Isolation, Characterization and Formulation Properties of a New Plant Gum Obtained from *Sida acuta*

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**ABSTRACT**

This study was carried out to determine the usefulness of *Sida acuta* gum (SAG) in tablet drug formulation and delivery. Physicochemical characterization of the gum was done by carrying out solubility tests, loss on drying, total ash/acid insoluble ash, pH and micromeritic properties. The moisture content of *Sida acuta* gum (SAG) was low, suggesting its suitability in formulations containing moisture sensitive drug tablets. The total ash and acid insoluble values of SAG were 2.0 and 1.0% w/w respectively. The swelling was highest in water, followed by phosphate buffer and least in 0.1N HCl, pH (6.0, 4.0 and 3.0 respectively). Study tablets prepared by incorporating an antiprotozoal drug, metronidazole, exhibited good mechanical strength and acceptable friability values. *In vitro* drug release studies carried out in simulated gastric and intestinal conditions revealed that SAG-formulated metronidazole release kinetics in simulated biological fluids varies between 2 to 8 hours, depending on the concentration of SAG used in the formulation. All the formulations used in this study, followed the zero-order kinetics with highest linearity (r² = 0.9709, 0.9743, 0.9716, 0.9727), via non-fickian (anomalous) 0.45<n>0.89 (n = 0.6, 0.7, 0.6, 0.6). All the formulations released the drug by diffusion in the hydrated matrix and polymer relaxation. There was no significant difference in drug release among the formulations, SAG 10%, SAG 20%, SAG 30%, and NACMC 30%, (P > 0.05). Release profile of SAG 30% and the reference gum, NACMC 30% were very similar, (P > 0.01). The findings of this study established the fundamental characteristics of *Sida acuta* gum. The matrices responded to changes in pH along the (gastrointestinal tract) GIT, implying that the gum’s potential is beneficial in tablet drug formulation and intestinal drug delivery.

**KEYWORDS:** Isolation, *Sida acuta*, gum, Metronidazole, Sustained drug release, Drug formulation.
INTRODUCTION

Excipients from natural products are of particular interest to the Formulation Scientists due to their minimal toxicity profile, cost effectiveness, and availability. In this respect, they serve as alternative to synthetic products. Natural gums from plants have diverse applications in drug delivery, emulsifying, suspending agents, and binders. They are also utilized in formulating immediate and sustained release preparations. Sida acuta Burm.f. (family MALVACEAE), is a shrub indigenous to pantropical areas, widely distributed in these regions and widely used in traditional medicine. The aerial part of the plant is the most frequently used part. In the United States, the plant is used to treat asthma, renal inflammation, colds, fever, headache, ulcers and worms. The plant is traditionally used in the treatment of malaria, diarrhea and many other diseases. Several phytochemical screenings resulted in the isolation of various compounds from the plant, including alkaloids and steroidal compounds. The alkaloids occurring in the plant belong to the indoloquinolines family. The main alkaloids are cryptolepine and its derivatives such as quindoline, quindolinone, cryptolepinone and 11-methoxy-quindoline, have been reported. The major steroids of the plant are ecdysterone. Beta-sistosterol, stigmasterol, ampesterol phenolic compounds such as evofolin –A- and B, scopoletin vomifoliol, loliolid and 4-ketopinoresinal have also been isolated.

The objective of this study was to isolate S. acuta gum, determine its physicochemical characteristics, and use its formulation in metronidazole to determine its benefits in tablet drug formulation and delivery.

MATERIALS

Metronidazole powder, lactose and maize starch were all purchased from BDH, England, UK. Magnesium stearate was purchased from Sigma Aldrich, USA. All other chemicals and reagents used were of analytical grade, obtained from our laboratory.

METHODS

ISOLATION OF GUM

Sida acuta gum (SAG) was extracted and purified as previously described by Wang et al., 2006

PHYSICOCHEMICAL CHARACTERIZATION OF THE GUM SOLUBILITY TEST

The separated gum was evaluated for solubility in water, acetone, chloroform and ethanol in accordance with the British Pharmacopoeia specification.

SWELLING INDEX

The method of Ohwoavworhua and Adekalum 2005 was used; 1.0g each of the sample was placed in each of 15ml plastic centrifuge tubes and the volume occupied was noted, with 10 ml of distilled water added from a 10 ml measuring cylinder and stopped. The contents were mixed on a vortex mixer (Vortex Gennie Scientific, USA) for 2 min. The mixture was allowed to stand for 10 min and immediately centrifuged at 1000 rpm for 10min on a bench centrifuge (GallenKamp, England, UK). The supernatant was carefully decanted and the volume of sediment measured. The swelling index
was computed using the equation (after triplicate determinations).
\[ S = \frac{V_2}{V_1} \]
Where: \( S \) = Swelling index
\( V_1 \) = Volume occupied by the gum prior to hydration
\( V_2 \) = Volume occupied by the gum after to hydration

**LOSS ON DRYING**

The method adopted as specified in the British Pharmacopoeia 2004 for acacia (13). A 1.0 g sample was transferred into each of several petri dishes and then dried in an oven at 105°C until a constant weight was obtained. The moisture content was then determined as the ratio of weight of moisture loss to weight of sample expressed as a percentage. The data presented here is for triplicate determinations.

**TOTAL ASH ACID INSOLUBLE ASH DETERMINATION**

Ash content was estimated by the measurement of the residual left after combustion in a furnace at 450°C (13). The ash obtained from the determination of the ash was boiled with 25ml of 2M hydrochloric acid solution (HCl) for 5min and insoluble matter was filtered and washed with hot water and ignited and the subsequent weight was determined. The percent acid insoluble ash was calculated (13). The data presented here is from triplicate determinations.

**pH DETERMINATION**

This was done by shaking a 1% w/v dispersion of the sample in water for 5min and the pH determined using a pH meter (Corning, model 10 England, UK), (14). The data presented here is from triplicate determinations.

**ANGLE REPOSE**

The static angle of repose, \( a \), was measured according to the fixed funnel and free standing cone method (13). A funnel was clamped with its tip 2cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

\[ \tan a = \frac{2h}{D} \]

The data presented here is from triplicate determinations.

**BULK AND TAP DENSITIES**

A 2.0g quantity each of the powder sample was placed in a 10ml measuring cylinder and the volume, \( V \) occupied by each of the samples without tapping was noted. After 100 taps of the table, the occupied volume \( V_{100} \) was read. After triplicate determinations, the bulk and tap densities were calculated as the ratio of weight to volume (\( V_0 \) and \( V_{100} \) respectively).

**HAUSNER’S INDEX**

This was calculated as the ratio of tapped density to bulk density of the samples.

**COMPRESSIBILITY INDEX (C%)**

This was calculated using the equation:

\[ \text{Compressibility} = \frac{(\text{Tapped density-bulk density})}{\text{Tapped density}} \times 100 \]
PREPARATION OF METRONIDAZOLE MATRIX TABLETS

Matrix tablets of metronidazole were prepared by a wet granulation method. Lactose was used as diluent and Magnesium stearate was used as lubricant. SAG was incorporated in the formulations in various proportions. The composition of different formulations used in the study containing 200mg of metronidazole in each case (Table 1). In all the formulations, SAG was sieved (<500µm) separately before use and mixed with metronidazole (<150µm) and lactose (<150µm) in a blender (Braun, Germany). The powder mixtures were mixed for 10 min using a tumbler mixer (Karl Kolb, D6072 Dreieich, Germany) and granulated with water for 5 min, using a granulator (Erweka, GmbH, Germany) fitted with 1.6 mm mesh screen. After passage through the screen, granules were dried at 50°C for 2h in a hot air oven (Salvis, Switzerland). The dried granules were rescreened through a 1.7 mm sieve and lubricated with 1.0% magnesium stearate for 5 min using the tumbler mixer. The final blend was compressed using a single station tablet press (THP Shanghai, Tianxiang and Chentai pharmaceutical Machinery Co. Ltd, China) equipped with 10.5 mm punch and die set. The tablet weight was adjusted to contain 200mg of the metronidazole per matrix tablet. Tablets weighing 300 mg each and containing 200 mg SAG formulated metronidazole were compressed at 23.75KN. They were tested for their hardness (n=5), drug content (n=10) and drug release (n=4) characteristics.

MICROMERITIC PROPERTIES OF GRANULES

The bulk and tapped densities for granulated powders was determined using standard methods as reported by Emeje et al (15) Compressibility and Hausners’ indices were calculated using the method of Carr 1965.

TABLET THICKNESS AND TENSILE STRENGTH

Tablet thickness and crushing strength were determined using the apparatus Pharmatest model PTB-311, Germany. Crushing strength was examined by placing a tablet between a stationary and moving spindle. Force was applied by turning the moving spindle until the tablet cracked diametrically. Tablet tensile strength was calculated according to the method reported by Emeje et al (15). Friability of the compacts was evaluated from the loss of 10 tablets tumbled for 100 revolutions (25 rpm for 4 minutes), using a friabilator (Erweka, Germany).

DETERMINATION OF DRUG CONTENT

Metronidazole matrix tablets were tested for their drug content. Ten tablets were finely powdered; 300mg of the powder was accurately weighed and transferred into a 100 ml volumetric flask. Distilled water, 50ml water was added and shaken on a vortex mixer for 10 minutes. The content of the flask was made up to 100 ml mark with more distilled water and allowed to stand for 2 hrs, with intermittent sonication to ensure complete solubility of the drug. The mixture was centrifuged at 1000 rpm for 10 min and the content of metronidazole in the supernatant liquid was analyzed spectrophotometrically at 270nm.

IN VITRO DRUG RELEASE STUDIES

The ability of SAG matrix tablets of metronidazole to remain intact in the physiological environment of the stomach and
small intestine was assessed by conducting drug release studies under conditions mimicking mouth to intestinal transit. Drug release studies were carried out using USP dissolution rate apparatus (Apparatus 1, 100 rpm, 37°C) for the first 2 h in pH 1.2 simulated gastric fluids (SGF) without enzymes (500 ml). Subsequently, the dissolution medium was changed to pH 7.4 simulated intestinal fluid (SIF) without enzymes (500ml) and tested for drug release for the remaining 6 h. Aliquots (5ml) of the dissolution medium were withdrawn at hourly intervals up to at least 8 h. The withdrawn amount was replaced with an equal volume of fresh dissolution medium kept at 37°C. The withdrawn samples were analyzed at 270 nm for metronidazole content using a Shimadzu UV Spectrophotometer (Shimadzu, Japan). The release data was analyzed for each dissolution profile after three repeats.

DRUG RELEASE KINETICS AND MECHANISM OF RELEASE

To investigate the drug release kinetics, data obtained from in vitro dissolution studies were plotted into various kinetic models (20 – 22). The mechanism of drug release was determined by plotting the data for the first 60% drug release in Korsemeyer et al equation (23).

RESULTS AND DISCUSSION

PHYSICOCHEMICAL PROPERTIES

Table 1 shows the physicochemical parameters of both the test and reference gums. The gum extracted from the stem of *Sida acuta* is slightly soluble in water and a dispersion of it yielded a brown, slimy solution. The gum was practically insoluble in ethanol, aceton and chloroform. Tragacanth which was used as a reference sample gave a similar solubility profile.

The swelling characteristic of SAG was studied in different media; 0.1N HCl, phosphate buffered saline (PBS, pH 7.4) and water. The swelling was highest in water followed by PBS and least in 0.1N HCl pH (1.2). Generally, the results show the SAG has high swelling index suggesting that the gum may perform well as binder, disintegrant, and matrixing agent. The gum is a pH responsive polymer. Hence a “smart polymer” that may find application in controlled release dosage formulations (19). The relatively higher swelling index obtained for SAG tablet formulations at pH 7.4, implies that unlike tragacanth, the gum may be useful as a matrix former in controlled drug release, since swelling is a primary mechanism in diffusion controlled release dosage formulations (16).

The moisture content of SAG was low, suggesting its suitability in formulations containing moisture sensitive drugs. Given suitable temperature, moisture will lead to the activation of enzymes and the proliferation of micro organisms thereby affecting the shelf life of most routine formulations. It is important to investigate the moisture content of a material because the economic importance of an excipient for industrial application lies not only on the cheap and ready availability of the biomaterial but the optimization of production processes such as drying, packaging and storage (17).

The total ash and acid insoluble value of SAG was found to be 2.0 and 1.0% w/w respectively. Ash values reflect the level of adulteration or handling of the drug. Adulteration by sand or earth is immediately detected as the total ash is normally composed of inorganic mixtures of carbonates, phosphate, silicates and silica. Therefore, the low values of ash and acid insoluble ash obtained in this study indicates low
levels of contamination during gathering and handling of crude drug (13).

The bulk and tapped densities give an insight on the packing and arrangement of the particles and the compaction profile of a material (18). The compressibility index and angle of repose of SAG was 16.67% and 23.50° respectively, implying that the SAG has a good flow with moderate compressibility unlike tragacanth with a poor compressibility index of 6.25% and angle of repose of 20.40°. It therefore implies that formulations containing this gum (SAG) will require minimal amount of glidants to improve flow properties than formulations containing the reference gum (tragacanth).

A 1% solution w/v solution of SAG in water gave a pH of 7.5 while that of tragacanth gave 5.2. The close to neutral pH of the former implies that formulations of uncoated tablets containing SAG will be less irritating to the gastrointestinal tract (GIT). Knowledge of the pH of an excipient is an essential parameter in determining its suitability in formulations since the stability and physiological activity of most preparations are pH dependent (19).

**TABLE PROPERTIES OF THE MATRICES**

*Table 2* shows some of the properties of Metronidazole matrix tablets. All of them were of good mechanical strength and acceptable friability values. Absolute drug content was within 97% and 99%. All the parameters investigated were comparable to the reference gum, (30% NaCMC). These values are within the USP specification for tablets (24). Content uniformity for all the formulations was found within acceptable limits (95-105%). Friability of less than 1% is usually acceptable. The low friability values indicate that the tablets can withstand the stress associated with transportation and dispensing processes.

**DISOLUTION PROFILES OF TABLETS**

All formulations were observed for physical integrity at different time intervals. All the formulations swelled and the outer layer of most of the tablets appeared to be hydrated after being placed in the dissolution medium, with progressive increase in the size of these hydrated matrices, there was also gel formation followed by gradual loss of integrity over a period, resulting from hydrodynamic stress induced by the dissolution apparatus. The quick hydration and subsequent gel formation is a foremost and important property of an excipient intended for use in sustained released formulations (26).

The time taken for 50% and 70% of the drug to be released (T50% and T70%) respectively and the maximum cumulative amount of drug release (Cmax) were used to characterize the release profiles of the matrix tablets (Tables 3). All the batches were able to retard the release of the drug beyond 6 hours. Formulation containing 10 % SAG had the lowest T50 of 4.6h while that containing 30% SAG had the highest T50 of 6.8h. The formulation containing 30% NaCMC released 50 % of the drug in 7.0 h, implying that 30% SAG is similar in retarding as NaCMC. There was no significant difference (P> 0.05) in release profile among the formulations (P> 0.05).

**DRUG RELEASE KINETICS AND MECHANISM OF RELEASE**

To investigate the drug release kinetics, data obtained from *in vitro* dissolution studies were plotted into various kinetic models (20 – 22). Ideally, a sustained release tablet should release the required quantity of a drug with predetermined kinetics in order to maintain an effective drug plasma concentration (25). To achieve this, the
tablet should be formulated so that it releases the drug in a predetermined and reproducible manner. (Table 4 shows the release Kinetics.)

Based on the results from Table 4, all the formulations follow zero order kinetics with highest linearity (\( r^2 = 0.9709, 9743, 9716, 9727 \)) via non-fickian (anomalous) 0.45<n>0.89 (23) (\( n = 0.6, 0.7, 0.6, 0.6 \)). The zero order rate describes a system where rate of drug release is independent of concentration. All the formulations released the drug by diffusion in the hydrated matrix and polymer relaxation. There was no significant difference in drug release among the formulations (\( P>0.05 \)).

### Table 1: Some physicochemical properties of *Sida acuta* gum and tragacanth powder

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sida acuta</em> Solubility</td>
<td>slightly soluble in water, insoluble in ethanol, chloroform and acetone</td>
</tr>
<tr>
<td><strong>Swelling ratio in:</strong></td>
<td></td>
</tr>
<tr>
<td>0.1N HCL</td>
<td>3.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>4.0</td>
</tr>
<tr>
<td>Buffer pH 7.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
<tr>
<td><strong>Loss on drying</strong></td>
<td>0.60%</td>
</tr>
<tr>
<td><strong>Total ash</strong></td>
<td>2.0%</td>
</tr>
<tr>
<td><strong>Acid insoluble ash</strong></td>
<td>1.0%</td>
</tr>
<tr>
<td>bulk density (g/cc)</td>
<td>0.65</td>
</tr>
<tr>
<td>Tapped density (g/cc)</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Compressibility index</strong></td>
<td>16.67%</td>
</tr>
<tr>
<td><strong>Hausner’s quotient</strong></td>
<td>1.20</td>
</tr>
<tr>
<td><strong>Angle of repose</strong></td>
<td>23.50</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>5.2</td>
</tr>
</tbody>
</table>

**Table 2:** Physical properties of SAG-formulated metronidazole tablets

<table>
<thead>
<tr>
<th>Batch</th>
<th>Weight (mg)</th>
<th>Thickness (mm)</th>
<th>Hardness (Kgf)</th>
<th>Friability (%)</th>
<th>Drug content (%)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAG10%</td>
<td>299±4.71</td>
<td>2.92±0.04</td>
<td>4.7±0.54</td>
<td>0.93±0.23</td>
<td>98.80±0.77</td>
<td>8.65±0.01</td>
</tr>
<tr>
<td>SAG20%</td>
<td>300.8±4.47</td>
<td>2.89±0.03</td>
<td>5.05±0.44</td>
<td>0.40±0.006</td>
<td>97.35±0.47</td>
<td>8.66±0.02</td>
</tr>
<tr>
<td>SAG30%</td>
<td>297.1±5.76</td>
<td>2.93±0.04</td>
<td>5.3±0.48</td>
<td>0.40±0.007</td>
<td>100.00±0.85</td>
<td>8.68±0.01</td>
</tr>
<tr>
<td>NACMC30%</td>
<td>297±6.25</td>
<td>2.92±0.07</td>
<td>5.5±0.41</td>
<td>0.40±0.006</td>
<td>99±0.46</td>
<td>8.69±0.02</td>
</tr>
</tbody>
</table>
**Table 3:** Maximum cumulative release of SAG-formulated metronidazole tablets

<table>
<thead>
<tr>
<th>Batch</th>
<th>$T_{50}$ (hrs)</th>
<th>$T_{70}$ (hrs)</th>
<th>$C_{\text{max}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAG10%</td>
<td>4.6</td>
<td>6.6</td>
<td>90.00</td>
</tr>
<tr>
<td>SAG20%</td>
<td>5.0</td>
<td>6.9</td>
<td>87.00</td>
</tr>
<tr>
<td>SAG30%</td>
<td>6.8</td>
<td>-</td>
<td>65.00</td>
</tr>
<tr>
<td>NACMC 30%</td>
<td>7.0</td>
<td>7.5</td>
<td>95.00</td>
</tr>
</tbody>
</table>

**Table 4:** Kinetics and mechanism of release for SAG formulated metronidazole matrix tablets

<table>
<thead>
<tr>
<th>formulation,</th>
<th>Zero – order</th>
<th>First – order</th>
<th>Higuchi</th>
<th>Korsmeyer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAG 10%</td>
<td>0.9709</td>
<td>0.8205</td>
<td>0.9194</td>
<td>0.9476</td>
</tr>
<tr>
<td>SAG 20%</td>
<td>0.9743</td>
<td>0.8352</td>
<td>0.9233</td>
<td>0.9549</td>
</tr>
<tr>
<td>SAG 30%</td>
<td>0.9716</td>
<td>0.9154</td>
<td>0.9529</td>
<td>0.9818</td>
</tr>
<tr>
<td>NACMC 30%</td>
<td>0.9727</td>
<td>0.9295</td>
<td>0.9576</td>
<td>0.9831</td>
</tr>
</tbody>
</table>

**Figure 1:** Release profile of SAG formulation of metronidazole

**CONCLUSION**

The results of this study established the fundamental characteristics of gum obtained from the stem of *Sida acuta*. The gum is pH sensitive, performs as a smart polymer, and may find its usefulness in intestinal drug delivery.

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