Pulp of *Picralima nitida* for Hypoglycemic Indication: Micromeritic and Stability Assessment

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ABSTRACT

The powder properties and the stability of granulated pulp of *Picralima nitida* with hypoglycemic activity were investigated. Pulp, a fleshy mesophyll material embedding the seed of *Picralima nitida* was processed into granules. The phytochemical, powder/particles and hypoglycemic properties were evaluated. The pulp contains glycosidic saponins; plant secondary metabolites responsible for hypoglycemic activity. The favorable micromeritic properties of the granulated pulp enable the pulp granules to be formulated into capsule dosage forms. The dosage form reduced postprandial blood sugar by 35.1% which is statistically significant at 1% α -level compared to the baseline value and the controls. The dosage form exhibit an appreciable stability with a shelf-life of about 68 weeks, suggesting its probable use as a standardized herbal dosage form.

Key words: *Picralima nitida*, pulp, powder properties, hypoglycemic activity, stability studies.

INTRODUCTION

Oral hypoglycemic drugs like the sulphonylureas, biguanides and α-glucosidase inhibitors have, for a long time, been available for treatment of patients with diabetes mellitus. However, herbal medicines are receiving increasing attention for the treatment of the disease [1, 2]. The herbal drugs often used are believed to have fewer side effects compared to synthetic drugs [3]. In many low resource countries with high incidence of the disease, adequate treatment is limited because of non-affordability of the required orthodox drugs. This necessitates the development of alternative strategies for management of NIDDM.

The effectiveness of extracts from *Picralima nitida*, in the ethnomedicals of diabetes mellitus [4-5], and other diseases [6-10] have been authenticated. Recent reports have shown that blood glucose reducing activity of the plant, also resides in the pulp [3, 11].

The pulp consists of complex combination of carbohydrates and has inherent binding ability. In the dried form, it can be reduced to a free-flowing, high density powder. From a pharmaceutical viewpoint, the powdered pulp can play multiple roles: providing sources of active principle(s), forming hydrogels with binding potentials and acting as bulking agents in solid dosage forms. In the present era when the standardization of herbal medicines and their conversion into preparations that simulate the conventional dosage forms are being advocated, a plant extract that manifest these multiple potentials would be valuable.

In the present study, therefore, the physicochemical properties of the dried powdered pulp of *P. nitida* have been examined, with an objective of converting it into granules or capsules for oral administration. It is expected that its hypoglycemic activity would be retained in the formulated forms and its stability improved.

MATERIALS AND METHODS

Processing of pulp

The ripe pod was harvested between the months of February and March. The pod was washed with clean water, dried with clean disposable paper tissue. It was then dissected longitudinally to expose the inner parts. The seeds were separated from the fleshy mesoderm. The mesoderm was scrapped off with a clean stainless spoon, and passed through a fine mesh of muslin cloth to get rid of the exudates. The resulting pulp was spread on a clean stainless steel tray and dried in an oven with forced air circulation at 40°C. It was later pulverized in a porcelain mortar. The resulting powder was screened to obtain a fraction with particle size of not more than 0.25 mm, which was then stored in an airtight glass container.

Precipitation of glycosidal saponins

Five hundred grams of the pulp were defatted by maceration in n-hexane in a ratio of 1:3 and dried. About 200 g of the defatted powder was macerated in methanol, filtered through Watman filter paper No 1 and concentrated to a resinous extract. The extract was stored in an amber coloured bottle and preserved in a refrigerator. Thereafter, the saponin glycoside was precipitated using a modified method of Schopke [12].

The defatted pulp powder was exhaustively extracted by maceration in 80% methanol in a ratio of 1:3. The hydromethanolic extracts were concentrated to one-fifth of their original volume through heating in a water bath at 40°C for five hours, resulting in aqueous portions. The aqueous portion volume was doubled with distilled water and extracted with n-buthanol in a ratio of 1:1. The n-buthanol fraction was thereafter concentrated to free flowing slurry in a water bath at 40°C. The slurry was gradually introduced into acetone in a ratio of 1:2 to obtain the precipitate of saponin glycosides. The precipitates were filtered off, dried and stored in Amber-coloured glass container in a refrigerator, at 2-4°C.

Preparation of granules and pulp capsules

The moistened pulp powder exhibited an inherent binding ability, and was wet granulated directly without any additional aid. The granulations were manually filled into capsule shells number 00 to a net mass of 400 mg on the average. Weight variations fell within ± 0.02 %.

Conditioning of rats

Normoglycemic Wister rats weighing between 200 and 250 grams were used for the study. The rats were housed in cages and allowed unlimited access to growers mash and water for two weeks. They were then removed from the feedlots and allowed to starve for two hours prior to drug administration.
Phytochemical analysis
The pulp was analyzed phytochemically using standard methods \cite{13} for the presence of secondary plant metabolites.

Acidity measurement
A 1 \% w/v dispersion of the pulp was made in de-ionized water. The mixture was allowed to hydrate for twelve hours. Triplicate pH values of the viscous dispersions were taken with a Labtech pH meter at a regulated temperature of 27 °C.

Moisture sorption capacity of pulp powder
A 2 g quantity of the dried pulp was spread on a glass plate of known weight. The glass plate was placed in a large desiccator containing distilled water in its reservoir at room temperature (27 °C). This arrangement was reproduced in triplicate. At daily intervals for a period of five days, the weight gained by the powder samples was recorded. The amount of water absorbed was calculated from the weight difference.

Spectra analysis of precipitated saponins
A 0.5 \% w/v mixture of pulp extract and precipitated saponin were made in 20 \% and 80 \% aqueous ethanol respectively and diluted 10-fold with the blank (20 \% and 80 \% aqueous ethanol). The mixtures were passed through filter paper (Whatman No 1). About 2 ml of the resulting solutions were scanned between 200-800 nm using a UV/VIS spectrophotometer (Unicon uv-2102 PC, USA).

Moisture content of pulp powder
A 10 g sample of the pulp powder was transferred to a weighed evaporating dish set up in triplicate. The dishes and their contents were placed in an oven at 110 °C for 4 h. The moisture content was calculated as the ratio of weight of moisture to the initial weight of the samples expressed as percentage.

Evaluation of powder/particle properties
A 30 g mass of the powdered pulp was measured into a 100 ml measuring cylinder. The initial volume after three gentle taps on a wooden surface was noted. Consolidated volume after five hundred reciprocal tapings was also recorded. The following densification parameters were evaluated: bulk density, tap density, true density, bulkiness, Carr’s Compressibility Index and Hausner’s quotient. A density bottle was used to evaluate the true density. Acetone, a non-solvent for the powder, was employed as the displacement fluid. The densification parameters were reevaluated using the pulp granules.

Hypoglycemic evaluation of granulated pulp powder
A 6.67 \% w/v aqueous suspension of the pulp granules was constituted. The resulting dispersion was allowed to hydrate for twenty-four hours. Prior to administration of the dispersion, blood glucose levels were taken at baseline time, using an electronic glucometer (One Touch model, Life Scan, USA). The dispersion was then administered to the animals using an oral intubations set. Blood glucose was again measured at intervals until the sixth hour after drug administration. Glibenclamide and metformin were used as positive controls.

RESULTS
The fleshy mesoderm, in which the seeds are embedded, on drying, gave a powder yield of 5.20 \%. The pulp extract yield was 9.5 \% while 35.9 \% by mass of total saponins was obtained from extract of the pulp. Phytochemical analyses of pulp of P. nitida showed that saponins and other glycosidic compounds occur in the pulp in moderate quantities. Alkaloids, steroids, flavonoids were recorded. The hydrated dispersion of the pulp presented a pH of 4.9, indicating that it is moderately acidic. However, no trace of tannins, terpenoids, resins, proteins, lipids and flavonoids were recorded. The hydrated dispersion of the pulp presented a pH of 4.9, indicating that it is moderately acidic.

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Shelf-life determination of capsule dosage forms
The encapsulated granules were subjected to shelf life studies. Prior to this, the initial concentrations of active principles were determined on the basis of total glycosidal saponins. The solution of the saponins was obtained by macerating the content of a capsule in 10 ml of aqueous ethanol (ratio, 20:80) for 30 minutes. The capsules were thereafter stored under temperature conditions of 40, 50 and 60 °C. At weekly intervals, the concentrations of total glycosidic saponins were determined.

RESULTS
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The spectra scan of the precipitated saponins showed a range of peaks occurring between 200-400 nm (Fig. 1).

There was no attempt to purify the pulp and its extract, since the primary

### Table 1: Powder properties of pulp.

<table>
<thead>
<tr>
<th></th>
<th>Powdered Pulp</th>
<th>Granulated Pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density (g/ml)</td>
<td>0.40</td>
<td>0.52</td>
</tr>
<tr>
<td>Tap density (g/ml)</td>
<td>0.55</td>
<td>0.56</td>
</tr>
<tr>
<td>True density (g/ml)</td>
<td>1.32</td>
<td>1.32</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.69</td>
<td>0.61</td>
</tr>
<tr>
<td>Bulkiness (ml/g)</td>
<td>2.50</td>
<td>1.92</td>
</tr>
<tr>
<td>Carr’s comp. Index (%)</td>
<td>27.30</td>
<td>7.14</td>
</tr>
<tr>
<td>Hausner’s quotient</td>
<td>1.38</td>
<td>1.08</td>
</tr>
</tbody>
</table>

* The reference values for Carr’s Compressibility Index and Hausner’s quotient are 5-10 \% and  \< 1.25 respectively \cite{13}.

### Table 2: Hypoglycemic effect of granulated pulp administered orally to rats.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose levels (mg/dL)</th>
<th>0 hour</th>
<th>1 hour</th>
<th>2 hours</th>
<th>3 hours</th>
<th>4 hours</th>
<th>6 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulated pulp</td>
<td>500.0</td>
<td>84.0±4.3</td>
<td>79.5±4.6</td>
<td>76.0±6.2</td>
<td>68.5±6.8</td>
<td>64.5±3.9</td>
<td>54.5±6.5</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.07</td>
<td>76.3±5.3</td>
<td>72.0±8.5</td>
<td>68.3±6.8</td>
<td>67.3±5.3</td>
<td>65.3±3.3</td>
<td>57.8±4.0</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>7.20</td>
<td>69.5±5.8</td>
<td>77.3±1.1</td>
<td>73.5±3.1</td>
<td>62.3±5.0</td>
<td>65.3±3.8</td>
<td>56.3±3.2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Nil</td>
<td>53.0±8.0</td>
<td>70.0±2.2</td>
<td>72.7±3.3</td>
<td>70.0±2.2</td>
<td>70.0±2.2</td>
<td>70.0±2.2</td>
<td></td>
</tr>
</tbody>
</table>
aim was to evaluate the crude plant extract for activity in order to probably justify the traditional mode of administration of the plant parts in ethnomedical practices.

The pulp powder has a high moisture sorption capacity approaching 45%. However, pre-sorption moisture content of the pulp was found to be 11.55% by weight.

Other micromeric properties of the powdered or granulated pulp are presented in table 1.

These micromeric properties provide insights into flow pattern of the powder if it is to be used as bulking agent in solid dosage forms. Carr’s compressibility index values (7.14 for granules and 27.30 for powder) indicate that the granulated pulp has much better flow tendencies than its powder. The Hausner’s quotient values also lead to the same conclusion.

The blood glucose reduction produced by the granulated pulp was consistent and sustained during the 6-hour duration of the tests (Table 2).

After six hours, the blood glucose reduction was 35.1% of the baseline value. Although the pulp granules were administered at a much higher dose than glibenclamide or metformin, their hypoglycemic effects were comparable. The activity of the pulp can be said to be considerably high, taking into account its crude nature.

Effect of temperature on the residual total saponins in encapsulated pulp is shown in Figure 2. Total saponins contents are apparently highest in capsules stored at 60°C than in those stored at 50 and 40°C. A common trend was, however, observed in which total saponin contents rose correspondingly under all conditions throughout the period of storage.

**DISCUSSION**

Efforts are on-going in various research institutions towards finding new and cheaper excipients as well as safe and active principles for use in pharmaceutical dosage formulations. In view of this, various complex carbohydrate derivatives have been evaluated as excipients in dosage form formulations[15 – 17]. In line with this objective, the incorporation and evaluation of the complex carbohydrate materials obtained from the pod of *P. nitida* in solid dosage forms is seen as a holistic utilization of components obtained from the plant. The pulp is rich in plant secondary metabolites. The presence of these metabolites may account for the wide ethnopharmacological activities associated with the plant[7 – 10, 11]. It has been indicated that saponin glycosides are responsible for the hypoglycemic activity[18].

The pulp extract has a maximum UV wavelength at 299 nm. When the scan was done with the precipitated total saponins at a narrower range, there were many bands occurring between 200-400 nm as shown in figure 1. Saponins are known to produce peaks at wavelength range of 200-350 nm in the UV region[19]. The broad peak at 299 nm appears to be encompassing the saponins responsible for the hypoglycemic activity. The concentrations of total saponins in the pulp were estimated using the calibration curve obtained from total precipitated saponins.

Evaluation of some of the micromeric properties of the pulp is shown in Table 1. It can be seen that the pulp powder do not conform to standards for powders with very good flow properties. However, when the powder was granulated, its flow properties were greatly improved as indicated by the Carr’s compressibility index and Hausner’s quotient[14]. With such improved flow parameters of the pulp powder when granulated, the powder can be used as bulking agent for compatible drugs.

The pulp formulated into a typical dosage form (capsules) exhibited two mechanisms of drug response. The immediate and sustained release of the active principles provide for better blood sugar control. A Nadir value of 35.1% was recorded for the pulp and was statistically significant at 1% a-level when compared to the baseline data using chi-test. Nadir value has been defined as the maximum lowering of blood sugar[20].
To evaluate the stability of the formulated pulp capsule, the capsules were subjected to accelerated degradation at temperatures of 40, 50 and 60 °C. There was a gradual surge in concentration of saponins in the capsule as shown in figure 2.

To explain this, it may be assumed that the saponins and the metabolites arising thereof, as a result of the degradation, absorb the UV radiation at the same wavelength, giving rise to the higher values recorded. However, similar studies conducted with the solution dosage form (data not shown) obtained from macerated pulp powder showed a consistent decrease in concentration indicating degradation.

The second and more probable assumption is that the saponins leach out from their storage vesicles to the periphery at the early stages of exposure to stress conditions. Peripherally accumulating saponins are then “dumped” into any available solvent. To verify this assumption, the content of the pulp was extracted with methanol, and the dried extract was converted into encapsulated granules. The washed dried residue serves as diluents with inherent binding ability. When the capsules were subjected to stress storage at the temperatures of 40 and 60 °C, there was an initial accumulation as the saponins migrated from the adhered powder particles to the periphery (Fig. 3).

Physical reduction of measurable saponins in the capsules over the four-week stability study period was gradual, and assumed a first order pattern. It would, therefore, be expected that the encapsulated pulp leached out its saponin content from the ergastic cells to the periphery. These cells serve as storage organs for most plants[21].

The sharp increases arising from the dumped saponins were consistent and were followed by decrease in concentration as saponin degradation progressed.

Based on this accelerated stability test, it was possible, using the Arrhenius principle, to estimate the shelf-life of the saponins in the granulated pulp. It was therefore estimated that at an ambient temperature of 27 °C, the total saponins in the encapsulated pulp granules would accumulate to a toxic level of 110 % at the rate of 0.0743 s⁻¹. This will give a projected shelf-life of about 68 weeks. This drug level of 110 % is the official specified limit for drugs above 250 mg active principles per unit of the label claim[21].

The proposed mechanism of release of active principles from the pulp would provide an impetus in blood glucose control, as the active compounds within the storage cells would serve as reserves for sustained drug release.

CONCLUSION

The pulp formulated into capsules, appears to be very promising in terms of hypoglycemic activity and stability. The pulp showed good micromeric properties when granulated. However, the capsule had a maximum blood sugar reduction of 31.5 % after 6 hours of treatment. The shelf-life value of the capsule dosage form is 68.2 weeks at 27 °C. The shelf-life value is well suitable for herbal drug formulations. The above findings suggest that the use of pulp component of Picralima nitida in the formulation of capsule dosage forms could improve utilization of the herbal drug in the treatment of diabetes mellitus.

Also the pulp from the plant could be used as excipients in formulating the plant extract and other compatible drugs used in the treatment of diabetes mellitus and other ailments. The long time safe use of the plant in ethnomedicine guarantees the safe use of the formulation.

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REFERENCES