Research Article

Antidiabetic Property of *Picralima nitida* Seed Extracts Entrapped in Chitosan Microspheres

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ABSTRACT

Diabetes mellitus has been managed using synthetic drug molecules. However, in the recent years search for potent antidiabetic molecules derivable from medicinal plants has been consistently explored. Typically, the performance of these phytomedicines may be enhanced using microparticulate drug delivery carriers. The present study was aimed at evaluating the antidiabetic potential of chitosan-based *picralima nitida* microspheres (PNM) on alloxan-induced diabetic wistar rats. Ethanolic and diethyl ether extracts of PN were obtained and dried. Phytochemical screening was conducted on the extracts for the presence of secondary metabolites. Chitosan microspheres containing the ethanolic extract was formulated, converted to powder and encapsulated in hard gelatin capsules. Powder properties, Loading efficiency and *in vitro* drug release study were carried out. Hypoglycemic properties of PNM and PN were evaluated in alloxanized wistar rats. For the first time, we spectrophotometrically determined the wavelength of maximum absorption (Λ max) and Beer Lambert´s constant (K) for PN in distilled water. The ethanolic and diethylether extracts showed the presence of alkaloids, glycoside, tannins, saponins, and fat and oil. Over 80 % of PNE was released in less than 2 h. Ultra violet spectral studies showed a Λ max of 320 nm and Beer-lambert´s constant of 0.0392 in distilled water. The drug Loading efficiency (% LE) was 67 %. Mean fasting blood sugar level reduction in hyperglycemic wistar rats by PNM, PNE and placebo (chitosan microspheres) was comparable ($p < 0.05$) to that of glibenclamide. We therefore conclude that *P. nitida* extract retained its antidiabetic property with the potential for enhanced activity when formulated into multi-particulate chitosan microspheres.

Keywords: *Picralima nitida*, microspheres, Chitosan, Carbosil®, hypoglycemia.

INTRODUCTION

Diabetes mellitus (DM) is a chronic hyperglycemic condition related to endogenous insulin deficit or absence. Type 2 DM is due to changes in carbohydrate, fat and protein metabolism that result to health complications. Although synthetic drug molecules have proved effective for the management of Type 2 DM, however the adverse effects associated with them impel the search for safer alternatives especially from natural sources. Folkloric use of medicinal plants for thousands of years has been a consistent guide to numerous drug discoveries and developmental prototypes. In spite of advances in herbal drug research, including the various health benefits accruing from plant-based bioactive compounds more scientific research voyages are still sought to proffer curative solutions for some diseases, including DM [1, 2, 3].

*Picralima nitida (*family: *Apocynaceae* and order *Gentianales)* is a widely distributed medicinal plant that grows in the tropical rainforests of Africa, as homesteads or bushes [4, 5]. *P. nitida* seed is commonly called Akuamma (Ghana), Osi-igwe in Igbo (Eastern Nigeria), Eso Abere in Yoruba (Western Nigeria) [6]. The extracts from different parts of the plant have been found to possess a broad range of pharmacological activities which lends credence to its ethnomedicinal uses. Indole alkaloids isolated from the seeds of *P. nitida* such as akuammine, akuammidine, akuammicine, akuammigine and pseudo-akuammigine are potent compounds with opioid analgesic activity. The

plant has been used as antimalarial, antifungal, analgesic and antidiabetic agents respectively [7, 8]. With established antidiabetic property, we thought it would be a step forward to incorporate the extract in a suitable dosage form with optimized excipient function to ascertain comparative and enhanced activity with the extract.

In the present investigation we have chosen the multiparticulate carrier (microspheres) as a potential drug delivery platform. Chitosan was selected due to its ease of microsphere formation through ionic interaction with sodium sulphate and potential antidiabetic property [9]. Chitosan is a polycationic polymer with versatile formulation excipient and sundry functions and uses [10, 11, 12]. Microparticulate formulations present as spherical (microspheres), vesicular (microcapsules) or nonspherical (microparticles) solid particles with size-range of 1-1000 µm. Their numerous uses include taste-masking, prevention of gastric irritation, avoidance of dose dumping, sustained release, drug targeting, etc [13]. The aim of this work was to evaluate the effect of chitosan microspheres on the antidiabetic property of *picralima nitida* extract.

MATERIALS AND METHODS

Materials

The following materials were used in this research work: ethanol 96 % (BDH, England), hydrochloric acid and diethyl ether (M&B, England), Tween® 80 (Merck, Damstadt, Germany), alloxan (SD Fine Chem Limited,

Mumbai), Carbosil® (Cabot Corporation, MA, USA), Chitosan (Acrous Organics, USA). All other chemicals and solvents used were of analytical grade.

Plant material

The seeds of *Picralima nitida* were purchased from the herbal material section of Owodo market in Offa, Kwara state, Nigeria. The plant sample was authenticated in the herbarium section of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka.

Experimental animals

Adult wistar rats of both sexes weighing between (100-285 g) were procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals used in this experiment were cared for and all treatment protocols were carried out in accordance with guidelines on animal ethics in Nigeria and University of Nigeria, Nsukka which complied with European Union directive for animal experiments. The animals were allowed to acclimatize for one week, fed with rodent pellet diet and water allowed *ad libitum* under strict hygienic conditions.

Extraction of antidiabetic constituent from *Picralima nitida* (PN) seed

A 1.379 kg of dried *P. nitida* seeds was separated from the shell and comminuted in a wooden mortar to coarse powder. The powder was extracted by cold maceration in ethanol (2 L) with intermittent shaking at 25 °C for 1 week. The menstruum was decanted and filtered off with Whatman filter paper while the marc was discarded. The menstruum was allowed to evaporate at 25 °C under the fan. The semi-solid mass was kept in a desiccator for one week. Thereafter, some portions of the dry solid ethanolic extract was pulverized and dispersed in diethyl ether in order to separate the polar from non-polar constituents. This non-polar solvent was meant to dissolve out lipophilic constituents while leaving behind the hydrophilic ones (diethyl ether insoluble). Upon filtration the insoluble hydrophilic constituents were separated from the lipophilic constituents. The diethyl ether was evaporated off to recover the extract. The extracts were stored in the desiccator for one week.

Determination of the Lambda max and Beer-Lambert constant of *P. nitida* extract

A 10 mg quantity of the *Picralima nitida* ethanolic extract was transferred into a beaker containing 100 mL of 0.1N HCl. It was stirred until the extract completely dissolved; the resultant mixture was filtered and served as the stock solution. Different serial dilutions were prepared to obtain 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg % concentrations respectively. From 0.4 mg % concentration the wavelength of maximum absorption (λ max) was determined using a UV/VIS Spectrophotometer (Spectrumlab, 752s, UK). Subsequently, the absorbance readings of 0.1, 0.2…1.0 mg % were determined at the above λ max and the absorbance plotted against

concentration. The slope of the graph was noted and used in further assay studies as the Beer-Lambert constant K.

Phytochemical Screening

The three extracts of *P. nitida* (ethanolic, diethyl ether and diethyl ether insoluble extracts) were screened for phytochemical contents using the methods already described[14, 15]. The plant metabolites tested for alkaloids, glycosides, flavonoids, saponins, tannins and fats and oil.

Preparation of Microspheres

A 0.4 g quantity of chitosan powder was dissolved in a beaker containing 2 mL of acetic acid and 2 mL of Tween® 80. This was made up to 200 mL with distilled water and stirred vigorously. A 3.5 mL quantity of sodium sulphate (Na_2SO_4) was added to the chitosan solution at 1mL per minute with continuous stirring. Sonication for 15 min and 30 min centrifugation at 5000 rpm respectively were subsequently carried out and the supernatant discarded. The sediment was re-suspended in distilled water 3 times to get rid of left-over acetic acid.

Droplet size and Zeta potential determination

One mL quantity (0.1g % w/v) of the chitosan microspheres in distilled water was introduced into a disposable plastic cuvette and the droplet size (DS) and polydispersity index (PDI) values determined using Zeta sizer (Malvern Instruments, Germany). The zeta potential readings were obtained using a cartridge cuvette. The mean and standard deviation values of triplicate readings were obtained.

Preparation of *P. nitida* microspheres (PNM)

Chitosan microspheres was produced and centrifuged as already described. The supernatant was decanted and the sediment stored. Ethanolic extract of PN was used instead of diethyl ether extract because it contained all the aqueous constituents. *P. nitida* powder was dispersed in equal weight of water prior to mixing with chitosan microspheres at a weight ratio of 1:6 and then sonicated for 30 minutes for complete interaction with the microspheres.

Wet granulation of *P. nitida* microspheres (PNM) and capsule filling

Granules were produced by wet granulation method using Carbosil® as a bulking agent. A 10 g quantity of *P. nitida* microspheres was mixed with 6 g of Carbosil® and blended together. The mixture was thoroughly kneaded with mortar & pestle, screened through sieve 1.7 mm and the wet granules dried in a hot air oven (Memmert, Schwabach, Germany) at 50°C for 1 h. The dry granulation was obtained by screening the granules through 1.0 mm sieve[16, 17]. Hard gelatin capsules (no. 2) were manually filled with 500 mg of granulation and stored in amber bottle.

Loading Efficiency

Twenty capsules were randomly selected and the mean weight of the 20 capsules determined. This was introduced into a mortar and triturated with 50 mL distilled water. The content was made up to 100 mL prior to filtration. 1 mL was further sampled and made up to 100 mL and assayed spectrophotometrically at 320 nm. Triplicate determinations were made and the drug content determined accordingly by dividing absorbance by the K value of *P. nitida*. From the drug content, the Loading Efficiency (LE) was calculated thus:

$$LE = \frac{Drug\ content}{Amount\ incorporated} \times 100 - - - - - (1)$$

Antidiabetic studies.

The effects of PNM, glibenclamide, *P. nitida* seed extracts (PNE) and placebo chitosan microspheres on mean fasting blood sugar of alloxanized wistar rats were determined. Twenty adult wistar rats of 100-285 g were used. The animals were intra-peritoneally injected with 150 mg/kg body weight of alloxan monohydrate (Sigma, USA), freshly prepared in water for injection. After 48-72 hours, the animals were fasted for 12 hours and their blood sugar levels determined using glucometer kit. Only animals with blood sugar levels above 219 mg/dL were selected for the experiment. The diabetic rats were divided into four groups of five animals per group. Group A, received 10 mg/kg glibenclamide, B received an amount of PNM equivalent to 300 mg/kg of PN (ethanolic extract), C received, 300 mg/kg PNE and D, received 300 mg/kg placebo (microspheres without *P. nitida*). The 300 mg/kg dose was selected based on a previous trial experiment. All administrations were via the oral route. At fixed time intervals (1, 3, 6, 9, and 12 h) post treatment, blood samples were withdrawn from the marginal tail of the animals and their blood sugar levels determined.

Uniformity of weight

The content of twenty capsules selected randomly was weighed individually and together and the mean weight calculated[18]. Then, the individual weights were compared with the mean weight to determine the percent deviation.

Bulk and Tapped densities

A 30 g quantity of the PNM granulation was introduced into a 100 mL measuring plastic cylinder. The volume occupied by the sample was noted as the bulk volume. The bulk density was obtained by dividing the mass of the sample weighed out by the bulk volume, as shown in Equation 2. To obtain the Tapped density the cylinder was tapped on a padded wooden platform by dropping the cylinder from a height of 2 inches at 2 seconds interval until there was no change in volume. Triplicate determinations were made in each case.

$$p_b = \frac{M}{v_b} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(2)$$

$$p_t = \frac{M}{v_t} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(3)$$

Where, M is the mass, while V_b and V_t are the bulk and tapped volumes respectively of the granulation.

Bulkiness

Bulkiness is the reciprocal of bulk density. It is calculated using the formula,

$$Bulkiness = \frac{1}{p_b} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(4)$$

Where p_b is the bulk density

Flow rate

A plastic funnel was clamped using a retort stand, with a plane sheath of paper positioned at the base of the Laboratory bench. A 50 g quantity of granulation was transferred to the funnel with a fiber board at its orifice. On removal of the board the time for the powder to flow through the funnel was recorded. The flow rate was calculated by dividing the mass of the sample by the time of flow in seconds. This was done in triplicate to get the mean flow rate (g/s).

$$Flow\ rate = \frac{m}{t} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(5)$$

Static angle of repose (θ)

The static angle of repose was determined using the fixed-base-cone method[19].

A 50 g quantity of granulation was emptied on a plastic base of known diameter. The height of the granulation was measured using a cathetometer. This was repeated 3 times to get the mean static angle of repose.

$$\theta = Tan\left(\frac{h}{r}\right) \dots\dots\dots\dots\dots\dots\dots\dots\dots (6)$$

Where, θ is the angle of repose, h is the height in cm and r is the radius in cm

Compressibility index and Hausner's quotient

Carr's compressibility index (%) and Hausner's Quotient of the granulation were obtained using the formula below:

$$Carr's\ Index = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \times 100 \dots\dots(7)$$

$$Hausner's\ Quotient = \frac{Tapped\ density}{Bulk\ density} \dots\dots\dots\dots\dots(8)$$

Dissolution study

Drug dissolution study was carried out with 3 capsules of PNM. Each capsule was introduced into a cylindrical basket dipped in 900 mL 0.1 N HCl in a beaker-magnetic

stirrer assembly. The temperature was 37 ± 1 ^{o}C and stirrer speed 100 rpm. Aliquots of 5 mL volume were withdrawn the first 5 min and subsequently at 10 min time intervals for 2 h. After each withdrawal, equivalent volume (5 mL) of the fresh dissolution medium was replaced and the drug content determined spectrophotometrically (Spectrumlab, 752s, UK) at the wavelength of 320 nm. Triplicate determinations were made.

Statistical analysis

The statistical analysis was carried out using Graphpad Instat (USA). The results were recorded as Mean ± SD. P<0.05 was considered significant.

RESULTS AND DISCUSSION

Extraction

The extraction process yielded 73.56 % w/w *P. nitida* extract. The high yield of extract showed that optimal extraction of constituents was obtained with the use of the polar solvent, ethanol. The ethanol extract was further subjected to extraction by dispersing it in diethyl ether. The non-polar constituents dissolved in it while the insoluble polar constituents precipitated and was separated through filtration. We evaluated the phytochemical distinction (if any) between the primary ethanolic extract and subsequent secondary diethyl ether extract.

Lamda (λ) max and Beer-Lambert´s Constant of *P. nitida* extract

The wavelength of maximum absorption of *P. nitida* was obtained at 320 nm (Figure 1) while the Beer-Lambert´s plot (Figure 2) gave a linear graph with R^2= 0.999 and y = 0.0392x. The slope, 0.0392 was noted as the Beer Lambert´s constant K. Preliminarily, we had introduced a single whole (uncrushed) *P. nitida* seed in 20 mL of distilled water for about 2 h in a beaker. This was to prompt the extraction of aqueous constituents since the hypoglycemic constituent was reported to be a glycoside (a hydrophilic constituent). The λ max was determined spectrophotometrically (UV). The value was the same with that obtained with the ethanol extract.
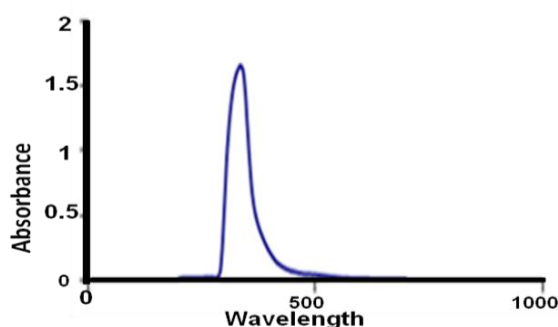

Figure 1: Lambda max for Picralima nitida

Every drug that has conjugated double bonds is amenable to UV spectrophotometric assay and quantitation. For

those without chromophores derivatization via hydrolysis may be carried out to achieve UV assay. For example, artemether does not have a chromophore in its structure, thus acid hydrolysis is required to yield a derivative that is assayable under UV wavelength [20]. The presence of a constituent in both aqueous and ethanolic extracts with the same λ max depicts that *P.nitida* contains a biomarker analytical property that portends ease of quantitative assay. Due to numerous bioactive constituents the quality control of medicinal plants requires the identification of appropriate biomarkers with reproducible characteristics[21]. The curious observation made was the single peak observed at 320 nm, which was devoid of spectral noises. Drug entities assayed by UV spectroscopy are observed within the wave length range 200-400 nm, while coloured APIs are detected within the wavelength range of 400-800 nm [22]. This result serves as a reference point for further detailed quantitation studies on *P. nitida*–based Pharmaceutical formulations.
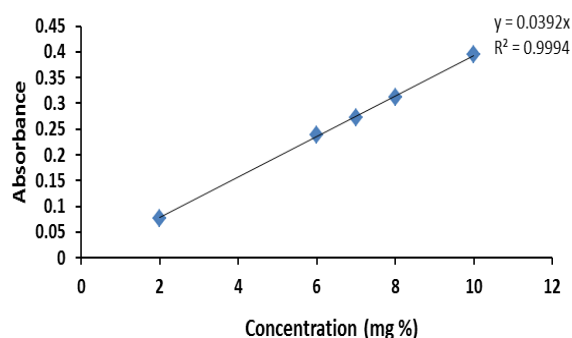

Figure 2: Plot of Absorbance vs concentration of picralima nitida

Phytochemical analysis

The results of the phytochemical screening of the *P. nitida* are shown in Table 1. The phytochemical constituents of ethanolic and diethyl ether extracts revealed the presence of the following secondary metabolites: flavonoid, saponins, glycosides, alkaloids, tannins, and fat and oil. This result agrees with earlier report [23]. However, other workers reported absence of glycosides [24]. The discrepancy could be attributed to the differences in plant species and the environmental conditions [25]. Since most alkaloids dissolve poorly in water but readily in organic solvents, such as diethyl ether or chloroform, diethyl ether was employed as one of the extracting solvents. The diethyl ether did not extract all the alkaloids contained in the *P. nitida* ethanol extract as the residual insoluble fraction still had high concentration of alkaloid, probably due to incomplete extraction. Similarly, the observed presence of lipophilic constituents like fats and oils in the diethyl ether insoluble extract further substantiates incomplete extraction by diethyl ether. It was normal to have higher quantities of saponin and glycosides in the ethanol extract than the diethyl ether extract since they are hydrophilic. The results were generally marked with highest content of alkaloids, tannins and fats and oils, followed by flavonoid, glycosides and saponins.

Particle size, Polydispersity index and Zeta potential

The particle size was 5.5±2.3 µm, Polydispersity index (PDI), 0.57±0.02 and Zeta potential, 12.6±1.9 mV. PDI increases from 0-1 and measures how individual particle sizes vary with each other. 0 indicates monodispersity while 1 shows high dispersity amongst particles. The marginal PDI observed with our microspheres indicates average particle size disparity. The low particle size may enhance the biopharmaceutical tight junction-opening potential of chitosan which may facilitate the paracellular absorption of *P. nitida* across the intestinal membranes [26].

Table 1: Phytochemical constituents of ethanol, diethyl ether and diethyl ether insoluble extracts of *P.nitida*

Tests	Ethanol extract	Diethyl ether Insoluble extract	Diethyl ether extract
Flavonoid	+	+	+
Glycosides	++	++	+
Fats and oil	+++	+	+++
Alkaloids	+++	+++	+++
Tannins	+++	+++	+++
Saponins	++	+	+

[+] Low concentration, [++]Moderate concentration, [+++] High concentration

Effects of *P. nitida* microspheres, *P. nitida* seed extract, Glibenclamide and placebo on mean fasting blood sugar of alloxanized rats.

Table 2 shows the anti-diabetic study results of the 4 batches on the wister rats. One-way ANOVA with posthoc Student-Newman-Keuls pair comparison tests showed that each of the four batches exerted significant (p<0.05) antidiabetic effect between the 1st and 12th h post administration. However, at the 12th h the blood glucose reduction did not significantly (p<0.05) differ amongst the batches.

The results obtained above depict similar pharmacodynamic performance in all the batches. Chitosan microspheres (the placebo batch) curiously lowered blood glucose level. Although its blood sugar lowering effect was less than that for glibenclamide, PNE and PNM (Table 2), the difference was not statistically significant (p<0.05). However, together with entrapped PNE chitosan microspheres demonstrated improved blood sugar lowering effect than the placebo, albeit the difference also lacks statistical significance [p<0.05]. Chitosan´s mechanism of antidiabetic action is attributed to activation of hepatic glucokinase and peripheral tissue uptake, increased pancreatic insulin secretion and enhanced skeletal muscle glucose uptake [27, 28]. Further molecular studies have revealed chitosan as having two mechanisms of action: inhibition of intestinal α-glucosidase and glucose transporters SGLT1 and GLUT2, and enhancement of adipocyte differentiation, PPARc expression and its target genes (FABP4, adiponectin, and GLUT4) [9]. In Ghana the traditional treatment protocol for D. mellitus using P. nitida seed extract, based on unpublished oral information, involves a 7-day maceration of de-hulled 25 seeds of *P. nitida* in 0.75 L of pre-boiled and cooled water prior to administration of 1-2 glasses daily. Standardization of the above regimen using our formulation may hold great promise in D. mellitus treatment.

Table 2: Effects of *P. nitida* microspheres, *P. nitida* seed extract, Glibenclamide and placebo on mean fasting blood sugar of alloxanized rats.

Group	Drug	Doses (mg/kg)	Blood sugar level (mean ±SD mg/dL) within 1-12 h				
			1	3	6	9	12
A	Glibeclamide	10	465±110	351±202	306±127	226±93	145±119
B	PNM	300	492±111	428±170	303±153	243±197	126±90
C	PNE	300	561±17	463±92	359±135	252±105	185±82
D	Placebo	300	441±76	429.8±80	360±112	292±158	250±177

PNM = *P. nitida* microspheres, PNE = *P. nitida* seed extract

Loading Efficiency

With the exception of antidiabetic studies, the rest of the evaluations were limited to granulated PNM. The Loading Efficiency LE (%) of PNM was 80 ± 1.1 %. This value was considered relatively high. It is an exciting observation that our plant extract could be evaluated using spectrophotometric assay technique with defined reproducible lambda max.

Uniformity of Weight

Table 3 shows the percent deviations of selected 20 capsules. Not more than 2 capsules deviated from the average weight by more than 7.5 %.[29] Therefore, the capsules are said to have met the standard requirement. Uniformity of weight is a standard test which has clearly stated limits with which solid dosage forms, such as tablets or capsules are evaluated. The test is designed to ensure dosage form homogeneity which may consequently affect uniformity of drug content.

Granulation properties of PNM

Table 4 shows the various properties of PNM granulations. Flow rate of 7.031 ± 0.04 and angle of repose of 26.04 ±0.51^o were recorded by PNM granulation. Carr's Index was excellent as it was within 5 – 15 %.[30] HQ value was less than 1.2 which also indicated acceptable particle interaction and flow behavior. Angle of repose value of $\leq 30^o$ also buttresses flow ability; poor flowing materials have values of $\geq 40^o$ [31, 32].

Table 3: The Uniformity of weight

| Capsule	% deviation ±SD (PMN)
1	2.145±0.48
2	7.252±1.63
3	6.027±1.35
4	2.962±0.67
5	1.124±0.25
6	2.145±0.48
7	3.166±0.71
8	0.102±0.02
9	0.102±0.02
10	0.102±0.25
11	0.102±0.21
12	1.124±0.44
13	0.919±0.02
14	1.941±0.67
15	0.102±0.21
16	2.962±0.25
17	0.919±0.21
18	1.124±0.21
19	0.9190.21
20	0.919±0.02

The inclusion of cabosil$^®$, a glidant may have contributed to improved flow. Bulkiness represents specific volume while bulk density is a measure of the powder density. The bulk and tapped densities indicate the packing arrangement of the particles and their compaction profile[31]. The evaluated parameters generally confirm good granulation flow characteristics.

Table 4: Granulation properties of PMN.

Parameters	Mean ±SD
Bulk density (g/Ml)	0.658 ±0.04
Tapped (g/mL)	0.748±0.03
Bulkiness	1.517±0.01
Hausner's Quotient	1.137±0.21
Compressibility index (%)	12.032± 1.02
Flow rate (g/s)	7.031 ± 0.04
Angle of repose (^{o})	26.04 ±0.51

Drug release studies

The i*n-vitro* drug release of *Picralima nitida* from the granulated microspheres (Figure 2) showed a consistent pattern. The T_{50} and T_{85} values were 42 and 96 min respectively. The presence of carbosil$^®$ and microspheric entrapment were responsible for the slight slow release. In our previous work involving solid lipid microparticles containing carbosil$^®$, T_{50} of over 40 min was also recorded[33].

Figure 3: Dissolution curve of Picralima nitida from wet-granulated chitosan microspheres
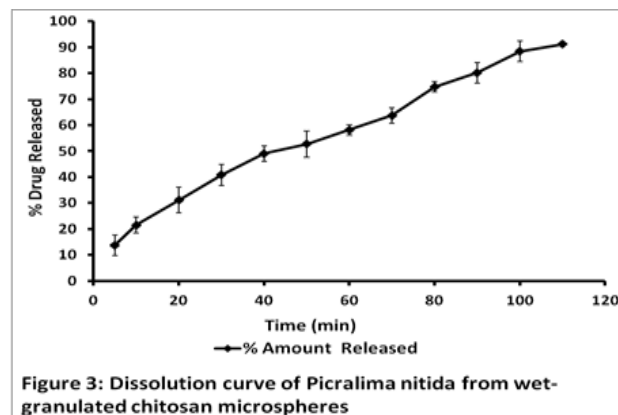
CONCLUSION

Certain phytoconstituent/s of *Picrallima nitida* has demonstrated blood sugar lowering effect. Bioactivity was retained when entrapped in chitosan microspheres. Placebo chitosan microspheres sufficiently lowered blood glucose level. For the first time we report the Lamda max of 320 nm for *Picrallima nitida* in distilled water under UV/VIS spectroscopy. We therefore conclude that P. *nitida* possesses relatively high blood sugar lowering potential when loaded in chitosan microspheres.

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