

**EVALUATION OF SUSPENDING PROPERTIES OF *CASSIA ROXBURGHII* MUCILAGE ON SULPHAMETHOXAZOLE SUSPENSION****K.S.G. ARUL KUMARAN*, D.CHRISTOPHER VIMALSON,
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ABSTRACT

It is evident that, plant gums and mucilage have been widely used in various industries like paper, textile, food, pharmaceuticals, ink, cosmetics, petroleum due to their abundance in nature, non-toxic, bio-degradable, ecofriendly and comparatively cheap. They are frequently used in pharmaceuticals as thickening, binding, emulsifying, suspending, and stabilizing agents and coating materials in micro encapsulation. Various plant gums, which have been used as suspending agents include, *Acacia*, Compound *Tragacanth*, *xanthan* gum. In view of importance of mucilage in pharmaceuticals for the manufacture of suspension, *Cassia roxburghii* seed gum (family Fabaceae/Leguminosae) were evaluated by comparing with *Acacia* and Compound *Tragacanth* gum at concentration 2.5 and 3% w/v in sulphamethoxazole suspension. The prepared suspension was evaluated for its sedimentation volume, rheology and particle size analysis. The *Cassia roxburghii* (filtered gum) at 2.5% w/v produced good suspendability compared to the *Cassia roxburghii* (Defatted gum), *Acacia* and Compound *Tragacanth* gum. The suspending ability of the suspending agents were in the order of *cassia roxburghii* (filtered gum) > Compound *Tragacanth* > *cassia roxburghii* (defatted gum) > *Acacia* gum. The results showed that sedimentation volume, viscosity and particle size were directly proportional to the concentration of the suspending agents. The reverse case was observed with the flow rate. The present studies indicate that *Cassia roxburghii* mucilage obtained by filtration method appeared to exhibit the best suspendability for sulphamethoxazole suspensions, compared with compound *Tragacanth*, *Cassia roxburghii* (defatted gum) and *Acacia* gum, and can be employed as stabilizer and thickener of choice in pharmaceutical suspension.

KEY WORDS*Cassia roxburghii*, Compound *Tragacanth*, *Acacia*, Sulphamethoxazole.**INTRODUCTION**

A Pharmaceutical suspension is a coarse dispersion in which internal phase is dispersed uniformly throughout the external phase. The internal phase consisting of insoluble solid particles having a specific range of size which is maintained uniformly

throughout the suspending vehicle with aid of single or combination of suspending agent. The external phase (suspending medium) is generally aqueous in some instance, may be an organic or oily liquid for non oral use¹⁻³. Like other disperse systems, it is thermodynamically unstable, thus, making it necessary



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to include, a stabilizer or suspending agent which reduces the rate of settling and permits easy redispersion of any settled particulate matter both by protective colloidal action and by increasing the consistency of the suspending medium⁴⁻⁶.

Galactomannans gum industry is a fast developing industry because of many uses of these gums in Pharmaceuticals, food, paper, textile, petroleum, cosmetics, paints, detergents, agriculture and a large number of related industries. A number of plant gums / hydrocolloids have been used as binding, suspending or emulsifying agents in solid or liquid dosage formulations⁷⁻¹⁴. Source of these gums are in the endosperm of plant seeds of the family Leguminosae. The leguminous crops owing to have the capacity to utilize the atmosphere for their growth generally do not require expensive nitrogenous fertilizers and increase the soil fertility and can be cultivated on marginal land. A great diversity of legumes is found in India, enough potential to cope with the increasing demand of seed gums in national and international markets. Extensive work to find out new sources of seed gums has been carried out at NBRI, Luck now. It includes the chemical investigation of about 2000 species belonging to different genera of leguminosae. It is found that almost all the species of Cassia, Croton, sesbania and indigofera occurring in India are rich in gum content whereas few species of Bauhinia, Caesalpinia and Desmodium contain appreciable amount of gum.¹⁵⁻²⁰

In view of importance of suspending agents in pharmaceuticals for the manufacture of suspension, *Cassia roxburghii* seed gum (family Fabaceae/Leguminosae) were evaluated by comparing with *Acacia* gum and Compound *Tragacanth* gum at concentration 2.5 and 3%w/v in sulphamethoxazole suspension

MATERIALS AND METHODS

The materials used for the formulation included were, Sulphamethoxazole BP (Himedia -Mumbai), Benzoic acid (SD Fine Chemicals-Mumbai), Amaranth (Himedia-Mumbai), Compound *Tragacanth* (Himedia-Mumbai), *Acacia* gum powder (SD Fine Chemicals-Mumbai), chloroform water double strength (Fisher Scientific-Mumbai) and *Cassia roxburghii* gum (From *Cassia roxburghii* seed, Thanjavur District, Tamil Nadu). All other solvents used were of analytical grade.

Sulphamethoxazole was chosen for this investigation because it is a typical representative of practically insoluble drugs which would require a suspending agent to be prepared as a liquid dosage form²¹.

ISOLATION OF SEED GUM²²:

Defatted seed gum: Three hundred grams of seed powder was 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 powder.

Filtered seed gum: Two hundred and fifty grams of seed powder was soaked in sufficient water, kept over boiling water bath for 30 minutes, with occasional stirring. It was left overnight and the filtered using eight folds of fine 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 powder.



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PHYSICO-CHEMICAL CHARACTERISATION OF SEED GUM²³⁻²⁷:

The separated gum was evaluated for solubility, swelling index, loss on drying, ash value, microbial load, density, angle of repose, compressibility index, porosity, hausner's ratio.

PHYTOCHEMICAL STUDIES²⁸⁻²⁹:

The extracted seed gum was subjected to some preliminary tests to confirm the nature of the obtained mucilage. The tests performed were to determine the presence of alkaloids, anthraquinone glycosides, tannins, cardenolides, carbohydrates, steroids, sterols, protein and amino acid.

EVALUATION OF TOXICITY³⁰:

The male albino rats of Wistar strain were sanctioned by the animal ethical committee. Rats weighing 160-200 gm were divided into different groups comprising of six animals each. The control group received normal saline 25ml/kg i.p. The other groups received 100, 200, 400, 600, 800, 1000, 2000, 3000 and 4000 mg/kg of gum suspension in normal saline orally. The animals were observed continuously for the behavioral changes for the first 4 hours and then observed for mortality if any for 24 hours. Since no mortality, no toxic manifestations were observed and behavioral pattern was unaffected.

PREPARATION OF SULPHAMETHOXAZOLE SUSPENSION³¹:

2.5 gm of *Cassia roxburghii* (filtered gum) and 10gm of sulphamethoxazole were triturated together with 20ml of Raspberry syrup to form a smooth paste. Benzoic acid 0.2gm and 0.01gm of amaranth were added gradually with constant stirring and then mixed with 50ml of chloroform water double strength. The mixture was transferred into a 100ml measuring cylinder, made upto volume with distilled water and then shaken vigorously for 2 min (thus making 2.5% w/v of the gum in the preparation). The

procedure was repeated using 3% w/v of *cassia roxburghii* (filtered gum). The above procedure was repeated with *Acacia* gum, Compound *Tragacanth* powder and *Cassia roxburghii* (defatted gum).

EVALUATION OF SUSPENSION PROPERTIES:

Sedimentation volume³²: Each suspension (50ml) was stored in a 50ml measuring cylinder for 7 days at 35°C. Observation were made at every hour for 7 hours and then every 24 hours for 7 days. The sedimentation volume (%) was then calculated using the following equation:

$$F = 100 \text{ VU/VO}$$

Where, VU is the ultimate volume of the sediment
VO is the original volume of the suspension

Rheology³³: The time required for each suspension sample to flow through a 10 ml pipette was determined and the apparent viscosity (η_a in mls^{-1}) was calculated using the equation:

$$\text{Flow rate} = \eta_a = \frac{\text{volume of pipette (ml)}}{\text{flow time (sec)}}$$

The viscosity (in poise) of the suspensions prepared using various gums were studied using BROOFIELD synchroelectric viscometer, spindle number S 00 was used since small sample (16 ml) was taken. Speed was set to 0.3 -0.6 rpm. All determinations were done in triplicate and the results are expressed as the mean values.

Particle size analysis³⁴:

After shaking, 10ml of each sample was separately transferred into 200ml cylinder. Distilled



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water (150ml) was then added, mixed, and 10 ml aliquot was removed at a distance of 10cm below the surface of the mixture and at 1, 5, 10, 15, 20, 25 and 30 min. This was transferred into an evaporating dish and evaporated to dryness in an oven at 105°C and the residue was weighed.

The particle diameter (d in cm) was then calculated using the Stokes equation:

$$D = 18 \eta h / (\rho_s - \rho_o)gt$$

Where, h is the distance of fall of the particle (cm),

t is the time (s)

η is the viscosity of the dispersion medium

(poise)

$\rho_s - \rho_o$ is the density grading between the dispersed particles and the liquid (g cm^{-3})

g is the gravitational constant (cm s^{-2})

RESULTS AND DISCUSSION

Two different laboratory methods were tried out for the isolation of seed mucilage from seed powder. Defatted method using soxhlet apparatus yielded 40% and filtration method yielded 24%. The mucilage powder obtained was a light brown fine powder. The mucilage obtained was subjected to physicochemical and microbiological tests (table: 1).

Table: 1

Physicochemical characterization of cassia roxburghii mucilage

PARAMETERS	OBSERVATIONS
Description	Light brown fine powder.
Solubility	Swells in cold water, dissolves in warm water forming colloidal solution. Insoluble in organic solvents
Loss on drying	9.3%
	DEFATTED GUM FILTERED GUM
Ash value(% W/W)	5.5 5.9
Water soluble ash	3.3 4.3
Acid insoluble ash	0.04 0.5
Sulphated ash	0.8 0.7
pH(1% W/V)	5.9 5.9
Swelling ratio	In distilled water
	10.4 13.7
Microbial Load	Bacteria(CFUs/g)
	125 129
	Fungi(CFU/g)
	68 73
Density of powder	Bulk density(g/cc)
	0.74 0.76
	Tapped density(g/cc)
	0.90 0.95
Angle of repose	35.34 ⁰ 37.38 ⁰
Carr's index	15.98 17.43



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Phytochemical tests on the *cassia roxburghii* gum (both filtered gum and defatted gum), confirmed the absence of alkaloids, anthraquinone glycosides, tannins, cardenolides, steroids, sterols and protein. It shows the presence of carbohydrates. On treatment of mucilage with ruthenium red showed red colour

confirming the obtained product as mucilage (table: 2). Toxicity study of the gum revealed no behavioral changes for first four hours and no mortality was observed even at the dose level 4000mg/kg body weight after 24 hours, indicating the safety of the gum

Table 2
Phytochemical investigation of Cassia roxburghii seed gum

PHYTOCHEMICAL TESTS	OBSERVATIONS	
	DEFATED GUM	FILTERED GUM
Mucilage(Ruthenium red test)	+	+
Proteins and amino acids (Ninhydrin Test, Biuret Test)	—	—
Alkaloids(dragendroff's test)	—	—
Anthraquinones(keller killiani test):	—	—
Carbohydrates(Molisch's test)	+	+
Tannins(ferric chloride test)	—	—
Sterols and steroids(Liebermann Burchard Test)	—	—
Test for reducing sugar(Fehlings test)	—	—

The sulphamethoxazole suspensions were prepared using *cassia roxburghii* gum (both filtered gum and defatted gum), compound *tragacanth* gum and *acacia* gum as suspending agent in concentrations 2.5% and 3% for each suspension and evaluated for the type and concentration of the suspending agents on sedimentation volume, flow rate, viscosity and particle size (Tables : 3 & 4). Sedimentation volume (Table 3), evaluation shows that the suspensions prepared using *cassia roxburghii* (filtered gum) showed better stability compared to the *cassia roxburghii* (defatted gum), compound *tragacanth* gum and *acacia* gum (Figure 1& 2).The suspending ability of the suspending agents were in the order of *cassia roxburghii* (filtered gum) >compound *tragacanth* > *cassia roxburghii* (defatted gum) >Acacia gum. The sulphamethoxazole suspension formulation prepared using *cassia roxburghii* (filtered gum) at concentration

2.5% showed better stability than the suspension prepared using 3% of the same gum and the other concentrations of *cassia roxburghii* (defatted gum), compound *tragacanth* and *acacia* gum. Rheology (Table 4) shows that the suspensions prepared using *cassia roxburghii* (filtered gum) 2.5% had better viscosity compared to the suspensions prepared using *cassia roxburghii* (defatted gum), compound *tragacanth* and *acacia* gum, of all concentrations. The viscosity of the suspensions were in the order of *cassia roxburghii* (filtered gum) >compound *tragacanth* > *cassia roxburghii* (defatted gum) >*acacia* gum.

The flow rate was inversely proportional to the viscosity of the suspension and were in order of *acacia* gum > *cassia roxburghii* (defatted gum) >compound *tragacanth* > *cassia roxburghii* (filtered gum). Particle



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size analysis showed that, all the formulations were observed to obey the Stoke's law.

The results showed that sedimentation volume, viscosity and particle size were directly proportional to the concentration of the suspending agents. The reverse case was observed with the flow rate. The present study

reveals that filtered *cassia roxburghii* mucilage at 2.5% concentration has excellent suspending properties in sulphamethoxazole suspension formulations, compared to the traditionally used gums like compound *tragacanth* and *acacia*.

Table: 3

Values of sedimentation volume (%) of suspension using different concentration of suspending agents.

Suspending agent	Conc (% w/v)	Sedimentation volume %														
		Time(Hours)							Time(Days)							
		0	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Blank	0	100	47	38	38	36	36	35	35	32	30	28	26	26	26	26
Cassia roxburghii (Filtered gum)	2.5	100	98	96	93	90	88	85	81	72	63	57	54	52	52	52
	3	100	100	100	100	99	99	99	99	98	98	98	97	97	97	96
Compound Tragacanth	2.5	100	94	91	88	82	74	63	50	48	47	47	46	46	46	45
	3	100	97	95	91	88	76	67	60	52	52	50	50	49	49	49
Cassia roxburghii (defatted gum)	2.5	100	86	73	61	54	46	42	40	36	36	35	35	34	34	34
	3	100	96	83	74	68	61	58	55	51	49	48	48	47	47	46
Acaia gum	2.5	100	89	76	65	53	46	30	25	22	22	21	21	20	20	20
	3	100	93	88	81	77	69	56	44	40	38	36	34	33	33	33

Table: 4

Effects of type and concentration of suspending agents on the flow rate and viscosity of sulphamethoxazole suspension.

Suspending agents	Conc (%w/v)	Flow rate ml s ⁻¹	Viscosity(poise)
<i>Cassia roxburghii</i> (Filtered gum)	2.5	0.88	2.21
	3	0.71	2.58
Compound <i>Tragacanth</i>	2.5	1.26	1.15
	3	1.11	1.34
<i>Cassia roxburghii</i> (defatted gum)	2.5	1.33	1.04
	3	1.16	1.19
<i>Acaia</i> gum	2.5	1.42	0.81
	3	1.21	0.92



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Figure 1

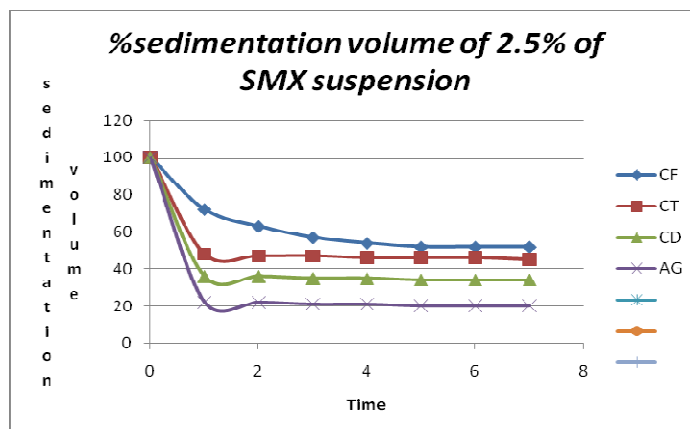
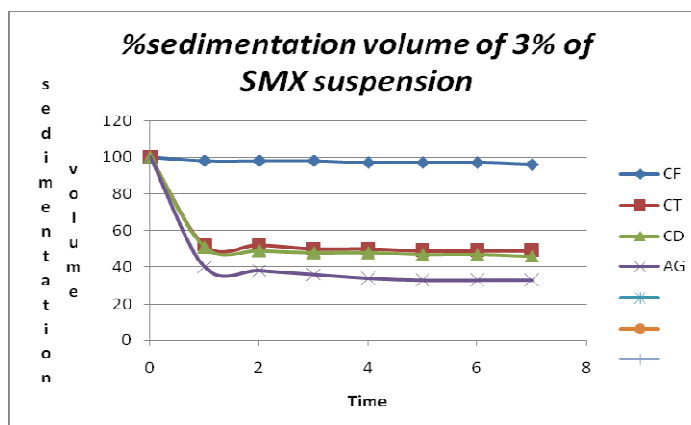


Figure 2



SMX = Sulphamethoxazole

CF = *Cassia roxburghii* gum by filtration method

CT = Compound tragacanth

CD = *Cassia roxburghii* gum- defatted

AG = Acacia gum



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CONCLUSION

The present study indicates that *Cassia roxburghii* mucilage obtained by filtration method appeared to exhibit the best suspendability for sulphamethoxazole suspensions, compared with compound *tragacanth*, *cassia roxburghii* (defatted gum) and *Acacia* gum. The results showed that sedimentation volume, viscosity and particle size were directly proportional to the concentration of the suspending agents. The reverse case was observed with the flow rate. The present study reveals that filtered *Cassia roxburghii* mucilage at 2.5% concentration has excellent suspending properties in sulphamethoxazole suspension formulations, compared to the traditionally used Compound *Tracaganth* and *Acacia* gums. On the other hand *Cassia roxburghii* (defatted gum) showed only slight suspending properties since the fat was removed. Therefore it may be concluded that *Cassia roxburghii* filtered mucilage as a novel suspending agent in the preparation of sulphamethoxazole suspensions and could be employed as stabilizer and thickener of choice in pharmaceutical suspension preparation.

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