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Formulation design and evaluation of the physicotechnical and hypoglycaemic properties of tablets containing *Dioscorea dumetorum* fraction in alloxanized diabetic rats

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ABSTRACT

Background & Aim: There is increasing interest in the quest for safe and relatively inexpensive medicines from natural products for the treatment of diabetes mellitus (DM). Thus, the main thrust of this study was to formulate and evaluate the anti-diabetic activity of tablets containing aqueous fraction of Dioscorea dumetorum (bitter yam).

Experimental: *D. dumetorum* was extracted using ethanol followed by aqueous fractionation. A rational amount (200 mg) of the fraction (drug) was blended used to produce seven batches of granules by wet granulation and their micromeritic profiles were determined. The granules were compressed into seven batches of tablets and quality control tests including weight uniformity, crushing strength, friability, drug content, disintegration, and dissolution profiles were undertaken. Anti-diabetic activity of the tablets was assayed using alloxan-induced diabetic rats.

Results: Results showed that the granules had good flow properties and compressibility. Tablets produced had good uniformity of weight (0.64 ± 0.01 to 0.66 ± 0.01 g), hardness (4.33 to 8.67 kgf), were less friable (0.05 ± 0.03 to 0.31 ± 0.04 %) with high drug content (98.91 ± 0.55 to 101.39 ± 0.92 %), had acceptable disintegration time (6.44 ± 0.46 to 14.05 ± 0.42 min) for normal release tablets, and recorded 70 – 100 % drug release in 60 min. The result of the anti-diabetic activity study showed that the tablets had blood glucose lowering ability similar to metformin tablet, and therefore could be used as an important alternative in the treatment of diabetes.

Recommended applications/industries: Aqueous fractions of *D. dumetorum* should be further purified and used in the preparation of tablets for the treatment of diabetes mellitus. Extraction and fractionation methods for the plant should be made reproducible for easier industrial application.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycaemia which occurs either due to the poor secretion of insulin by the pancreas or decreased effective utilization of insulin by the body. In 2014, 422 million cases were recorded, and it accounted for 1.6 million mortalities in 2016 in low- and middle-income countries (WHO, 2021). Most diabetic patients receive oral antidiabetic agents which are used for the management of Type 2 or non-insulin-dependent DM (Tripathy, 2013). However, these antidiabetic drugs are synthetic, very expensive, and

produce adverse effects (Gadhiya *et al.*, 2018). Hence, there is a yearning need for green antidiabetic agents that are eco-friendly, with reduced adverse effects, are relatively cheap and readily available, and with the potential for outright healing of diabetes.

Medicinal plants are the oldest sources of drug molecules known to man. They are processed for the treatment of ailments, and literature evidence showed that over 800 medicinal plants have been applied in the treatment of diabetes in different regions of the world (Pujari and Jadhav, 2019). *Dioscorea dumetorum* (bitter yam) is one of the medicinal plants that belong to the family, Dioscoreaceae, used for the treatment of DM (Iwu *et al.*, 1990). In Nigerian folk medicine, it has also been reportedly used as an antioxidant and anti-cholinesterase agent (Salawu *et al.*, 2017), analgesic and anti-inflammatory agent (Ukwueze *et al.*, 2015), for the control of hyperlipidaemia, hypercholesterolaemia, and hyperketomaemia (Malviya *et al.*, 2010; Obidiegwu *et al.*, 2020). In addition, it has been reported that *D. dumetorum* starch has been utilized to produce chloroquine phosphate tablets with high mechanical strength and prolonged release (Okunlola and Odeku, 2011). However, the direct use of the yam extracts of *D. dumetorum* to treat DM is crude, may lead to intoxication due to extract contamination, does not enjoy wide acceptability, does not support prolonged storage of extract, and has become obsolete. Hence, it is important to develop an eco-friendly, inexpensive, reliable, acceptable, convenient, sustainable, easy-to-use, and safe dosage form using extracts of *D. dumetorum* for the treatment of DM.

This is based on the hypothesis that formulation and delivery of extracts of *D. dumetorum* as tablets for oral administration would enhance its acceptance and use as an anti-diabetic agent. This is because the oral route of drug administration is the most common and convenient method of drug administration and formulating *D. dumetorum* extracts as tablets will enhance its oral delivery as an anti-diabetic dosage form in the appropriate amount over the appropriate period of time, and have its physical and chemical integrity protected up to the point of ingestion. Furthermore, preparation of the extracts as tablets will endow them with aesthetics, portability, ease of use, unit dosing, and high acceptability. It will facilitate determination of important technical aspects such as standardization and quality control of the herb, which will be of immense benefit for enhanced overall utility. Therefore, the aim of the study is to formulate tablets containing *D. dumetorum* aqueous fraction and evaluate the physicotechnical properties of the tablets for use in the treatment of diabetes mellitus.

2. Materials and Methods

2.1. Materials

The following materials were obtained directly from the manufacturers and used without further purifications: ethanol, n-hexane (JHD Chemicals, People's Republic of China), polyvinyl pyrrolidone, PVP (Merck KGaA, Darmstadt, Germany), microcrystalline cellulose, MCC (Avicel® PH 102, FMC Biopolymer, PA, USA), acacia, and magnesium stearate (Peter Greven Fett Chemie, Muenstereifel, Germany).

2.2. Methods

2.2.1. Collection and preparation of plant material

Fresh tubers of *Dioscorea dumetorum* were purchased from a local market at Mbaukwu town, Awka, Eastern Nigeria. The plant was authenticated by a taxonomist in our institution and specimen was deposited in our herbarium. The tubers were washed under running tap water and allowed to dry over 24 h. The bark of the tubers was peeled off and the flesh thinly sliced, air-dried at ambient temperature and milled (JCT Thakur, Hoshiarpur, Punjab, India). The powdered material (500 g) was defatted by cold maceration using n-hexane for 48 h with continuous agitation. The marc was recovered and allowed to concentrate for 24 h (Tiwari *et al.*, 2017). Defatted material was extracted by cold maceration using ethanol with continuous agitation for 48 h. Then, the extract was recovered and concentrated to dryness using rotary evaporator (Stuart, Barloworld Scientific, Essex, UK) under reduced pressure to yield *D. dumetorum* extract (DDE), which was stored in an airtight container until use.

2.2.2. Screening for phytochemical constituents

The presence of secondary metabolites in the recovered extract was tested to determine the presence or absence of phytochemical constituents following standard protocols (Umeyor *et al.*, 2016).

2.2.3. Acute toxicity study

Acute toxicity, LD_{50} value of the extract was determined using Lorke's method (1983). A total of 13 mice, weighing 30 g were used in two phases. Phase one comprises 3 groups of 3 mice each which were administered the extract at 10, 100 and 1000 mg/kg, and observed for 24 h. Absence of death in the first phase led to the administration of 2000, 3000, 4000 and 5000 mg/kg dose of extract in 4 groups of 1 mouse each. Animals were examined again for another 24 h. The number of death was noted for each group and the LD_{50} was calculated as follows;

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where: D_0 = Highest dose that gave no mortality and D_{100} = Lowest dose that produced mortality.

2.2.4. Fractionation of crude extract

Fractionation was carried out using distilled water. An amount (50 g) of the crude extract was dispersed in 250 ml of distilled water, transferred into a separating funnel and was shaken thoroughly to mix and form two clear layers. The aqueous portion was collected and concentrated to dryness using water bath set at 40 °C and stored in an airtight plastic container placed in a desiccator for further studies.

2.2.5. Preparation of granules using aqueous fraction of D. dumetorum

Different batches, B1 – B7 of granules were prepared using aqueous fractions of *D. dumetorum* with different binders: MCC, acacia, PVP and their blends (at ratios of 1:1:0, 1:0:1, 0:1:1, and 1:1:1) by wet granulation technique using the formula as shown in Table 1. Appropriate amounts of the fraction and starch were physically blended for 5 min using mortar and pestle followed by incorporation of binder solution prepared with different concentrations of the selected binders, massed separately with adequate quantity of water. This was continued for 5 min after which the wet mass was granulated using 2.0 mm sieve and dried in a hot air oven at 40 °C. Dried granules were sieved through a 2.0 mm sieve and granules were collected stored in an air tight container for further studies (Okunlola, 2015).

Table 1. Formula for preparing the granules.

Ingredients (mg)	B1	B2	B3	B4	B5	B6	B7
Fraction	200.0	200.0	200.0	200.0	200.0	200.0	200.0
PVP	65.0	-	-	32.5	32.5	-	21.67
MCC	-	65.0	-	32.5	-	32.5	21.67
Acacia	-	-	65.0	-	32.5	32.5	21.67
Maize starch	372.0	372.0	372.0	372.0	372.0	372.0	372.0
Magnesium stearate	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Talc	6.5	6.5	6.5	6.5	6.5	6.5	6.5

PVP: Polyvinyl pyrrolidone, MCC: Microcrystalline cellulose, B1 – B7: tablet batches 1 – 7.

2.2.6. Characterization of granules

2.2.6.1. Angle of repose

The angle of repose of the granules was determined using a glass funnel clamped on a retort stand which was 5 cm away from a flat surface. An amount (30 g) of the granules was placed into the funnel and allowed to flow freely through the orifice of the funnel to the flat surface forming a conical heap. The height (h) of the powder cone was determined and the mean radius (r) of the base of the powder cones was determined and the tangent of the angle of repose calculated using the equation (Kipo *et al.*, 2014).

$$\text{Angle of repose, } \text{Tan}\,\theta = \frac{h}{r} \quad \text{..... Eqn. 1}$$

2.2.6.2. Bulk and tapped density

Bulk and tapped densities were determined by measuring the volume (V_p) occupied by a known (30 g) weight (W_p) of the granules in a dry measuring cylinder. The process was repeated in triplicate. The bulk density (Bd) was calculated using the formula (Kipo *et al.*, 2014).

$$\text{Bulk density, } B_d = \frac{Wp}{Vp} \quad \text{..... Eqn. 2}$$

The measuring cylinder was tapped 200 times on a wooden surface from a height of about 2 cm and the tap volume (T_v) was recorded. The process was repeated in triplicate. The tapped density (T_d) was calculated using the formula (Kipo *et al.*, 2014).

Tapped density, $T_d = \frac{Wp}{Tv}$ ..... Eqn. 3

2.2.6.3. Carr's Compressibility index

Carr's compressibility index (CI) was calculated from the results obtained from bulk and tapped densities using the relation (Kipo *et al.*, 2014).

Carr's Compressibility index, $CI = 100 \frac{Td - Bd}{Td}$ ..... Eqn. 4

2.2.6.4. Hausner's ratio

Hausner's ratio was determined using the results obtained from the bulk and tapped densities. It was calculated using the formula (Kipo *et al.*, 2014).

Hausner's ratio, $HR = \frac{Td}{Bd}$ ..... Eqn. 5

2.2.6.5. Scanning electron microscopy (SEM) of powders

The morphological characteristics of *D. dumetorum*-loaded granules from each batch were determined using a PhenomProx scanning electron microscope (SEM) (PhenomWorld, Eindhoven, Netherlands). Granules were added on the sample holder of the SEM at 50 °C to freeze-fracture the granules. Following instrument equilibration, surface properties of the granules was carried out at 15 kV, and images were captured using an in-built digital camera and transferred to Phenom suite software for analysis (Umeyor *et al.*, 2021).

2.2.6.6. Preparation of tablets

The granules were mixed with talc and magnesium stearate prior to compression. The granules were compressed into tablets using Rimek tablet press (UNIK I FC, India) using die and flat punch set of diameter 13 mm to produce circular tablets. The tablets were kept in air tight containers for 48 h prior to further analysis.

2.2.6.7. Evaluation of tablets weight uniformity

From each batch of the *D. dumetorum* tablets, twenty (20) tablets were selected randomly and weighed individually using an electronic balance (Ohaus Adventurer, China) in triplicate and the mean individual weight of the tablets was recorded. The weight variation of each batch was determined using the average weight of the 20 tablets and the standard deviation and percentage deviation for each batch was determined (BP, 2012).

2.2.6.8. Hardness test

The Mosanto hardness tester (VinSyst, India) was used in measuring the hardness of the tablets. Six tablets were selected at random and each tablet was in turn placed between the anvil and the spindle of the instrument and test pressure was increased by clockwise movement of the knob at constant rate resulting in crushing of tablets. The value of the pressure applied was taken as the crushing strength of the tablet. The mean of six determinations were taken (Dheeraj *et al.*, 2014)

2.2.6.9. Friability test

Twenty tablets were selected randomly, dusted, weighed accurately, placed inside the drums of the friability tester (Erweka, Germany), and the equipment was run for 5 min at 25 rpm. The intact tablets were removed from the drum, dusted and re-weighed. The percentage loss in weight was calculated and recorded as the percentage friability (Okunlola, 2015).

2.2.6.10. Disintegration time test

Six tablets were randomly selected and placed individually in the six tubes of the rack of the disintegrating machine (Erweka ZT-71, Germany). The rack was raised and lowered at constant rate in 900 ml of distilled water contained in a glass beaker suspended in a water bath at 37 ± 1°C and the disintegration time was recorded in triplicate (Okunlola, 2015).

2.2.6.11. In vitro dissolution test

The *in vitro* dissolution test was carried out using the paddle method (Erweka DT 700, Germany). The dissolution medium used was 900 ml 0.1N HCl at 37 ± 0.1 °C. The paddle rotating at 50 rpm provided agitation. One tablet was placed into each glass beaker. A sample of the dissolution medium (5 ml) was withdrawn at specified time intervals of 15, 30, 45 and 60 min respectively and analyzed at a pre-determined wavelength of 350 nm using UV Spectrophotometer (6405 UV/Vis Barloworld Scientific, UK). After each sample withdrawal, same volume of fresh dissolution medium was replaced to maintain sink condition. The amount of drug release was determined in triplicate.

2.2.6.12. Content of active ingredient

Ten tablets were randomly selected from each batch and used for the study. The tablets were crushed and a quantity of powder equivalent to 650 mg was accurately weighed and transferred to 100 ml volumetric flask to which phosphate buffer (pH 7.4) was added to disperse the contents and later adjusted to 100 ml. The dispersion was stirred for 2 h using magnetic stirrer and then allowed to settle. Solution was filtered using Whatman's filter paper (No.41). Drug content was determined in triplicate using the calibration curve for *D. dumetorum* already prepared (Dheeraj *et al*., 2014).

2.2.6.13. Animal care and use protocols

Randomly selected Wistar rats of both sexes weighing 220 – 250 g were procured from the animal breeding centre, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria. Animals were treated according to National Institute of Health (NIH Publications no. 8023, as revised in 1978) guidelines for animal care. They were housed in a regulated environment (25 ± 2 °C, 12 h light/dark cycle, light cycle starting at 7 am), acclimatized for 1 week prior to study, fed standard rodent pellets (Guinea feeds Ltd, Nigeria) and allowed free access to clean, fresh water in glass water bottles *ad libitum*. Animal experiments conformed to the ARRIVE guidelines and in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/ EU for animal experiments. Animal use protocols were approved by the Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences (ethical approval number: FP/PT/018/A/005).

2.2.6.14. Induction of experimental hyperglycaemia

Alloxan monohydrate was used to induce experimental diabetes in rats as previously described (Hemamalini and Vijusha, 2012). Animals were fasted for 24 h, followed by injection of single dose of 150 mg/kg body weight of alloxan monohydrate intraperitoneally. The alloxanized rats were kept for 3 days with free access to feed and water for hyperglycaemia to develop. Baseline fasting blood glucose levels were determined using one Touch Glucometer (Lifescan, USA). Rats with blood glucose levels (BGL) above 200 mg/dl were recruited for the study.

2.2.6.15. Hyperglycaemia treatment protocols

Fifty rats consisting of 45 diabetic rats and 5 non-diabetic rats were randomized into 10 groups of five rats each as follows: Group 1 – Normal control, 5 % Tween®80, Group 2 – Negative control (Diabetic), 5 % Tween®80, Group 3 – Positive control (Diabetic) + metformin 500 mg/kg, Group 4 – Diabetic + B1 formulation 50 mg/kg, Group 5 – Diabetic + B2 formulation 100 mg/kg, Group 6 – Diabetic + B3 formulation 150 mg/kg, Group 7 – Diabetic + B4 formulation 200 mg/kg, Group 8 – Diabetic + B5 formulation 300 mg/kg, Group 9 – Diabetic + B6 formulation 400 mg/kg, Group 10 – Diabetic + B7 formulation 500 mg/kg. Doses were chosen considering the LD_{50} of the extract. However, since rats were treated with tablets containing fractions of the crude extract, we decided to start with a low dose of the fraction within the LD_{50} safety window of the extract and increase in a graded manner. Baseline pre-treatment BGL (zero hours) of rats was taken. Then, diabetic rats were treated with the stated doses. Daily administration of metformin and formulations was carried out for a period of 14 days (2 weeks) on the animals. BGL was measured on day 2, 4, 6, 8, 10, 12 and 14. After administration of the last dose, animals were fasted overnight and final blood glucose level was taken (Ahmed *et al*., 2010).

2.2.6.16. Stability study

Stability study was carried out on all the batches at 25 ± 2 °C and 65 % relative humidity in a humidified chamber for six months. Thereafter, the samples were analyzed for drug content in triplicate.

2.3. Statistical analysis

Data were expressed as the mean values ± standard deviation (SD). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests for multiple comparisons. Statistically significant differences were defined using the figure legends * ($P< 0.05$, significant), ** ($P<0.01$, very significant), and *** ($P<0.001$, extremely significant).

3. Results and discussion

3.1. Phytochemical constituents

Result of phytochemical screening of the extract and aqueous fraction is presented in Table 2. The result indicates the presence of saponins, reducing sugars, flavonoids, alkaloids, fats and oils, and proteins in the extract while tannin was absent. In contrast, the aqueous fraction contains all the above listed phytochemicals except fats and oils, tannins and proteins. The secondary metabolites present in the extract and the fraction provide a strong scientific basis for the ethnopharmacological use of the plant in the treatment of hyperglycaemia (Sparg *et al.*, 2004; Anioke *et al.*, 2017).

Table 2. Phytochemical composition of crude extract and fraction of *D. dumetorum*.

S/N	Phytoconstituent	Crude extract	Aqueous fraction
1	Saponins	+	+
2	Tannins	-	-
3	Reducing Sugars	+	+
4	Flavonoids	+	+
5	Alkaloids	+	+
6	Glycosides	+	+
7	Fats and oils	+	-
8	Proteins	+	-

+ = Detected; - = Not detected

3.2. Acute toxicity test

Acute toxicity test of the extract was evaluated to predict its margin of safety through measurement of its LD_{50} value. Result of the study indicates that the LD_{50} of the plant extract was > 5 gkg^{-1} suggesting that the plant was very safe on oral administration because animal mortality was not recorded. In addition, cage-side clinical observations did not show any unusual changes in the behaviour and locomotor activities of the animals. This shows that possible *in vivo* evaluation of the extract might not produce lethal effects in the animals.

3.3. Granules flowability

Result of the micromeritic properties of the granules shown in Table 3 revealed that angles of repose of the granules ranged between 21.96 and 24.87° indicating that the powders had low interparticulate friction and hence, excellent flow property (Reginald-Opara *et al.*, 2015). Inter-batch comparisons showed that B1 with angle of repose of 21.96° had the best particle flow followed by B3 with angle of repose of 22.36°, while B5 with angle of repose of 24.87° showed the least flow in that order. This is very significant (p-value<0.01) compared to pharmacopoeial poor flow rating of powders at angle of repose of 46 – 55° (USP 32, 2009). Typically, powders with good flow property have bulk and tapped densities in the range of 0.32 – 0.40 gm/cm^3. The bulk and tapped densities of the granules were within acceptable ranges of 0.34 to 0.48 gm/cm^3 and 0.39 to 0.54 gm/cm^3 respectively, indicating good packing and sphericity properties. In addition, the acceptable densities showed that the granules had high porosity and deformation potential which will promote excellent particulate intimacy during compression to tablets (Onyishi *et al.*, 2013). It has been reported that CI values of ≤10 % indicates excellent flow, 11 – 15 % indicates good flow, 16 – 20 % fair flow, and above 38 % very poor flow. Similarly, HR 1.00 – 1.11 shows excellent flow, 1.12 – 1.18 indicates good flow, while values 1.35 – 1.45 shows poor flow (USP 32, 2009). Therefore, the CI values between 2 and 13 % and HR values of < 1.25 recorded in Table 3 for all batches connote good flowability of the powders.

Table 3. Micromeritics profile of *D. dumetorum* granules.

Batch	Angle of repose (°)	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Compressibility index (%)	Hausner ratio
B1	21.96 ± 1.07**	0.48 ± 0.01*	0.53 ± 0.01	9.49 ± 0.11	1.10 ± 0.01
B2	22.42 ± 1.62	0.34 ± 0.01*	0.39 ± 0.02*	11.12 ± 3.53	1.09 ± 0.02**
B3	22.36 ± 1.55**	0.44 ± 0.01	0.47 ± 0.02	4.92 ± 2.30**	1.05 ± 0.03**
B4	23.09 ± 1.82	0.36 ± 0.01*	0.37 ± 0.01*	2.73 ± 0.05***	1.03 ± 0.00**
B5	24.87 ± 1.28**	0.44 ± 0.01	0.46 ± 0.01	2.91 ± 1.24***	1.03 ± 0.01**
B6	23.77 ± 1.16	0.38 ± 0.01	0.43 ± 0.01	13.06 ± 2.49*	1.15 ± 0.03
B7	23.85 ± 1.49	0.47 ± 0.01	0.54 ± 0.02*	12.87 ± 4.42	1.15 ± 0.06

B1 – B7: Granules batches 1 – 7. Data represents Mean ± Standard deviation for triplicate independent determinations (n = 3). Statistical significance is indicated by * (P< 0.05, significant), ** (P<0.01, very significant), and *** (P<0.001, extremely significant).

3.3. Granules morphology

Shape and surface morphology of the granules are shown in Fig. 1. Visual examination indicates that the granules were powdery, consistent, and brownish-white in colour. SEM images revealed that the particles were mainly spherically and regularly-shaped micro-structures with smooth surface and topology that are

uniformly distributed (homogenously packed) throughout all the batches. The result connotes proper and adequate blending and compatibility between *D. dumetorum* fraction and the granules excipients.

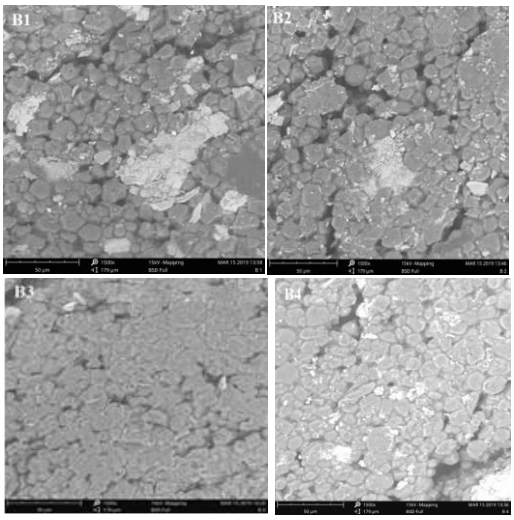

Fig. 1. Scanning electron micrographs (SEM) of granules loaded with *D. dumetorum* fraction showing spherically and regularly-shaped microstructures with smooth surface and topology. B1 – B7 represents granules batches 1 – 7.

3.5. Weight uniformity of tablets

The result of the weight uniformity of the tablets is shown in Table 4. It could be seen that the weight of the tablets ranged from 0.64 ± 0.01 to 0.66 ± 0.01 g and the calculated percentages deviations (data not shown) were non-significant (P>0.05) because the values did not exceed pharmacopoeial prescription as confirmed from the very narrow standard deviation values. Inter-batch comparison showed that batches B1 and B6 had same weight of 0.64 ± 0.01 g while B2 and B5 had same weight of 0.66 ± 0.01, and hence, could be considered the most uniform batches. Therefore, all the tablets passed the test since they met BP specifications which states that for tablets having mean weight greater than 250 mg, not more than 2 tablets are permitted to deviate from the mean weight by greater than ± 5% and no tablet by ± 10 % (BP, 2012).

Table 4. Physicotechnical properties of *D. dumetorum* tablets

Batch	Hardness (kg/cm^2)	Weight variation (g)	Friability (%)	Disintegration time (min)	Drug content (%)
B1	4.47 ± 0.15	0.64 ± 0.01	0.07 ± 0.02**	13.33 ± 0.12*	99.05 ± 0.32
B2	4.33 ± 0.15**	0.66 ± 0.01*	0.12 ± 0.01	9.28 ± 0.15	98.91 ± 0.55
B3	7.77 ± 0.15	0.65 ± 0.02	0.12 ± 0.01	12.78 ± 0.51	99.31 ± 0.48*
B4	6.67 ± 0.15	0.65 ± 0.01	0.05 ± 0.03**	9.28 ± 0.37	99.08 ± 0.71
B5	8.67 ± 0.15*	0.66 ± 0.01	0.31 ± 0.04*	14.05 ± 0.42*	99.13 ± 0.66
B6	5.46 ± 0.21	0.64 ± 0.01*	0.11 ± 0.02	6.44 ± 0.46***	99.27 ± 0.28*
B7	4.77 ± 0.15	0.64 ± 0.03	0.20 ± 0.01	8.47 ± 0.58**	101.39 ± 0.92

B1 – B7: Tablets batches 1 – 7. Figure represents Mean ± Standard deviation. Data represents Mean ± Standard deviation for triplicate independent determinations (n = 3). Statistical significance is indicated by * (P< 0.05, significant), ** (P<0.01, very significant), and *** (P<0.001, extremely significant).

3.4. Hardness test

Tablet hardness or crushing strength was evaluated because it shows tablets ability to withstand shock and stress during manufacturing, packing and transportation, and while used by the patient. From Table 4, tablet hardness ranged from 4.33 to 8.67 kgf suggesting that the mechanical strength of the tablets might not be compromised due to handling since hardness value of ≥ 4 kgf is considered the acceptable minimum standard for normal uncoated tablets (Onyishi *et al.*, 2013). Inter-batch consideration in order of increasing mechanical strength is B2<B1<B7<B6<B4<B3<B5 in that order. Batches B5, B3 and B4 with the best crushing strengths of 8.67 ± 0.15, 7.77 ± 0.15, and 6.67 ± 0.15 kgf could be attributed to higher values of porosity, acceptable bulk and tapped densities.

3.6. Friability test

The upper acceptance limit for friability prescribed in British and United States Pharmacopoeia ≤ 1(BP, 2012; USP 32, 2009). Friability values ranging from 0.05 ± 0.03 to 0.31 ± 0.04 % were obtained for the different batches of tablets as shown in Table 4. Generally, all tablet batches passed the test. However, it was observed that the batches (B5, B3, and B4) with the highest crushing strength or hardness were also the least friable. Batch B5 formulated with PVP and acacia as binder blends recorded the least friability value of 0.05 ± 0.03 %, B3 prepared with acacia recorded 0.07 ±

0.02 % and B4 formulated with PVP and MCC as binders had friability value of 0.11 ± 0.02 %. This connotes that the lower the friability of tablets, the higher their mechanical strength.

3.7. Disintegration time study

The result of the tablet disintegration time test shown in Table 4 indicates that all the batches complied with pharmacopoeial specifications for uncoated tablets (BP, 2012; USP 32, 2009). Table 4 showed that disintegration time for all the batches ranged from 6.44 ± 0.46 to 14.05 ± 0.42 min. However, inter-batch comparison showed that Batch B6 formulated using a blend of equal amounts of MCC and acacia gave the least disintegration time of 6.44 ± 0.46 min followed by batch B7 prepared using a blend of equal amounts of PVP, MCC and acacia with disintegration time of 8.47 ± 0.58 min while batch B5 formulated with equal amounts of PVP and acacia recorded the highest disintegration time of 14.05 ± 0.42 min. The result also showed a positive correlation between friability and disintegration time as batch B6 with the highest friability value disintegrated faster than batch B5 with the least friability and high disintegration time value (Ngwuluka *et al.*, 2010).

3.8. In vitro dissolution test

The results of the release profile of the API from the tablets shown in Fig. 2 revealed that all the tablet batches exhibited good release property, perhaps due to good wettability of the tablets granules. Precisely, batch B4 recorded drug release of about 70 % in 60 min, batches B2 and B3 showed 93 and 96 % drug release respectively, batch B7 recorded 100 % drug release in 30 min followed by batch B1 which also recorded 100 % drug release but in 40 min while batches B5 and B6 exhibited drug releases of 100 % in 60 min. This shows that batch B7 formulated with a blend of all binders – PVP, MCC, and acacia gave the best release profile by recording the maximum drug release in the shortest time compared with other batches. Thus, batch B7 conformed to the US-FDA guideline which provides that normal release drug products should release 85% (T_{85}) of labeled amount of drug within 30 min of study (Obitte *et al.*, 2008).

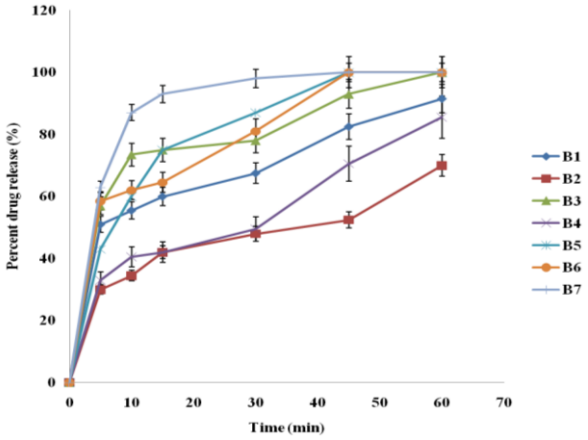

Fig. 2. *In vitro* release profile of tablets containing *D. dumetorum* fraction showing percentage (%) drug release in 0.1N HCl. B1 – B7 represents tablets batches 1 – 7.

3.9. Content of active ingredient

The results of assay of drug content in the tablet batches shown in Table 4 indicate that the tablets passed the test for content of active ingredient. The content of API for the tablets ranged from 98.91 ± 0.55 to 101.39 ± 0.92 %. The very low standard deviation values obtained suggest that the *D. dumetorum* fraction was sufficiently entrapped in the granular core of the tablets and incorporation of the API did not change the original structure and physiochemical properties of the API. All the tablet batches conformed to pharmacopoeial limits of acceptance or rejection as seen in BP 2012 which prescribes a lower limit of not less than 92.5 % and an upper limit of not more than 107.5 % for drug content (BP, 2012).

3.10. Anti-diabetic activity

The result of the anti-diabetic activity study of the tablets shown in Fig. 3 indicates that the formulations (B1 – B7), following oral administration, had blood glucose lowering ability similar to the standard metformin tablet. This shows that the tablets could release therapeutic quantities of *D. dumetorum* sufficient to stimulate insulin release and utilization resulting in lower BGL in the rats to achieve normoglycaemia, thus providing pharmacological evidence for its folkloric claim and use as an anti-diabetic agent. Activation of insulin effect by the plant

was very evident from day 2 and lasted to the last day (day 14) of the study in the diabetic and treated groups. Group 1 animals (non-diabetic and normal control) group maintained their normoglycaemic state (BGL ≥ 100 mg/dl) throughout the study. Hyperglycaemia (BGL ≥ 200 mg/dl) was sustained in group 2 rats (diabetic and negative control) for the duration as 5 % Tween®80 did not reduce blood glucose concentration. Anti-diabetic effect of the metformin standard was more pronounced than in group 4 rats *D. dumetorum* tablets, perhaps due to its high (500 mg/kg) dose of administration. However, control of blood glucose by metformin was similar to the effect produced in groups 6 and 7 rats at 400 and 500 mg/kg of *D. dumetorum* tablets, respectively. It is possible that *D. dumetorum* might have potentiated the insulin effect of plasma by stimulating insulin release from the pancreatic β-cells, enhanced peripheral glucose utilization with concomitant decrease in glycogenolysis and gluconeogenesis (Mahomed *et al.*, 2003; Pareek *et al.*, 2009). Furthermore, the hypoglycaemic activity of *D. dumetorum*-enriched tablets could be due to the synergistic effects of its secondary metabolites such as flavonoids, saponins, and alkaloids, which have been associated with hypoglycaemia (Onyegeme-Okerenta *et al.*, 2013).

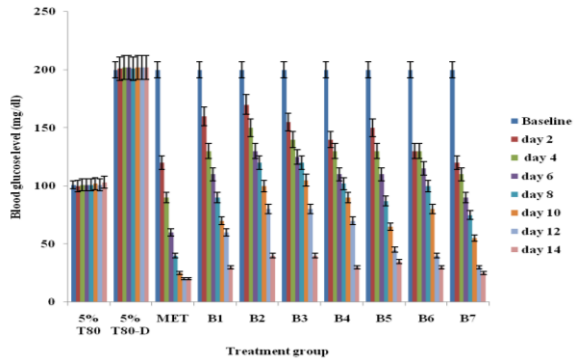

Fig. 3. Blood glucose lowering profile of *D. dumetorum* tablets in alloxan-induced diabetic rats. B1 – B7 represents tablet batches 1 – 7, MET – metformin, 5% T80 – 5 % Tween®80, 5% T80-D – 5 % Tween®80 diabetic.

3.11. Stability study

Result of stability study of the tablets after six months of storage shown in Table 5 indicates that all batches of the tablets were stable and recorded insignificant ($p > 0.05$) changes in drug content at the experimental conditions after six months. For instance, drug content for batches B1, B4 and B7 tablets immediately after formulation was 99.05 ± 0.32, 99.08 ± 0.71, and 101.39 ± 0.92 % respectively while 98.05 ± 0.54, 98.25 ± 0.44, and 99.68 ± 0.35 % respectively were recorded for these batches after six months of storage.

Table 5. Comparative drug content analysis.

Formulation	Drug content (%)	Drug content (%) after 6 months
B1	99.05 ± 0.32	98.05 ± 0.54
B2	98.91 ± 0.55	97.26 ± 0.84
B3	99.31 ± 0.48	97.92 ± 0.26*
B4	99.08 ± 0.71	98.25 ± 0.44
B5	99.13 ± 0.66	98.09 ± 0.31
B6	99.27 ± 0.28	97.05 ± 0.19*
B7	101.39 ± 0.92	99.68 ± 0.35*

B1 – B7: Tablets batches 1 – 7. Figure represents Mean ± Standard deviation. Statistical significance is indicated by * ($p < 0.05$, significant)

4. Conclusion

Dioscorea dumetorum (bitter yam) granules containing its aqueous fraction had good micromeritic and compressibility profiles. Tablets were stable, uniform in weight, and had API content within pharmacopoeial limits. They had acceptable hardness, were less friable, and had good disintegration and dissolution profiles. The tablets had blood glucose lowering property in vivo similar to the standard metformin tablets used as control. Due to these very good physicotechnical and anti-diabetic profiles, aqueous fraction of *D. dumetorum* should be formulated as tablets through wet granulation technique for possible clinical trials.

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