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Article

In Vitro Evaluation of *Dalbergia sissoo* and *Acacia modesta* gum as Pharmaceutical Binders for Drug Delivery System

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The present study aimed to compare the crude, modified and hydrolyzed gums of *Dalbergia sissoo* and *Acacia modesta* as a biodegradable binder for drug delivery system using acetaminophen as a model drug. The physiochemical properties such as pH, fluorescence analysis and swelling index were determined. The gums were hydrolyzed and modified. Acetaminophen tablets were prepared using wet granulation technique and the gum solutions were used as a binder. Hydroxypropyl methylcellulose was used as a synthetic binder. Different properties of granules and tablets were evaluated. Results showed that both gums were acidic in nature, while *D. sissoo* and *A. modesta* showed light brown and creamy color in fluorescence analysis. The swelling ratio was the highest in water followed by 0.1N HCl and least in phosphate buffer. The prepared tablets showed faster and slower dissolution profiles in the same dissolution system. The crude gums have the highest dissolution rate, and this rate was decreased in the case of modified and hydrolyzed gums samples. The crude gums showing slower release can be useful in sustained-release tablets, while the modified gums having faster release rate are helpful in conventional tablet formulation. Taken together, the selected gums could be a good model for evaluation as a binder or hydrophilic polymer in tablet formulation.

Keywords: Binder. Granulation. Tablet. Polymer. Modification.

INTRODUCTION

Binders are one of the important ingredients added to the tablet formulation to impart cohesiveness (Chaudhari, Patil, 2011). These are also used in the formation of the tablet to give it mechanical properties by promoting the bonding among the different components of a powder mixed in the tablet formulation, and improve the flow and compaction properties of granules (Odeku, 2005; Odeniyi, Babalola, Ayorinde, 2013). Binders have also been used as solutions in the formulation and the method of preparation. A variety of natural, semi-synthetic and synthetic substances such as starch, cellulose, and gums have been used in the formation of the tablet as binders. Gum extracts of *D. sissoo* and *A. modesta* possesess significant antibacterial potential and are found to be non-mutagenic and non-hemolytic (Munir *et al.,* 2016).

Similarly, plant gums are widely used as suitable binders in the preparation of pharmaceutical solid dosages (Adetogun, Alebiowu, 2009). Natural gums as binders have preference over synthetic binders due to their low cost, abundant availability, non-toxicity, non-irritating and emollient nature (Selvi *et al.,* 2010).

Gums are the byproduct of metabolic mechanisms of plants and are either water-soluble or absorb water to form a viscous solution. Natural gums are economic and found to be useful as tablet binder (Singh, Selvam, Sivakumar, 2010). The physicohemical properties of wet granulate, and the tablets are influenced by binders (Tavakoli *et al.,* 2008). The gum of *Acacia*

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modesta is present in the form of angular fragments or small tears and vermiform. It is translucent and yellow color gum (Nussonovitch, 2010). *A. modesta* is used as 'Miswak' (the chewing stick as a natural toothbrush to clean teeth) in many parts of Pakistan. The fruits, leaves, wood, and bark are mostly used as medicinal, fuel and timber purposes. It has shown potential against chronic stomach disorders, gastric troubles and dental diseases (Asghar *et al.,* 2003; Murad *et al.,* 2011). The gum is also used as a tonic and to cure dysentery (Sher, Aldosari, Ahmad, 2012). *D. sissoo* has been used as abortifacient, aphrodisiac, anthelmintic, antipyretic and expectorant (Shah, Mukhtar, Khan, 2010). Its extract was reported as anti-inflammatory (Kumar, Kumud, 2010), analgesics (Hajare *et al.,* 2000) and antidysenteric (Brijesh *et al.,* 2006). It is used in conditions like ulcers, emesis, dysentery, leukoderma, skin diseases, and stomach troubles. *D. sissoo* is antidiarrheal as it affects bacterial virulence. Roots, leaves, and bark of *D. sissoo* can be used as astringent and stimulant (Hussain, Shahazad, Hussnain, 2008).

Several drawbacks of native and unmodified gums solution such as lack of clarity, free-flowing properties, uncontrolled rates of thickening and hydration, decrease in viscosity on storage, bigger microstructure formation and microbial contamination impede their use for industrial applications. The above-mentioned drawback can be overcome by the modification of physicochemical properties of these gums (Prabaharan, Jayakumar, 2009; Rana *et al.*, 2011; Kumar, Ahuja, 2012). Therefore, the present investigation was carried out to investigate the modification and hydrolysis of *A. modesta* and *D. sissoo.* The natural gums were compared to determine their effectiveness as a bio-binder in tablet formulations by using acetaminophen and hydroxypropyl methylcellulose as a candidate drug and standard binder, respectively.

MATERIAL AND METHODS

Acetaminophen was used as a study drug (33.104 gm) as it has poor compression properties. Microcrystalline cellulose (44.139 mg), magnesium stearate (2.468 mg) and lactose (163.74 mg) served as a disintegrant, lubricant and filler respectively. Hydroxypropyl methylcellulose (HPMC) and gum (6.550 mg) solutions were used as binder solutions. Same concentrations of HPMC and gum samples were used. All the chemicals used were of high quality grade and purchased from Sigma-Aldrich (U.S.A) and Fluka (U.S.A).

Collection of crude gums

After the review and market survey, the gums were procured from the local market of Faisalabad, Pakistan. The selected gum samples were identified and confirmed from the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Purification of gums

The crude gums were purified using a method described earlier (Shahid *et al.,* 2013). Briefly, the dried gum (the crude gum) was dissolved in distilled water at room temperature. The resulting suspension was allowed to swell overnight and forming a viscous solution. The viscous gum solution was stirred on a mechanical stirrer for 6 h at room temperature, filtered using a muslin cloth, and slowly added to absolute ethanol yielding white amorphous precipitates. The precipitates were filtered, washed with absolute ethanol, and dried in a hot air oven at 40 °C. The dried gum was ground to a fine powder using a mortar and pestle and stored in a tightly closed container by labeling as 'the purified gum' until used further.

Physiochemical properties
pH determination and fluorescence analysis

The 1% w/v solution of crude gums in water was prepared and allowed to stand for 5 min, and the pH was determined using a digital pH meter (3BW, microprocessor, pH/mv/temperature meter), (Singh *et al.,* 2010). The fluorescence of selected crude gums was recorded under UV light as reported earlier (Chase, Pratt, 1949; Jahan *et al.,* 2008).

Swelling test

The 1.0 g of each crude gum sample was placed in 15 mL plastic centrifuge tubes and the occupied volume was noted (Akpabio *et al.,* 2011). Distilled water (10 mL), phosphate buffer (pH 7.4) and 0.1 N HCl were used for the swelling test and poured into falcon tubes containing samples. They were vortex mixed for 2 min followed by incubated for 10 min. The mixture was centrifuged (10 min at 1000 rpm), supernatant was collected and the volume of swollen gum was measured. The swelling index was calculated by using the following equation:

$S = V2/V1$

$S=V2/V1$
S =Swelling index
$V1$ = Volume occupied by the gum prior to hydration
$V2$ = Volume occupied by the gum after to hydration

Modification and hydrolysis of the gum

Acacia modesta and *Dalbergia sissoo* were chemically modified and hydrolyzed to alter their properties.

Acidic hydrolysis

Acidic hydrolysis of gums was performed by using the method of Grobl *et al.,* (2005). Purified gum samples ranging from 0.2-4 mg were taken up in 2 m trifluoroacetic acid (100 µL/0.2 mg sample) and hydrolyzed in capped glass vials for 2h at 110 °C. After hydrolysis, the hydrolyzed gum samples were collected through ethanol precipitation method, and dried in an oven.

Partial basic hydrolysis

For partial basic hydrolysis of gums, the method of Beltran *et al.,* (2008) was used. Purified gum samples (5g) were hydrolyzed with 200 mL of saturated solution of barium hydroxide at 100 ˚C for 8 h. The hydrolyzed sample was neutralized with 1 m H_2SO_4. The resultant product was precipitated, filtered and oven-dried.

Enzymatic hydrolysis

For enzymatic hydrolysis of purified gums method of Tester and Sommerville (2003) was used with some modifications. Distilled water (0.5 mL) was added to the samples (10 mg ± 0.1) and mixed thoroughly with a clean plastic rod. Tubes were placed in a water bath for 30 min at 40-80 °C. The resulted gels were loosely dispersed with plastic rod and 1.5 of acetate buffer having pH 4.7 was added to the tubes containing gum solution. Afterwards, 5 mL of mannanse was added, and the final volume was made 2.5 mL using distilled water. The mixture was incubated at 30 °C for 15 min. After hydrolysis, the sample was precipitated using ethanol, filtered and oven-dried for further analysis..

Modification of gums

Polyacrylamide grafting

Chemical modification of both selected gums was performed by using a polyacrylamide grafting method as reported by Singh, Srivastava, Tiwari (2009). Purified gums were dissolved in distilled water. Acrylamide (16×10^2 M), $AgNO_3$ (8.0×10^5 M) and ascorbic acid (22×10^3 M) were added to the solution. The solution was thermostated at 35 ± 2 ºC in water. $K_2S_2O_8$ (8.0×10^3 M) was added after 30 min, and the reaction was allowed for 1 h. Using ethanol, the polyacrylamide modified gum was separated from polyacrylamide by precipitating the reaction mixture. The final product was oven-dried for further use.

Carboxymethylation

For carboxymethylation of selected gums, an earlier procedure was followed (Dodi, Hritcu, Popa, 2011) with slight modifications. Purified gums were dissolved in distilled water. NaOH and Chloroacetic acid were added to gum solution under continuous stirring. Using ethanol, the reaction product was extracted, and the precipitated gum was repeatedly washed by ethanol. Modified gum was oven-dried for further use.

Preparation of granules

Wet granulation method (Singh *et al*., 2010) was used for the preparation of granules. The wet mass was granulated by passing them from a sieve of 12 mesh sieve. The granules were dried at 50 ºC in a hot air oven for 30 min. After drying of granules, they were screened from number 30 mesh sieve.

Analysis of granules

Bulk and tapped densities

Granules (10 g) were weighed and transferred in a 50 mL measuring cylinder and the volume occupied by granules was noted (W), which is the bulk volume (V_B). The bulk density (P_B) was calculated from the equation:

$$P_B = W/V_B \text{ (g/mL)}$$

The tapped volume (V_t) was determined by tapping the cylinder from a fixed height on a soft base until there was no further reduction in volume. The tapped density (P_t) was calculated from the following equation from Onunkwo (2010):

$$P_t = W/V_t$$

The data generated was used in calculating the Carr's compressibility index (CI) and Hausner's ration (HR).

$$CI = 100(TD-BD)$$
$$HR = TD/BD$$

BD= bulk density
TD= tapped density (Basawaraj *et al.,* 2010).

Preparation of tablets

For the formulation of tablets, the granules were compressed in a tableting machine (Model F_3, Manesty, England), fitted with 8 mm convex faced tools at a tablet target weight of 250 mg.

Tablet evaluation

Uniformity of weight

Twenty tablets (Ahuja *et al.,* 2013) were randomly selected from each batch and weighed individually and collectively using an electronic balance.

Hardness test

The hardness of 10 tablets from each batch was taken randomly for determining their hardness by using a hardness tester model TBH 200. The mean hardness was calculated (Ahuja *et al.,* 2013).

Friability

The 20 tablets were randomly selected from each batch and their weight was noted collectively as initial weight, WA. These tablets were placed in a friabilator (Copley FR-1000, UK) at speed of 25 rpm for 4 min. This device subjects the tablets to the combined effect of abrasions and shock in a plastic chamber revolving at 25 rpm and dropping the tablets from a height in each revolution. After the completion of a run the tablets were de-dusted and weighed (WB). Using the following formula, the friability was calculated:

$$F = (WA-WB)/ WA \times 100$$

Disintegration time

The disintegration time of tablets was determined according to the method described in the British Pharmacopoeia (2010). One tablet was placed in each six component of the disintegration apparatus (Model: Copley, ZT 34, UK). The disintegration medium was 0.1 M HCl, maintained at 37 ± 1 ºC. The disintegration time was taken as the mean time needed for the tablets to break into particles small enough to pass through the screen into the disintegration medium. The tablets were considered to have passed the test after the 6 tablets passed through the mesh of the apparatus in 15 min.

Dissolution rate

Drug release from tablets was determined using USP II dissolution apparatus (PTWS3C, PharmaTest, Hainburg, Germany), employing British Pharmacopoeia method for acetaminophen. One tablet was placed in the apparatus and paddle speed set at 100 rpm. The dissolution medium was 900 mL 0.1 N HCl, maintained at 37 ± 0.5°C. After every 5 min interval, 5 mL of sample was withdrawn from the dissolution medium, and replaced with an equivalent fresh dissolution medium, till 90 min. The collected samples were analyzed at 243 nm using a UV/VIS spectrophotometer. The amount of drug present in the samples was calculated.

Statistical analysis

All the experiment were performed in triplicate. Mean and Standard deviation of results were calculated.

RESULTS AND DISCUSSION

Physiochemical properties

The crude *D. sissoo* and *A. modesta* gums were purified, and their physiochemical properties were determined with respect to the crude gum.

pH determination

A 1% w/v dispersion of crude *A. modesta* and *D. sissoo* gave acidic pH (Table I). Jahan *et al.* (2008) has reported, 5.1 and Singh *et al.,* (2010) reported 5.0, the pH of gum acacia. Information regarding the pH of the gum is an essential parameter to determine its appropriateness for the formulation process. Further, the physiological properties and stability of the preparation depend on pH (Singh *et al.,* 2010). It also has an influence on the surface active molecules and interactions between the proteins and gum polysaccharides (Mahfoudhi *et al.,* 2012).

TABLE I - Physiochemical properties of gum *D. sissoo* and *A. modesta*

Parameters	*Dalbergia sissoo*	*Acacia modesta*
pH	5.8±0.04	5.7±0.02
Fluorescence analysis	Light brown	Creamy
Swelling Test:		
Distilled water	1.8±0.01	2.0±0.02
Phopshate buffer	1.3±0.02	1.2±0.04
0.1 N HCl	1.6±0.03	1.7±0.01

Fluorescence analysis

The fluorescence of crude gum samples was noted under UV light (Table I) to determine the purity and standard. Creamy color of gum acacia was also reported by Jahan *et al*. (2008).

Swelling test

The swelling ratio of *D. sissoo* and *A. modesta* was studied in different media. Results showed that the swelling ratio was highest in water followed by 0.1N HCl and least in phosphate buffer. Both gums were found to have a high swelling index. The stability and tableting properties of pharmaceutical formulations can be influenced by the excipient's moisture content. Water absorption or retention capacity is the main cause of any polysaccharide's swelling ability (Kumar *et al.,* 2011). Swelling is the prime procedure of diffusion controlled release (Akpabio *et al.,* 2011).

Granule analysis

The granules were prepared by using gum *D. sissoo*, *A. modesta*, their derived forms, and HPMC. The pre-compression parameters were bulk and tapped density, Hausner's ratio and Carr's index.

The bulk and tapped densities possessed by the used gums showed nearly the same result as HPMC have. The bulk and tapped densities of granules produced using various gum samples and HPMC showed that they have good flowing property (Table II). The compressibility

index is a measure of flow ability and compressibility of a material. Its value up to 15% results in good to excellent flow properties and indicate desirable packing characteristics. Compressibility index more than 25% are usually sources of poor tableting qualities (Kumar *et al.,* 2011). The lower the Carr index of a material, the better the flow ability and the poorer compressibility of the material. The Hausner's ratio and Carr's index were calculated using bulk and tapped densities. According to literature, granules having carr's index between the range of 5 – 15 % and Hausner's ratio of less than 1.25, exhibit good flow properties. Hausner's ratio is related to interparticle friction and could be used to predict powder flow properties (Lachman, Lieberman, Kanig, 1987). All the tested formulations have the Hausner's ratio of less than 1.25. While the carr's index was up to 15 % which was excellent. Thus, all the granules possessed good flow properties.

TABLE II - Pre-compression parameters using gum *Dalbergia sissoo* and *Acacia modesta*

Tablets using:	Bulk density (g/mL)	Tapped density (g/mL)	Hausner's ratio	Carr's index (%)
		Dalbergia sissoo		
Crude	0.50	0.58	1.16	8.0
Purified	0.50	0.62	1.25	12.5
Acidic hydrolysis	0.52	0.62	1.18	9.9
Basic hydrolysis	0.50	0.62	1.25	12.5
Enzymatic hydrolysis	0.47	0.58	1.23	11.2
Polyacrylamide grafting	0.50	0.62	1.25	12.5
Carboxymethylation	0.50	0.62	1.25	12.5
		Acacia modesta		
Crude	0.52	0.62	1.18	9.9
Purified	0.55	0.62	1.12	7.0
Acidic hydrolysis	0.52	0.62	1.12	14
Basic hydrolysis	0.55	0.62	1.12	7.0
Enzymatic hydrolysis	0.55	0.62	1.12	7.0
Polyacrylamide grafting	0.47	0.55	1.16	7.9
Carboxymethylation	0.55	0.66	1.20	11.1
		HPMC		
	0.45	0.55	1.21	9.6

Preparation of tablets

The acetaminophen tablets were prepared using *A. modesta* and *D. sissoo* gum and HPMC as the binder. There were 15 batches of tablets with the different binder solution.

Tablet analysis

Weight uniformity

To study the weight uniformity of all the prepared batches of tablets, twenty tablets were randomly selected

from each batch and then weighed individually. Data given in Table III portrays that there was no major variation shown by the acetaminophen tablets in mean weights. The tablets standard deviation of less than 2 % reflects good uniformity (Ahuja *et al.,* 2013).

TABLE III - Tablet analysis formulated by *D. sissoo* and *A. modesta* and Hydroxypropyl methylcellulose as binder

Tablets using:	Weight uniformity (mg)	Hardness (N)	Friability (%)	Disintegration time (min)
Dalbergia sissoo				
Crude	250.77 (1.21)	94.5 (0.22)	0.88	16
Purified	251.99 (1.32)	81.0 (0.70)	0.92	10
Acidic hydrolysis	252.77 (1.27)	69.1 (0.82)	0.95	6.0
Basic hydrolysis	252.82 (0.80)	67.8 (0.76)	0.13	14
Enzymatic hydrolysis	253.31 (0.93)	141.4 (0.27)	0.49	21
Chemical modification	252.29 (1.31)	54.5 (0.68)	0.96	3.0
Carboxymethylation	252.89 (0.91)	154.1 (0.14)	0.35	19
Acacia modesta				
Crude	257.86 (1.83)	99.2 (0.49)	0.47	16
Purified	255.04 (2.69)	90.8 (0.97)	0.60	15
Acidic hydrolysis	249.97 (0.88)	87.4 (0.67)	0.54	15
Basic hydrolysis	254.83 (1.90)	58.2 (0.84)	0.65	2.0
Enzymatic hydrolysis	252.82 (1.06)	109.3 (0.51)	0.36	17
Chemical modification	252.22 (0.86)	94.3 (0.94)	0.80	11
Carboxymethylation	253.04 (1.05)	113.9 (0.08)	0.62	16
HPMC				
	251.18 (1.12)	90.4 (0.89)	0.88	3.0

Hardness

From each batch, 10 tablets were taken randomly for determining their hardness and data presented as mean ± S. D was calculated (Table III). The purified, acidic hydrolyzed and chemical modified gum samples possess the hardness near to hardness value of HMPC. Whereas, the crude, basic and enzymatic hydrolyzed, and polyacrylamide grafted gum samples have a high value of hardness as compared to HMPC. The gum solutions having high viscosity possess high hardness value.

Friability

Tablets (20) were randomly selected from each batch for the evaluation of friability. The friability of a tablet should be less than 1 %. Tablets from all 8 batches shows the friability less than 1 % (Table III).

Disintegration time

According to the method (British Pharmacopeia), the tablet should disintegrate within 15 min. For this, six tablets were placed in each component of the disintegration apparatus and the time was noted. Some of the prepared tablets were disintegrated within 15 min and some did not (Table III). The basic hydrolyzed sample shows the minimum disintegration time i.e., within 2 min.

Dissolution rate determination

In 0.1 N Hydrochloric acid medium, the dissolution rate of the tablets was analyzed for 90 min in triplicate. The dissolution rate of *D. sissoo* and *A. modesta* is shown in Figure 1 and 2, respectively. Gums of *D. sissoo* and *A. modesta* were tested to evaluate their role as a binder and compared with HPMC. The 75 % of tablets should be dissolved in 30 min.

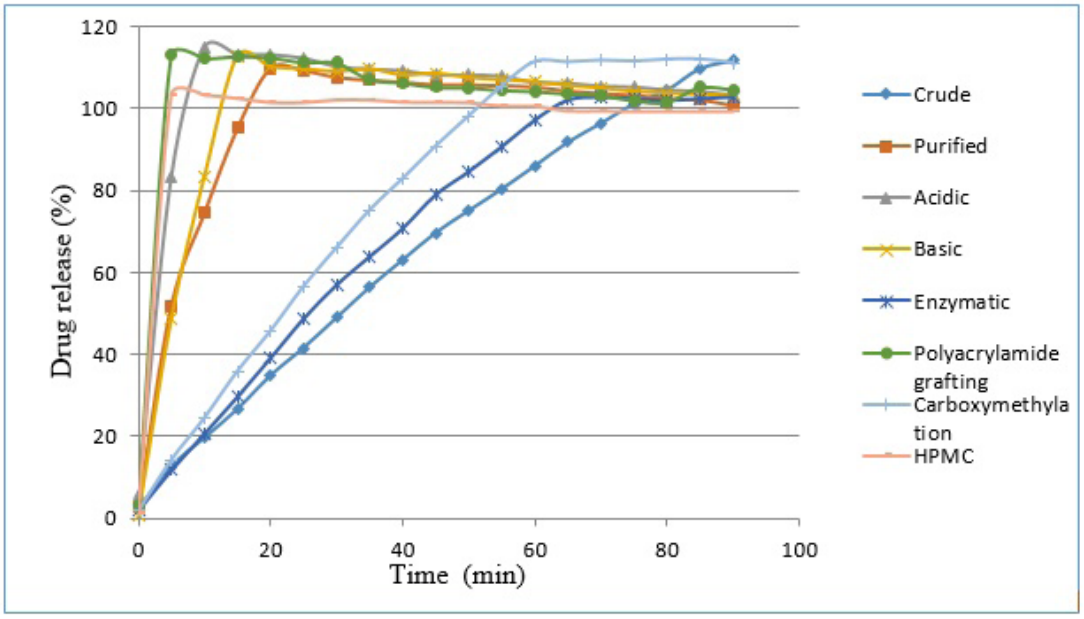

FIGURE 1 - *In-vitro* dissolution profile of paracetamol tablets prepared with *D. sissoo* gum as binding agent.

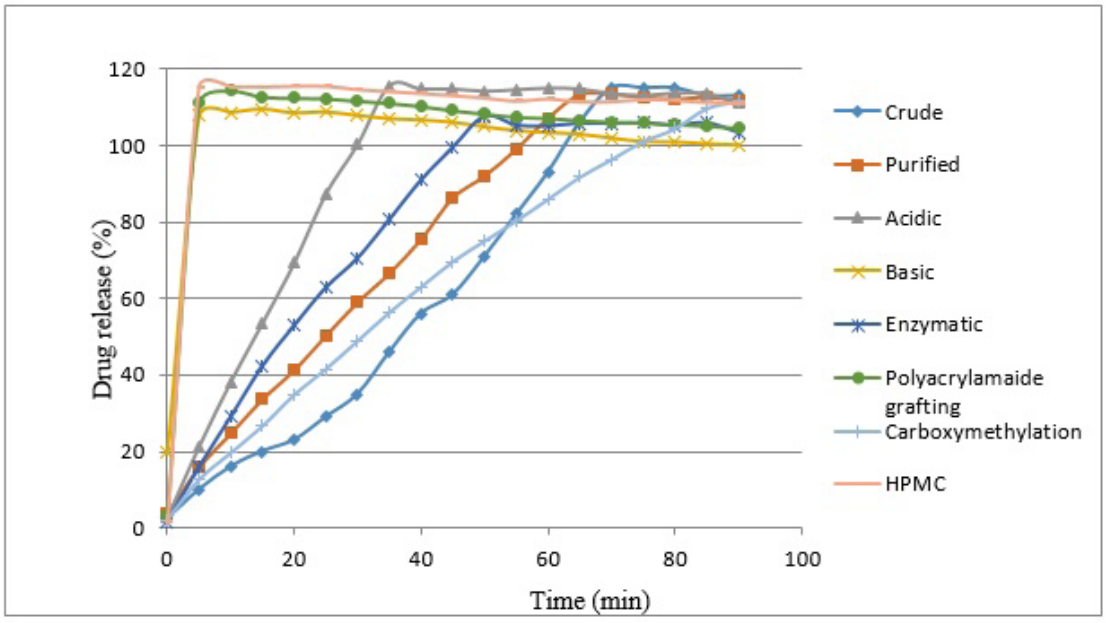

FIGURE 2 - *In-vitro* dissolution profile of paracetamol tablets prepared with *A. modesta* gum as binding agent.

The tablets prepared from crude *D. sissoo* do not dissolve within 30 min and displays a delay in the release. Also, the enzymatically hydrolyzed and carboxymethylated gum do not dissolve up to 75 % within 30 min, due to the viscosity of the binder (gum) used. Rest of the modified, hydrolyzed and purified gums tablets were dissolved within the 30 min. In case of gum *A. modesta*, the crude, purified and carboxymethylated gums show a delay in dissolution analysis, as 75 % of the tablet was not dissolved in 30 min. Except for the above-mentioned gums, all the other gums showed good release profile in paracetamol tablets as binders. These tablets show a similar release profile as of HPMC, the model binder used. In a study, Ahuja *et al.,* (2013) investigated the release profile of paracetamol tablets using *Mimosa pudica* by using different concentrations their release profile of drug was similar with our findings.

Based on the results, the present study demonstrates that both gums and their modified samples could be used in the pharmaceutical formulation as they have different release pattern. The evaluation of tablets reveals that the binding efficacy of tablets prepared using *A. modesta and D. sissoo* is comparable with the tablets prepared using HPMC as a standard binder. All tablet formulations show faster and slower dissolution profiles. After performing the above-stated parameters, it is concluded that the hydrolyzed and polyacrylamide-grafted gums had a similar binding ability as HPMC possess and has the potential to be used as a pharmaceutical tablet binder with faster release rate. The tested tablets showed faster and slower dissolution profiles. The gums showing slower release can be useful in sustained-release tablets as a binder, and those exhibiting faster release rate are helpful in the conventional tablet formulation.

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