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Original Research Article

Shelf-Life of Formulated Herbal Liquid Dosage Forms of *Picralima nitida* Seed Extracts for Anti-hyperglycaemic Indication

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ABSTRACT

Herbal remedies are widely used all over the world as alternative to conventional medicine. *Picralima nitida* has gained medicinal importance because of its use for treating wide range of ailments. Preliminary studies showed that its seeds and pods have hypoglycaemic effects in albino wistar rats. The aim of this study was to subject the dosage forms of the plant materials to stability studies using conventional method to establish their shelf life. The powdered dry seeds of *Picralima nitida* were extracted with 95% ethanol. The pod's pulp obtained after the removal of the seeds was dried and powdered. The products were prepared into conventional dosage forms; oral solution (500 mg/kg) and suspension (500 mg/kg) were compared against standard glucose-lowering drugs, glibenclamide (0.07mg/kg) and metformin (7.20 mg/kg) for their hypoglycaemic activities. The formulations were subjected to stability studies under stress conditions of temperature. The oral solutions produced from the ethanol seed extract reduced postprandial blood glucose in the rats by an average of 14.0% within 2 h of administration. The oral suspension reduced postprandial blood glucose by an average of 29.2% within 6 h. The suspension was followed in performance by glibenclamide (24.2%), metformin (19.0%) and lastly, the oral solution of the seed extract (14.0%) in that order.The shelf-life values of the oral solution and suspension are 6.0 and 5.5 weeks respectively at 27^{o}C. These shelf-life values are encouraging considering the herbal nature and the aqueous environment of the formulation.

Keyword: Herbal liquid dosage forms, *Picralima nitida*, Hypoglycaemic activity, Shelf life, Anti-hyperglycaemic activity.

Introduction

Picralima nitida (famApocynaceac) is a deciduous tree with wide distribution in the tropical rain forests of Africa. The plant is gaining increasing scientific and medicinal importance because of its use for treating wide range of ailments. Scientific reports have confirmed its use in folklore medicine for antibacterial control.[1-8] Its antiviral activity has been noted in the laboratory; [9]also, the anti-protozoan activity has been reported.[10] It has been shown to possess blood sugar-reducing activity both in hyperglycaemic and normoglycaemic wistar rats; confirming its use in folklore medicine for treating diabetes mellitus.[11-13] The extracts of the plant have been shown to be very safe for human consumption.[14]In the practice of traditional medicine, the plant products are prepared under unhygienic conditions into several crude dosage forms. In these forms, the safety and stability remain undetermined. In the present study, the extract of the plant seeds and the pulp, obtained after the removal of the seeds, were formulated into standard dosage forms and evaluated for hypoglycaemic activity. The dosage forms produced from the plant materials were subjected to stability studies using conventional method often employed for orthodox drugs to establish their shelf-life.

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Materials and Methods

Plant collection and taxonomy
The samples of *Picralima nitida* seeds used for this work were collected from a local traditional doctor in May, 2019. A specimen of the plant was identified at Department of Pharmacognosy and Traditional medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. A voucher specimen (PCG/474/A/025) was deposited at the herbarium for future reference.

Processing of Picralima nitida seed
The pods were washed with distilled water, dried with clean disposable tissues and dissected longitudinally. The seeds, embedded in the pulp,were removed and dried in an air circulated oven at 40^{o}C. The seed testa was hand-peeled and further drying carried out at 40^{o}C until the seeds turned dark-brown and hard. Pulverization into smaller particles was done with hand-operated grinder (Corona model, Mexico). Further size-reduction was done with glass mortar and pestle. The resulting powder was screened through a 0.25mm sieve and packed in a glass container.

Processing of pulp
The pulp, scrapped off from the rind after seeds removal, was spread onto a clean stainless steel tray and dried in the oven at 40^{o}C. It was later packed into a desiccator over anhydrous calcium chloride for 1 week. Pulverization was done on a glass mortar and the resulting powder screened to a particle sizes of 0.25mm. The powder was stored in an amber glass container.

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Preparation of seed extract
Approximately 500g of the seed powder was defatted in 1500 mL of n-hexane for 24 h with intermittent agitation. The residue was dried in a hot air oven at 40^{o}C.Thereafter, the residue was packed into a flatbottom flask of 1000 mL capacity. Ethanol (95%) was poured into the flask with the powder to ethanol ratio in 1:3. The flask was agitated occasionally and the maceration was allowed for 24 h. The mixture was passed through a muslin cloth and finally filtered through suction pump. The resulting filtrate was poured into a stainless tray and allowed to dry in an oven at 40^{o}C. The resulting resinous extract was packed into a clean dried wide mouthed glass container and stored in a refrigerator.

UV absorption spectra and calibration curve of seed extract solution
A 0.2% w/v of seed extract was made in 80:20 aqueous ethanol. Further dilution was done with the blank. The mixture was filtered using filter paper (Watman No1). About 2ml of the resulting solution was scanned using UV/VIS spectrophotometer (Uncon, USA).
Thereafter, oral solution concentrations (90 mg/ml, 100 mg/ml, 110 mg/ml, 120 mg/ml, 130 mg/ml, 140 mg/ml and 145 mg/ml); and suspension concentrations (125 mg/ml, 150 mg/ml, 175 mg/ml, 200 mg/ml and 250 mg/ml) of the seed extract were made and their absorbance's read in the spectrophotometer.

Preparation of seed extract into suspension
Preliminary formulation and evaluation of 12 batches were done with varying amount of pulp and sodium carboxylmethyl cellulose (NaCMC) as suspending agents as well as equal amounts of seed extract (100 mg/mL). propylparaben (0.02%), sodium benzoate (0.5%), sodium metabisulphate (0.5%), mannitol (5%) and water (to 5mL) were also employed. The batch containing 2% pulp and 1% NaCMC was chosen after evaluation using viscosity, sedimentation and content of active principle parameters. This batch was thereafter reformulated by triturating all the ingredients (except the seed extract) with aliquot quantity of water. The seed extract (200 g) was equally triturated separately with 200 mL of water. The two mixtures were thoroughly mixed using Silverson homogenizer (Gallenkamp, England). The suspension was stored in an amber glass container.

Preparation of seed extract into oral solution
Exactly the same constituents and method used for the preparation of the suspension were employed for this purpose. However, the suspension was finally filtered through a fine sieve cloth to obtain a clear solution.

Hypoglycemic activities of prepared oral dosage forms
Normoglycaemic albino wistar rats were used for the evaluation. The rats weighing between 200 and 250 g were housed in metallic cages under ambient temperature (25±3°C) and 12-hour light and dark periodicity. They were fed *adlibitum* on growers' chicken mash with unrestricted access to water. The animals were allowed to acclimatize for 2 weeks before commencing the experiment. Prior to the administration of the formulations, the rats were removed from feedlots and allowed to starve for 2 h before administration of the formulations. Blood glucose reduction was monitored from tail nips under anesthesia at 0-, 1-, 2-, 3-, 4- and 6 h after administration of the formulations. One touch glucometer (life Scan, USA) was used for blood sugar measurement. The experimental protocol was approved by the institution animal ethics committee of the Nnamdi Azikiwe University Teaching Hospital (NAUTH/CS/66/Vol.11/48).

Shelf-life determination of prepared dosage forms
The oral dosage forms were subjected to stability studies. The initial concentrations of the dosage forms were determined with the aid of a calibration curve obtained from the solutions of the precipitated saponin glycosides. A solution of the suspension was achieved by filtration of the suspension. At weekly intervals, the concentrations of total saponins in the solution and filtered suspension dosage forms, stored at temperatures of 40^{o}C, 50^{o}C and 60^{o}C, were determined for 4 weeks. The data obtained were used to establish the shelf life using the appropriate Arhenius equation.

Statistical analysis
Data analysis was done using GraphPad prism version 7.0 (GraphPad, San Diego, CA, USA). The level of significance was tested using one-way analysis of variance (ANOVA), followed by the Tukey post hoc analysis. Probability levels less than 0.05 ($p<0.05$) was considered significant.

Results and Discussion

Currently, research is on-going in various institutions towards formulating existing herbal drugs into pharmaceutical dosage forms. Preparation of medicinal plant products into conventional dosage forms is of urgent importance to preserve the potency and enhance the stability and safety of these herbal drugs. Formulation will also add value to the plant products by increasing their durability and acceptability, while retaining their pharmacological activities. This is necessary since herbal medicines now receive increasing amount of end user patronage.
In line with this objective, the formulation and evaluation of some components obtained from the pod of *Picralima nitida* into some dosage forms will go a long way to achieving a holistic utilization of these components from the plant. The plants' excellent agronomic potentials will ensure sustainability of inputs for the formulations.
Conventional orthodox anti-diabetic drugs correct hyperglycemia through one or more of the following mechanisms; supplementing insulin, improving insulin sensitivity, increasing insulin secretion from the pancreas and/or glucose uptake by tissue cells.[15]The effectiveness of hypoglycaemic herbs has been shown to be mediated by increasing insulin secretion (ginseng, bitter melon, aloes, B*iophytum sensitivum*), enhancing glucose uptake by adipose and muscle tissues (ginseng, bitter melon and cinnamon), inhibiting glucose absorption from intestine (myrcia and sanzhi) and inhibiting glucose production from hepatocytes (berberine, fenurgreek leaves).[15] However, mechanisms of action of *Picralima nitida* have not been elucidated but could be through any of the aforementioned means and more likely through increasing insulin secretion from the pancreas as seen in glybenclamide and/or glucose uptake by tissue cells as seen in metformin.It has been shown that the sugar reducing effect of *Picralima nitida* resides in the saponins (Okonta and Aguwa 2007).[16]The plant seed extract showed maximum UV absorption at 299nm (Figure 1).

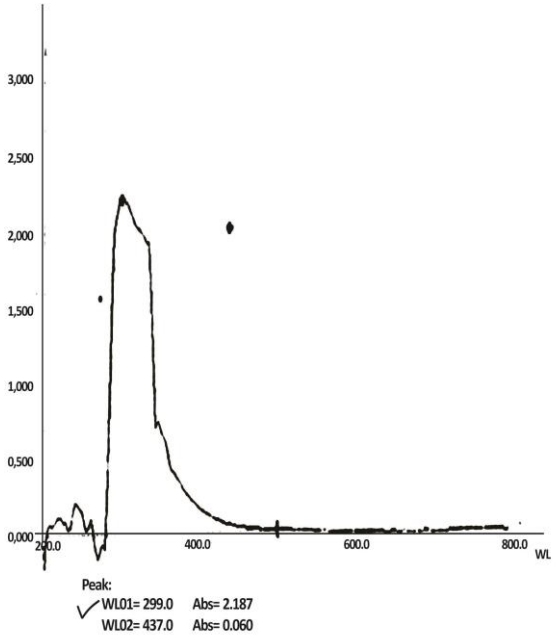

Figure 1: Spectra of seed extract in 80:20 aqueous ethanol

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This absorption range houses the entire saponins responsible for the hypoglycemic activity.[17,18] With this peak, the concentrations of total saponins in the solution and suspension dosage forms were estimated using a calibration curve obtained from total precipitated saponins (Figure 2). Studies have shown that the seed extract and the pulp displayed hypoglycemic activity; with the activity of the seed extract lasting for three hours and the pulp showing sustained release effect.[18] Preliminary formulations of 12 batches of the suspension were carried out using NaCMC and the pulp as suspending agents. The best performing suspension dosage forms were reformulated as well as the formulation of the solution dosage form of the seed extract. The reformulated suspension and solution were evaluated for anti-hyperglycemic property using glibenclamide and metformin as positive controls. The suspension's N_{adir} value of 29.2% is statistically significant at 5 % α-level (Table 1).

The suspension is followed in performance by glibenclamide (24.2%), metformin (19.0%) and lastly, the oral solution of the seed extract (14.0%) in that order.The formulated oral solution and suspension were subjected to storage at temperatures of 40°C, 50°C and 60°C for the purpose of determining their stability. The concentrations of the saponins contained in the solution and suspension, determined at weekly intervals are shown in Figures 3 and 4. While the solution was degrading steadily (Figure 3), the suspension showed an initial rapid decline in concentration of total saponins as the solubilized saponin degrades (Figure 4). Then a gradual decline followed as those stored in the pulp begin to leach into the solution and undergo degradation.

Some calculated stability parameters of the formulations are shown in Table 1. The shelf life values of the suspension and oral solution were similar and are shown to be 6.0 and 5.5 weeks respectively at 27°C using conventional shelf life determination procedures. The calculated shelf lives of the oral suspension and oral solution dosage forms were encouraging considering the herbal nature of the formulations in an aqueous environment. The shelf lives in aqueous conditions suggest that the saponins are relatively unstable in an aqueous formulation compared to orthodox drugs. It can be suggested that, instead, the products be formulated in dry forms for reconstitution prior to administration. However, research work is needed in this regards to further enhance and extend the shelf lifes of these dosage forms.

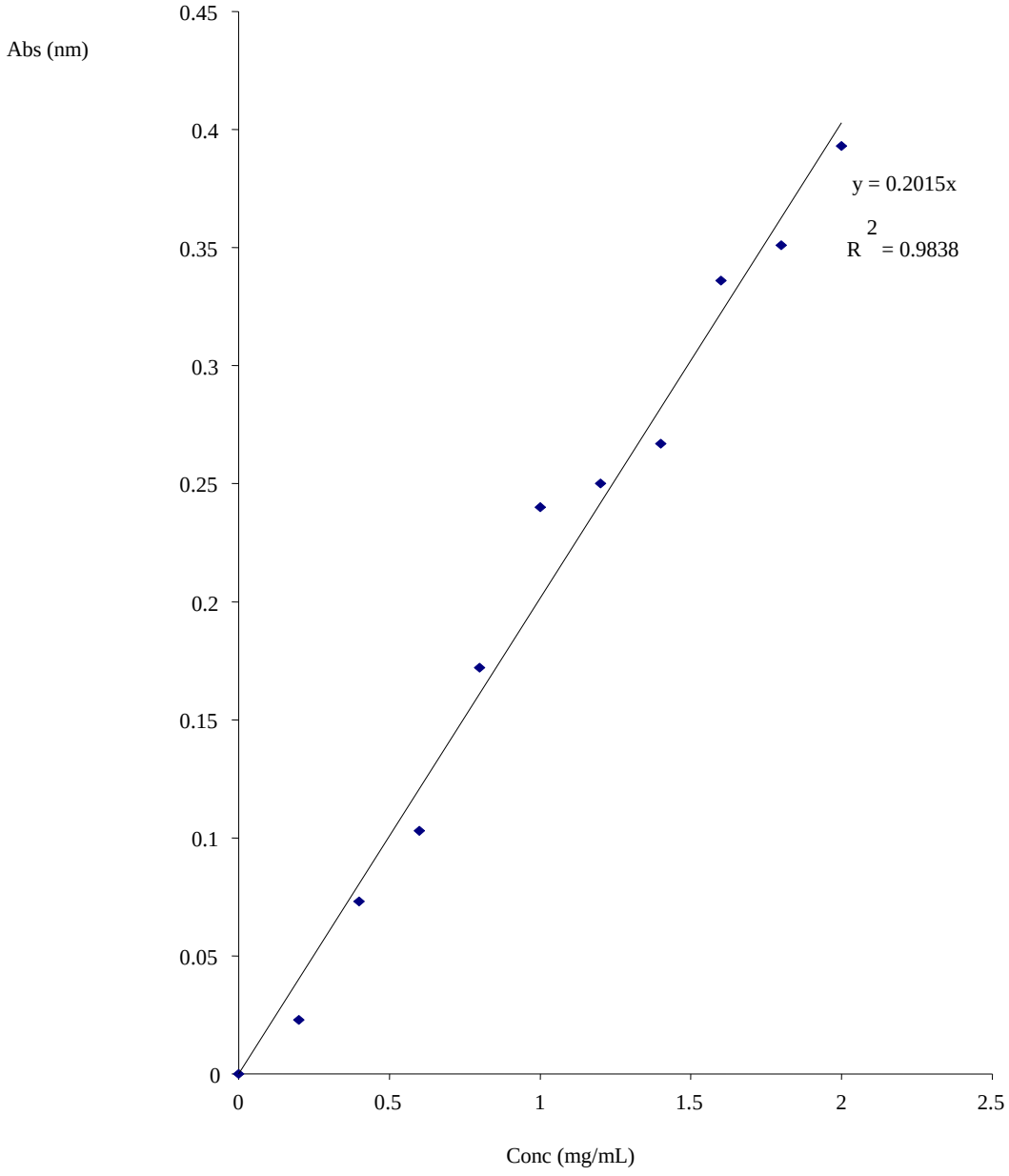

Figure 2: Calibration curve of the precipitated saponins

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Table 1: Dosage and stability parameters of formulated liquid dosage forms of *P. nitida*

Preparation	**Dose rate (mg/kg)**	**N_{adir} (% of baseline)***	**T_{nadir} (hour) ****	**K(s^{-1})**	**Shelf-life**	**(weeks)**
					25°C	**27°C**
Suspension	500	29.2	6	0.0191 (0.0173)***	6.1	5.5
Solution	500	14.0	2	0.0178 (0.0158)***	6.7	6.0
Glibenclamide	0.07	24.2	6			
Metformin	7.20	19.0	6			

* N_{adir} effect is the percentage maximum lowering of glucose level.**$T_{nadir}$is the time taken to achieve Nadir effect *** = K_{25}

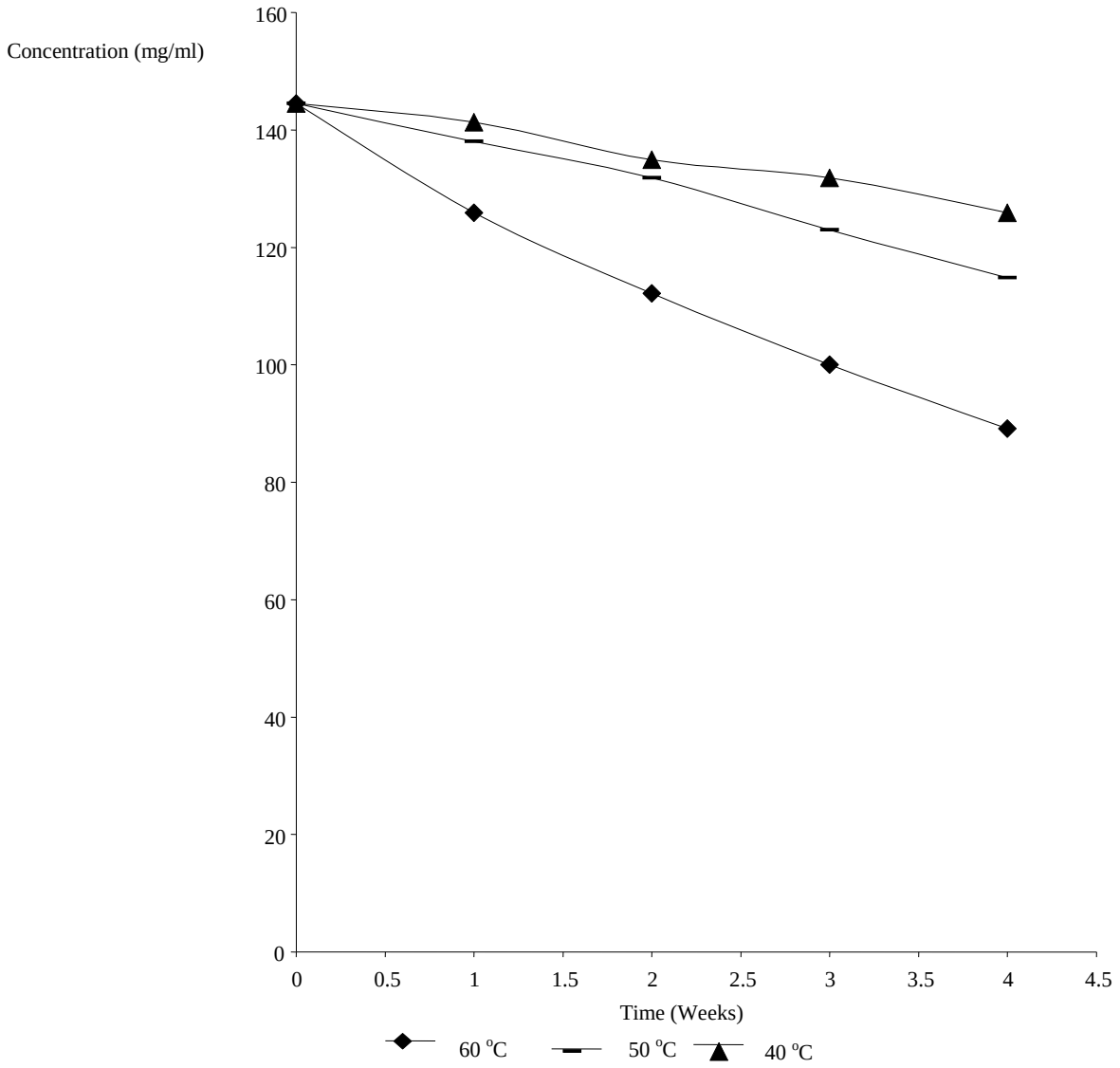

Figure 3: Concentration of total saponins Vs time for oral solution stored at different temperatures

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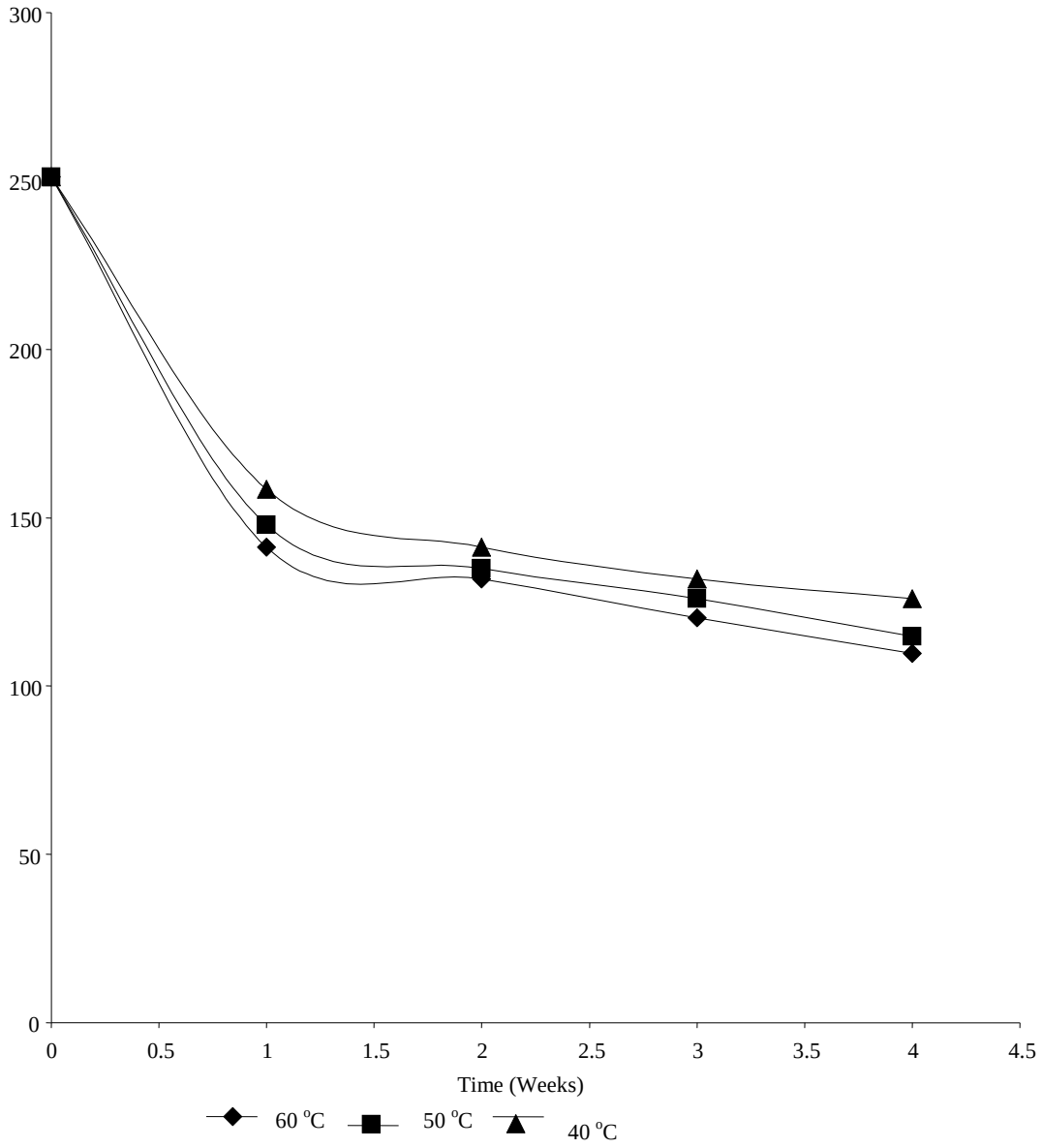

Figure 4: Concentration of total saponins Vs time for the suspension at different temperatures

Conclusion

The various components of the *Picralima nitida* which have previously been shown to possess hypoglycaemic activity were used to formulate suspension and solution dosage forms for hyperglycemic indications. The suspension dosage forms with blood glucose reduction of 29.2% performs better than the two positive controls employed which includes glibenclamide with 24.2% blood glucose reduction and metformin with 19.0 % blood glucose reduction. The solution dosage form has the least blood glucose reduction of 14%. Conventional stability study protocols were employed to establish their shelf lives. The suspension dosage form was found to have a shelf life of 6 weeks while the solution dosage form has 5.5 weeks as its shelf life. These findings are promising considering the herbal nature of the formulations.

Conflict of interest

The authors declare no conflicts of interest.

Authors' declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Opoku-Agyemang T, Amponsah MP, Bonnah SG, Mensah KB, Jarikre TA, Emikpe BO. Field Evaluation of Some Selected Medicinal Plants for the Treatment of Piglet DiarrhoeaIn Ghana. Afr J Biomed Res. 2020; 23(1):107-110.
2. Onyechi KK and Igwegbe CA. Determination of shelf life of Picralima nitida, ciprofloxacin and pefloxacin using bio-based

concentration-activity relationship technique. AJRMPS. 2019; 14:1-7.
3. Feyisayo AK and Victor AC. Assessment of antioxidant and antidiabetic properties of *Picralima nitida* seed extracts. J Med Plant Res. 2019; 13(1):9-17.
4. Amaeze OU, Aderemi-Williams RI, Ayo-Vaughan, MA, Ogundemuren DA, Ogunmola DS, Anyika EN. Herbal medicine use among Type 2 diabetes mellitus patients in Nigeria: understanding the magnitude and predictors of use. Int J Clin Pharm. 2018; 40(3):580-588.
5. Ndukwu MC, Bennamoun L, Anozie O. Evolution of thermo-physical properties of Akuama (P*icralima nitida*) seed and antioxidants retention capacity during hot air drying. Heat Mass Transfer. 2018; 54(12):3533-3546.
6. Dapaah G, Koffuor GA, Mante PK; Ben IO. The possible mode of antitussive and expectorant activity of the ethanol seed extracts of *Picralima nitida* ((Stapf) Th. & H. Durand). JTradCompl Med. 2017; 7(1):133-140.
7. Igboasoiyi AC and Essien EE. Antimicrobial properties of the seed extract and fractions of *Picralima nitida* (Stapf) T. Durand and H. Durand. WJPPS. 2017; 6(7):39-46.
8. MacDonald I, Oghale OU, Ikechi EG, Orji OA. Hepatoprotective potentials of *Picralima nitida* against in vivo carbon tetrachloride-mediated hepatotoxicity. J Phytopharmacol. 2016; 5:6-9.
9. Oluremi BB and Adeniji JA. Anti-viral Activity Evaluation of Selected Medicinal Plants of Nigeria against Measles Virus. BMRJ. 2015; 7(5):218-225.
10. Antwi AN, Owusu KBA, Amoa-Bosompem M, Williams NB, Ayertey F, Akyeh L, Agyapong J, Tetteh T, Djameh GI, Botchie SK, Azerigyik F. Anti-microbial activities of selected Ghanaian medicinal plants and four structurally similar anti-protozoan compounds against susceptible and multi-drug resistant bacteria. Eur J Med Plants. 2017; 20(2):1-14.
11. Oguntibeju OO. Hypoglycaemic and anti-diabetic activity of selected African medicinal plants. Int J Physiol Pathophysiol Pharmacol. 2019; 11(6):224.
12. Ndukwu MC, Ohia A, Anozie O. Influence of Moisture Content and Compression Axis on Mechanical, Physical, and Phytochemicals Properties of Akuamma (*Picralima nitida*) Fruits and Seeds. J InstEng India Ser A. 2019; 100(3):417-426.
13. Adjoumani PE, Mea A, Gohi PKB, Bi JSI, Koffi JN, Kouakou JCA. Effect of *Picralina nitida* on the glycemia and intestinal absorption of glucose in rat. GSCBPS. 2018; 5(3):106-114.
14. Feyisayo AK and Victor AC. Assessment of antioxidant and antidiabetic properties of *Picralima nitida* seed extracts. J Med Plant Res. 2019; 13(1):9-17.
15. Hui H, Tang G, W Go V. Hypoglycemic herbs and their action mechanisms. Available at: www.suntenglobal.com/news/img/Hypoglycemic%20herbs_09212009.pdf.;Accessed December 15, 2019.
16. Okonta JM andAguwa CN. Evaluation of hypoglycemic activity of glycosides and alkaloids extract of *Picralimanitida* Stap (Apocynaceae) seed. Int J Pharmacol.2007; 3(6):505-509.
17. Scott AI. Interpretation of the Ultraviolet Spectra of Natural Products: IntSerMonogr Org Chem. 2013, (Vol. 7). Elsevier.
18. Calistus DN and Vincent CO. Pulp of *Picralima nitida*for hypoglycemic indication: micromeritic and stability assessment. J Pharm Res. 2012; 5(12):5253-5256.