Critical Diluent-Binder Choice in the Tablet Formulation of the Deliquescent Crude Leaves Extract of *Vernonia galamensis* (Asteraceae)

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ABSTRACT
Tablet formulation of the crude aqueous extract of the leaves of *Vernonia galamensis,* used for the treatment of diabetes mellitus in folk medicine was carried out using the wet granulation method. The dried extract is deliquescent and hygroscopic, therefore efflorescent diluents were carefully selected for the formulation. *Aim:* To establish suitable diluents and binders for good quality tablet formulation of the deliquescent extract of *Vernonia galamensis. Materials and Methods*: The selected diluents and binders were; calcium phosphate (Hopkins and Williams, UK), aerosil® 200 (GmbH, Meggle, Germany) and avicel® PH 101 (FMC Corporation, USA), polyvinylpyrrolidone (BDH chemicals Ltd, Poole, England), maize starch and gelatin (May and Baker, Germany). Crushing strength, friability, disintegration and dissolution times of tablets were determined as specified in BP 2007. The mechanical strengths of tablets were assessed using the crushing strength-friability, disintegration time ratio (CSFR:DT) and the drug release properties using dissolution times. *Statistical analysis:* GraphPad Prism® version 5.03 software was used. *Results and conclusion:* Drug release profiles show that only formulations containing calcium phosphate as diluent and polyvinylpyrrolidone as binder met the BP 2007 specification for uncoated tablets, which states that 70% of the active medicament should be released within 45 min.

Key words: Diabetes mellitus, *Vernonia galamensis,* tablets, mechanical strength, dissolution

INTRODUCTION

Diabetes mellitus is a disease marked by elevated blood glucose and urinary glucose excretion. It is caused by a faulty production of insulin or its tissue response[1]. The prevalence of diabetes mellitus is rising at an alarming rate and is projected to more than double by 2030. The disease currently afflicts 171 million people worldwide. Treatment strategies that are aimed at reducing these events have embraced both optimal medical therapy (lifestyle intervention, vigilant glycemic control, and aggressive secondary prevention) and interventional management[2].

The decoction of leaves of *Vernonia galamensis* (Asteraceae) have been used in folk medicine for ages in the treatment of diabetes mellitus. This information was revealed through oral communication with traditional herbalists in northern Nigeria during a search for antidiabetic herbal remedies. But folkloric medicines have no standard dose or acceptable method of formulation, and most plant extracts are hygroscopic and deteriorate quickly[3], Therefore there is the need for standardization and formulation into suitable pharmaceutical dosage forms. Tablets are by far the most frequently used dosage form due to their advantages for both manufacturer and user. Ease of administration and accurate dosing make tablets a versatile and popular dosage form[4].

The objectives of the present study were: (i) to extract the leaves of *Vernonia galamensis, (ii)* investigate and quantify the antidiabetic activity of the extract and (iii) to formulate same into conventional tablets by wet granulation method. The crude aqueous leaves extract of *Vernonia galamensis* (EVG) was found to be highly hygroscopic and deliquescent; therefore, efflorescent pharmaceutical diluents were carefully selected in combination with officially approved selected binders for the tablet formulation.

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MATERIALS AND METHODS

Materials

These include Aerosil® 200 (GmbH, Meggle, Germany), Avicel® PH 101 (FMC Corporation, USA),

Maize starch and Gelatin (May and Bayker, Germany); Polyvinylpyrrolidone (Aldrich Chemical company, USA) and the leaves of *Vernonia galamensis* (collected from the natural habitat of Ahmadu Bello University, Zaria, Nigeria and identified in the herbarium unit of the Department of Biological Sciences of the University where a sample was deposited with a voucher specimen number V16).

Methods

i). Preparation of the extract

Leaves of *Vernonia galamensis* were washed, air dried, milled to a coarse powder (particle size ≤ 1000 µm) and macerated in distilled water for 24 h at room temperature and the liquid extract filtered through a calico cloth and concentrated to a ratio of 5:1 using a rotary evaporator. The concentrated filtrate was then transferred into a tray and dried in an oven at 40 °C, pulverized using a mortar and pestle and then passed through a 150 µm sieve.

ii). Phytochemical screening

The crude aqueous leaves extract of *Vernonia galamensis (EVG)* was quantitatively analysed to ascertain the presence or absence of saponins, glycosides, alkaloids, carbohydrates, flavonoids and tannins according to standard procedures as adopted by Konkon *et al* (2010)[5].

iii). Evaluation of hypoglycemic activity on alloxan-induced diabetic rats

Diabetes was induced in male albino rats weighing about 180 g – 220 g by intraperitoneal administration of 150 mg/kg body weight aqueous alloxan monohydrate[6] and the animals monitored for 72 hours. Alloxan treated rats with fasting blood glucose levels ≥ 100 mg/dl were considered hyperglycemic[7]. Samples of the extracts were administered orally to each of four groups of male albino rats at stepping up doses (200, 500, 750 and 1000 mg/kg) at suitable time intervals (0, 1, 3, 5, and 7 h). The fifth group served as control and was administered normal saline (sodium chloride 0.9% w/v). Metformin 42.9 mg/kg (3000 mg/70kg), per oral was used as the standard antidiabetic agent for comparison. Blood samples were squeezed out from the tale (after a slight cut) into a glucometer with the test strip (One Touch Basic, Johnson & Johnson Company Inc., California, USA) and the reading taken. The data presented here were for triplicate determinations. All procedures involving use of the animals were approved by the ethical committee of the Faculty of Pharmaceutical Sciences of the Ahmadu Bello University, Zaria, Nigeria.

Table 1: Stepwise fractionation procedure

Steps	Fractionation of Mixtures	Separated Fractions
I	Powdered leaf + Petroleum ether	Pet ether extract and F_1
II	F_1 + Ethanol	Ethanol extract and F_2
III	F_2 + Diethyl ether	Diethyl ether extract and F_3
IV	F_3 + Ethyl acetate	Ethyl acetate extract and F_4
V	F_4 + n-butanol	n-butanol extract and F_5

F_1 to F_5 depict Fractions 1 to 5 respectively

iv). Acute Toxicity studies to determine the Lethal Dose (LD_{50}) of extract

Acute Toxicity studies (LD_{50}) by oral route was conducted according to the Organization for Economic Cooperation Development (QECD) guideline 423 as adopted by Chandra et al (2010)[8]. Forty rats divided into eight groups of five were used for assessing the LD_{50}. Varying doses of the extract (500, 1000, 1500, 2000, 5000 mg/kg body) were given to the animals as single doses and a control group received Normal Saline solution. All animals were observed for mortality over two weeks including once daily cage side observation of changes in skin, fur, eyes, nasal mucous membrane and some detectable autonomic and central nervous system changes.

Table 2: Tablet formula for respective batches

Material	Quantity per tablet (mg)
Dried Aqueous Extract	115
Diluent (aerosil® 200, avicel® PH 101 or calcium phosphate)	155
Endodisintegrant (Maize starch 6.8%w/w)	20.4
Binder (MS, PVP or GLT 5% w/w)	Qs
Talc (3.0% w/w)	9.0
Magnesium Stearate I (0.2% w/w)	0.6
Theoretical tablet weight	**300 ± 7.5**

v). Bioassay-guided fractionation by partitioning

Separation of crude extract components was done using the bioassay-guided fractionation by

partitioning according to their polarity[9]. Five gram of the powdered leaf was weighed into a 250 ml stoppered conical flask and 100ml petroleum ether was added and shaken in a mechanical shaker for 6 h and allowed to stand for 18 h. The mixture partitioned into the organic portion known as the petroleum ether portion at the top of the flask and the aqueous fraction known as fraction 1 (F_1) at the bottom. The petroleum ether portion was evaporated to dryness and kept for test of antidiabetic activity whereas F_1 was partitioned with ethanol (step II) and subsequent fractionation and partitioning continued as shown in Table 1. All the various solvent extracts obtained from the stepwise fractionation were evaporated to dryness and their antidiabetic properties determined on alloxan-induced diabetic rats.

Table 3: Effects of different doses of crude aqueous extract of V. galamensis

Time (hrs)	Percentage change in blood glucose levels (%)					
0	0	0	0	0	0	0
1	1.1± 0.1	-4.5 ± 0.1	-57.9 ± 1.4	-40.7 ± 1.0	-62.5 ± 1.5	-18.3 ±1.1
3	5.2 ± 0.1	-16.7 ± 0.6	-56.8 ± 1.6	-71.6 ± 1.7	-73.9 ± 1.7	-55.2 ± 1.3
5	6.8 ± 0.1	-13.5 ± 0.9	-65.5 ± 1.5	-84.5 ±1.5	-80.1 ± 1.4	-63.8 ± 1.2
7	6.6 ± 0.1	-12.1 ± 0.4	-65.8 ± 1.1	-82.7 ± 1.8	-81.7 ± 1.5	-73.6 ± 1.6

Batch I is Normal saline, batches II-V are extract doses of 200, 500, 700 and 1000 mg/kg body weight respectively and batch VI is metformin 28.6mg/kg body weight. Results were expressed as mean ± SD of three runs and at 95% confidence level, p values ≤ 0.05 were the limit of significance.

Table 4: Effects of different fractions of V. galamensis leaves extract obtained from stepwise fractionation procedure compared with that of the whole crude extract on diabetic rats

Time (hrs)	Percentage change in blood glucose levels (%)						
	I	II	III	IV	V	VI	VII
0	0	0	0	0	0	0	0
1	1.5 ± 0.1	-40.7 ± 1.0	-25.6 ± 1.4	-2.3 ± 0.1	-2.6 ± 0.1	-2.3 ± 0.1	-1.3 ± 0.1
3	4.0 ± 0.1	-71.6 ± 1.7	-33.2 ± 1.6	-3.6 ± 0.1	-4.6 ± 0.1	-3.6 ± 0.1	-2.6 ± 0.1
5	5.6 ± 0.1	-84.5 ±1.5	-31.2 ± 1.5	-7.4 ±0.1	-6.8 ± 0.1	-5.4 ±0.1	-4.4 ±0.1
7	8.9 ± 0.1	-82.7 ± 1.8	-33.2 ± 1.1	-9.6 ± 0.1	-9.1 ± 0.1	-6.6 ± 0.1	-4.7 ± 0.1

Batch I = Normal saline, batch II = crude aqueous extract, batches III - VI are the extracts of ethanol, pet ether, diethyl ether, ethyl acetate and n-butanol respectively. Results were expressed as mean ± SD of three runs and at 95% confidence level, p values ≤ 0.05 were considered significant

Table 5: Values of granule size, moisture content and crushing strength-friability, disintegration time (CSFR/DT) ratio values for V. galamensis granules and tablets prepared using selected binders (MS, PVP and GLT).

Diluent	Binder (5%w/v)	Mean Granule size (um)	CS (kgf)	FR (%)	DT (min)	CSFR/DT
	MS	250±1.0	5.4±0.03	0.01±0.005	12.01±0.19	44.96
AR	PVP	250±1.7	4.6±0.05	0.01±0.003	12.50±0.24	36.80
	GLT	250±5.2	5.3±0.01	0.01±0.003	12.54±0.22	42.26
	MS	500±1.5	5.4±0.03	0.01±0.002	6.16±0.25	97.95
AV	PVP	500±3.2	7.8±0.08	0.01±0.003	9.67±0.18	80.66
	GLT	250±4.1	9.0±0.02	0.01±0.003	9.48±0.21	94.94
	MS	1000±.2.7	4.0±0.01	0.01±0.003	5.75±0.21	69.57
CP	PVP	1000±1.4	4.1±0.07	0.02±0.001	3.43±0.18	119.34
	GLT	600±7.2	4.2±0.04	0.01±0.001	5.65±0.14	74.34

Results were expressed as mean ± SD of three runs and at 95% confidence level, p values ≤ 0.05 were considered the limit of significant

vi). Preparation of granules

The wet granulation method of massing and screening was used. Appropriate quantities of the dry extract and the diluent ratio 1:1.4 were mixed in a mortar for 5 minutes. Disintegrant (maize starch, 6.8% w/w) was added and mixing continued for another 5 minutes. A liquid binder prepared using 5.0 % w/v Maize Starch (MS), polyvinylpyrrolidone (PVP) or gelatin (GLT) powder was added in 1-mL portions and mixed with a pestle. The moistened mass was forced through a 1000µm sieve, dried at 40 °C for 2 h to give a moisture content of 4% – 6%, determined on an Ultra X moisture balance (August Gronert Co., Germany). The granules were again passed through a 1000 µm screen to break up agglomerates.

vii). Preparation and analysis of tablets

Tablets equivalent to 300mg of granules were produced by compressing the granules for 60 seconds at 26.25 KN (303 MNm^{-2}) using a single punch tablet machine (Tianxiang and Chentai Pharmaceutical Machinery Co Ltd, Shanghai, China) fitted with 10.5 mm flat punch and die set. After ejection, the tablets were stored over silica gel in a desiccator for 24 h to allow for elastic recovery and hardening. The tablet diametral crushing strength (CS), friability (FR), disintegration time (DT) and dissolution time were determined using the Erweka GmbH models hardness tester, friability tester, disintegration apparatus and dissolution machine respectively. The dissolution was determined using the Jenway 6405 model UV/Visible-Spectrophotometer at a wavelength of 216nm (the principal absorption maximum for the EVG) and the linear regression equation for the plot of absorbance versus concentration was given as y = 0.1734x – 0.0043.

ix). Data analysis

The graphs were plotted and data analyzed using the nonlinear regression of XY analyses in the GraphPad Prism® version 5.03 software. The data used to plot the graphs were the mean of three readings ± SD. At 95% confidence interval, p values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Phytochemical analysis Phytochemical studies show that the aqueous extract of *Vernonia galamensis* contains saponins, glycosides, carbohydrates, flavonoids and alkaloids.

Toxicity studies (Determination of LD_{50})

As much as 5000 mg/kg body weight of extract was administered orally to the animals and there was no record of any toxic effects within two weeks. LD_{50}'s greater than 5000 mg/kg body weight are of no practical interest, therefore the crude extract is considered relatively safe[8].

Determination of minimal effective dose extract

Table 3 presents the effects of increasing doses of the aqueous extracts (200, 500, 700 and 1000 mg/kg body weight respectively) and that of the standard maximum dose of metformin on alloxan-induced diabetic rats (the usual standard maximum single dose of metformin for a 70 kg adult is 2000 mg i.e. 2000 mg/70kg = 28.6 mg/kg). A dose of 700 mg/kg of the crude extract was observed to cause the maximum reduction in blood glucose level by 82.7% which was found to be even more pronounced than that of metformin (73.6%). This confirms that the extract can be used as an antidiabetic as claimed in folk medicine.

Hypoglycemic activity of solvent extracts obtained from stepwise fractionation procedure

Table 4 presents the effects of different solvent extracts obtained from the stepwise fractionation by partitioning on the alloxan-induced diabetic rats. Batch I was the negative control (sodium chloride 0.9% w/v), Batch II was the positive control which was the total crude aqueous extract (EVG) before partitioning (700 mg/kg,) and Batches III to VII were the ethanol, pet ether, diethyl ether, ethyl acetate and n-butanol extracts 700 mg/kg each respectively after partitioning. It was observed that the hypoglycemic effects of the individual organic solvents extracts (Batches III - VII) were lower than that of the EVG before partitioning, eliminating the need for further isolation and purification. It could be concluded that the best antidiabetic effect of EVG is provided by a group of compounds that exist together and provide synergistic effect.

Mechanical strength of the tablets

Table 5 presents the values of mean granule size, moisture content, CS, FR, DT and CSFR:DT of *V. galamensis* tablets produced using selected diluents (AR, AV, CP) in combination with selected different binders (MS, PVP, GLT) at 5% w/v. Kuntz *et al* [10] have observed that increase in granule size leads to increase crushing strength of tablets and that this is as a result of increased surface irregularity of the

larger granules, leading to an increased number of binding surface areas. It was difficult to ascertain this

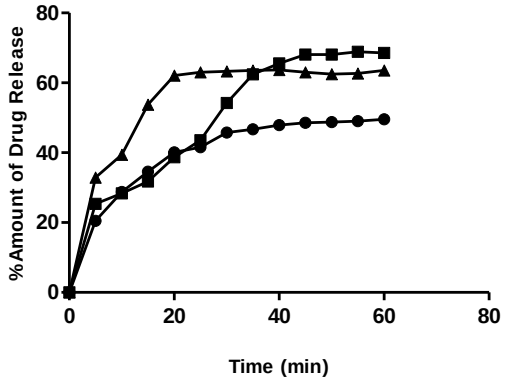

Fig. 1: Graph of Percentage amount of drug release Vs Time of Vernonia galamensis tablets produced using selected diluents and maize starch (MS) 5%as Binder

AR AV CP

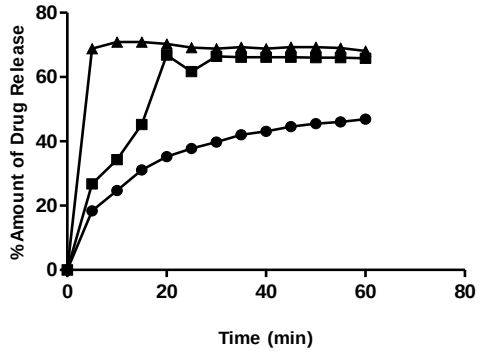

Fig. 2: Graph of Percentage amount of Drug Release Vs Time of Vernonia galamensis tablets produced using selected Diluents and Polyvinylpyrrolidone (PVP) as Binder

AR CP AV

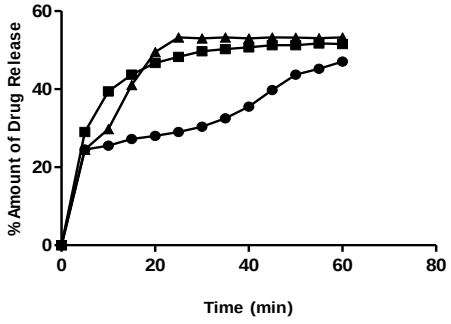

Fig. 3: Graph of Percentage amount of Drug Release Vs Time of Vernonia galamensis tabletsproduced using selected diluents and gelatin (GLT 5%w/v) as Binder

AR AV CP

in our study. Instead, we observed what almost a complete reverse of that hypothesis was. The effect of binder type could be explained as the reason for the discrepancies. For example gelatin and maize starch have been found to have higher binding effects than polyvinylpyrrolidone[11]. So if a binder of lower binding capacity is used, the crushing strength will be low, regardless of granule size.

The British Pharmacopoeia 2007 specifies the following values for uncoated tablets; crushing strength ≥4 kgf and ≤15 kgf, friability <10%, disintegration time ≤15 min, and for dissolution time; 70 – 100% of the active ingredients should be released within 45 min. Results in table 5 show that all the batches passed the crushing strength, friability and disintegration tests as expected of standard uncoated tablets.

Crushing strength-friability ratio (CSFR) which is the quotient of the crushing strength (CS) value divided by the friability (FR) value, has been the index used as a measure of mechanical strength of tablets. But the CSFR:DT ratio which is a later index has been suggested as being better for measuring tablet quality. This is because in addition to measuring tablet strength (crushing) and weakness (friability), it simultaneously evaluates all negative effects of these parameters on disintegration time. Higher values of the CSFR:DT indicate a better balance between binding and disintegration properties[12]. On a general note, the rank order of CSFR:DT values for the three diluents, using the selected binders at 5% w/v was as follows; AV > CP > AR, except for the CP/PVP tablets which recorded the highest CSFR:DT value of 119.34 (Table 5). The higher CSFR:DT and dissolution rate values obtained for tablets produced using CP and AV as diluents as presented in Table 5 and figure 3 respectively, may be explained by the presence of crystalline components in the CP and AV since crystals would normally disintegrate more easily in water than amorphous materials. CP and AV contain crystalline components while AR is completely amorphous[13]. Comparing CP and AV however, tablets formulations with CP have higher CSFR:DT and dissolution rate values than those with AV (Figs. 4-6). This could be as a result of a decrease surface irregularity, leading to decrease number of binding surface areas for binders in CP[14].

The significance of binder types in determining the overall quality of tablets has already been mentioned in this study including the work of Varshosaz *et al* (1997)[14] who found out that PVP has

lower binding effect than GLT and MS. Naturally, the lower the binding effect, the shorter the disintegration time and consequently, the higher the CSFR;DT value. Our study directly agrees with this hypothesis when we used the PVP with CP as diluent where the highest CSFR;DT value of 119.34 was obtained(Table 5). But we could not achieve the hypothesis when we used the PVP with AR and AV as diluents. This may be due to the overwhelming efflorescent effects of the two diluents since they were used in large quantities and the binders in very small amounts as shown in the tablet formula in Table 2. PVP can therefore be conclusively recommended as the binder of choice when CP is the diluent in the tablet formulation of EVG.

Drug release properties of tablets

The result of spectrophotometric analysis shows that the EVG exhibited a principal absorption maximum at 216 nm typical for saponin alkaloids with a diene chromophore[15]. Thus the calibration curve to assess the release properties of the tablets were determine at a wavelength of 216nm and the linear regression equation for the plot of absorbance versus concentration was given as y = 0.1734x – 0.0043. The amount of drug (saponin alkaloid) released was plotted against time and the representative plots for tablets containing AR, AV and CP as diluents and MS, PVP and GLT as binders were presented (Figures 1-3). The rank orders for both disintegration time (Table 5) and dissolution time (Figure 1-3) were found to be the same as follows CP < AV < AR. This further confirms CP as the diluent of choice in EVG tablet formulation. But the BP 2007 specifies that conventional tablets should release 70% of active components within 45 min and only the CP containing formulations produced using PVP as binder meets this specification (Figure 2). Conclusively therefore, good quality tablets of the deliquescent EVG can be produced using CP as diluent and PVP as binder.

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