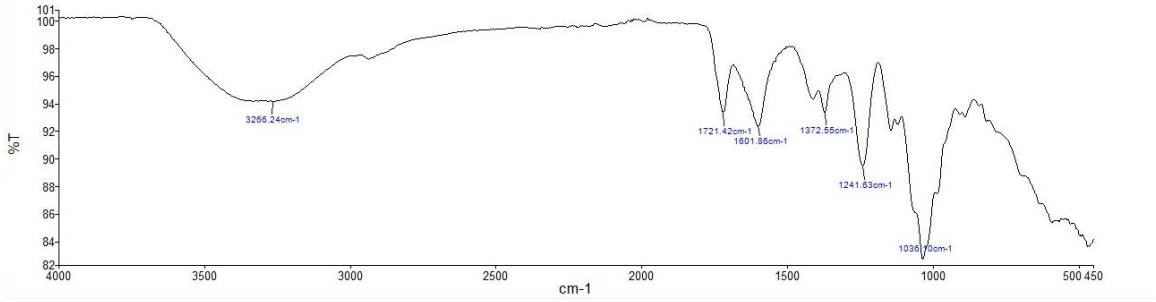

Figure 3. FTIR analysis of the Karaya gum extract

Antibacterial activity

For the negative control (water) there was no zone of inhibition observed. For The positive control (Ampicillin antibiotic discs), the zone of inhibition was observed for both *S.aureus*, but no zone of inhibition for *E.coli*. The Karaya gum extract inhibition zone was also observed against both *S.aureus* and *E.coli*. The zone of inhibition indicates that the gum extract was able to inhibit and kill the bacterial growth, which shows an antibacterial activity. Minimal inhibitory concentration of the Karaya gum extract was found to be 5µl/ml. while for 20µl/ml concentration it showed higher antibacterial activity than the

positive control (ampicillin antibiotic discs) (Table 2). In a study it has been reported that the antibacterial activity of the greenly synthesized silver nanoparticles using gum ghatti showed zone of inhibition (ZOI) of around 12.25 mm diameter against S. *aureus* and nearly 9 mm ZOI for *E.coli*[21]

Table 2 Antibacterial Activity of Karaya gum extract against *E.coli* and *S.aureus*

Organism	Zone of inhibition (cm)					
	Positive Control	Negative Control	Concentration			
			20µg	15µg	10µg	5µg
Escherichia coli	10mm	-	23 mm	17 mm	-	10 mm
Staphylococcus aureus	10mm	-	18 mm	12 mm	9 mm	5 mm

Antioxidant Activity

Based on the calculation, the Karaya gum were found to have an increased radical scavenging activity against DPPH with the increase of the concentration. At 250 µl, the scavenging activity was 70% which indicates a good antioxidant activity by the gum extract (Table 3) (Figure 4). The absorbance value decreased as the concentration of the Karaya gum increased, which indicates an increased Antioxidant activity. Bouaziz et al.[20] have reported that the antioxidant activity of flaxseed gum was very higher with the IC50 value of 2.5 $mg \cdot mL^{-1}$

Table 3 Antioxidant activity evaluation using DPPH assay

Concentration (µl/ml)	Scavenged Activity of the sample (%)	Scavenged Activity of the standard (%)
50	50%	71%
100	55%	76%
150	59%	82%
200	65%	87%
250	70%	90%

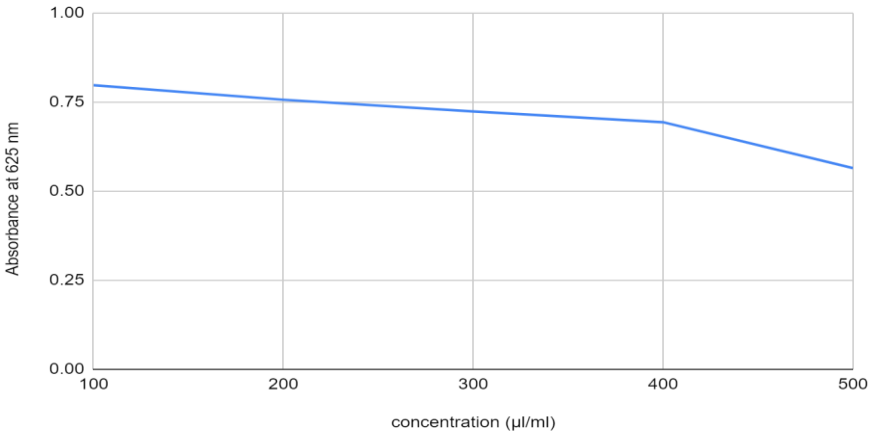

Figure 4. Phosphomolybdenum Assay of Karaya gum extract

CONCLUSION

In conclusion, the quality of well characterized materials are directly linked to their physical and chemical characters. In this study, Karaya gum extract was subjected to several characterization tests; Bioactivities were performed, Karaya gum were found to have a good antioxidant property and also good antibacterial activity against *E.coli* and *S.aureus*. On the other hand Karaya gum extract was analyzed spectroscopically and found to have sugars like sucrose glucose and xylose etc. The gum was also characterized by TLC, Phytochemical Screening and FTIR, and it was found to contain several compounds such as Alcohol, Phenols, Aldehydes, Ketones, etc. Plant gum-based polysaccharides are natural components which are readily available, biocompatible and biodegradable. Therefore, production of nanocarriers from these non-toxic natural materials can be a convenient choice for drug delivery agents.

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