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Characterization, Formulation and Evaluation of the Suspending Properties of Achi (*Brachystegia eurycoma)* Seed Gum In Sulphamethoxazole Suspension

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ABSTRACT

The purpose of the study is to search for new, cheap, readily available and effective excipient for suspension which can effectively compete with standard suspending agents like *Acacia* and *Tragacanth* gums. The suspending properties of *Brachystegia eurycoma* (*Fabaceae*) gum were evaluated and compared with *Acacia* and *Tragacanth* at concentrations 0.2%, 0.5%, 1.0%, 2.0% and 3.0% in sulphamethoxazole suspension. Sulphamethoxazole was chosen as a model drug because it is an in-diffusible powder and require among other thing a suspending agent. Physicochemical and Organoleptic analyses were carried out on the pure gum extract. Sedimentation volume, re-dispersion, crystal growth and flow rate were employed as evaluation parameters of the suspension. The values obtained were used as basis for comparison. The gum extract was found to have better suspending properties at concentrations evaluated. The gum extract at high concentrations has a high viscosity and can be used as stabilizer where high viscosity is desired and can also serve as thickening agent. Therefore, the gum can be used as an alternative suspending agent to both *Acacia* and *Tragacanth* gums.

Keywords: *Brachystegia eurycoma*, Sulphamethoxazole, Suspending agent, *Acacia* and *Tragacanth*

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INTRODUCTION

A pharmaceutical suspension is a coarse dispersion in which the internal phase is dispersed uniformly throughout the suspending vehicle with the aid of a single or combination of suspending agents. The external phase (suspending medium) generally aqueous in some instance may be an organic or oily for non-oral [1].

Suspensions are classified based on the route of administration (oral suspension e.g. antacids, externally applied suspension e.g. lotions, parenteral suspension e.g. intramuscular contraceptives), proportion of solid particles (dilute suspension (2 to 10%w/v solid), concentrated suspension (50% w/v solid)), electro kinetic nature of sediments produced (flocculated suspension, deflocculated suspension), the size of solid particles (colloidal suspension (<1 micron), coarse suspension (>1 micron), nano suspension (10 nano grams)), dispersed phase suspensions (diffusible solids, in-diffusible solids, poorly wettable solids, precipitate forming liquids, products of chemical reactions) [1].

Suspending agents, thickeners or colloids are excipients which are adsorbed on insoluble particles, increase the strength of hydration layer formed around suspended particles through hydrogen bonding and molecular interaction. Suspending agents commonly used are cellulosics (examples are microcrystalline cellulose, starch, methylcellulose)’ clays (examples are bentonite, kaolin, silicondioxide), gums (examples are agar, algins, guar, pectin, acacia), polymers (examples are povidone, carbomers, polyvinyl alcohol), sugars (examples are dextrin, sucrose, maltitol). Others include aluminum monostearate, gelatin and emulsifying waxes [2].

Gums are considered to be pathological products formed following injury to the plant or owing to unfavorable conditions, such as drought, by a breakdown of cell walls (extra cellular formation; gummosis)[3]. Gums are widely employed in pharmacy as thickeners, suspending agent, emulsifying agent, binders and film formers [4] Gums obtained from *Ocimum gratissimum* [4], tamarid seed [5], *Ocimim basilicum* and tomato powder[6], *Grewia mollis*[7], *Sesamum indicum* [8], *Abelmoschus esculentus*[9] and *Khaya senegalensis* [10] have been evaluated as suspending agents and were found to be useful as suspending agents.

Brachystegia eurycoma Harms, family *Fabaceae*, is a woody plant mostly found in the forest zone. It is native to tropical Africa; it is indigenous to Nigeria and Cameroon [11.] The seed is called different names by natives; some of these names are *Achi* in *Igbo, Akalado or Eku in* Yoruba, *Akpakpa or Apaupann in Ijaw, Dewen in Bini, Okwen in Edo, Otigbo and Okung in Efik.* The result of chemical analysis of raw seed *Brachystegia* showed 12.2 - 23.2% crude protein, 4.9 – 12.0% fat [12.] Analysis on the floor of *Brachystegia* showed that it contains 10.25% of moisture, 12.77% of crude protein, 10.52% of crude fat, 1.48% of total ash, 2.2% crude fiber and 58.77% starch content, while the starch contains 0.61% protein, 0.25% fat, 0.69% crude fiber, 0.79% ash, 84.28% starch [13]. Its uses range from food to medicine. In West Africa, the edible seed is used in soup making as a thickener. The seed flour has good gelation properties and imparts a gummy texture when used in soup [14]. The seeds help in softening bulky stools and have been associated with the protection against colon and rectal cancer. Due to their absorption capacity, they are useful as functional agents in fabricated foods such as bakery products and meat formulations [12].

The aim of the study is to characterize, formulate and evaluate a new, effective and natural suspending agent from *Brachystegia eurycoma* seed that can be used as an effective alternative for the formulation of pharmaceutical suspension.

MATERIALS AND METHODS

Acacia gum(Poleron Equipment Ltd. Watford, England), *Tragacanth* gum (BDH Chemicals Poole England), Sulphamethoxazole powder (BDH Chemicals Poole England) All solvents used were of analytical grade. *Achi* gum powder extracted from *Brachystegia eurycoma* collected from a local market in Orlu; Imo state. It was identified by Prof. S. S. Sanusi a taxonomist in the Department of Biological Science of University of Maiduguri.

Extraction of the Seed Gum

The *Brachystegia* seeds were weighed, roasted and soaked in water. This was allowed to stand for 2 hrs and the outer cotyledons were washed with water and dried. The dried seeds were milled; this was done for size reduction. The powder materials obtained were collected and dispersed in water. The powder dispersed gave a good viscous mixture which hydrated properly within 24 hours. The mixture was stored for 24 hours to aid hydration. This was boiled for thirty (30) minutes and allowed to stand for one (1) hour. It was then passed through a multilayer muslin cloth. Acetone was added to the supernatant at a ratio of 1:1, this ensured a complete precipitation of the gum. The precipitated gum was then dried to flakes. The dry flakes were pulverized and size reduced. It was then stored in an air tight container. The percentage yield was obtained by dividing the weight of the milled seed and the weight of the gum extracted.

PHYSICOCHEMICAL PROPERTIES

The powdered gum was evaluated for the following properties;

Identification test

DETERMINATION OF DENSITY

Bulk density

This was done by weighing 30.0g of the powder, it was then put into a graduated cylinder and the volume noted. The bulk density was calculated by this formula;

$$\text{Bulk density} = (\text{weight of powderedgum})/\text{volume occupied by the gum}$$

Tapped density

A 30.0g weight of the powders (Achi, Acacia and Tragacanthgums) were put in a graduated cylinder and tapped from a height of 2 cm until the powder bed volume reached a constant value. The tapped density was then calculated in g/ml.

Compressibility Index (Carr's Index)

This was calculated from the result obtained from bulk and tapped densities and can be written as;

$$\text{Compressibility index} = (\text{Tapped density} - \text{bulk density})/(\text{Bulk density})$$

Hausner Ratio

This is the ratio of the tapped density to the bulk density and this was calculated using the formula

$$\text{Hausner's ratio} = (\text{tapped density})/(\text{bulk density})$$

Determination of hydration capacity

One gram (1.0g) of the sample was weighed and transferred into a dry test tube and this was re-weighed (W1). The powder was then dispersed in 50ml of distilled water. The slurry was centrifuged at 2200 rpm for 15 min to separate the gel and supernatant. The sediment was separated and the weight of the swollen sediment determined (W2);

$$\text{Hydration capacity} = (W2 - W1)/(\text{weight of dry sample}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Determination of swelling power

The tapped volume occupied by 5.0g of the starches each was noted. The starches were then dispersed in 85ml of distilled water and the volume made up to 100ml with more distilled water. It was allowed to stand for 24hrs. The volume of the sediment was then determined and the swelling capacity calculated from the difference in volumes.

Determination of moisture sorption

Two grams (2.0g) of powders of Achi, Acacia and Tragacanth were measured and evenly distributed on tarred Petri dishes. The sample was placed in a desiccators containing distilled water in a reservoir at room temperature. The samples were periodically weighed until a constant weight was attained

DETERMINATION OF FLOW PROPERTY

Angle of repose

This is used to predict the flow characteristic of the powder. A funnel was attached, 5cm from the base of the retort stand, with the tip closed. 10.0g of the powder measured and poured into the funnel. The orifice was opened and powder flowed freely. The angle of repose (θ) was determined with this formula;

$$\theta = \tan^{-1}\left(\frac{h}{r}\right)$$

Where; h is the height of the heap

r is the radius of the heap

Determination of moisture content

A 3.0g sample of gum was weighed into the pan of a moisture analyzer (Sartorius, Germany), set at 130ºC for 5 minutes. The test was repeated twice and the mean of the three recorded

Determination of ash value

A 2.0 g weight of the powder was placed in a Porcelain crucible and this was placed in a blast furnace (Lento furnace, England) set at 650^{o}C for 10 hours until the residue free from carbon (grayish white) was obtained. The sample was removed from the furnace and cooled in desiccators. The weight of the crucible and residue was noted. The percentage ash was calculated by;

$$\text{Ash value} = (ash\ weight)/original\ sample\ weight \times 100$$

Microbial load

A 1.0g weight of the powdered gum was dissolved in 10ml of sterile distilled water and 1 ml of the dissolved extract was put into a universal bottle containing 9ml of sterile distilled water to make a 1:10 dilution. This was repeated for 1:100 and 1:1000 dilutions. The diluted solutions were incubated at 37^{0} for 18 hours prepared plates and the numbers of colonies formed were counted.

Preparation of Sulphamethoxazole Suspension

Suspensions of 0.2, 0.5,1, 2, and 3%w/v Sulphamethoxazole in water using *Brachystegia eurycoma* seed gum, *Acacia* gum, and *Tragacanth* gum powder as suspending agents were formulated. The suspensions were evaluated using the following:

Sedimentation volume

The sedimentation volume of the suspension was evaluated using graduated cylinder. The suspension formulations (50ml) were poured separately into 100ml calibrated measuring cylinder and the volume of the sediment was measured after 1, 2, 3 and 7 hours then every 24 hours for 7 days. It was computed by the ratio of the equilibrium volume of sediment, Vu, to the total volume of suspension, Vo, the sedimentation volume F, was then calculated

$$F = Vu/Vo$$

Re - dispersion

Fixed volume of 30ml of each suspension was kept in calibrated tubes which were stored at room temperature for 7 days. Each tube was removed and shaken to redistribute the sediment and the presence of deposit if any was recorded. Re-dispersion was recorded as the number of inversions required for re-suspension to occur.

Rheology

The time required for each suspension sample to flow through a 2 ml pipette was determined and the flow rate ($\eta\alpha$ in mls^{-1}) was calculated using the equation:

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

Statistical analysis

Analysis was done in triplicate. Statistical analysis was carried out using a statistical software SPSS version 16 and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

The percentage yield of *Brachystegia eurycoma* gum extraction was found to be 52.6%. The seed gum obtained was subjected to physicochemical analysis and the results so obtained are summarized in Table 1.

Table 1: Results of physicochemical analysis of mucilage

Parameters	Tragacanth	Acacia	Achi
Angle of Repose(^{0})	40.86 ± 1.69	20.73 ± 3.5	26.73 ± 0.23
Bulk Density (g/ml)	0.56 ± 0.02	0.77 ± 0.02	0.64 ± 0.06
Tapped Density (g/ml)	0.73 ± 0.02	0.90 ± 0.00	0.74 ± 0.01
Hydration Capacity	1.17 ± 0.24	0.70 ± 0.54	5.28 ± 2.00
Hausner's Ratio	1.28 ± 0.04	1.17 ± 0.00	1.16 ± 0.02
Carr's Index (%)	23.25 ± 2.10	14.44 ± 0.00	13.89 ± 1.35
Moisture Sorption	0.06 ± 0.01	0.36 ± 0.01	0.10 ± 0.23
Moisture Content (%)	5.87 ± 0.33	7.48 ± 0.26	7.86 ± 0.56
Ash Value (%)	2.70 ± 0.10	1.23 ± 0.06	4.60 ± 0.58
Swelling Power (%)	15.69 ± 3.40	36.02 ± 9.09	26.39 ± 18.58
Microbial Load (CFU/g)	312	61	18

Angle of repose indicates the measure of the flow properties of powders (i.e. the ease with which powders are able to flow over each other. Powders with angles of repose greater than 50° have unsatisfactory flow properties, whereas angles of repose close to 25° correspond to good flow properties [15]. From Table 1, it can be seen that *Tragacanth* has the poorest flow while *Acacia* has the best flow (with angle of repose of 20.73^0). *Achi* also has a good flow property with angle of repose of 26.73^0. The flow ability can be arranged in an ascending order as *Acacia>Achi>Tragacanth,* this might be as a result of individual particle sizes of the gum.

The swelling and hydration capacity of the gums (Table 1), *Achi* has the highest swelling power and hydration capacity while *Tragacanth* had the lowest. The most effective disintergrants act via swelling mechanism which acts by swelling after sorption of water leading to the rupture of tablets [16.] Statistically, there was no significant difference between *Acacia* and *Achi* gums while *Acacia* and *Achi* gums were significantly higher than *Tragacanth* gum ($p<0.05$).

The Hausner's Ratio and Carr's Index are both measures of the flow properties of powders. A Hausner's Ratio of < 1.25 indicates a powder that is free flowing whereas >1.25 indicates poor flow ability [15]. *Achi* has Hausner's Ratio of 1.16 which is less than 1.25 and indicates a good flow property. *Acacia* with Hausner's Ratio of 1.17 also indicated good flow property while *Tragacanth* with Hausner's Ratio of 1.28 indicating poor flow.

The smaller the Carr's Index the better the flow properties of the powder[17]. *Achi* has the lowest of 13.89% and *Acacia* a density of 14.44% while *Tragacanth* had the highest (with density of 23.35%).

Therefore, when the flow properties are combined together, *Achi* and *Acacia* have excellent flow rate while Tragacanth has a poor flow. The flowability of the three gums can be ranked as *Tragacanth* < *Acacia* < *Achi* gums.

The moisture content of *Acacia*, *Tragacanth* and *Achi* gums are shown in Table 1, *Tragacanth* has the lowest moisture content, therefore is the least liable to microbial contamination. The ranking was *Tragacanth* < *Acacia* < *Achi* gums.

The result of microbial load (table 1) indicated by their microbial load shows that *Tragacanth* supported the growth of microbes more than *Acacia* and *Achi* gums but fell within the standard of less than 1000 CFU/g [18]

The greater the sedimentation volume of suspensions, the more stable they are. Figure 1, 2 and 3 show the sedimentation volumes of *Achi*, *Tragacanth* and *Acacia* gums. It can be seen that the sedimentation volume increases as the concentration of the suspending agent increases. Figure 4 shows the sedimentation volume of the three gums at 1.0%. It can be deduced that achi had the highest sedimentation volume (stability) indicating that at lower concentrations, *Achi* gum is a better suspending agent than *Tragacanth* and *Acacia* gums.

The number of inversions required to re-disperse the suspension completely as presented in Table 2 indicates that at 0.5% and 1.0%, showed the best suspending agent was *Acacia* gum, at 0.2% *Achi* gumshowed good suspending property while at 2% and 3%, *Tragacanth* gum exhibited the best suspending property. The poor re-dispersibility of achi gum can be attributed to the viscosity of the gum while high re-dispersibility may be due to its low viscosity.

Table 2: Re-dispersion time of the suspension formulated with the various gums

Concentration (%)	Tragacanth	Acacia	Achi
0.2	4.40 ± 1.30	3.60 ± 1.68	5.07 ± 4.65
0.5	5.67 ± 1.15	2.33 ± 0.58	9.67 ± 0.58
1	3.67 ± 0.58	2.33 ± 0.58	10.33 ± 0.58
2	3.33 ± 0.58	5.33 ± 0.58	0.00 ± 0.00
3	3.67 ± 0.58	5.67 ± 0.58	0.00 ± 0.00

Table 3: Flow rate of the suspensions at varying concentration

	Flow rate (ml/sec)		
Gums Concentration (%)	Tragacanth	Acacia	Achi
0.2	0.20 ± 0.00	0.20 ± 0.00	0.17 ± 0.00
0.5	0.19 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
1	0.18 ± 0.12	0.10 ± 0.02	0.06 ± 0.01
2	0.17 ± 0.02	0.07 ± 0.02	too viscous
3	0.17 ± 0.00	0.03 ± 0.01	too viscous

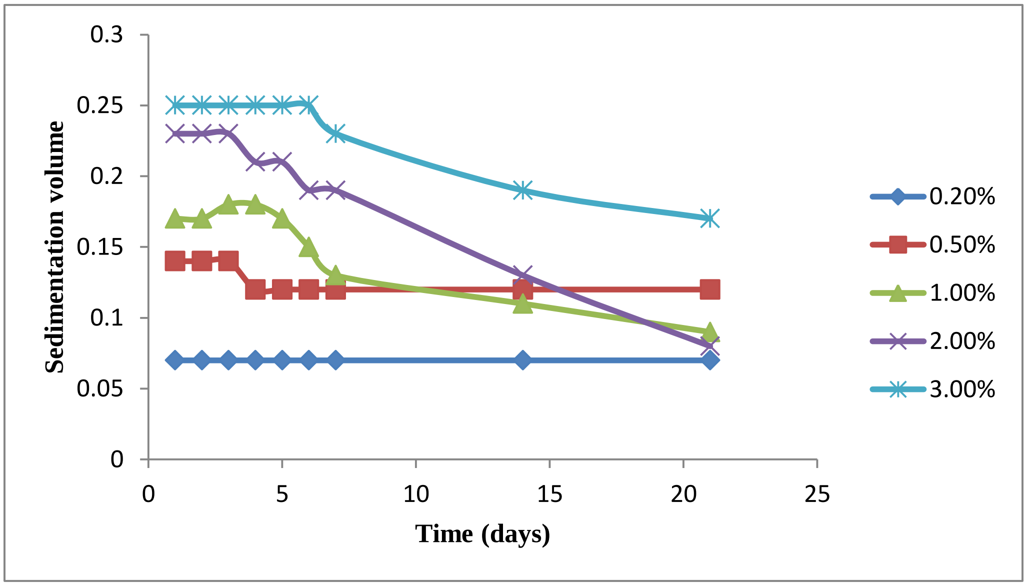

Figure 1: Graph of sedimentation volume versus time of sulphamethoxazole suspensions using different concentrations of *Achi* gum

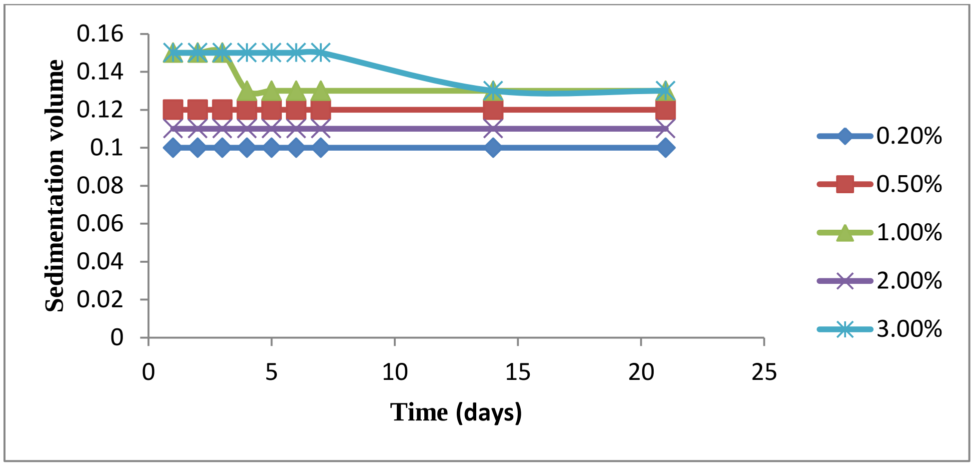

Figure 2: graph of sedimentation volume versus time of sulphamethoxazole suspensions using different concentration of *Tragacanth* gum

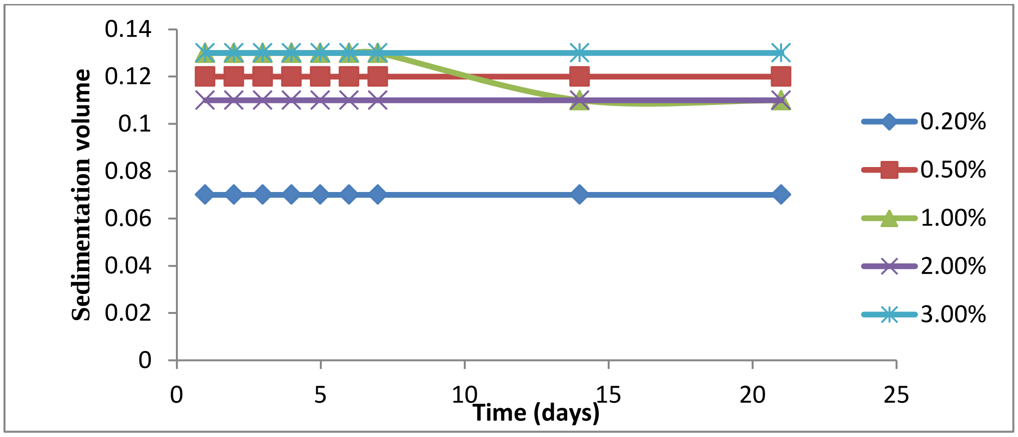

Figure 3; graph of sedimentation volume versus time of sulphamethoxazole suspensions using different concentrations of *Acacia* gum

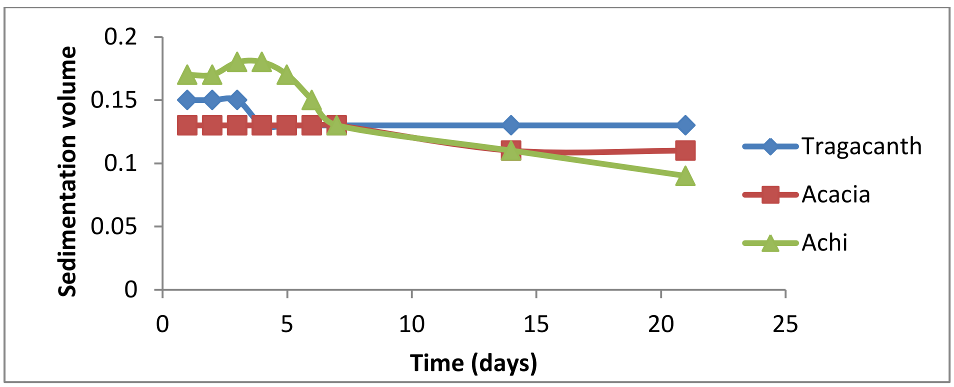

Figure 4: graph of sedimentation volume versus time of sulphamethoxazole suspensions at 1.0% concentration of the gums.

The flow rate(Table 3) indicates that the flow rate of the suspensions increases with increase in concentration of the suspending agent, and this may be due to the increase in viscosity as concentration increases.The flow rate of suspensions formulated with *Tragacanth* gum was significantly higher than *Achi* gum at all concentrations and also *Acacia* gum except at concentration 0.2% where there was no significant difference ($p > 0.05$).

CONCLUSION

In conclusion, the research shows that *Achi* gum has good suspending qualities at low concentration which is better than the standards used. It can be used for those formulations where very high viscosity is required as stabilizer or thickening agent. Since it is readily available locally and has a high yield it will reduce the cost of importation of other gums thereby enhancing the economy of the country.

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