Isolation, Evaluation and Toxicity Studies of Natural Edible Mucoadhesive Polymers from Various Parts of Plants and Compared with Synthetic Mucoadhesive Polymers

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ABSTRACT

The objective of the present work was isolated, evaluated and performed the toxicity studies and results were compared with marketed available synthetic mucoadhesive polymers. In this study, we used four different plant sources, namely Pithecellobium dulce (PD), Prosopis juliflora (PJ), Acacia arabica (AA) and Abelmoschus esculentus (AE). From the plants, isolated water soluble mucoadhesive materials by cold/hot aqueous extraction. The isolated mucoadhesive materials were proved to be safe and free from toxic or adverse effects. The yield value of four species for (PD), (PJ), (AA) and (AE) was 5.49, 4.91, 3.46 and 3.87 % w/w. We evaluated the swollen volumes, moisture sorption capacities, shear, tensile strengths, compatibility, interaction, and studied toxicity studies with a comparison of synthetic mucoadhesive polymer. Results indicate that, the isolated natural mucoadhesive polymers are generally regarded as safe (GRAS) category polymers and higher than the other natural polymers such as sodium alginate and Guar gum.

Keywords: Mucilages of plant, Mucoadhesive polymers, Natural mucoadhesive polymers, Sodium alginate, Guar gum.

INTRODUCTION

Mucoadhesive materials from natural sources are nowadays accounting more

importance for broad placement of mucoadhesive dosage devices. If they are

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biodegradable and biocompatible this provides added advantages for formulating various controlled release pharmaceutical formulations and avoids patient noncompliance, with special reference by chronically ill patients. The advantages of such materials include their natural origin, ready availability, low cost, biodegradability etc.¹

Pithecellobium dulce (Roxb.) Benth (PD), is a species of flowering plant in the pea family of *Mimosaceae* that is native to Mexico. Central America southern South America as shown in figure number 17. It is introduced and extensively naturalized in the Caribbean, Gum and southeast Asia like the Philippines. It is considered an invasive species in Hawaii. It is familiarly by the name "Madras thorn" but it has not originated in Madras. The name "manila tamarind" is misguiding that it is neither analogous to tamarind nor origin in Madras. It is usually known as "Seema chintakaya" in Telugu consumed as food, the seed pods consists of a sweet pulp that can be taken orally in the raw form ^{2,3}

Prosopis juliflora (Sw.) DC. (PJ), is a spiny tree grows up to 12 meters height and has branches with a diameter of up to 1.2 meters. It is broad leaved, bipinnate with 12 to 20 leaflets. The length of flowers is 5-10 cms, has green-yellow cylindrical spikes, which occurs in a group of 2 to 5 at the extreme part of the branches. The length of pods between 20 to 30 cms and consist of 10 and 30 seeds per pod. Seeds remain germinate for up to 10 years. The tree reproduces sexually by the way of seeds, not vegetatively. Seeds are scattered by cattle and other animals that ingest the seed pods and excrete the seeds in their droppings. The roots can penetrate to a great extent, up to a depth of 53 meters.⁴

Acacia Arabica willd. (AA), is occurring naturally in Sindh located in

Pakistan. It exists wild in India and Africa. It is cultivated for its bark. The tree produces a gum, generally known as acacia Arabic gum. The bark, leaves and fruits of acacia Arabica tree contain tannin and Gallic acid that have healing power and curative properties. The leaves and the bark of the tree have medicinal values and also useful in preventing the secretion or bleeding. The pods aid to remove catarrhs matter and phlegm from the bronchial tubes. The gum alleviates any irritation of the skin and eases the inflamed membranes of the pharynx, alimentary canal and genitor-urinary organs.

The bark, fruit and oleo gum resin are used in various Ayurvedic preparations. Acacia Arabica bark finds its primary applications in oral and dental hygiene products, burn injuries and in skin diseases. Being an astringent, twig of *Acacia Arabica* has been used in India as natural toothbrushes in the prevention of bleeding gums. In burn injuries, *Acacia Arabica* powder has been stimulating the healing process of burn injuries and controls the scar formation.⁵

Abelmoschus esculentus (L.) Moench (AE), familiar to many English-speaking countries as lady's fingers, Bhindi or gumbo, is a flowering plant belonging to the family of Malvaceae. It has significance for its edible green seed pods. The geographical location of abelmoschus esculentus Moench is controversial in South Asian, Ethiopian and African countries. The plant is grown in tropical, subtropical and warm climatic regions around the world. The name abelmoschus esculentus monarch is most oftenly pronounced in the United States with alternative names, English caribbeanokro. The word okra comes from Nigeria is located in west Africa. Okra is usually known as "lady fingers". It is called by different names in European countries like in Portugal (quiabo), Spain (quimbombo or guigambo). Dutch and French (gumbo). In India and Pakistan and frequently in the united kingdom, it is called by variant names such as Bhindi, bhendi, bend or behind. In East Asian countries, it is called with regional names like dherosh (Bangladesh), qui kui (china), bamia or bamyeh (middle east asia), etc. unspecified parts of the plant possess diuretic properties, that is mentioned in various sources associated with traditional and herbal medicine.^{6,7}

Present days, mucoadhesive agents are precisely studied for buccal drug delivery to enhance bioavailability, sustain drug release, by pass first pass metabolism and improve patient compliance by lowering the frequency of administration.⁷ isolated mucoadhesive agents, purificated seed from pithecellobium dulce (Roxb)Benth(PD), Prosopis juliflora (Sw.) (PJ), Gum of Acacia Arabica Willd (AA) and Fruit Abelmoschus esculentus (L.) Moench (AE), were evaluated for various in vivo toxicity studies and various in vitro mucoadhesive studies.

In various studies, natural substances were reported mucoadhesive property due to the presence of the carbonyl group, thiol group, sugars, proteins, carbohydrates, hydroxyl groups, hydrogen bond, amide groups, cations and anions in their composition. Therefore the use of natural mucoadhesive agents for the purpose of keeping the drug for a prolonged period of time in Buccal region should be of great interest. The present research work was mainly focused on isolation, purification and evaluation of natural mucoadhesive agents using different *in vitro* mucoadhesion methods and *in vivo* toxicity studies.

MATERIAL AND METHODS

Plants materials were authenticated and specimens were stored at Department of Botany, Osmania University, Hyderabad-500007, Telangana, India wide voucher numbers 0044,0130,0249,0301, Dated:26-

11-2013. Chemicals and Reagents used in the present study were of analytical grade.

Isolation and purification of mucoadhesive agents

The mucoadhesive agents were Isolated and perfected by the method adopted by Kulkarni *et al.* ¹⁰

Isolation and purification of mucoadhesive agents from *Pithecellobium dulce* (Roxb) benth (PD)

Pithecellobium dulce (Roxb) Benth seeds were collected from the Jannaram Village, Adilabad district of Telangana, India in April month. 100 gm of the seeds was soaked in one liter of distilled water for 12 hrs. The tegmens (an outer covering of the seeds) were removed and the white coverings as well as the white portion of the kernels were separated. They were ground to a fine paste and 500 ml of water was added. Stir vigorously for a few minutes and kept for 12 hours. The slurry was filtered through a muslin cloth. The filtrate was collected and kept undisturbed in refrigerator for 12 hrs. The upper clear solution was collected by decantation. The precipitated by formed by the addition of 3 volumes of acetone and filter it and continuously stir for 15 min and the precipitated mucoadhesive material was washed thrice with acetone and dried in a vacuum dried and powdered. The powder was passed through the sieve no 120 and kept in a desiccator for further studies.

Isolation and purification of mucoadhesive agents from *Prosopis juliflora* (SW.) (PJ)

Dried pods of *Prosopis juliflora* (SW.) were collected from the Thiryani Village, Adilabad district of Telangana, India in June month. The seeds were segregated from the pods and the white mucilaginous covering was isolated from the cleaned seeds by soaking 100 gm in 200 ml of warm water. The seeds were stirred

mechanically for 6 hrs at 300 RPM using a common Laboratory stirrer, so as to detach mucoadhesive material from the kernel and the tegmen.

The mucilaginous portions were picked up manually and the aqueous extract of the same was prepared by continuously stirring for 6 hrs. Then it was poured to thrice the volume of acetone. Precipitated material was redispersed in water and precipitated again with acetone to get the purified product. Finally the precipitate was dried in a vacuum dried and powdered. The powder was passed through the sieve no. 120 and kept in a desiccator for further studies.

Isolation and purification of mucoadhesive agents from *Acacia arabica* Willd (AA)

100 gm of the gum obtained from the market was powdered and 500 ml of water was added and stirred well with a Laboratory magnetic stirrer for 6 hrs and set aside for 12 hours. Then the liquid was filtered through a muslin cloth and allowed to stand. By decantation the clear supernatant liquid was obtained and the sediments were rejected. The volume was reduced to half by heating on a rotary vacuum evaporator.

The concentrated extract was precipitated with 3 volumes of acetone, purified by redispersing in water and precipitating with acetone. The precipitate was dried under vacuum desiccators, powdered and passes through sieve no. 120 and kept in a desiccator for further studies.

Isolation and purification of mucoadhesive agents from *Abelmoschus esculentus* (L.) Moench (AE)

Tender fruits were collected from the market in the month of May and washed well with water. They were cut into small pieces. To this, triple the volume of water was added and heated at 60 0 C for 4 hrs on a

water bath and set aside for 12 hours. Then filtrate the liquid through a muslin cloth and allowed to stand. By decantation the clear supernatant liquid was separated and the sediments were rejected. The volume was reduced to half by heating on a rotary vacuum evaporator. The concentrated extract was precipitated with 3 volumes of acetone and purified by redispersing in water and precipitating with acetone. The precipitate was dried under vacuum desiccators, powdered and passes through sieve no. 120 and kept in a desiccator for further studies.

Toxicity studies

Acute toxicity studies

Wistar rats of 8 to 10 weeks old weighing 200 – 250gms, were individually housed in polypropylene cages lined with husk renewed every 24 h in well-ventilated rooms at 22±3°C and RH between 50 to 60, under artificial lighting12: 12 h light and dark cycle in hygienic condition for at least five days prior to the study. The rats were fed with a pellet diet and water. The studies were performed according to OECD Guidelines 420 and the protocol was approved by the Institutional Animal Ethics Committee.

Sighting study

Fasted animals are over-night prior to dosing and weighed. The test substance was administered to single animals in an order following the flow charts in Annex 2 of OECD 420. The starting dose in the siting study was selected from the fixed dose levels of 300 mg/kg. The next dose used for this study was 2000 mg/kg. The test substances were made in the form of suspension and administered orally in a constant volume of 2 mL/100g body weight. After the substance has been administered, food was withheld for a further 3-4 h. A period of at least 24 hours was allowed

without dosing. All animals were observed for two weeks

Main study

A total of five Wistar rats was used for each dose and the animals were selected one animal from the siting study dosed at the selected dose level together with an additional four animals. The time interval between dosing at each level was 3 or 4 days.

Observations

Animals were observed after dosing at once during the first 30 min, periodically during the first 24 h with special care given during the first 4 h and daily thereafter, for a total of two weeks. All observations were systematically noted for each animal. Observations include changes in skin, fur, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity, Observations done to tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Individual weights of animals were determined before the test substance was administered for a week. Weight changes were calculated and noted. At the end of the test surviving animals were weighed and then humanely killed. All animals were subjected to gross necropsy and pathological changes were observed. With microscopic examination of organs was also done for evidence of gross pathology in all animals surviving 24 or more hours after the initial dosing.11

Acute toxicity studies

Two groups of animals selected 3 in each group. The group one was treated with vehicle as distilled water and marked as a control. Group II was treated with 5000 mg/kg dose with consideration of body weight. Blood/tissue was collected at the end of the second week. Hematological and

biochemical parameters were measured in the both. The organs were blotted and weighed in a digital balance, then gross necropsy of heart, liver and kidney were observed.¹²

Sub-acute toxicity studies

Mucoadhesive polymers at the dose of 250, 500 and 1000 mg/kg body weight was administered orally to four groups of six rats, respectively, to every 24 h for four weeks and control received vehicle at the same volume. The toxic effect such as body weight, mortality and food and water intake was observed. After four weeks all surviving animals were fasted overnight anesthetized with ether. The heparinised samples collected blood were determining hematological parameters and the serum from non-heparinised blood was carefully collected for determining clinical blood chemistry. After blood collection Animals were humanely killed and removed internal organs, then weighed to determine the relative organ weights and observed for gross lesions. Removed internal organs were preserved in 10% buffered formaldehyde solution for histological examination.¹³

Predetermination studies

All the Predetermination studies were conducted as described below and the results are represented in Table 1.

PH

pH of 1% w/v aqueous solutions of isolated mucoadhesive substances were measured by Toshniwal pH meter.

Determination of swollen volume

Swellability studies was done by dispersing 1 GM of mucoadhesive substance with a few drops of ethanol in a graduated measuring cylinder and were then made up to 50 ml with water. Swollen volume was

noted after 24 hours. Swelling capacity was calculated by the following equation:¹⁴

$$S = (V_2 - V_1) / V_1 \times 100$$

Where,

S = % swelling capacity

 V_1 = Tapped volume of the material prior to hydration.

 V_2 = Volume of the hydrated or swollen material.

Moisture sorption capacity

Accurately weighed 2 g of mucoadhesive substance and distributed evenly over the surface of a 70 mm-tarred *Petri* dish. The sample was then placed in a humidity chamber at room temperature and relative humidity of 100%. The weight gained by the exposed samples at the end of a five-day period was calculated and the amount of water absorbed was calculated from the weight difference.¹⁵

Loss on drying

Weigh the 5 g of powder sample of mucoadhesive material in a *Petri dish* and dried it in an oven at 105°C until a constant weight was obtained. The % moisture content was determined as the ratio of the weight of moisture loss to weight of the sample expressed as a percentage. ¹⁶

Measurement of mucoadhesive strength of polymer

Thumb's test

Thumb's test is useful in initial screening test parameters. The test is being carried out by means of the force required or the difficult to pull out the thumb from another finger, when kept in contact with the mucoadhesive material in particular concentration and volume respect to contact them.¹⁷

Shear stress method

Several methods have been reported and in most of the cases, *in vitro* models are

based on the measurement of shear or tensile strength. Two smooth, polished plexiglass plates of 2.5 (7.5 cm were fixed with the help of an adhesive (Araldite). A nylon thread was sandwiched in between the glasses. Another glass plate of same dimension has been taken and one end was fixed with another nylon thread, which was then passed on a pulley and at the end, and provision was provided to add weight. The sandwiched plate was fixed on a flat table as shown in figure 1. Another glass plate fixed with nylon thread was kept in contact between the sandwiched plate by placing appropriate concentrations like 0.5%, 1.0% and 1.5% w/v of mucoadhesive material, in particular volume of 0.5 ml and allowed at particular time intervals of 5,10,15, 20 and 30 minutes. The force required to detach the plates were measured as a means of adhesive strength. This represents the adhesion strength, i.e. shear stress required to measure the contactness and repeated the same procedure for three times. 18

Park and Robinson method

Tensile strength measured by this method with the help of a modified instrument as shown in figure 2. A section of tissue having the mucous side exposed was secured on a glass vial placed in a beaker containing a phosphate buffer of pH 6.6. The another section of the same tissue was placed over a rubber stopper, secured with a vial cap and with the mucous side exposed. A drop of polymer solution (1.0%) was placed between two mucosal tissues. How much force used to detach the polymer from mucosal tissue was recorded. The results of the study provided important experimental conditions such as pH, ionic strength. applied and pressure bioadhesion 19

FTIR studies

The I.R. Spectrum of mucoadhesive substances, were calculated individually. The disc was made using 1 mg of sample in 100 mg potassium bromide and the spectra were recorded between 4000 cm⁻¹ - 400 cm⁻¹ using Shimadzu FTIR Spectrophotometer and are shown in Figures 9-12. ²⁰

Differential scanning calorimetry

DSC Thermographs of Natural Edible Mucoadhesives polymers and were recorded between 30.0°C to 300.0°C at the rate of 20.0°C per minute under the environment of nitrogen and the results are provided in Figures 13-16.²¹

RESULTS AND DISCUSSION

Mucilages or mucopolysaccharides of plant origin have been used widely as demulcent because of their unique properties to bind to the mucus membrane. The selection of the materials for the current investigation was based on their edibility, blandness, availability and the economics.

Isolation mucoadhesive ofsubstances from the natural edible sources was carried out by cold and hot aqueous extraction process followed by the organic solvent precipitation. The selection of the process was based on previous literature giving utmost importance to preserve the components against thermal, enzymatic and hydrolytic degradation. The organic solvents used for precipitation can be recovered back by fractional distillation, making the process more economical. The processes used were found to be effective in selective isolation purification and ofthe interested constituents and the yielded components possessed good handling properties.

The Table 1, represents the details of the extraction processes, respective yields and their physical properties such as pH, swollen volume, swelling capacity, moisture sorption capacity, loss on drying etc.

The yields of PD, PJ, AA and AE were ≈5. 49, 4.91, 3.46 & 3.87 % w/w respectively to the initial weight. The pH values of 1% w/v solutions of PD and PJ were found to be 5.67& 6.68 respectively. which are very close to the pH of saliva ($^{\approx}$ 6. 6) Suggesting its non-irritability to the buccal mucosa. Swelling is the primary characteristic of any material to be a mucoadhesive substance, but over hydration causes slippery surface. Excessive swelling also causes loss of mechanical strength that is required to maintain the structural integrity of the solid dosage forms.²² Hydration of swollen volumes after 24 hours were found to be 12.1, 12.4, 13.3 & 18.3 indicating their moderate swell ability compared to 27.4 of CP 934 P, 25.7 of sodium alginate, 31.2 of guar gum and 6.4 of HPMC.

The swelling was also assessed by the determination of swelling capacity and moisture sorption profile. Study of moisture sorption is also of considerable importance since it reflects the relative physical stability of dosage forms when stored under humid conditions. In all, this property showed that the AA powder is sensitive to atmospheric moisture and should therefore be stored in airtight containers. But it was found that the moisture sorption capacities of PJ, AA and PD are very less. The loss on drying of PJ, PD, AA & AE were less than the official limit of 6% stated in British Pharmacopoeia 2004.²³

The acute and subacute toxicity studies of such extracted sample profile showed that the Natural Mucoadhesive Polymers did not cause any toxic effects on animals. After the observation for 14 days, in the case of sighting study, the data confirmed no hypersensitization of skin and irritation to eyes. No ulceration or inflammation was observed in mucosal membrane and respiratory system respectively. On circulatory system, no sign

of cardiac toxicities like increased heart rate, force of contraction or elevated blood pressure was observed. Abnormal toxic effects like neurotoxicity, anxiety or depression was also not observed. The motor coordination and body weight were observed to be normal. Hematological and biochemical parameters showed no changes in the normal blood counts. The heparinised and non-heparinised blood samples also showed a normal profile and no gross lesions.

Figures 3-8, represent the weight required to detach the blocks/tissues attached together by the mucoadhesive solutions after specified contact time The results suggest that each periods. isolated mucoadhesive material possessed comparable shear and tensile strengths to the commercially available GRAS (generally regarded as safe) category polymers and higher than the other natural polymers such as sodium alginate and guar gum. Further, these strengths were increased with the increase in concentration. but considerable increase was observed after 15 minutes of contact time, irrespective of polymers Strengthening studied. bioadhesion may be due to the formation of more number of secondary bonds as time progresses.

Figures 9-12 represent the FTIR Spectra's of mucoadhesive polymers under investigation. Results suggest that Natural Mucoadhesive polymers isolated from the natural edible sources have shown similar peaks as compared with the synthetic mucoadhesive polymers which are earlier used in buccal formulations.

Figures 13-16 represent the DSC thermographs of Natural Mucoadhesive Polymers under investigation. The thermographs suggest that the melting point of mucoadhesive polymers under investigation with compared with Synthetic mucoadhesive Polymers.

SUMMARY AND CONCLUSION

Natural mucoadhesive agents were isolated from the natural edible sources by cold/hot aqueous extraction followed by organic solvent precipitation. The methods were found to give satisfactory yields and are reproducible. The physical properties of the substances such as pH, swelling, moisture sorption capacity, loss on drying etc. were evaluated. The mucoadhesiveness of aqueous solutions of natural polymers were evaluated by shear stress, Park and Robinson methods and compared with the commercially available polymers such as HPMC, CP, sodium alginate and guar gum. From these findings, it was evident that the natural mucoadhesive agents possess good comparable handling properties and bioadhesive strengths.

The acute and subacute toxicity studies of extracting samples showed that the mucoadhesive agents did not cause any toxic effects on animals. Hematological and biochemical parameters showed no changes in the normal blood counts.

In the light of the above results it can be concluded that,

- 1. All the materials isolated from natural sources were found to possess good physical characteristics that are essential for utilization as a Mucoadhesive polymer for Buccal formulations.
- 2. The pH values of the mucoadhesive substances were nearer to buccal pH, suggesting non-irritability to the mucosa.
- 3. The isolated mucoadhesive materials obtained from natural sources were proved to be safe and free from toxic effects
- 4. The FTIR and DSC studies indicated that this has not given remarkable interaction between the drug which are used in buccal formulations and the Mucoadhesive polymers isolated from natural edible sources.

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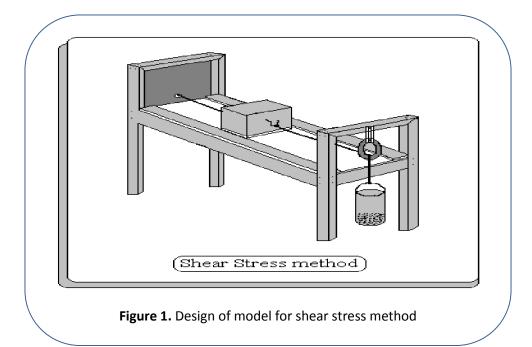
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propranolol through porcine buccal epithelium. *Mil MED*. 161, (1996), 669-672.

Table 1. Physical properties of mucoadhesive materials

Mucoadhesive substance	Bilogical source	Part used	Organc solvent	Yield %W/W	рН	Swollen volume (ml)	Swelling capacity (%)	Moisture sorption capacity (%)	Loss on drying
PD	Pithecellobiu m dulce	Seeds	Acetone	5.49	5.67	12.1 ± 0.4	186.8 ± 5.28	7.6	2.3
PJ	Prosopis juliflora	Seeds	Acetone	4.91	6.68	12.4 ± 0.5	156.1 ± 8.17	6.8	1.2
AA	Acacia arabica	Gum	Acetone	3.46	3.57	13.3 ± 0.7	167.3 ± 7.18	7.3	4.9
AE	Abelmoschus esculanthus	Fruits	Acetone	3.87	4.08	18.3 ± 1.5	387.3 ± 13.78	18.2	5.4
НРМС	**	**	**	**	7.21	6.4 ± 0.7	87.3 ± 3.10	11.2	2.6
CP 934p	**	**	**	**	2.86	27.4 ± 1.1	521.3 ± 10.08	24.1	7.2
SA	**	**	**	**	6.16	25.7 ± 1.6	512.4 ± 11.34	11.3	2.9
GG	**	**	**	**	6.54	31.2 ± 1.5	611.9 ± 18.51	8.7	1.4
±SD (n=3)									



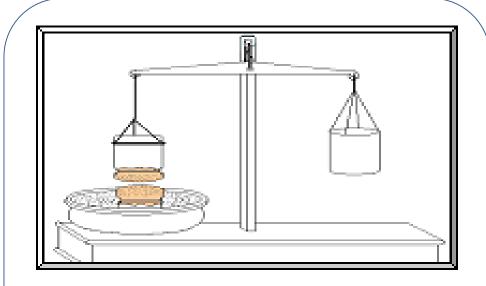


Figure 2. Instruments for measuring bioadhesiveness by Park & Robinson method

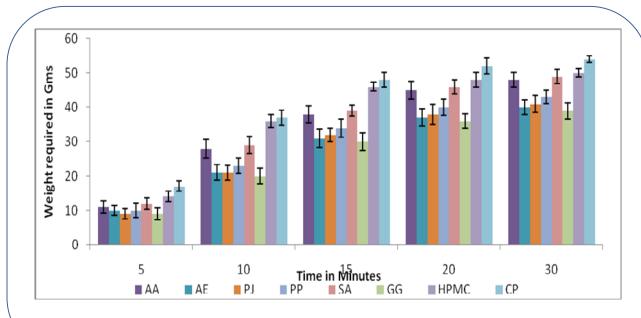


Figure 3. Mucoadhesive strength of polymer solutions (0.5%w/v) by shear stress method

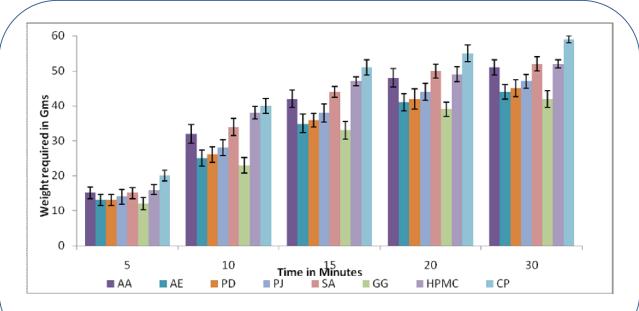


Figure 4. Mucoadhesive strength of polymer solutions (1%w/v) by shear stress method

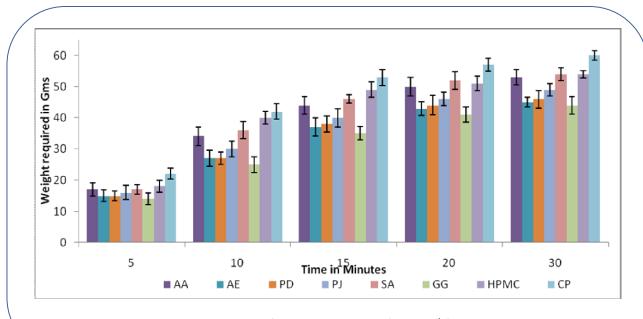


Figure 5. Mucoadhesive strength of polymer solutions (1.5% w/v) by shear stress method

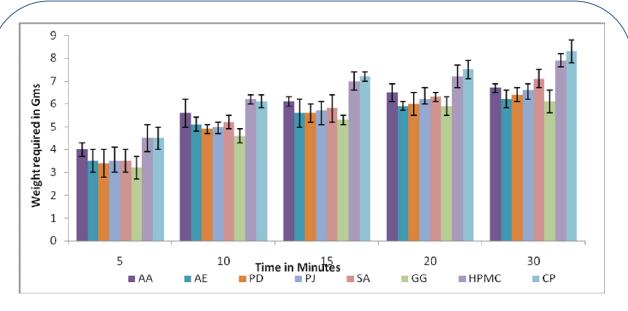


Figure 6. Mucoadhesive strength of polymer solutions (0.5% w/v) by Park & Robinson method

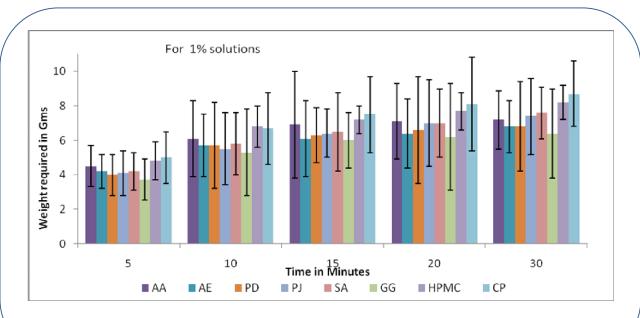


Figure 7. Mucoadhesive strength of polymer solutions (1%w/v) by Park & Robinson method

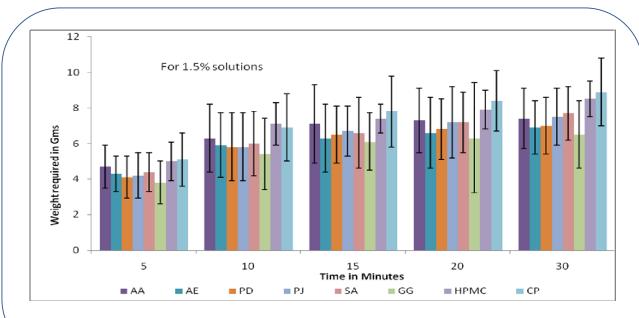
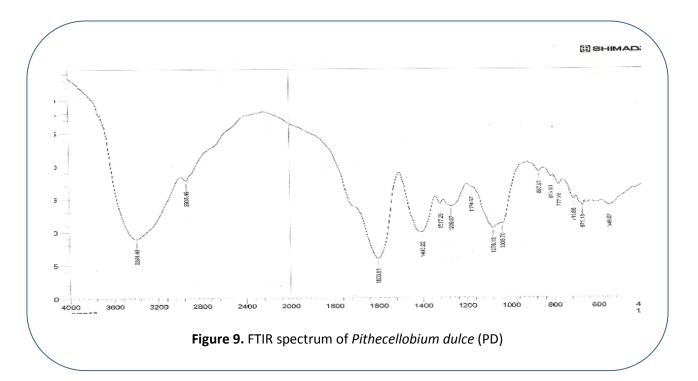
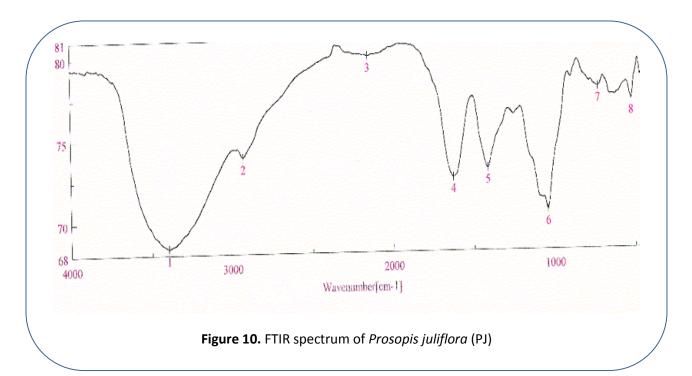
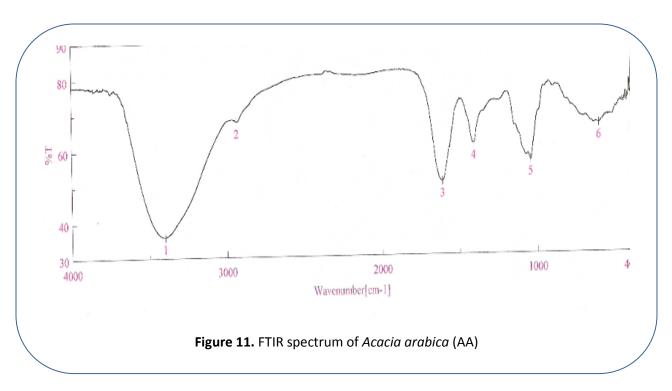


Figure 8. Mucoadhesive strength of polymer solutions (1.5%w/v) by Park & Robinson method







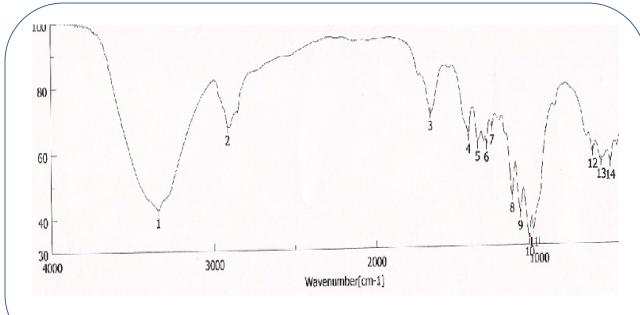


Figure 12. FTIR spectrum of Abelmoschus esculentus (AE)

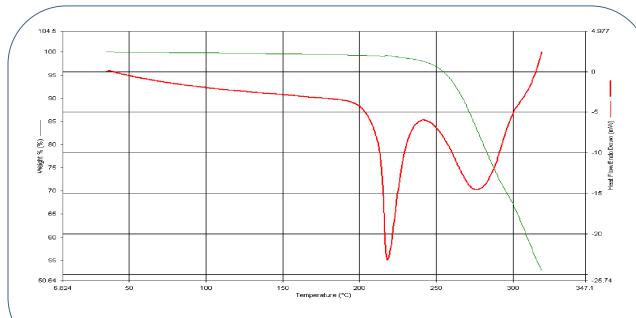
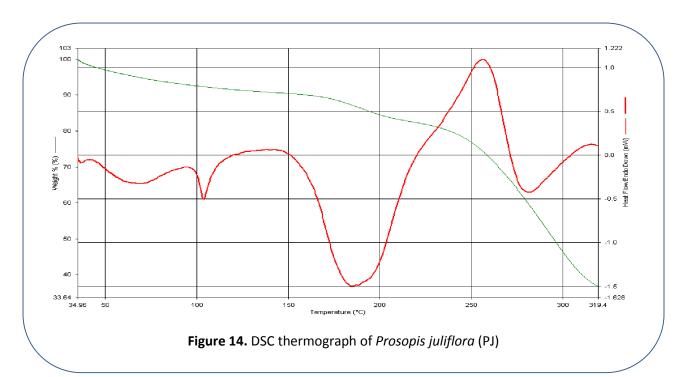
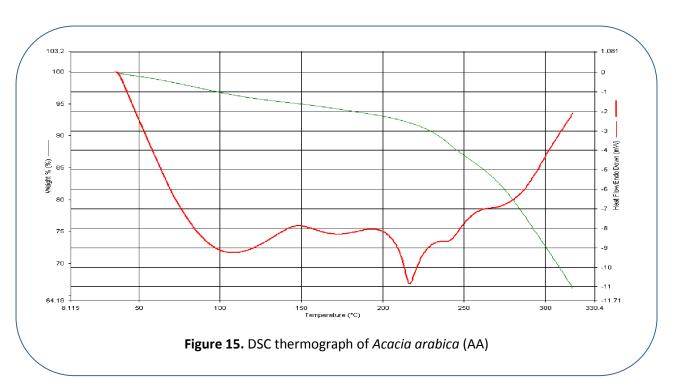


Figure 13. DSC thermograph of Pithecellobium dulce (PD)





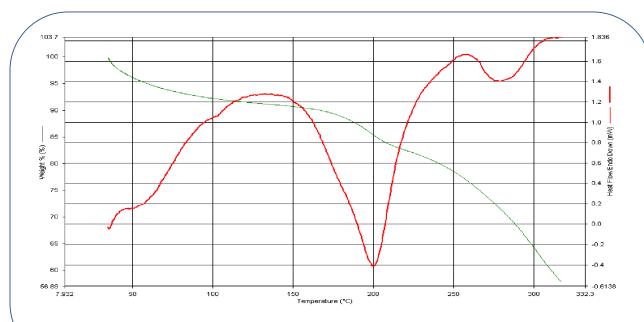


Figure 16. DSC thermograph of Abelmoschus esculentus (AE)



Figure 17. Pithecellobium dulce (roxb.) Benth. plant (PD)



Figure 18. Prosopis juliflora (SW.) DC. plant (PJ)



Figure 19. Acacia arabica willd. plant (AA)



Figure 20. Abelmoschus esculentus (L.) moench plant (AE)