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Formulation Development and Optimization of Herbo Synthetic Lipospheres Based on Solidified Reverse Micellar Solutions for Therapeutic Management of Diabetes Mellitus

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ABSTRACT

The therapeutic efficacy of bioactives from *Anogessius leiocarpus*, a medicinal plant widely used in folkloric medicine in Nigeria in the management of diabetes mellitus (DM), can be improved using novel drug delivery systems. The objective of this study is to evaluate the antidiabetic potentials of *Anogessius leiocarpus* root bark extracts and lipospheres delivery system loaded with *A. leiocarpus* root-bark methanol extract compared with glibenclamide, a standard antidiabetic. The root bark was powdered and then extracted using methanol, 95% ethanol, and a combination of 95% ethanol and trona using a Soxhlet extractor. Preliminary antidiabetic properties of *A. leiocarpus* root-bark extracts were determined in alloxanized rats, and thereafter the optimized methanol extract was formulated into lipospheres containing 1, 2 and 3%w/w of the extract by high-shear homogenization using 10%w/w lipid matrix composed of 30% Phospholipon® 90H in 70% beeswax. Physicochemical properties, *in vitro* drug release in simulated intestinal fluid (SIF, pH=7.4) and simulated gastric fluid (SGF, pH=1.2) and anti-diabetic properties were determined. The phytochemical screening revealed the presence of saponins, alkaloids, glycosides, steroids, reducing sugars, flavonoids and tannins. Spherical particles with particle size range 135±1.58 - 195±2.24µm, which were stable over four weeks were obtained. Higher drug release in SIF (up to 100%) than SGF (<50%) and a mixed order release mechanism were obtained. The formulations caused significant (P<0.05) reduction in blood glucose level which was comparable with that obtained with glibenclamide. Lipospheres are a potentially safer and cheaper alternative therapeutics for DM given the numerous side effects associated with conventional glibenclamide.

Keywords: *Anogeissus leiocarpus* root-bark methanol extract, glibenclamide, lipospheres delivery system, anti-diabetic property, beeswax, Phospholipon® 90H

Introduction

Diabetes mellitus (DM), characterized by elevated blood glucose levels and anomalies in the metabolism of protein and fat, is a chronic multisystem illness having effects on the body's chemistry and anatomy.[1] Type 1 diabetes is associated with absence of insulin, while type 2 diabetes is associated with inadequate pancreatic insulin secretion.[2] Diabetes mellitus can present as glycosuria, polyuria, polyphagia, and polydipsia, among other clinical symptoms.[3-5] Diabetes mellitus has an epidemic impact on the populace. According to estimates, 21% of adults over 60 suffer from diabetes, while 9.6% of those over 20 have the condition. In the US, diabetes ranks as the fourth most common cause of death from disease. Formerly called as non-insulin dependent diabetes mellitus (NIDDM), type 2 diabetes accounts for approximately 98% of all diabetes diagnoses in adults over 45, 18% in adults 65 to 75 years of age, and 40% in adults over 80 years of age.[6] Over the next 30 years, the World Health Organization (WHO) predicts that the number of people with diabetes will double.[7]

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In addition to medication, diet and exercise are crucial for managing blood sugar levels and diabetes overall.[8, 9] However, long-term use of antihyperglycemic medications, which are the mainstay for managing diabetes and include metformin, sulphonylureas, and others, has been linked to a variety of side effects, including hypoglycemia, hepatotoxicity, diarrhea, flatulence, and stomach pain.[10, 11] Following extended treatment, reports of drug resistance to these medications have also been made.[12] For diabetes therapy, the creation of safe, natural anti-diabetic drugs is critically needed.in order to avoid or at least lessen these difficulties. With the enormous function that phytomedicines have contributed to the advancement and finding of drugs aimed at alleviating various ailments, the identification of novel phytoconstituents via local use or ethnobotanical data seems to be a particularly interesting technique. The main advantage of employing medications derived from plants is that they are generally safer than synthetic substitutes, providing significant therapeutic benefits and more reasonably priced therapies.[13–15] It is believed that 50% of Western medications now on the market contain plant ingredients or have used them as models.[16] Researchers and diabetes patients are increasingly looking for medicinal herbs with anti-hyperglycemic properties as a substitute therapy. Many plants have been found to have antidiabetic properties; it has been shown that these plant ingredients can reduce hunger, gluconeogenesis in the liver, blood sugar levels, weight of the body, and intestinal sugar absorption. They can also stimulate the pancreatic beta-cells' production of insulin in response to glucose, which may be helpful in the prevention and management of diabetes mellitus.[17–23] Moreover, some active principles and parts thereof of plants with anti-diabetic activity have been formulated into various dosage forms for better administration and efficacy.[24]

Anogessius leiocarpus (Figure 1) root bark (DC Guill & Perr, Fam. Combretaceae) is a tiny to medium-sized tree or a slow-growing evergreen bush that grows to a height of 15 to 30 meters and is primarily found in humid tropical forests and valleys in East and West Africa. Its leaves are simple, entire, and alternate to nearly opposite, densely silky and hairy.[25, 26] Traditional healers employ several components of this plant, including the leaves, roots, and trunk bark, to cure and manage helminthiasis, trypanosomiasis, malaria, dysentery, diarrhoea, fever, coughs, rheumatism, leprosy, dental caries and periodontal diseases, wounds and skin diseases as well as diabetes mellitus.[25, 26]

Over the years, tremendous improvements have been achieved on the creation of innovative drug delivery systems (NDDS) for extracts and active ingredients found in plants.[27-31] The creation of innovative herbal remedies, such as microspheres, ethosomes, and lipospheres, has several advantages for herbal drugs in phyto-formulation research. These advantages include improved distribution of tissue macrophages, continuous administration, and defense against chemical and physical deterioration, and enhanced solubility and bioavailability.[27-31] Particulate dispersions of solid, spherical particles with a diameter ranging from 0.2 to 100 µm, lipospheres are supported by a monolayer of phospholipids and include a solid hydrophobic fat core, such as triglycerides or derivatives of fatty acids.[32] The medication is dissolved or distributed in a solid fat matrix within the lipospheres' interior core.[28–41] Many plant extracts, such as ginseng, kushenin, marsupsin, kushenin, green tea, and curcumin, as well as plant-derived bioactives like flavonoids, terpenoids, saponin, catechins, and flavolignan from herbal remedies, have been prepared as lipospheres. as well.[35, 42] This approach has severally been shown to improve the physicochemical properties, stability, bioavailability and efficacy, and enhance administration of the encapsulated bioactives. Meanwhile, the literature on lipospheres delivery system of *Anogeissus leiocarpus* contains little information. Formulating *Anogeissus leiocarpus* into lipospheres delivery system would improve the physicochemical, biological and pharmacotherapeutic properties of the plant extract.

Consequently, this study's goals were to determine *Anogeissus leiocarpus* root bark extract's antidiabetic qualities in order to authenticate the veracity of the claim regarding its ethnomedical use in the management of diabetes, to make lipospheres with methanol extract from the root bark of *Anogeissus leiocarpus* and scrutinize plain (drug-free) as well as methanol extract loaded lipospheres for physicochemical performance, and to investigate the anti-hyperglycemic effect of methanol extract loaded lipospheres in alloxanized animals.

Figure 1: *Anogeissus leiocarpus* tree showing the trunk and part of the root.

Materials and Methods

Beeswax (BDH, England), Poloxamer® 188 (BASF AG, Ludwigshafen, Germany), sorbic acid (Sigma Aldrich, USA), alloxan monohydrate (Sigma, USA), dimethylsulphoxide, distilled water, monobasic potassium phosphate, hydrochloric acid, sodium chloride and Phospholipon® 90H (Phospholipid GmbH, Koln, Germany), were the materials used as obtained without further purification. In our lab, we processed methanolic root bark extract from *Anogeissus leiocarpus*.

Plant collection, identification and extraction

A herbal practitioner in Uzo Uwani Local Government Area, Enugu State, collected the root of *Anogeissus leiocarpus* in March 2010. Mr. Ozioko, an analyst in the Botany Department of the University of Nigeria Nsukka, identified the root, and the voucher specimen (voucher number PHE-07-2010) was deposited in the Department of Pharmacognosy of our University. *Anogeissus leiocarpus* root was chopped into little pieces and dried in the shade. After being dried and powdered into a fine powder, 95% methanol, 95% ethanol, and a mixture of 95% ethanol and trona each was used to extract the bark using a Soxhlet extractor (Gallenkamp, UK). A rotary evaporator (Gallenkamp, UK) was used to further filter and concentrate each extract.

Analysis of the root bark of Anogeissus leiocarpus using phytochemistry

Using known standard protocols, phytochemical analysis was performed on all extracts of *A. leiocarpus* root bark to determine the presence of phytoconstituents.[43] Positive (+) or negative (-) was the qualitative expression for each test.

Acute toxicity studies on the extracts

Animals used in the experiment

The study used Swiss albino mice (18–25 g) of both sexes. The animals were obtained from the animal home of the University of Nigeria, Nsukka's Faculty of Veterinary Medicine. Two weeks were given to the animals to become used to their new surroundings before the study began. Throughout the trial, the mice were housed in polypropylene cages at ambient temperature and humidity levels, with a 12-hour light/dark cycle. They were fed normal rodent diet and had unlimited access to water. The Ethical Guidelines of Animal Care and Use were followed during the conduct of this investigation and was approved by the Research Ethics Committee of our institution (approval no. FPSRE/UNN/14/00043).

Studies on acute toxicity

A modified Lorke's method was used to determine the oral acute toxicity (LD_{50}),[44] which allowed for an assessment of the extracts' safety for the mice. Appropriate markings and weights were applied to the treatment and control animals. Six oral dosages of the plant extracts (10, 100, 1000, 1600, 2900, and 5000 mg/kg body weight) were used in the acute toxicity test on mice. There were two stages of the investigation. In the first phase of the trial, nine mice were randomly assigned to three groups of three mice each, and each group was given oral doses of 10, 100, and 1000 mg/kg body weight of the extract. The mice were monitored for paw licking, salivation, stretching of the entire body, weakening, sleeping, breathing difficulties, coma, and death during the first four hours and then daily for the following seven days. In the second part of the investigation, nine additional mice were randomly assigned to three groups consisting of three mice each, and 1600, 2900, and 5000 mg/kg b. wt. of each extract were given orally based on the results of the first phase. The animals were observed daily for the next seven days, and during the initial critical four hours, for signs of toxicity and death. The square root of the product of the lowest fatal dose and the maximum non-lethal dose—that is, the geometric mean of the successive doses for which survival rates of 0% and 100% were noted in the second phase—was then used to compute the LD_{50}. Equation 1 was used to compute the dosage of oral median fatality.

LD_{50} = √Minimum toxic dose x Maximum tolerated dose Equation 1.

The extract's in vivo anti-diabetic assessment

Initial *in vivo* anti-diabetic effects of each of the extracts of *Anogeissus leiocarpus* root bark (MeAL - methonol extract of *Anogeissus leiocarpus*, EtAL - ethonol extract of *Anogeissus leiocarpus*, Et(T)AL - ethanol plus trona extract of *Anogeissus leiocarpus*) was carried out in rats so as to ascertain the veracity of earlier claims on the ethnomedicinal use of the plant in the management of diabetes mellitus.

Rats used in the experiment

For the experiment, 200 ± 10 g of clinically normal albino rats were prepared. A time of two weeks was given to the animals to get used to their new surroundings before the trial began. They were initially allowed free access to tap water and food. Before the animals were given diabetes, their body weights, water and food intake, urine volumes, and serum glucose levels were measured. They were kept apart in metabolic cages. Light and dark cycle was also maintained as stated ealier in the preceding section. Five groups, each consisting of six rats, were created from the rats. At the University of Nigeria, Nsukka's Faculty of Pharmaceutical Sciences, the animal study complies with the ethics of using animals for research.

Diabetes mellitus induction

Before causing diabetic mellitus, the rats were starved for the entire night. Blood was drawn in order to measure the baseline level of glucose. A fresh alloxan monohydrate solution (Sigma, USA) was made right before the injection. Alloxan was dissolved in standard saline with 0.9% w/v NaCl at a dosage of 100 mg/kg to create a stock solution of alloxan monohydrate. After administering a volume equal to 1 milliliter of the stock solution intraperitoneally, blood sugar levels were monitored regularly for several days using a glucometer (ACCU-CHECK, Roche, USA). After three days of administering Alloxan®, diabetes was verified. [21–23] A blood glucose level of 200 mg/dl was deemed hyperglycemic.

Assessment of antidiabetic activity

We employed thirty albino rats, split up into five groups, each with six rats. Group I received 400 mg/kg of methanolic extract, Group II received 400 mg/kg of ethanolic extract, Group III received 400 mg/kg of ethanolic+trona extract, Group IV received 5 mg/kg of glibenclamide (GL), and Group VI received distilled water (2.0 ml/kg) based on the results of the LD_{50} determination. The rats with alloxan-induced diabetes were given 400 mg/kg of the extracts after they had been dissolved in 3% DMSO. The study was extended to the lipospheres formulations (PL - Plain or unloaded lipospheres, ND - no drug or negative control group that received no drug, and *Anogeissus leiocarpus*-loaded lipospheres containing 1, 2 and 3 % w/w of *Anogeissus leiocarpus* root bark methanol extract, (AL (1 %) or AL_1, AL (2 %) or AL_2, and AL (3 %) or AL_3, respectively).

Establishment of spectral characteristics

Based on the results of the preliminary anti-diabetic studies, the methanol extract was chosen for formulation studies. The spectral characteristics was studied following a reported procedure [42]. With distilled water, simulated intestinal fluid without enzyme (SIF), and simulated gastric fluid without enzyme (SGF) as solvents, the maximum wavelength of absorption of *A. leiocarpus* root bark methanol extract was determined. Freshly made SIF, SGF, distilled water, and ethanol were used to create stock solutions for the extract. To get diluted concentrations of the extract, tenfold dilutions of each stock solution were prepared. The spectrophotometer was calibrated using the solvents as a blank. Each diluted solution was then put into a quartz cuvette, and the spectral readings were taken using an automated UV/VIS spectrophotometer (JENWAY 6405, UK) that scanned the range of 200 to 700 nm. The wavelength of maximum absorption was recorded for each solvent.

Beer-Lambert plots

In order to calculate the Beer-Lambert's plot for *A. leiocarpus*, diluted extract solutions containing distilled water, SGF, and SIF at concentrations between 1 and 10% were prepared. The absorbance of each diluted solution was calculated using the predefined maximum absorption wavelength to see if Beer-Lambert's law was followed in terms of a linear connection between sample concentration and absorbance. Beer-Lambert's plot was also established for pure sample of glibenclamide in relevant solvents.

Lipid matrix preparation

Using the previously described procedure,[37] phospholipid at a concentration of 30% w/w of P90H in beeswax was combined with beeswax to create the lipid matrix. In a crucible, 30 g of P90H was precisely weighed, combined with 70 g of beeswax, and heated to approximately 70ºC in a temperature-regulated steam bath (Memmert, England). After giving the molten lipid matrix a good shake to guarantee sufficient mixing, it was left to solidify at room temperature (30°C).

Formulation of unloaded lipospheres

The lipospheres were prepared using the high-shear homogenization technique.[36] After carefully weighing 10 g of the lipid matrix, it was put in a 250 mL beaker and melted at 70 °C in a steam bath using a thermometer (Memmert, England). The molten lipid matrix was mixed with the aqueous phase of the surfactant, which was composed of 1.5% w/w Poloxamer® 188, 0.05 % w/w Soric acid, and enough distilled water to make 100% w/w at the identical temperature. The hot primary dispersion was created by dispersing the mixture using a homogenizer (Ultra-Turrax, T25 basic, Ika, Germany) at 5000 rpm for five minutes. After that, it was gathered into a heated container and given time to recrystallize at ambient temperature. Table 1 shows the amounts of each excipient that was utilized.

Table 1: Formulation composition of the lipospheres

Code	Poloxamer®188 (% w/w)	Lipid matrix (30% P90H in 70% Beeswax (% w/w)	Sorbic acid (% w/w)	Drug (% w/w)	Distilled water (q.s. to % w/w)
AL_1	1.5	10	0.05	1	100
AL_2	1.5	10	0.05	2	100
AL_3	1.5	10	0.05	3	100
GL	1.5	10	0.05	1.6	100
PL	1.5	10	0.05	0	100

Key: AL_1, AL_2 and AL_3 are *Anogeissus leiocarpus*-loaded lipospheres containing increasing concentrations (1, 2 and 3 % w/w) of methanol root extract of *Anogeissus leiocarpus*; GL means glibenclamide-loaded lipospheres while PL stands for plain or unloaded lipospheres.

Preparation of drug loaded lipospheres

This was carried out using the method previously employed in developing herbal antidiabetic lipospheres.[45] After carefully weighing 10 g of the lipid matrix, it was put in a 250 ml beaker and cooked on a steam bath until it melted. After adding one percent of the methanol extract from *Anogeissus leiocarpus* (AL), the melted lipid was well-mixed. Carefully weighed out, sorbic acid (0.05 % w/w) and poloxamer® 188 (1.5 % w/w) were dissolved in sufficient distilled water to form 100%. The molten lipid containing the dispersed AL methanol extract was combined with the solution at the same temperature. The mixture was homogenized for five minutes at 5000 rpm to create a hot primary dispersion, which was gathered and let to recrystallize at ambient temperature in a heated container. For AL methanol extract concentrations of 2% and 3% w/w, the same procedure was carried out twice. Using the same process, a batch of lipospheres loaded with glibenclamide was generated as the positive control. Table 1 displays the matching formulas for each ingredient as well as the batches used in the formulation.

Characterization of the lipospheres
Analysis of particle size and morphology
Within a week of manufacturing, the lipospheres were subjected to a particle size analysis utilizing a digital photomicroscope (Leica Germany) and Moticam camera (USB 1000, USA) to capture the photos. For capture and viewing under a microscope, a drop of each batch of lipospheres was positioned in-between a cover slip and a glass slide. Image analysis of the lipospheres was used to identify the sizes and shape of the particles.

Time-resolved pH-dependent stability studies
The pH of the lipospheres was determined using a pH meter (Jenway, 3510 UK). Additionally, a time-dependent approach was used for this (72 hours, 2 weeks, and 4 weeks after preparation). For every measurement, replicates were made.

Determination of drug encapsulation efficiency
Anogeissus leiocarpus extract or glibenclamide-loaded lipospheres containing around 6 ml of each medication was centrifuged for 45 minutes at 1500 rpm in Gallenkamp, England. A UV/Vis spectrophotometer (Jenway 6405 UK) was used to properly examine the supernatant at preset wavelengths of 270 nm for glibenclamide and 290 nm for the extract of *Anogeissus leiocarpus*. Equation 2 was used to determine the encapsulation efficiency (EE) of each drug encapsulated in each liposphere formulation.

$$\boldsymbol{EE}\ (\%) = \frac{\textit{Total quantity of the drug} - \textit{Quantity of drug in the supernatant}}{\textit{Total quantity of the drug}}\ \boldsymbol{X\ 100}$$

Equation 2.

Determination of loading capacity
The loading capacity was ascertained using the same process that was utilized to ascertain the encapsulation efficiency. The third equation provides the loading capacity (DL).

$$DL = \frac{\textit{Total quantity of the drug} - \textit{quantity of drug in the supernatant}}{\textit{Total quantity of the lipid base}}\ X\ 100$$

Equation 3.

Studies on drug release
The prepared liposphere was suspended in 250 ml of physiological fluids in a 10 mL volume inside a dialysis membrane with a hole size of 0.30 µm that was knotted at both ends. SIF (pH 6.8) was added to the receptor compartment and stirred with a magnetic stirrer bar at 50 rpm to keep the temperature constant at 37 ± 1 ºC. The water bath was thermostatically controlled. Within eight hours, at intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours, a 5 ml volume was withdrawn and replaced with an equal volume of the receptor phase (SIF). The drug content of the samples was determined using a spectrophotometer (Jenway 6405, UK) set to 270 nm. Additionally, the drug release assessment for glibenclamide and *Anogeissus leiocarpus* was carried out in SGF (pH 1.2) at 290 and 270 nm, respectively. Beer-Lambert's calibration was used to calculate the drug content at each time.

Drug release kinetics and mechanism
To investigate the kinetics and processes of release, the findings from the *in vitro* investigations were fitted into a number of release models. Equations 4–7, which describe the zero order, first order, Higuchi, and Korsenmeyer–Peppas models, respectively, were utilized.

$Q_t = K_0 t$ Equation 4.
$\text{In } Q_t = \text{In } Q_0 - k_1 t$ Equation 5.
$Q_t = K_H.S\ \sqrt{t}$ Equation 6.
$M_t/M\infty = Kt^n$ Equation 7.

Q_0 represents the starting dose of medication in the lipospheres, and Q is the amount released at time t. The rate constants for the Huguchi rate equations, first order, and zero order are denoted by K_0, K_1, and K_H, respectively. The drug release amounts are denoted by M_t and M_∞, respectively, whereas $t = \infty$ represent an infinite time, the power law constant is represented by K. The diffusional exponent n represents the drug release mechanism. The fraction of drug released is therefore M_t/M_∞. When n is less than 0.43, a Fickian diffusion (Case 1) occurs; when n is greater than 0.89, a non-Fickian transport occurs; and when n is greater than 0.89, a Case II transport, or zero order drug release mechanism, predominates.[45]

Statistical analysis
In order to ensure the validity of the statistical analysis, each experiment was run three times. The findings were presented as mean ± SD. The data sets created with the Statistical Package for Social Sciences (SPSS) version 16.0 underwent an ANOVA. p-values ≤ 0.05 were used to determine the significance of differences.

Results and Discussion

Anogeissus leiocarpus root bark phytochemical analyses
The phytochemical analysis result is shown in Table 2. There have been reports of hypoglycemic activity in plant elements such as alkaloids, flavonoids and related chemicals, glycosides, steroids, terpenoids, polysaccharides, and proteins.[46] It has been shown that flavonoids can reduce the risk of diabetic complications by acting as insulin secretagogues or insulin mimetics on their own, most likely via affecting pleiotropic processes. Additionally, it has been discovered that they increase the absorption of glucose in peripheral tissues and control the expression and/or activity of the rate-limiting enzymes in the pathway involved in the metabolism of carbohydrates. Because of this, bio-flavonoids are currently seen to be important and promising natural compounds that can improve the available treatment choices for diabetics.[47] Polyphenols have the potential to impact glycemia via many mechanisms, such as impeding the intestinal absorption of glucose or its assimilation by peripheral tissues. Moreover, saponins and tannic acid inhibit glucose transport mediated by S-glut-1. Furthermore, saponins postpone the passage of glucose from the stomach into the small intestine.[48] By preventing glucose from moving from the stomach into the small intestine and by blocking glucose transport at the small intestine's brush boundary, glycosides demonstrate their hypoglycemic action.[49] Given that *A. leiocarpus* ethanol, ethanol plus trona, and methanol root bark extracts include some of the above-mentioned phytoconstituents, it can be assumed that these phytoconstituents may be in charge of the plant extracts' antidiabetic effects.

Acute toxicity studies
No mortality was observed in mice after oral administration of the *Anogeissus leiocarpus* root bark ethanol extract, *Anogeissus leiocarpus* root bark ethanol plus trona extract as well as *Anogeissus leiocarpus* root bark methanol extract, even at doses as high as 5000 mg/kg signifying that the oral LD_{50} was greater than 5000 mg/kg for the two root bark extracts. The extracts did not produce any major clinical signs of toxicity in mice during a 4-day observation period. The result of acute toxicity test showed that *Anogeissus leiocarpus* root bark ethanol extract, *Anogeissus leiocarpus* root bark ethanol plus trona extract as well as *Anogeissus leiocarpus* root bark methanol extract each has a wide range of effective doses.
The absence of death following oral administration of each of the extracts at 5000 mg/kg b.wt. observed in the animals is indicative of no acute toxicity of each extract.[44] Its extensive application in various ethnotherapeutic therapies may have been facilitated by this. The authors selected the dose for the initial antidiabetic and formulation tests based on the acute toxicity data, nevertheless.

Table 2: Phytochemical analyses of *Anogeissus leiocarpus*

Constituents	AL root bark ethanol extract	AL root bark ethanol plus trona extract	AL root bark methanol extract
Flavonoids	+	+	+
Alkaloids	+	+	+
Saponins	+	+	
Tannins	-	-	+
Resins	+	+	+
Reducing sugar	+	+	+
Glycosides	+	+	+

Keys: (+) indicates presence, (-) Indicates absence, AL- *Anogeissus leiocarpus*

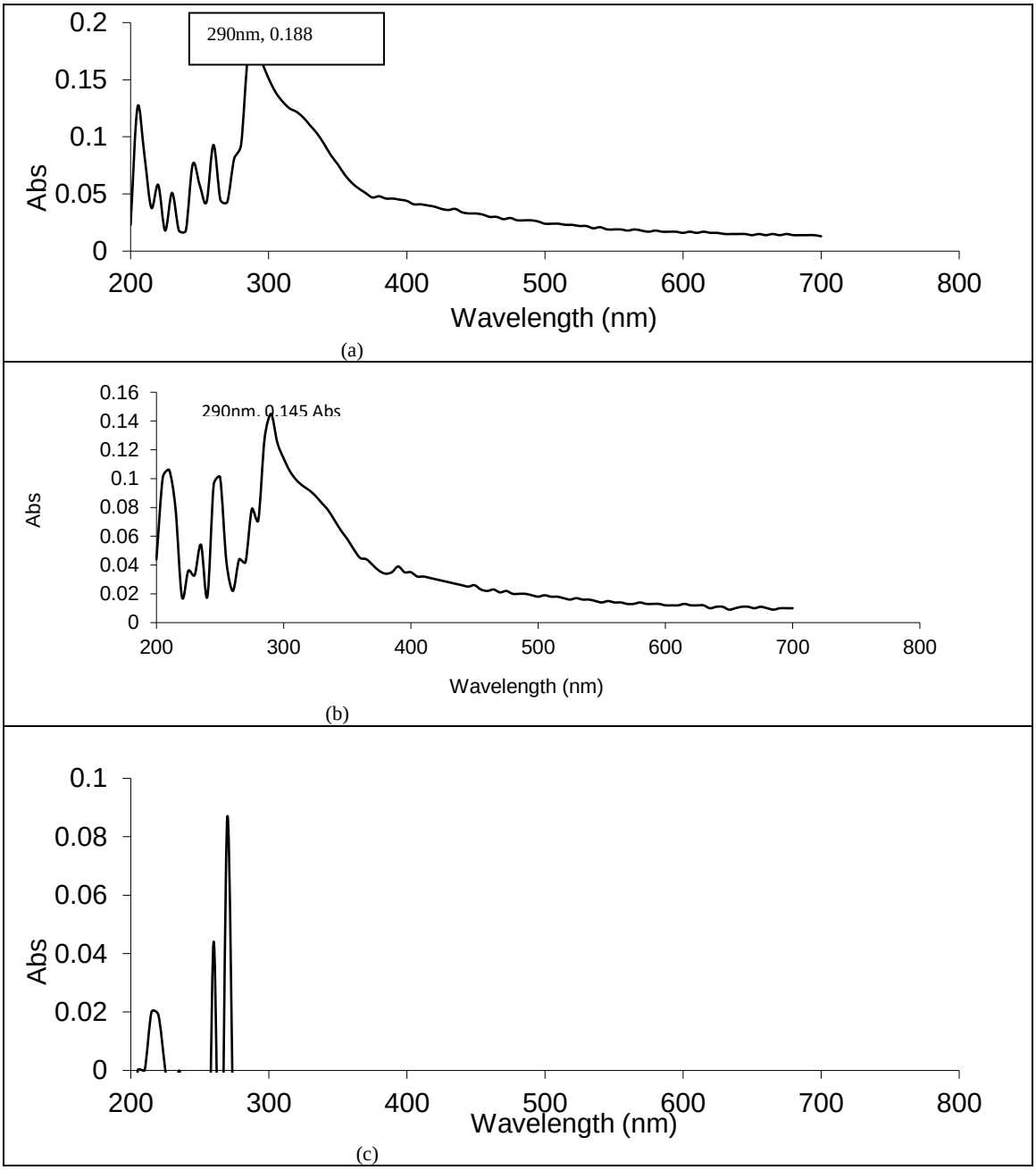

Figure 2: UV-Absorption spectrum of *Anogessius leiocarpus* root bark methanol extract in (a) water (pH 7.0), (b) SGF (pH 1.2) and (c) SIF (pH 6.8).

Spectral characterization studies
The absorption spectra of the methanol extract of *Anogeissus leiocarpus* root bark in water (pH 7.0), SIF (pH 6.8), and SGF (pH 1.2) are displayed in Figures 2 (a–c). In the release investigations of the extract-drug-loaded lipospheres, the maximum wavelength of absorption (λ_{max}) was utilized as a marker to ascertain the presence and content of the methanol plant extract.

pH measurements
Because it helps the formulation scientist choose the right ingredient and stabilizer for a given preparation, pH is a powerful predictor of the stability of pharmaceutical goods. The pH analysis would be used to evaluate the drug's shelf life as well as the excipients' characteristics of degradation. The results (Figure 3) showed that although the pH values measured after the lipospheres were prepared for 72 hours increased somewhat at the 2-week mark, they remained within the acidic range.
The sorbic acid employed as a preservative may be the cause of the formulations' minor pH increase during storage. The pK_a of sorbic acid is 4.8, and its antibacterial activity is limited to its acid forms. As a result, acid values are where it is most active. Sorbic acid is susceptible to light and air, which could account for the rise in pH levels during the fourth week of storage. Stability may be increased by chilling or the addition of an antioxidant. The plain lipospheres displayed a more stable pH with a negligible difference, while the glibenclamide-loaded lipospheres displayed a slight difference in pH that may have been caused by an interaction between the active pharmaceutical ingredient (API) and the formulation's excipients.

Particle size and morphological analyses
The particle size distribution of the lipospheres is presented in Table 3, while the morphological features of the lipospheres are depicted in Figure 4. The extract-loaded lipospheres had a mean particle size distribution of 135.00 ± 1.58 µm to 195.00 ± 2.24 µm, and the unloaded lipospheres had a mean particle size of 111.00 ± 3.18 µm. The lipospheres were spherical. As the amount of extract used in the formulation increased, a rise in particle size was noted. The average particle size of the 1% AL lipospheres was 135.00 ± 1.58 µm, the 2% AL lipospheres were 165.00 ± 23.67 µm, and the 3% AL lipospheres were 195.00 ± 2.24 µm on average. The average particle size of the glibenclamide-containing lipospheres, which served as the control, was 160.00 ± 21.08 µm.
All of the lipospheres' diameters fell between micrometers. Because of the increased viscosity of the dispersion created by the amount of lipid employed in preparation,[38] the average size of the particles increased as the fat to phospholipid molar ratio rose. This suggests that the particle size was affected by the amount of lipid and phospholipid included in the formulation. Particle size characteristics of lipospheres are significant because they impact the drug formulation's pharmacological, chemical, and physical properties, including pharmacokinetics, drug release from the matrix, and liposphere flow. The syringeability of lipospheres for parenteral delivery is similarly influenced by particle size.[35] The way topical treatments are absorbed via the skin is also influenced by particle size. The pace at which the medication is absorbed from the digestive system and the matrix
are influenced by the particle size in this formulation, which was designed for oral delivery.

Entrapment efficiency
Figure 5 displays the entrapment efficiency values of glibenclamide lipospheres (control) and methanol extract from the root bark of *Anogeissus leiocarpus*. The outcome demonstrates that the entrapment efficiencies of the lipospheres encapsulated with *Anogeissus leiocarpus* extract at concentrations of 1, 2, and 3%w/w were 22.53, 43.88, and 46.56%, respectively. This demonstrates that the amount of medication entrapped increases with the addition of AL extract. It is noticeable, nonetheless, that the entrapment efficiency has only increased by 3% from 2% to 3%, whilst the EE% has decreased from 1% to 2%.
The EE% results show that the medication that is entrapped increases with the amount of AL extract supplied. Increases in *A. leiocarpus* extract exceeding 3% in the liposphere formulation process led to instability and phase separation of the lipospheres, which may have been caused by system saturation, which can also result in separation of the drug melt and lipid melt.[35] This could be explained by the fact that the chemical and physical structure of the solid lipid matrix, the drug's solubility in melted lipid, and the drug's loading capacity in lipid carriers all affect these factors.[50] The preparation method, the drug's physicochemical characteristics, and the formulation variables all affect this parameter.[51] Furthermore, instability in the dispersions may also result from insufficient concentration of the stabilizers such as surfactants or a breakdown in its chemical structure and by extension its function.

In vitro drug release
The drug release profiles are displayed in Figures 6(a & b). The outcome demonstrates a moderate release of the drug that was entrapped in SGF and a highly significant *in vitro* release in SIF up to 100%. In the first 30 to 60 minutes of the release experiments, a burst effect was seen in both the SIF and SGF. The drug release profile in SIF demonstrated a burst effect at first, followed by a continuous release of the API to 100% for AL (1 %w/w). The initial burst effect and the extended release of the API (*Anogeissus leiocarpus* root bark methanol extract) were likewise observed in the release profiles of AL (2 %w/w) and AL (3 %w/w).
In SIF media, glibenclamide lipospheres exhibited the similar trend. The kind of the dissolving media affected the drug release from the lipospheres. The drug release in SIF was 100% but in SGF it was less than 50%, indicating this. The release profile of the extract-loaded lipospheres in AL (3%), however, showed plateaus and spikes.

Table 3: Mean particle size of the lipospheres.

Sample	Mean particle size (µm)
AL_1	135.00 ± 1.58
AL_2	165.00 ± 23.67
AL_3	195.00 ± 2.24
GL	160.00 ± 21.08
PL	111.00 ± 3.18

Keys: AL_1 refers to 1% w/w of *Anogeissus leiocarpus* root bark methanol extract, AL_2 refers to 2 % w/w, AL_3 refers to 3% w/w, GL refers to glibenclamide 1.6% w/w and PL refers to unloaded lipospheres

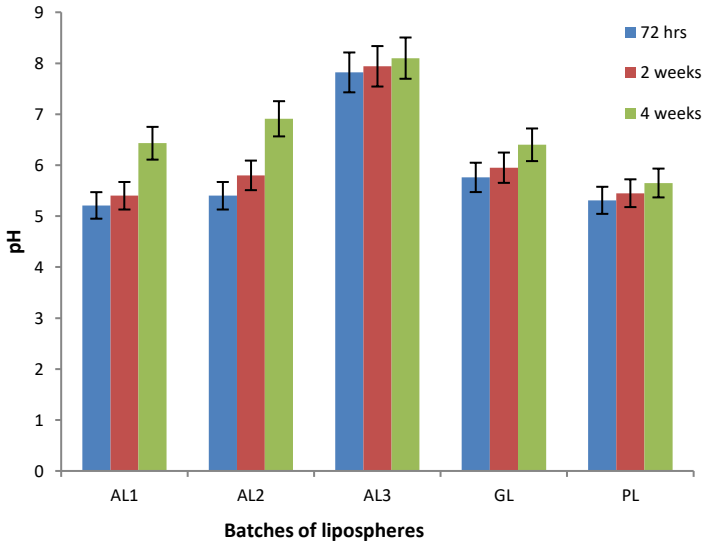

Figure 3: pH analyses of drug-loaded and unloaded lipospheres.
Keys: AL 1 refers to 1% w/w of *Anogeissus leiocarpus* root bark methanol extract, AL 2 to 2 % w/w, AL 3 to 3% w/w, GL to glibenclamide 1.6% w/w and PL to unloaded lipospheres.

In order to forecast the release of the extract or medication from the lipospheres in the gastrointestinal tract or lumen, the release profiles of the prepared *A. leiocarpus* methanol extract and glibenclamide lipospheres were assessed in SGF (pH 1.2) and SIF (pH 6.8) as release media. One possible explanation for the initial burst release is either the drug's rapid release from the surface or the direct exposure of the lipid matrix to the media.[52] The drug's therapeutic plasma concentration may be reached quickly with the help of the observed initial release, and the plasma concentration may be maintained for a longer amount of time after that. This aligns with the established benefits of lipospheres as a medication delivery technology with extended release.[30] The kind of dissolving medium affected the extract's release from the lipospheres, but it was not capacity-limited. This implies that the drug's release from the prepared lipospheres is facilitated by an alkaline environment. This might be explained by the drug accumulation in the dissolving solvent over time. Batch AL (3%) showed peaks and plateaus, which suggest that the drug release did not follow a regulated release profile.

Drug release kinetics and mechanism

The *in vitro* drug release study's outcomes were fitted into a number of kinetic equations, including Korsenmeyer-Peppas (log of cumulative percent drug released versus log time), Higuchi (cumulative percent released versus √t), and first order (log cumulative percent drug retained versus time). Table 4 presents the kinetic parameters. A comparison analysis of the R^2 in SGF (as indicated in Table 4) reveals that the release profile mostly adhered to Higuchi's model, while in SIF the release adhered to both Higuchi and Korsenmeyer-Peppas models.

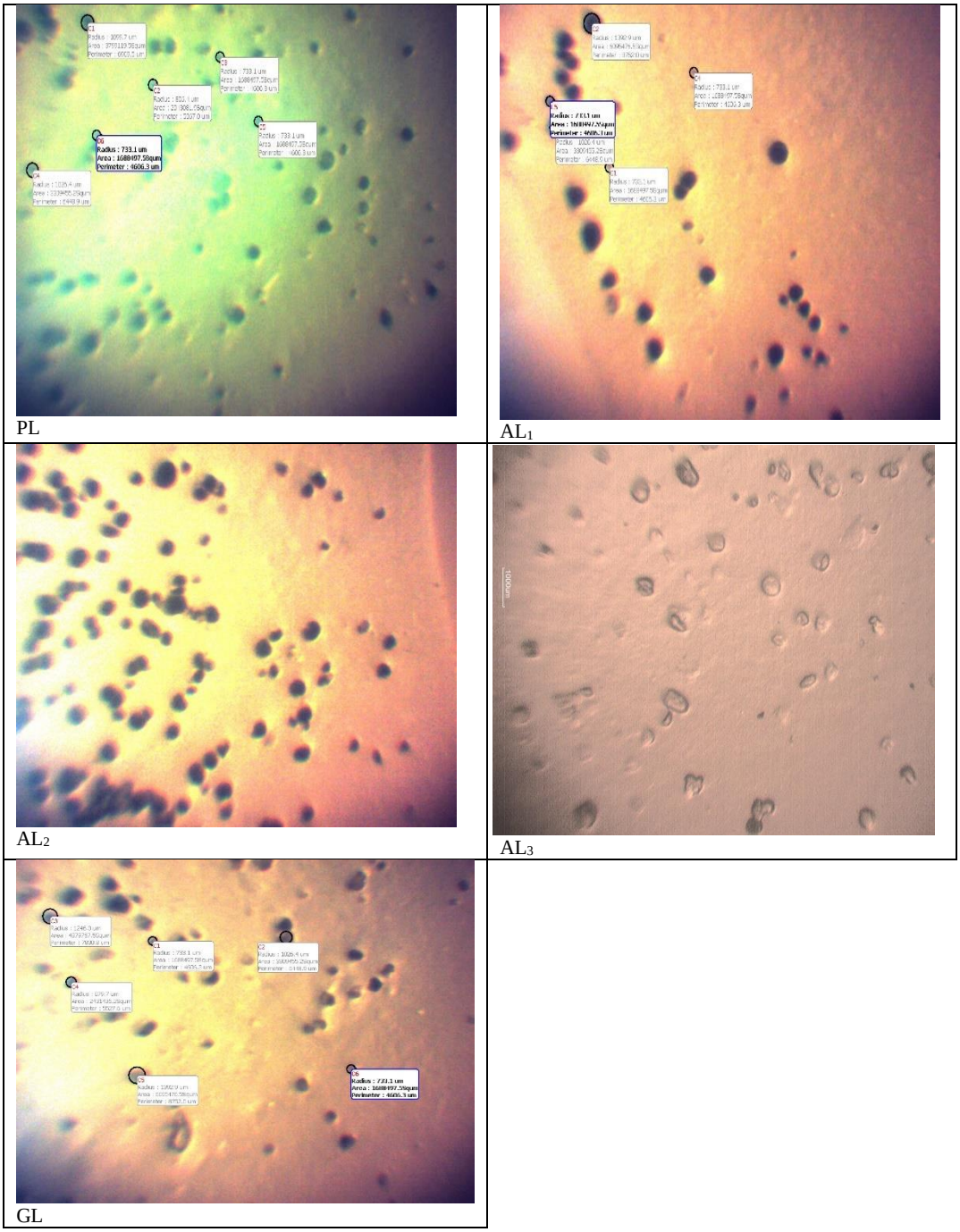

Figure 4: Photomicrograph of extract loaded lipospheres containing 1 % (AL_1), 2 % (AL_2) and 3 % (AL_3) of *Anogessius leiocarpus*; plain or unloaded lipospheres (PL) and glibenclamide-loaded lipospheres (GL) (Mag x 100).

Regression coefficient (R^2) values from the plots of different models were compared to determine which kinetic model best fit the drug release data. The release exponent, or "n" value, which indicates the mechanism of release, and the kinetic constant, or "K," which takes into account the geometric and structural properties of the release device, are used in the Peppas (Fickian diffusion) model to define drug release processes. Zero-order release kinetics (Case-II transport) are indicated by a "n" value of 1.0; an anomalous (non-Fickian) diffusion release model is indicated by a value of $0.48 < n < 1$; Fickian diffusion is indicated by a value of 0.48, and a super case II transport release is indicated by a value of $n > 1.0$.[45]

The method of drug release in SGF is diffusion, wherein the dissolving fluid penetrates the shell, dissolves the core, and seeps out through the interstitial channels or pores. The entire release was determined by the speed at which the drug dissolved in the dissolution medium, the speed at which the dissolved drug leaked out and dispersed from the surface, and the speed at which the dissolution fluid penetrated the liposphere wall.[53] The values of the release exponent, n, varied from 0.1 to 0.37, with $n \leq 0.48$, according to the examination of the release profile in SIF. This implies that the release of drug-loaded lipospheres (AL 1%, AL 2%, AL 3%, and GL) was significantly influenced by Fickian diffusion. Drug release by diffusion is one of the processes of drug release from the extract or drug-loaded lipospheres, according to examination of the release profile, which also shows that the Korsenmeyer-Peppas model was also followed.

Assessment of antidiabetic activity

The results of the first antidiabetic evaluation of the methanol (MeAL), ethanol (EtAL), and ethanol plus trona [Et(T)AL] extracts from the root bark of *Anogeissus leiocarpus* are displayed in Figure 7(a). Their effect on the blood glucose levels of diabetic albino rats was assessed during a 24-hour period. Their effect on the blood glucose levels of diabetic rats was assessed during a 24-hour period. All extracts showed a maximum drop in blood glucose up to the 12th hour, while the Et(T)AL and EtAL extracts showed an increase in blood glucose after that. In comparison to EtAL root extract, which provided a 65% decrease in the preliminary blood glucose level, 25% for Et(T)AL, 62.4% for MeAL, and 80% for glibenclamide, GL, diabetic albino rats' preliminary blood glucose levels were reduced over the course of 24 hours by MeAL extract and glibenclamide. The complete set of drug-loaded lipospheres showed a significant ($p < 0.05$) decrease in blood glucose levels (Figure 7(b), which was dose and treatment time related. The findings showed that the groups receiving lipospheres containing 1, 2, and 3 mg% of AL root bark methanol extract, respectively, experienced a reduction in blood glucose levels of around 34.5, 56.5, and 62.9% percentage at 8 hours after the formulations containing AL were administered. At two hours, batch AL(3%) formulation showed a substantial ($p<0.05$) anti-hyperglycemic impact that was equivalent to the antihyperglycemic effect of glibenclamide, the standard medication. Unloaded lipospheres preparation (PL) (serving as negative control) showed a general increase in the blood glucose level.

The extracts' effectiveness was assessed by calculating the percentage decrease in initial glycemia. The initial glucose level, or mean blood glucose baseline, was used as the 100% level, with subsequent levels being determined by the initial basal blood glucose level. The lipospheres were formulated using methanol root bark extract (MeAL), which demonstrated a prolonged decrease of the diabetic rats' initial blood glucose levels comparable to that of glibenclamide (positive control). *Anogeisus leiocarpus* root bark extract was used to make the lipospheres, and their efficacy was evaluated by measuring how well they were able to lower blood glucose levels in diabetic rats that had been given alloxan.[24]

The amount of the herbal medication released from the lipospheres delivery system and absorbed into the systemic circulation was demonstrated by the percentage reduction of the initial glucose level,[45] which in turn caused the blood glucose to drop. Overall, the doses utilized in this investigation were based on the weight of the animals, and the oral administration of conventional glibenclamide and normal saline (or unloaded lipospheres) served as positive and negative controls, respectively.

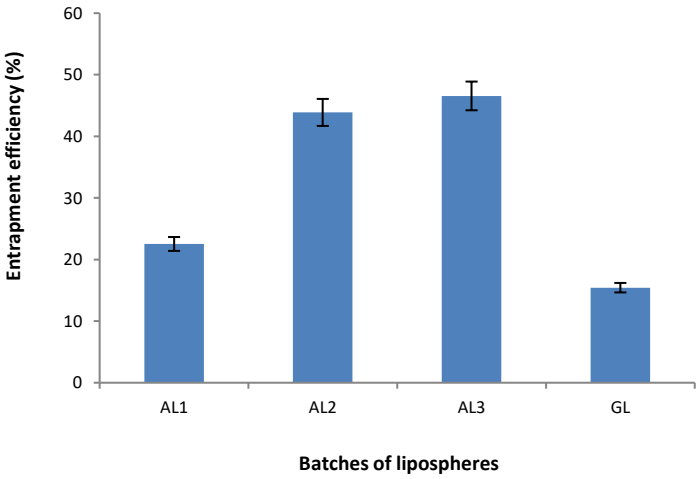

Figure 5: Entrapment efficiency of the drug-loaded lipospheres. Keys: AL_1 refers to 1 % w/w of *Anogeissus leiocarpus* root bark methanol extract, AL_2 to 2 % w/w, AL_3 to 3 % w/w, and GL to glibenclamide 1.6% w/w lipospheres.

Table 4: Kinetics of drug release from the lipospheres in SGF and SIF

Sample	Media	Zero order R^2	First order R^2	Higuchi R^2	Korsenmeyer-Peppas R^2	n
AL_1		0.6449	0.3251	0.5888	0.4000	0.3
AL_2	SGF	0.4002	0.3875	0.6912	0.2000	-0.28
AL_3		0.7500	0.6495	0.9030	0.0400	-0.15
GL		0.8395	0.7193	0.9464	0.2120	-0.03
AL_1		0.1185	0.6130	0.9336	0.9336	0.16
AL_2	SIF	-0.069	0.5383	0.8847	0.7889	0.15
AL_3		0.2860	0.5013	0.78889	0.9018	0.10
GL		0.5388	0.8091	0.8461	0.8461	0.37

Keys: AL_1 refers to 1 % w/w of *Anogeissus leiocarpus* root bark methanol extract, AL_2 to 2 % w/w, AL_3 to 3 % w/w, and GL to glibenclamide 1.6% w/w lipospheres

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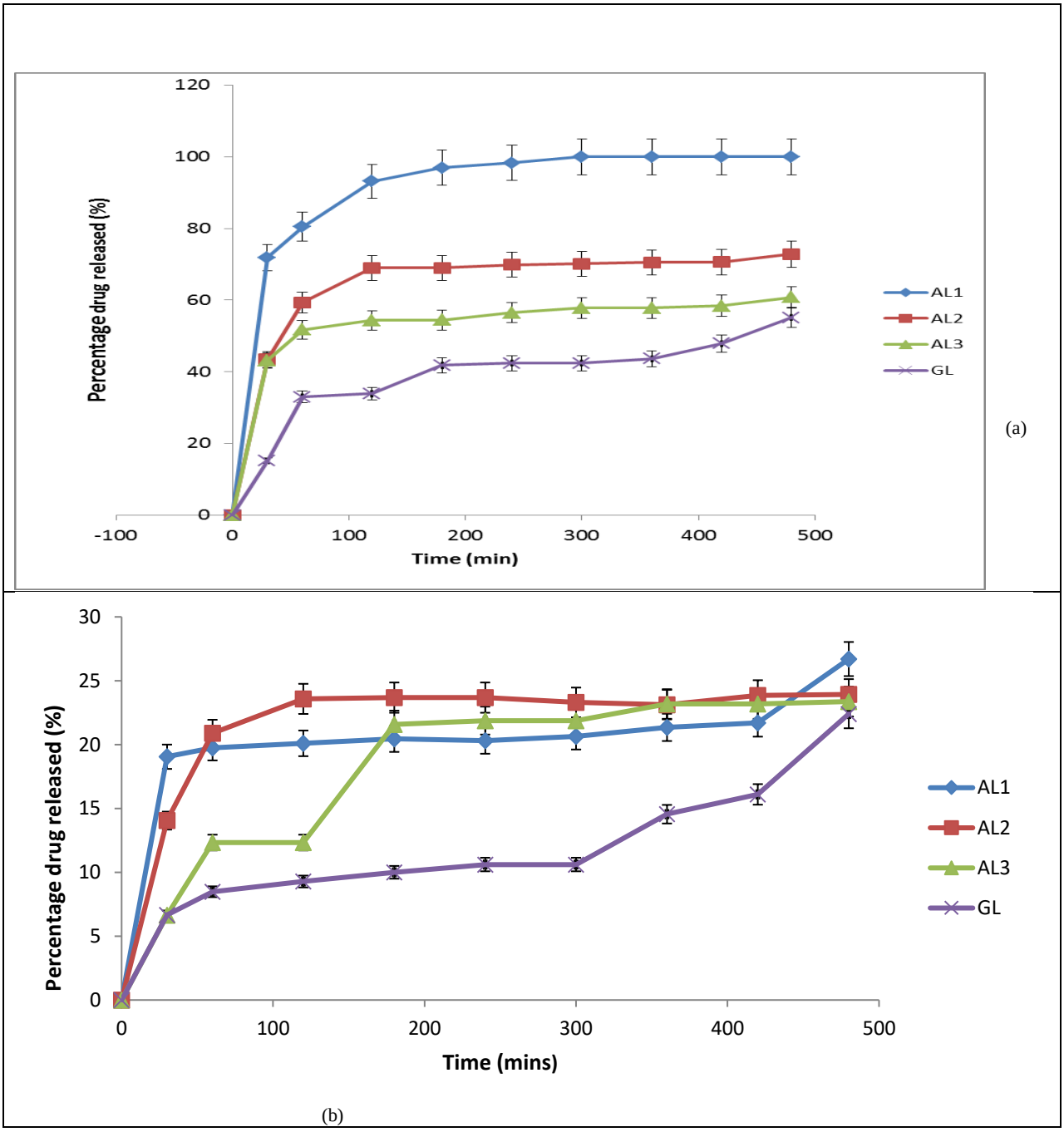

Figure 6: *In vitro* drug release profiles of the loaded liposphereres in (a) SIF (pH 6.8) and (b) SGF (pH 1.2).
Keys: AL_1 refers to 1 % w/w of *Anogeissus leiocarpus* root bark methanol extract, AL_2 to 2 % w/w, AL_3 to 3 % w/w, and GL to glibenclamide 1.6 % w/w liposphereres.

The results show that all of the formulations with AL loaded significantly lower blood glucose. Stated differently, different batches of AL-loaded liposphereres successfully reduced the rats' fasting blood glucose levels. The formulations demonstrated varied degrees of blood glucose decrease, however there was an initial increase in the first hour following batch AL (2%) treatment. This increase may have been caused by the animals receiving the batch being under stress during the liposphere administration.[11] In other animal groups that got different samples, this early increase may have been concealed by a more effective drop in blood glucose levels. The data also show that, with the exception of batch AL (2%) the glucose decrease effect occurred quickly, between 0.5 and 1 hour following oral administration of the formulations. This action's potential cause could have something to do with the drug or bioactives that were trapped and released early, adhering to the liposphereres' surface through the burst effect. Furthermore, the unloaded formulation and NS-treated groups did not show any evidence of a glucose-lowering impact; on the contrary, their blood glucose levels remained elevated for the duration of the trial. This is because neither the loaded liposphereres nor the NS contain any medication or bioactive. This suggests that the antidiabetic principle was actually released by the extracts, the standard drug, and the AL-loaded liposphereres. This suggests that the release of the antidiabetic principle from the extract, pure drug, or formulation stimulated the production of insulin from the Langerhans islet cells, which is why the animal groups that received these samples showed an observed reduction in glucose levels. Given the many side effects associated with glibenclamide, such as potentially fatal hypoglycemia, kidney damage, cholestatic jaundice, agranulocytosis, aplastic anemia, hemolytic anemia, gastrointestinal symptoms, and allergic skin reactions, among others[2], methanol extract-loaded liposphereres derived from the root bark

of *Anogeissus leiocarpus* are a superior choice for the treatment of diabetic mellitus due to their similar anti-hyperglycemic activity. Additionally, the primary cause of the anti-hyperglycemic actions seen in this investigation is the samples' stability in the stomach's acidic environment.[54] It is noteworthy that the evaluation of the antidiabetic properties of the root bark of this plant, as well as the formulation of its lipospheres, was prompted by the global socio-economic and health implications of this serious disease (diabetes), in addition to the possibility of using various parts of ethnomedical plants, like AL, in the treatment of diabetes. The study's findings supported the ethnomedical claim that the root bark of AL has antidiabetic potential. They also showed that the integrity and antidiabetic qualities of the extract were preserved during the creation of lipid-based formulations like lipospheres, which encouraged the formulation's continued development. Previous studies have shown that lipid-based excipients can influence oral absorption via various physiological effects, such as retarded gastric emptying,[43] stimulating bile flow and secretion of pancreatic juice, increasing the membrane lipid fluidity or acting directly onto enterocytes-based drug transport and disposition,[55] and one or more of these could account for the mechanism of the anti-hyperglycemic effect of AL in this study. A recent study also suggested that the release of the herbal antidiabetic drug from a lipospheres delivery system stimulated the production of insulin from islets cells of Langerhans.[45]

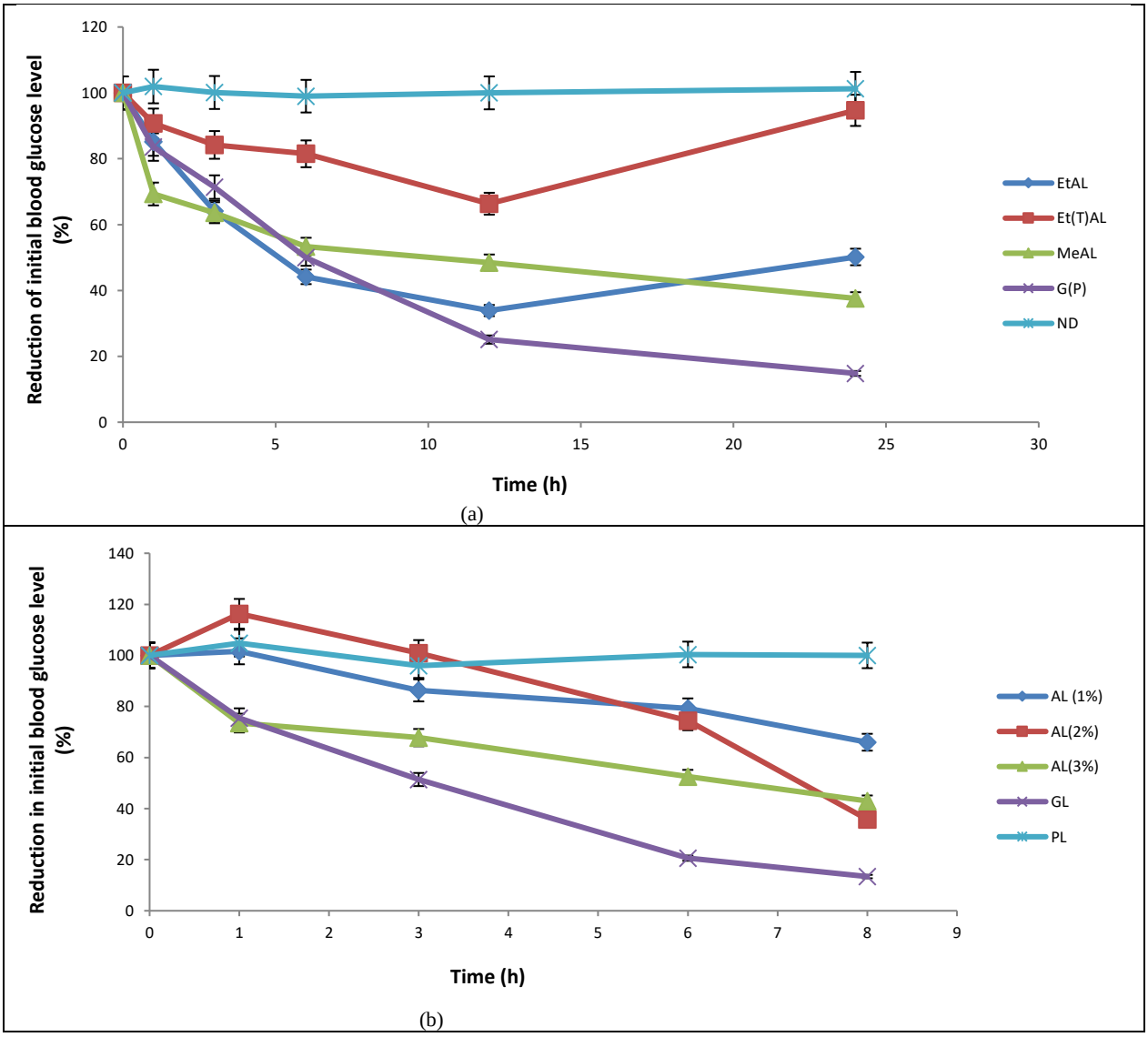

Figure 7: Percentage reduction of initial blood glucose level of diabetic albino rats treated with (a) *Anogeissus leiocarpus* extracts and (b) drug-loaded lipospheres.

Keys: EtAL represents ethanol *Anogeissus leiocarpus* extract; Et (T)AL, ethanol plus trona *A. leiocarpus* extract, MeAL, methanol *A. leiocarpus* extract; G(P), glibencalmide; ND, no drug); AL_1 refers to 1 % w/w of *Anogeissus leiocarpus* root bark methanol extract, AL_2 to 2 % w/w, AL_3 to 3 % w/w, GL to glibenclamide 1.6% w/w and PL to unloaded lipospheres.

Conclusion

When it comes to improving the bioavailability and efficacy of poorly water-soluble medications, lipospheres offer a potentially effective lipid-based carrier system. In this work, the root bark of *Anogeissus leiocarpus*, which has historically been used to treat diabetic mellitus, was formulated into lipospheres, a novel drug delivery system, for better management of diabetes mellitus. Different extracts of the plant's root bark were scrutinized pharmacologically in order to substantiate the veracity of ethnomedical claim of their antidiabetic potentials. The methanol extract was further developed into lipospheres, a novel drug delivery system that has demonstrated great capability to improve bioavailability and efficacy of various drugs and bioactives, based on positive results obtained with the preliminary anti-hyperglycemic evaluation. This was done in order to assess the effect of using this novel carrier on the antidiabetic efficacy of this plant extract. The lipospheres were characterized after being created using 30% w/w of

beeswax in 70% w/w of Phospholipon® 90H as the lipid foundation. The results indicated the production of stable, primarily spherical lipospheres with good physicochemical properties. Diffusion and dissolution were the main processes of API release, according to drug release and kinetics. Pharmacodynamic assessment of the antidiabetic impact in rats demonstrated a noteworthy decrease in blood glucose levels, akin to the outcome achieved with glibenclamide. Considering the side effects associated with conventional antidiabetics, including glibenclamide, AL-loaded lipospheres formulation developed in this study portends safer and cheaper alternative for the management of diabetes mellitus. Thus, this study has demonstrated that, in order to enhance the therapeutic benefits of drugs, plant materials with low hydrophilicity can be formed into lipospheres as a drug delivery vehicle.

Conflict of Interest

The authors declare no conflict 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. Ahmed I, Goldstein B. Diabetes mellitus. Clin Dermatol. 2006;24: 237 – 246.
2. Koda-kimble MA, Young YL, Allderdge BK, Corelli LR, Guglielmo JB, Kradjan WA, Williams BR. Applied therapeutics: The Clinical Use of Drugs. 9thedition, Lippincott Williams and Wilkins, 50.5-50.35, (2009).
3. Owens DR. New horizons—alternative routes for insulin therapy. Nat Rev Drug Discov. 2002;1: 529 – 40.
4. Evans M, Schumm-Draeger PM, Vora J, King AB. A review of modern insulin analogue pharmacokinetic and pharmacodynamic profiles in type 2 diabetes: improvements and limitations. Diabetes Obes Metab. 2011;13: 677–84.
5. Akhter DT, Nijhu RS. Diabetes mellitus: a journey of insulin. Int Curr Pharm J. 2012;1: 32–42.
6. Harris MI. Diabetes in America. National Institute of Health, National Institute of Diabetes, and Digestive and Kidney Diseases. NIH (95-1468) 2nd edition. New York, pp.631–659, (1995).
7. World Health Organization (WHO). General guidelines for methodologies on research and evaluation of traditional medicines. World Health Organization, Geneva, Switzerland, (2002).
8. Morishita M, Peppas NA. Is the oral route possible for peptide and protein drug delivery? Drug Discov Today. 2006;11: 19–20.
9. Pamnani D. Reality check on oral insulin. Pharma Express. 2008;3: 16 – 31.
10. Pavlakis M, Khwaja K. Pancreas and islet cell transplantation in diabetes. Curr Opin Endocrinol Diabetes Obes. 2007;14: 146.
11. Momoh MA, Kenechukwu FC, Nnamani PO, Umetiti JC. Influence of magnesium stearate on the physicochemical and pharmacodynamic characteristics of insulin-loaded Eudragit entrapped mucoadhesive microspheres. Drug Deliv. 2015;22(6): 837 – 848.
12. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. New Engl J Med. 2007;356: 2457 – 2471.
13. Ajali U, Okoye FBC. Antimicrobial and anti-inflammatory activities of *Olax viridis* root bark extracts and fractions. Int J Applied Res Nat Prod. 2009;2(1): 27–32.
14. Dahanuka SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol. 2002;2: 508–512.
15. Iwu MW, Duncan AR, Okunji CO. New antimalarials of plant origin. In: Janick J, ed. Perspective on new crops and new uses. Alexandria, VA: ASHS Press, pp.457–462, (1999).
16. Robbers J, Speedie M, Tyler V. Pharmacognosy and pharmacobiotechnology. Baltimore:Williams and Wilkins. pp.1–4, (1996).
17. Venkidesh R, Dilipkumar P, Ashok KCK, Saravanakumar A, Subhash C. Studies on antidiabetic potential of *Symplocos racemosarox* bark extract in streptozotocin induced diabetic rats. Int J Phytopharmac Res. 2012;3(1): 1 – 5.
18. Patiel A, Nirmal S, Pattan S, Tambe V, Tare M. Antidiabetic effect of polyherbal combinations in streptozotocin-induced diabetes involve inhibition of α-amylase and α-glucosidase with amelioration of lipid profile. Phytopharmacol. 2012;2(1): 46 – 57.
19. Kumudhavalli MV, Jaykar B. Evaluation of antidiabetic activity of *Costus igneus* leaves on streptozotocin induced diabetic rats. Der Pharmacia Sinica. 2012;3 (1): 1-4.
20. Nina A, Taufik F, Akhmad D. Bioactivities evaluation of indonesian mistletoes (*Dendrophthoe pentandra* (L.) Miq.) leaves extracts. J Appl Pharm Sci. 2012;2(1): 24–27.
21. Mizanur SK, Islam R, Rahman S, Mosaiah T, Rasheda A, Khatun T, Dilruba N, Nahar N, Ahsan S, Rahmatullah M. Antihyperglycemic studies with methanol extract of *Annon areticulate* L. (Annonaceae) and *Carissa carandas* L. (Apo cynaceae) leaves in swiss albino mice. Adv Nat Appl Sci. 2011;5(2): 218 – 222.
22. Lanjhiyana S, Garabadu D, Ahirwar D, Bigoniya P, Rana AC, Patra CK, Lanjhiyana S K, Karuppaih M. Antidiabetic activity of methanolic extract of stem bark of *Elaeodendron glaucum* in alloxanized rat model. Adv Appl Sci Res. 2011;2(1): 47-62.
23. Priya RM, Krishna SM, Padmakumari KP, Raghu GK, Sundaresan A. *Zingiber officinale* extract exhibits antidiabetic potential via modulating glucose uptake, protein glycation and inhibiting adipocyte differentiation: an *in vitro* study. J Sci Food Agric. Abstract, 2012;10.1002.
24. Momoh MA, Chime SA, Kenechukwu FC. Novel drug delivery system of plant extract for the management of diabetes: An antidiabetic study. J Diet Suppl. 2013;1-12. DOI:10.3109/19390211.2013.822454.
25. Mann A, Barnabas BB, Daniel II. Phytochemical and antibacterial screening of *Anogessius leiocarpus* against some microorganisms assiociated with infectious wounds. Afri J Microbiol Res. 2008;2: 60 – 62.
26. Ibrahim MB, Owonubi MO, Onaopo JA. Antibacterial effect of extract leaf, stem and root bark of *Anogessius leiocarpus* on some bacterial organism. J Pharm Res Dev. 1997;2(1): 20-23.
27. Saraf AS. Applications of novel drug delivery system for herbal formulations. Fitoterapia. 2010;81: 680 – 689.
28. Esposita ER, Cortesi SJ, Nastruzzi C. Production of lipospheres for bioactive compound delivery. In: Claudio N, ed. Lipospheres in drug targets and delivery: Approaches, methods and applications. Florida: CRC press, pp.23-40, (2007).
29. Domb AJ, Bergelson L, Amselem S. Lipospheres for controlled delivery of substances. In: Benita S, ed. Microencapsulation: Methods and industrial applications. New York: Marcel Dekker, p.377, (1996).
30. Bekerman T, Golenser J, Domb A. Cyclosporin nanoparticulate lipospheres for oral administration. J Pharm Sci. 2004;93: 1264–1270.

31. Domb A. Lipospheres for controlled delivery of substances. In: Benita S, ed. Microencapsulation: Methods and industrial applications. 2nd ed. Boca Raton: Taylor and Francis, pp.297–316, (2006).
32. Mangenheim B, Levy MY, Benita S. An *in vitro* technique for evaluation of drug release profile from colloidal carriers – ultrafiltration technique at low pressure. Int J Pharm. 1993;94: 115 – 120.
33. Singh RM, Singh D, Swarnlata S. Development and *in vitro* evaluation of polar lipid based lipospheres for oral delivery of peptide drugs. Int J Drug Deliv. 2009;1: 15 – 26.
34. Domb AJ, Maniar AB, Manoj MD. Liposphere for controlled delivery of substances. European Patent. EP0502119, (1996).
35. Chime SA, Kenechukwu FC, Onunkwo GC, Attama AA, Ogbonna JDN. Recent advances in lipospheres drug delivery system. J Pharm Res. 2012;5(3): 1743 – 1748.
36. Attama AA, Okafor CE, Builders PF, Okorie O. Formulation and *in vitro* evaluation of a PEGylated microscopic lipospheres delivery system for ceftriaxone sodium Drug Deliv. 2009;16: 448 – 616.
37. Kenechukwu FC, Momoh MA, Nnamani PO, Ogbonna JDN, Umeyor CE, Attama AA. Improved bioactivity of gentamicin from novel solid lipid microparticles based on beeswax. Nig J Pharm Res. 2014;10(1): 35-45.
38. Attama AA, Muller-Goymann CC. Effect of beeswax modification on the lipid matrix and solid lipid nanoparticle crystallinity. Colloids Surf A: Physicochem Eng Aspects. 2008;315: 189-195.
39. Porter JH, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6: 231 – 48.
40. Phospholipid. Phospholipon® 90H. Phospholipid GmbH, Nattermannallee, American lecithin company, (2007).
41. Hitesh RP, Rakesh PP, Patel MM. Poloxamers: A pharmaceutical excipient with therapeutic behaviors. Int J PharmTech Res. 2009;2: 299 - 303.
42. Barbosa CMS, Morais HA, Deivivo FM, Mansur HS, Oliveira MCD, Silvestre MPC. Papain hydrolysates of casein: Molecular weight profile and encapsulation in lipospheres. J Sci Food Agric. 2004;84: 1891 – 1900.
43. Trease GE, Evans MC. Textbook of Pharmacognosy. 12th edition, London: BailliereTindall, London. pp.343 – 383, (1983).
44. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54: 275 – 287.
45. Chime SA, Onyishi IV, Attama AA, Onunkwo GC, Ajaraonye MC. Lipospheres: A potential delivery system of herbal extract for the treatment of diabetes mellitus. Am J PharmTech Res. 2013;3(4): 479 – 491.
46. Lambia SS, Buch KY, Lewis JR. Phytochemicals as potential hypoglycemic agents. Studies Nat Prod Chem. 2000;21: 457 – 479.
47. Goutam B. Bio-flavonoids with promising antidiabetic potentials: A critical survey. Opportunity, challenge and scope. Nat Prod Chem. ISBN: 978-81-308-0448-4, pp.187-212, (2011).
48. Kanti BP, Syed IR. Plant polyphenol as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5): 270 – 278.
49. Matsuda H, Yuhao LI, Murakami T, Matsumura N, Yamahara J, Yoshikawa M. Antidiabetic principles of natural medicines. III. Structure-related inhibitory activity and action mode of oleanolic acid glycosides on hypoglycemic activity. Chem Pharm Bull. 1998;1399-1403.
50. ZurMuhlen A, Scwarz C, Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery: drug release and release mechanism. Eur J Pharm Biopharm. 1998;45: 149 – 155.
51. Kenechukwu FC, Attama AA, Ibezim EC, Nnamani PO, Umeyor CE, Uronnachi EM, Gugu TH, Momoh MA, Ofokansi KC, Akpa PA. Surface-modified mucoadhesive microgels as a controlled release system for miconazole nitrate to improve localized treatment of vulvovaginal candidiasis. Eur J Pharm Sci. 2018;111: 358 – 375.
52. Ram CD. Variables affecting the drug entrapment efficiency of microspheres: A pharmaceutical review. Pharmacia Letter. 2010;2(5): 102-116.
53. Singh MN. Microencapsulation: A promising technique for controlled drug delivery. Res Pharm Sci. 2010;5(2): 65 – 77.
54. Hauss DS. Oral lipid-based formulations. Adv Drug Del Rev. 2009;57: 667 – 76.
55. Guruswami S, Kumar V, Mishra DN. Characterization and *in vitro* dissolution studies of solid systems of valdecoxib with chitosan. Chem Pharm Bull. 2006;54: 1102–6.