Evaluation of *Basella alba* L. Mucilage as a Suspending Agent in Metronidazole Suspension

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ABSTRACT

In the quest for natural biodegradable, non-toxic polymers for use as excipients in pharmaceutical formulations, mucilage of *Basella alba L* (BAM) stem was isolated and evaluated as a suspending agent in metronidazole suspensions at different concentrations (0.5% - 2% w/v) in comparison to tragacanth (TCG) and gelatin gums (GLT). The micromeritic properties of the mucilage powder were determined and the metronidazole suspension was characterized using flow rate, redispersion number, sedimentation volume, viscosity and pH. The degree of flocculation was also determined. BAM powder has good flow property with minimal swelling. The order of flow rate of metronidazole suspension was BAM=TCG>GLT while sedimentation volume ranking was TCG>BAM>GLT. There was no significant difference ($p>0.05$) in the redispersion number of BAM and TCG formulations. The viscosities of formulations containing BAM and TCG at concentrations of 0.5%-1.0% w/v were the same. The pH of the suspensions ranged from 5 to 8. The degree of flocculation was in the order GLT>BAM>TCG. From our findings, BAM can be used as an alternative suspending agent in suspension formulation.

Keywords: *Basella alba*, Suspensions, Suspending agents, Metronidazole.

INTRODUCTION

A Pharmaceutical suspension is a two-phase system with uniform dispersion of finely divided solid drug particles in a continuous phase of solid, liquid or gas in which the drug has minimal solubility. The insoluble solid remain in equilibrium with a saturated solution of the solid in the continuous phase. Drugs are formulated as suspension to enhance the stability of drugs that are unstable in aqueous medium and also to provide a means of formulating poorly soluble and indiffisuble drugs in liquid preparations. This system is thermodynamically unstable; hence, there is need for a stabilizer in form of a suspending agent to reduce the settling rate of the suspended particles [1]. Suspending agents can be classified into three groups namely synthetic e.g. carbopol, semisynthetic e.g. methyl cellulose and natural agents such as the polysaccharides[2-4].

Basella alba L. (Family Basellaceae) is a widely cultivated cool season vegetable and its native to tropical Southern Asia[5]. It is highly abundant in tropical Africa, Malaysia, the Caribbean, Philippines and tropical South America[6]. *Basella alba* is also known as Indian spinach, Ceylon spinach, vine spinach and Chinese spinach[7,8]. It is widely grown in the coastal area of southern Nigeria as a vegetable for food[9]. Extracts from this plant has been shown to have wound healing activity, anti-inflammatory and antiviral properties[10-12]. Mucilage from Basella leaves has been isolated and used as a suspending agent[13], a

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binder in paracetamol formulation[14] and a disintegrant[15]. Literature search showed that mucilage from the stem was characterised[16], but there was little or no formulation studies. Therefore, this present work isolated the mucilage from the stem of *Basella alba* and its suspending properties evaluated in metronidazole suspension.

Materials and Methods

Materials

Metronidazole was a gift from Bond Chemicals, Gelatin (Type B) and tragacanth (MW 840 kDa) were obtained from BDH chemicals, UK. Indian spinach stems were obtained from a local farm in Osun State, Nigeria and authenticated at the herbarium of the Pharmacognosy Department, Olabisi Onabanjo University. All other materials were analytical grade.

Extraction of mucilage

The stems of *Basella alba* L were collected and sun dried for 10 days. The dried stems were pulverised and defatted using petroleum ether (60-80ºC). The defatted powder was soaked in distilled water for 6 hours and refluxed on water bath at 70ºC for 2 hours. It was filtered with a muslin cloth and the filtrate was precipitated with acetone and dried in hot air oven at 40ºC. Dried mucilage was pulverised and passed through sieve size No 60 (250 µm).

Characterisation of Basella mucilage

The powdered mucilage was visually examined for colour, odour and texture.

Percentage yield

The percentage yield was calculated from the equation:

$$\%\ Yield = \frac{weight\ of\ dried\ mucilage}{weight\ of\ dried\ stem} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Hydration capacity

One gram of powdered mucilage was weighed into a 10 mL test tube, tapped and the volume (V_1) noted. Distilled water was added and made up to 10 mL mark. It was shaken for 2 minutes and allowed to stand for another 10 minutes. Volume occupied by the sediment was noted (V_2). The tube was centrifuged at 3000 rpm for 10 minutes and the supernatant was decanted. The weight of the sediment was used in calculating the hydration capacity using equation 2[17,18]: Determinations was done in triplicate

$$Hydration\ Capacity = \frac{(weight\ of\ tube + sediment) - weight\ of\ tube}{Sample\ weight\ (dry\ basis)} \quad \ldots\ldots\ldots\ldots 2$$

Determination of bulk and tapped densities

Five grams of powdered mucilage was weighed into a 50 mL measuring cylinder. The volume (bulk) occupied was noted. The measuring cylinder was subjected to 100 times of tapping manually and the tapped volume was noted. Measurements were done in triplicate. Hausner's ratio was determined from the values of bulk and tapped densities [19].

$$Bulk\ Density\ (g/mL) = \frac{Weight\ of\ mucilage\ (g)}{Bulk\ volume\ (mL)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

$$Tapped\ Density\ (g/mL) = \frac{Weight\ of\ mucilage\ (g)}{Tapped\ volume\ (mL)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

$$Hausner's\ Ratio = \frac{Tapped\ Density}{Bulk\ Density} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 5$$

Angle of repose

Five gram of *Basella* mucilage was poured into a glass funnel held in place. The powder was allowed to flow through the funnel orifice (clamped with its tip 2 cm above a paper placed on a flat horizontal surface) to form a conical heap. The height of the cone and radius were determined. Angle of repose was calculated from the equation:

$$Tan\ \emptyset = \frac{height}{radius} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 6$$

All determinations was in triplicate

Determination of flow rate of suspending agents

Five gram of sample was poured into a glass funnel held in place. The powder was allowed to flow through the funnel orifice and the time taken for the flow was determined. This was done in triplicate.

Preparation of Metronidazole suspensions

Two hundred (200) mL batch sizes of 40 mg/mL metronidazole (10 µm) suspensions were prepared by dispersions method using different concentrations (0.5%-2% w/v) of the powdered mucilage as suspending agent. 0.01% w/v benzoic acid was used as a preservative. Gelatin and tragacanth gum were also used as suspending agents to compare with *Basella alba m*ucilage (Table 1).

Table 1: Formula for Preparation of Metronidazole Suspensions

Formulation	Metronidazole (g)	Basella (g)	Tragacanth (g)	Gelatin (g)	Benzoic acid (g)	Water (mL)
F1	4	0.5	-	-	1	100
F2	4	1.0	-	-	1	100
F3	4	1.5	-	-	1	100
F4	4	2.0	-	-	1	100
F5	4	-	0.5	-	1	100
F6	4	-	1.0	-	1	100
F7	4	-	1.5	-	1	100
F8	4	-	2.0	-	1	100
F9	4	-	-	0.5	1	100
F10	4	-	-	1.0	1	100
F11	4	-	-	1.5	1	100
F12	4	--	-	2.0	1	100

Determination of pH

The pH of each suspension was determined with a pH meter after preparation

Determination of sedimentation volume

50 mL of metronidazole suspension was poured into a measuring cylinder and left standing undisturbed at room temperature. At predetermined time intervals, the sedimentation volume was determined. The sedimentation volume (F) was calculated from the equation:

$$F\ (\%) = \frac{V_U}{V_O} \times 100 \quad \text{.....7}$$

V_U is ultimate volume of sediment, V_O is the original volume of suspension

Determination was in triplicate

Determination of flow rate and viscosity of suspensions

The time taken for 5 mL suspension to flow through a 5 mL pipette was determined and used in calculating the flow rate.

$$Flow\ rate\ (mL/\text{sec}) = \frac{Volume\ of\ suspension\ (mL)}{Flow\ time\ (Seconds)} \quad \text{.....8}$$

The viscosity of the suspension was determined with a Brookfield viscometer Model DV-II+Pro (Brookfield Engineering Laboratories, INC, Middleboro, MA, USA), at 25ºC using spindle 3 at 50 rpm. Determinations were made in triplicate.

Table 2: Physicochemical Properties of Suspending Agents

Properties	Basella	Tragacanth	Gelatin
Swelling index	0.85±0.04	1.80±0.56	None
Hydration capacity	3.70±0.15	4.2±0.28	None
Bulk density(g/mL)	0.357±0.01	0.385±0.02	0.385±0.01
Tapped density(g/mL)	0.385±0.01	0.455±0.02	0.500±0.01
Hausner's ratio	1.08	1.19	1.30
Angle of repose	24.90	19.65	19.65
Flow rate(g/sec)	0.1	0.2	0.2

Data are presented as the mean ± SD; n=3

Redispersion Number

The metronidazole suspensions were left to stand for one week, after which the bottles were inverted manually to allow the suspensions to re-disperse. The number of times the containers were inverted before the bottom of the bottles was free of sediments was recorded as the redispersion number.

Degree of flocculation

The method of Kumar *et al.*[20] was used for the determination of degree of flocculation. Potassium dihydrogen phosphate (0.004 mol) was added to the suspension as a flocculating agent. The sedimentation volume of the flocculated suspensions was compared with those without a deflocculating agent.

$$\beta = \frac{Sedimentation\ volume\ of\ flocculated\ suspension}{Sedimentation\ volume\ of\ deflocculated\ suspension} \quad \ldots\ldots\ldots\ldots 9$$

Statistical analysis

Statistical analysis was carried out using analysis of variance with computer software GraphPad Prism® 4 (GraphPad Software Inc. San Diego, USA).

Results and discussion

Micromeritic properties of suspending agents

The *Basella* mucilage powder was dark brown in color with a coffee-like odour. The yield was 4% w/w. The physico-chemical properties of the different suspending agents are presented in Table 2. Bulk density is the ratio of weight of powder to volume occupied which includes the inter-particulate space. The ranking of bulk density was TCG = GLT > BAM. Hausner's ratio and angle of repose are indirect ways of measuring powder flow. The ranking of Hausner ratio was BAM < TCG < GLT. Hausner ratio of less than 1.20 is indicative of good flow[21]. This indicates that BAM has good flow properties with Hausner ratio of 0.93 (Table 2). The swelling index and hydration capacity of BAM was lower than that of TCG, while gelatin did not hydrate nor swell. The ranking of flow rate of the suspending agents was BAM<TCG=GLT

Table 3: Flow rate and viscosity of suspensions

Suspending agent	Concentration (%w/v)	Flow rate ($mlsec^{-1}$)	Viscosity(centipoise)
BAM	0.5	1.11±0.19	600
	1.0	1.11±0.27	600
	1.5	0.83±0.06	600
	2.0	0.83±0.08	600
TCG	0.5	1.11±0.17	600
	1.0	1.11±0.19	600
	1.5	1.00±0.09	700
	2.0	1.00±0.09	800
GLT	0.5	1.00±0.10	400
	1.0	1.00±0.08	600
	1.5	1.00±0.09	600
	2.0	1.00±0.07	700

Evaluation of suspension

The pH of the metronidazole suspensions produced with the different suspending agents was in the range of 5 and 8. There was no change in pH with change in concentration of suspending agents. The ranking of the pH was BAM>TCG>GLT. The pH of BAM was 8.0, indicating alkaline pH. This suggests that the gum is basic. World Health Organisation recommended a pH of 5-6.5 for metronidazole suspension[22].

The flow rate of the suspensions was observed to decrease with increase in concentration of suspending agents. Similar trend was observed by Bamiro *et al.* [23] when terminalia gum was used as a suspending agent in magnesium carbonate formulation. Oppong *et al.*[24] also observed the same trend with shea tree gum in paracetamol suspension formulation. The ranking of flow rate at 0.5% and 1.0% w/v concentration was BAM=TCG>GLT. This indicates that BAM suspensions have better flow ability than GLT suspensions. A good suspension must be easily pourable from a container, therefore from this result; we can infer that BAM has the quality of a good suspending agent.

The suspensions were observed to sediment rapidly during the first 24 hours, after which the sedimentation became steady. This can be seen in the representative plot shown in figure 1. The ranking of sedimentation volume was TCG>BAM>GLT. A suspension with sedimentation volume ratio of 1 or 100% is said to be a good suspension[25]. High sedimentation volume is an indication that even though the particles have settled, as expected with suspensions, the interparticle attraction and bonding were loose and not strong enough to form hard cake during the study period[26]. Suspensions containing GLT had the least sedimentation volume. A good suspension is expected not to sediment rapidly. When sedimentation volume is near to 1 or 100%, the particles tend to flocculate easily[4].

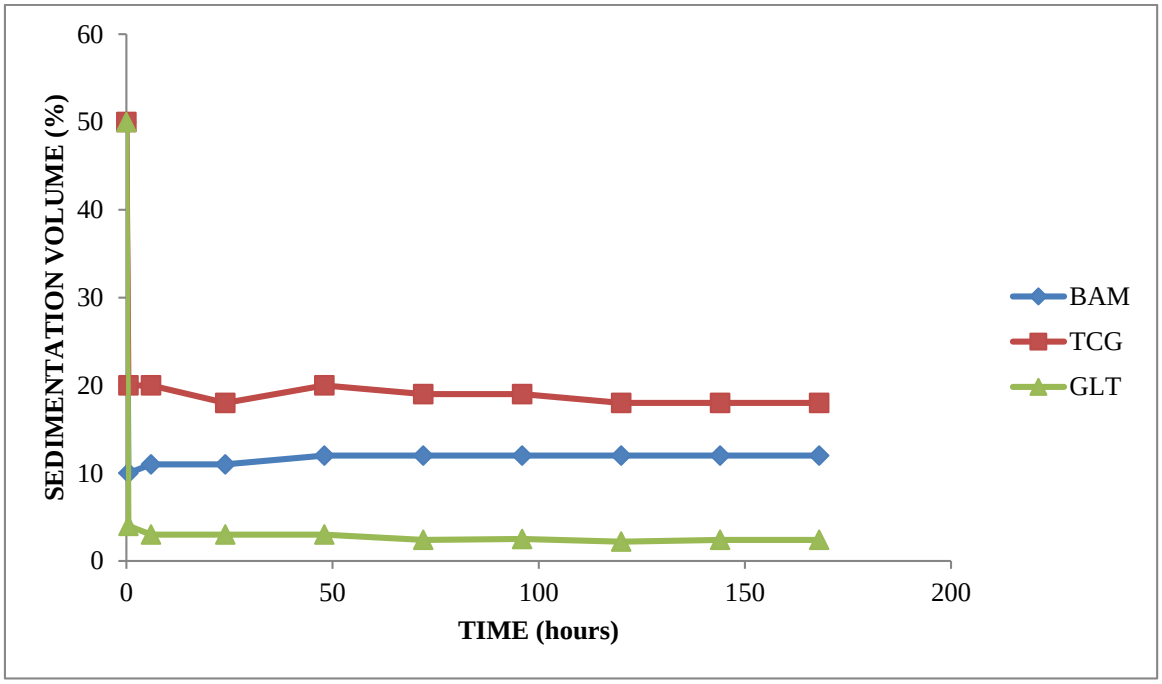

Figure 1: Representative plot of sedimentation volume against time at a concentration of 1.5% w/v suspending agent

The number of times taken to shake the suspension bottles before redispersion is presented in Table 4. There was no significant difference ($P > 0.05$) in the suspensions containing BAM and TCG. The low redispersion number could have been due to loose flocs formed by the sedimented suspensions. However, suspensions containing GLT with low sedimentation volume was observed to have significantly high ($p<0.05$) redispersion number. This could have been due to the formation of hard cake on settling, which made it difficult for redispersion. The suspension could have been a deflocculated one. Redispersion is an important aspect of the pharmaceutical quality of a dilute suspension since they tend to settle on standing (storage)[27,28]. Dose uniformity is dependent on the homogeneity of the suspension during administration; therefore, a suspension with low redispersion number will be desirable. The degree of flocculation was in the order GLT>BAM>TCG (figure 2).

Table 4: Redispersion number of suspensions at different concentartions

Suspending agent	Concentration(%w/v)	Redispersion number
	0.5	3
BAM	1.0	3
	1.5	3
	2.0	3
	0.5	3
TCG	1.0	3
	1.5	3

Suspending agent	Concentration(%w/v)	Redispersion number
GLT	2.0	3
	0.5	5
	1.0	5
	1.5	10
	2.0	15

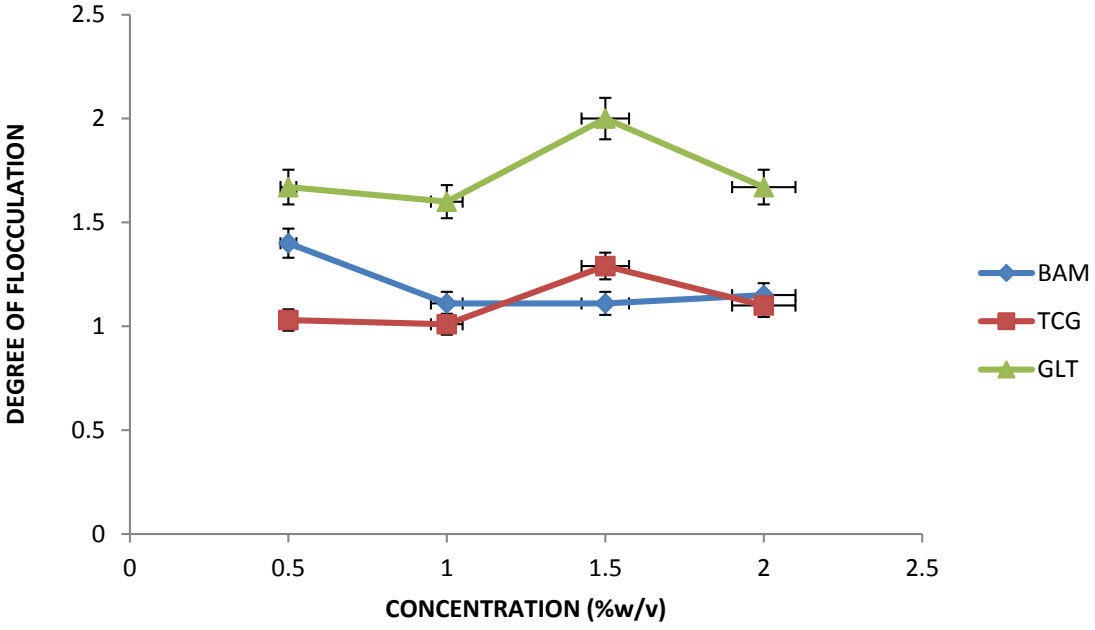

Figure 2: Plot of degree of flocculation against concentration

Conclusion

Formulated metronidazole suspension containing BAM mucilage has same flow rate as the formulations containing TCG. There were no changes in the viscosity of suspensions containing BAM with increase in concentration. There was no significant difference in the sedimentation volume of suspensions containing BAM and TCG. Therefore, BAM can be used as an alternative suspending agent in formulation.

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تقييم *Basella alba* L Mucilage كعامل تعليق في تعليق ميترونيدازول

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ملخص

في البحث عن بوليمرات طبيعية قابلة للتحلل وغير سامة لاستخدامها كسواغات في المستحضرات الصيدلانية، تم عزل الصمغ من جذع *Basella alba* L (BAM) وتقييمه كعامل معلق في معلقات الميترونيدازول بتركيزات مختلفة (0.5٪ – 2٪ وزن / ت) بالمقارنة مع تراجاكانث (TCG) ولثة الجيلاتين (GLT). تم تحديد الخواص الميكروميريتية لمسحوق الصمغ وتم تمييز معلق الميترونيدازول باستخدام معدل التدفق وعدد إعادة التشتت وحجم الترسيب واللزوجة ودرجة الحموضة. تم تحديد درجة التندف أيضًا. مسحوق BAM له خاصية تدفق جيدة مع الحد الأدنى من التورم. كان ترتيب معدل تدفق معلق الميترونيدازول BAM = TCG> GLT بينما كان ترتيب حجم الترسيب TCG> BAM> GLT. لم يكن هناك فرق كبير ($p> 0.05$) في عدد إعادة تشتت تركيبات BAM و TCG. كانت لزوجة التركيبات المحتوية على BAM و TCG بتركيزات 0.5٪ –1.0٪ وزن / حجم هي نفسها. تراوح الأس الهيدروجيني للمعلقات من 5 إلى 8. كانت درجة التلبد بالترتيب GLT> BAM> TCG. من النتائج التي توصلنا إليها، يمكن استخدام BAM كعامل تعليق بديل في صياغة التعليق.

الكلمات الدالة: *Basella alba*، معلق، عوامل تعليق، ميترونيدازول.

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