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ISOLATION AND EVALUATION OF *CASSIA AUNGOSTIFOLIA* SEED MUCILAGE AS GRANULATING AGENT

Sudarshan Singh*¹ and Sangeeta Singh²

Shree HNS Institute of Pharmaceutical Education and Research ¹, Rajkot, (Gujarat), India

CPS Mahuda College of Pharmaceutical sciences ², Bhermpur, (Orissa), India

ABSTRACT

Keywords:

Cassia aungostifolia,

Mucilage,

Binder

Cassia aungostifolia is a common herbaceous belongs to the family of *Caesalpiniaceae*. Seeds of plant contain gluco-mannose hence attempt to evaluate the seeds for suitability as tablet binder is considered and the present exploration reports the isolation of mucilage of *Cassia aungostifolia* seed. The DSC and FTIR thermograms of drug and mucilage indicated no chemical interaction. Phytochemical and Physiochemical characteristics of mucilage were studied which confirmed the mucilage nature. The mucilage was evaluated for its granulating and binding properties in compressed tablet using Diltiazem HCl as model drug. The granules prepared by mucilage were compared with xanthan gum, which was used as standard binder. The tablets had good physiochemical properties and the drug release was more than 95% within 2hr. It was observed that increasing the concentration of mucilage increases hardness and decreases the disintegration time. All the formulations were subjected to stability studies for three months as per ICH guidelines all four formulation showed stability with respect to release pattern and other parameters which confirm the use of mucilage as excipient.

Correspondence to Author:

Sudarshan singh

Shree H.N.S.I.P.E.R., C/O BM
Kiayda Campus,
Nr. Lal Pari Lake, B/H Marketing
Yard, Amargardh - Bichari,
Rajkot, 360002 (Gujarat.) India
E-mail:
sudarshansingh83@gmail.com

INTRODUCTION: Seed gums are important agrochemical used in various industries worldwide. The growing industrial utility of these gums in the field of paper, textile, petroleum recovery and pharmaceutical industries has resulted in an impetus in India for intensified research on new sources of gums and their modified products. *Cassia aungostifolia* mucilage derived from the seeds of *Cassia aungostifolia* Linn. is a common herbaceous annual occurring weed throughout India commonly known as Tinnevely Senna or Tanner's cassia, is a well known source of sennosides¹.

This herb contains anthraquinones, flavonoids and flavan-3-ol derivatives¹. *Cassia aungostifolia* has been used as natural medicine for the treatment of Anti-viral, anti-cancer and hypoglycemic³. The whole plant possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice².

Although, some work had already been carried out on seed but it seems that no work has been done on the suitability of *Cassia aungostifolia* mucilage as a binding agent in tablet preparation as compared to the relatively common natural agents as Acacia, guar gum and xanthan gum, using disintegration time, rheology and *in vitro* analysis as assessment parameters. Hence the present work was attempted to evaluate binding properties of seed mucilage of *Cassia aungostifolia*.

MATERIALS AND METHODS: *Cassia aungostifolia* seeds were procured from the forest of KORIA, Chhattisgarh, India. Diltiazem HCl was obtained as gift sample from Arvind remedies pvt. Ltd., Chennai. All other ingredient was of analytical grade and purchased from Loba chemicals, Mumbai.

METHODS:

Method A (Isolation of Mucilage from *C. aungostifolia* Defatted seed, MCAD): *C. aungostifolia* seeds Kernel's powder (20g) were defatted by soxhlet extraction using petroleum ether as a solvent at temperature 60-70°C this was repeatedly extracted using hot water till the complete mucilage was extracted. The mucilaginous solution was then filtered through eight folds of muslin cloth. The mucilage was then precipitated by adding sufficient acetone. The extracted mucilage was then dried in microwave oven till it was completely dried. The obtained powder was then sieved to get fine gum powder³.

Method B (Isolation of Mucilage from *C. aungostifolia* filtered seeds, MCAF): *C. aungostifolia* seeds Kernel's powder (20g) was soaked in cold distilled water (200 ml) and slurry was prepared. Then slurry was mixed with 800ml of boil distilled water. The solution was boiled for 20 minutes under stirring condition in water bath. The resulting thin clean solution was kept overnight for settling protein and fibers. The solution is centrifuge at 5000rpm for 20 minutes. The supernatant was separated and poured in to twice the volume of absolute ethanol by continues stirring to precipitate the polysaccharides. The precipitate was washed with absolute diethyl ether and petroleum ether and then dried at 40-45°C and passed through sieve #120 and stored in desiccators until used for further studies⁴⁻¹⁰.

Drug- Excipient Compatibility Studies: This study has been done to check whether there is any compatibility related problems are associated with drug and excipients used for the formulation of tablet. The drug and excipients must be compatible with one another to produce a product that is stable, efficacious, attractive and

easy to administer and safe. If the excipients are new and not been used in formulations containing the active substance, the compatibility studies are of paramount importance. Thermal analysis and FTIR can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients.

FTIR Spectroscopy: The IR spectral analysis of a drug and other excipients were taken using Press pellet technique (using KBr). The IR spectra's were determined by using Jasco FT-IR 410¹¹⁻¹⁴.

Differential Scanning Calorimeter Studies (DSC): DSC was performed on a Shimadzu DSC-60 (Shimadzu Limited Japan). A 1:1 ratio of drug and excipient was weighed into aluminum crucible and sample was analyzed by heating at a scanning rate of 10°C/min over a temperature range 20⁰-300⁰C under a nitrogen flow of 40ml/min. Reproducibility was checked by running the sample in triplicate¹⁵.

Preliminary Phytochemical Screening of Isolated Mucilage: The phytochemical properties such as presence of carbohydrate, protein, flavonoids, sterols, alkaloids, tannins, saponins, glycoside, resin, phenol, diterpines and terpenoids were determined¹⁶.

Physicochemical Properties of Dried Mucilage: The physicochemical properties such as solubility, pH and viscosity of dried mucilage were determined at 20°C. The loss on drying, total ash content, acid insoluble ash and water soluble ash were determined according to Ayurvedic Pharmacopoeia of India (API)¹⁷.

Microbiological Properties:

Microbial Content Determination: 1 gm of MCAD and MCAF dissolved in 9ml of sterile distilled

water. Serial dilutions were made and viability assessed using pour plate method. For detection of fungal growth in sample, sodouraud dextrose agar medium was used. The plates were incubated at 27°C for 72 hr¹⁸⁻¹⁹. For detection of bacteria growth Casein digest agar medium was used. The plates were incubated at 37°C for 24 hours²⁰.

Acute Toxicity Study: The method was performed according to the OECD test guide line for testing of chemical TG 423²¹. Healthy Wistar female rats fasted over night, but allowed access to water *ad libitum*. Animals were randomly divided in to five group (n=3). The controlled received water. Group I-IV were orally treated with MCFD and MCFE at dose of 50, 300, 2000, 5000mg/kg respectively the animals were observed at 15, 30, 60, 120 and 240 minutes with no intake of food and water and thereafter over a period of 24hrs. The rats were further observed for 14 days with food and water intake animals were monitored shown in **table 1**.

Preparation and Evaluation of Granules: Diltiazem HCl (DTZ) was used as model drug to formulate the granules. Microcrystalline cellulose was used as disintegrant, were lactose and aerosil was used as diluents and lubricant respectively, the composition is shown in table 2. Binder solution was prepared by dissolving the MCAD and MCAF in water at 1.0%, 2.0%, 3.0% and 4.0% w/v concentrations. The batch size was 100gm. The drug, lactose, aerosil, and MCC were mixed thoroughly and sufficient volume of 20 ml of 1.0%, 2.0%, 3.0% and 4.0% w/v MCAD and MCAF was added slowly to powder blend, and kneading was performed for near about 10 min until the formation of wet mass with enough cohesiveness. The wet mass forced through the sieve # 16 and dried at 40-45°C in hot air oven for 2hr. The dried granules were received through sieve # 20.

TABLE 1: BODY WEIGHT OF FEMALE RATS IN ACUTE TOXICITY OF THE *C. AUNGOSTIFOLA* SEED MUCILAGE

	Body Weight (gm)					
	MCAD			MCAF		
	Days 0	Days 7	Days 14	Days 0	Days 7	Days 14
Control	222±1.4	230±1.9	242±1.6	220±1.3	218±1.5	231±1.3
Group I	214±1.5	228±1.7	249±2.1	216±1.4	212±1.0	234±1.5
Group II	223±1.6	234±1.9	247±1.2	219±1.9	219±1.6	230±1.2
Group III	219±1.1	236±2.1	251±1.1	222±1.8	220±1.3	229±1.4
Group IV	218±1.7	231±1.4	246±1.4	221±1.1	221±1.9	233±1.9

TABLE 2: COMPOSITION OF TABLET FORMULATION

INGREDIENT	MCAD as binder				MCAF as binder				Xanthan Gum
	F1	F2	F3	F4	F5	F6	F6	F7	F8
DTZ	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg	50 mg
<i>C. aungostifolia</i> (%w/v)	1.00%	2.00%	3.00%	4.0%	1.00%	2.00%	3.00%	4.0%	4.0%
MCC	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Lactose	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Aerosil	4 mg	4 mg	4 mg	4 mg	4 mg	4 mg	4 mg	4 mg	4 mg
Total weight	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg	200 mg

The prepared granules were then evaluated for percentage of fines, particles size and flow properties by measurement of angle of repose²³⁻²⁵. The bulk and tapped densities of the granules were then assessed in accordance with the USP XXV tapped volume meter apparatus compressibility index of the granules was determined by Carr's compressibility index²⁶⁻²⁸.

Preparation and Evaluation of Tablet; The lubricated granules were compressed into tablet using 8 mm biconcave punch with 10 station single rotary Clit (Jemkay) machine and keeping average weight 200 mg. The prepared tablets were evaluated for content uniformity, hardness, disintegration time and *in vitro* dissolution profile using method specified in Indian pharmacopeia²².

Accelerated Stability Studies: Formulation were stored at various temperature *viz.* 25°C/60% RH, 30°C/65% RH and 40°C/75% RH as per ICH guidelines and various physicochemical

parameter (appearance, percentage drug content and release profile) were monitored periodically for 3 months²⁹.

RESULT AND DISCUSSION:

Drug- Excipient Compatibility Studies: The dried and coarsely powdered seeds of *C. aungostifolia* yielded high percentage using MCAD and MCAF method; 6.69% w/v and 10.30% w/v respectively of mucilage using acetone and ethanol as mucilage precipitating solvent. The thermograms of drug and mucilage of *C. aungostifolia* shows that there is no change in melting point which confirms that there is neither change in crystallinity of the drug nor any interaction **fig. 1**. Further drug polymer interaction was checked by comparing the IR spectra of the physical mixture of drug with the Mucilage of *C. aungostifolia* used with the IR spectrum of pure drug.

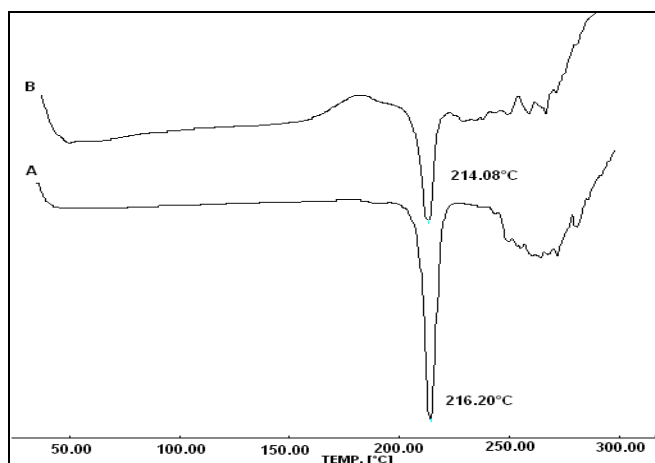


FIGURE 1: DSC OF, A) PURE DRUG DILTIAZEM HCL AND B) DRUG WITH MUCILAGE (MCAD AND MCAF)

Frequencies of functional groups of pure drug remained intact in physical mixture containing *C.aungostifolia* **fig. 2** so it was concluded that there was no major interaction occurred between the drug and *C. aungostifolia* used in the study.

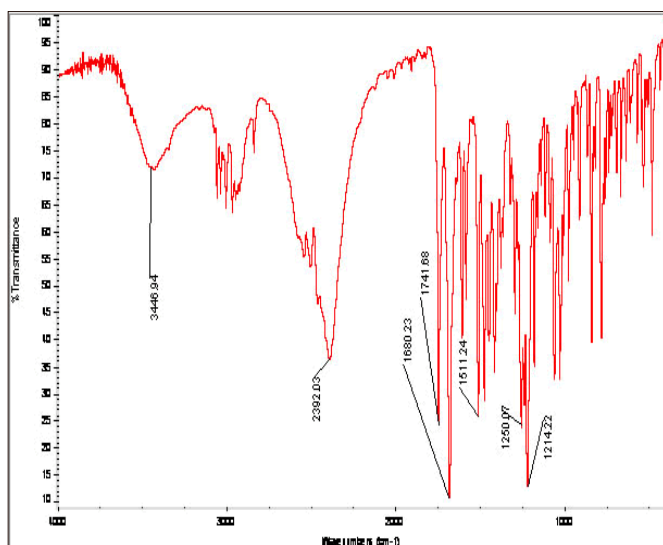


FIGURE 2: FTIR SPECTRUM OF PHYSICAL MIXTURE OF DTZ WITH MUCILAGE USED

Preliminary Phytochemical Screening of Isolated Mucilage: The phytochemical screening of natural mucilage confirmed polysaccharides in nature **table 3**.

TABLE 3: DATA SHOWING, PRELIMINARY PHYTOCHEMICAL SCREENING OF ISOLATED MUCILAGE

ACTIVE CONSTITUENT	MCAD	MCAF
Carbohydrate	+	+
Protein	-	-
Flavonoids	+	+
Tannins	-	-
Saponins	-	-
Sterols	-	-
Alkaloids	-	-
Triterpenes	-	-
Glycosides	-	-
Fats & Oil	-	-
Resins	-	-
Phenols	-	-
Diterpenes	-	-

+ Present, - Absent

The physicochemical and microbiological properties of mucilage of *C. aungostifolia* were determined, results shows mucilage were under microbial limit. The results are presented in **table 4** and **5** respectively.

TABLE 4: TECHNOLOGICAL CHARACTERIZATION PHYSICOCHEMICAL PROPERTIES OF *C. AUNGOSTIFOLA* MUCILAGE

PARAMETER	MCAD	MCAF
Test for mucilage (Ruthenium red)	Positive	Positive
Solubility	Soluble in cold water and hot water forming viscous colloidal solution	Soluble in cold water and hot water forming viscous colloidal solution
Moisture content (%)	10.01	10.09
Swelling index (%)	33.0 ± 0.15	35±0.12
pH	5.6-6.5	5.5-6.5
Viscosity (1.0%w/v solution)	20-100cps	20-100cps
Specific gravity (g/ml of 1.0%w/v solution)	0.9975	0.9978
Loss on drying (%)	8.2 ± 0.02	8.4±0.04
Total ash (%)	7.72± 0.13	7.70±0.11
Acid insoluble ash (%)	0.57± 0.05	0.51±0.03
Water soluble ash (%)	6.532± 0.08	6.823±0.02

*All values are mean ± S.D. for n=3

TABLE 5: TECHNOLOGICAL CHARACTERIZATION OF MICROBIAL LOAD

NATURAL GUM	MICROBIAL LOAD	
MCAD	Bacteria	Absent
	Fungi	
MCAF	Bacteria	Absent
	Fungi	

Acute Toxicity Study: Acute toxicity studies were conducted according to OECD guidelines no. 423. The results shows that there is neither a behavioral change nor change in body weight which show no toxicity induced from mucilage (MCAD and MCAF).

Physicochemical Properties of Dried Mucilage:

The prepared granules were evaluated for percentage of fines, flow properties, the result are shown in table 6. It was observed that percentages of fines were reduced as the concentration of mucilage of *C. aungostifolia* was

increased. The percentage of fines was little higher in granules prepared using 1.0% of mucilage as binder. The flow properties of granules were determined by angle repose which was found to be 32° to 27°. Hence all the granules exhibited good flow properties. Bulk densities of the prepared granules were found to decrease slightly by increasing the concentration of MCAD and MCAF. This result may be due to the formation of larger agglomerates and decrease in fines in the granules, as increasing mucilage of *C. aungostifolia* concentration. The result of compressibility index indicates decrease in flow ability with increasing mucilage of *C. aungostifolia* concentration. However, all formulation showed good flow properties. In general, compressibility index values up to 21% result in fair to passable flow properties. All these result indicates that the granules possessed satisfactory flow properties and compressibility.

TABLE 6: TECHNOLOGICAL CHARACTERIZATION OF GRANULES USING MCFD AND MCFE AS BINDER

PROPERTIES	MCAD				MCAF				XANTHAN GUM
Concentration	1.0%	2.0%	3.0%	4.0%	1.0%	2.0%	3.0%	4.0%	4.0%
Percentage of Fines (%)	23.31	23.74	22.86	20.91	24.50	23.40	21.10	19.40	18.06
Angle of Repose	31.60°	32.01°	31.20°	29.40°	29.05°	28.05°	32.80°	27.60°	26.40°
Hausner's Ratio	1.28	1.31	1.21	1.26	1.24	1.33	1.26	1.23	1.19
Mean particle size (mm)	0.31	0.34	0.30	0.32	0.34	0.31	0.33	0.32	0.34
Percentage friability (%)	0.79	0.66	0.51	0.48	0.75	0.62	0.54	0.46	0.35
Disintegration Time (in min.)	1	2	3	3	2	3	4	3	6
Loose Bulk density (g/ml) ±SD	0.39±0.05	0.39±0.03	0.36±0.06	0.35±0.01	0.56±0.05	0.55±0.03	0.52±0.06	0.51±0.01	0.52±0.01
Tapped Bulk Density (g/ml) ±SD	0.50±0.04	0.52±0.01	0.45±0.02	0.44±0.01	0.69±0.04	0.73±0.01	0.66±0.02	0.68±0.01	0.64±0.01
True density (g/ml) ±SD	1.55±0.06	1.54±0.05	1.65±0.01	1.61±0.08	1.42±0.04	1.45±0.03	1.52±0.06	1.57±0.03	1.55±0.06
Compressibility Index (%)	21.88±0.78	22.81±0.24	17.67±0.05	19.03±0.04	18.92±0.78	22.66±0.24	20.83±0.05	20.90±0.04	20.64±0.04
Content Uniformity (%) ± SEM	99.6±0.44	100.2±0.54	100.1±0.52	101.4±0.51	99.8±0.34	98.2±0.04	99.1±0.52	100.4±0.51	98.0±0.70
Hardness (kg/cm ²) ± SEM	3.29±0.13	4.13±0.16	5.18±0.17	6.23±0.13	3.41±0.44	4.02±0.04	5.48±0.08	6.57±0.14	6.63±0.56
Weight Variation (%)	202.4±2.68	200.5±2.01	204.0±2.38	199.8±2.22	202.9±2.41	201.3±2.29	200.1±2.54	200.3±2.39	204.5±2.60

*All values are mean ± S.D. for n=3

In vitro Evaluation of Tablet: To understand the release profiles of the drug from the tablets, eight batch of tablet were prepared using mucilage of *C. aungostifolia* (MCAD and MCAF) at each four different concentration (1.0%, 2.0%, 3.0% and 4.0% w/v); xanthan gum mucilage (4.0%w/v) was used as standard binder for comparison. The prepared tablets were evaluated for content uniformity, hardness, friability, disintegration time, dissolution profile. All the batches of tablet exhibited good uniformity in content. Hardness of tablet increased with increase in concentration of mucilage.

The tablet prepared with 4.0%w/v MCAD and MCAF showed the hardness nearly equal to the tablet prepared by using 4.0% w/v of xanthan gum. The percentage friability values were slightly decreased as increase in concentration of mucilage. Through increase in hardness of tablet, increase in concentration interestingly showed decreased in disintegration time of tablet. *In vitro* dissolution study showed that drug release from the tablets prepared by using mucilage at four different concentrations was more than 95% in 2hr (fig. 3).

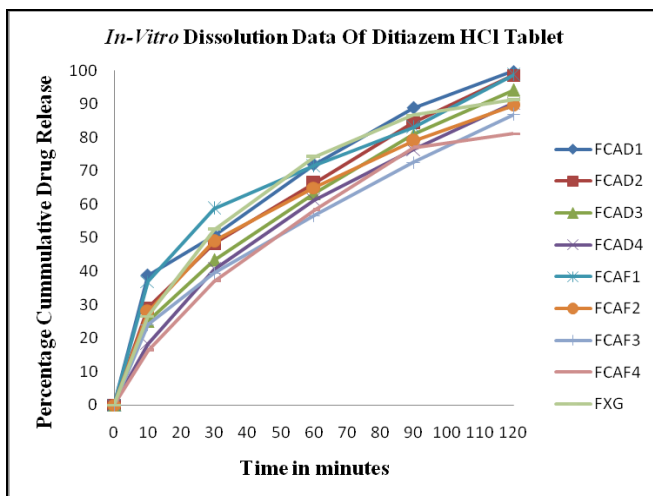


FIGURE 3: IN VITRO DISSOLUTION PROFILE OF DTZ TABLETS PREPARED WITH *C. AUNGOSTIFOLIA* SEED MUCILAGE (MCAD AND MCAF) AS BINDING AGENT

Accelerated Stability Studies: The stability study of optimized batch was carried out at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH as per ICH guidelines. The tablets of all formulation were found to be stable at such condition and other parameters were found to be unaffected and were under Pharmacopoeial limits.

CONCLUSION: The present study indicates that *C. aungostifolia* gum may be better binder for tablet formulation since it minimized the capping tendency without adversely affecting other crucial properties tablets. Considering the mechanical and binding property of tablet formulation, MCAD was found to be better than MCAF. *C. aungostifolia* seed mucilage produces tablet stronger mechanical properties and can enhance the disintegration time. This suggests that *C. aungostifolia* seed mucilage could be useful as binding agent, especially when higher mechanical strength and slower dissolution rates are desired. As per pharmacopeia, disintegration time of uncoated tablets should be < 15 min. It was found that the tablets prepared using 4.0% w/v concentration of isolated *C. aungostifolia* seeds mucilage exhibited disintegration time and Hardness within the standard limit.

Formulations of 1.0, 2.0, 3.0% w/v mucilage exhibited less hardness and disintegration time, when compared with 4.0% w/v xanthan gum binder formulation, but the formulations have enough hardness to withstand the mechanical shocks of handling in manufacturing and packing. Taking all the above parameters into consideration, the study has revealed a good potential of *C. aungostifolia* mucilage as a binder for conventional tablet formulations and as per OECD guideline it doesn't show any toxicity. So it can also be used for sustaining the drug release from tablets at higher concentration, since the prepared tablets using *C. aungostifolia* seed mucilage produces a sticky

film of hydration on the surface. Moreover it may prove economical as binding property of 4.0% w/v *C. aungustifolia* mucilage is almost equivalent to 4.0% w/v xanthan gum.

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