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NANO-ENCAPSULATION OF *Gongronema latifolium* (UTAZI) LEAF EXTRACT USING PLURONIC F-127 FOR TREATMENT OF DIABETES

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ABSTRACT

Diabetes is a debilitating disease placing a huge burden on the body as a result of its inability to break down glucose. In this study, Gongrenema latifolium (Utazi) leaf were air-dried, macerated with ethanol, and encapsulated with pluronic F-127. Standard analytical methods were used for the quantitative phytochemical analysis, Uv-Visible spectrophotometry was used to determine the time-release profile of the encapsulated G. latifolium leaf extract and metformin (a standard-anti-diabetic drug) at 295 nm. FTIR, SEM, TEM, BET and EDX analysis were conducted on the encapsulated particles to establish its surface area, morphology and elemental composition. The results of the phytochemical analysis indicated that tannin (22.001% ± 4.712), was contained highest in the plant leaf, followed by flavonoids (13.011% ± 0.611), cardiac glycoside (11.912% ± 0.911), and the least was oxalate (0.194mg/100 ± 0.202). The time-release profile at 0.5h, 1h, 2h, 3h and 4h released (0%, 4.80%, 18.50%, 30.60% and 40.30% respectively of the encapsulated G. latifolium leaf extract), and 0%, 7.20%, 18.40%, 30.60% and 40.30% respectively of the metformin, into the body at equal rates and quantities. FTIR results showed C≡C or C≡N bond stretching, 1647.77 cm^{-1} of C=O stretching, 1540.06cm^{-1} of N-H bending and C-N stretching vibrations for amides in alkaloids, and 875.13cm^{-1} of C-H out of plane bending. The presence of these peaks confirmed the successful encapsulation of the G. Latifolium leaf extract components by the pluronic F-127 without altering the chemical compositions. The FTIR spectra for the metformin showed peaks for alcohol (OH stretch at 3666.99cm^{-1}), amines and amides (NH symmetric stretch at 3126.00cm^{-1}), sulfonamide (SO_2 symmetric stretch at 1189.54cm^{-1}), sulfoxide (S=O stretch at 1057.41cm^{-1}), aldehydes (CHO bending at 2738.18cm^{-1}. The SEM and TEM results gave a monograph of a good interconnectivity of the G. latifolium leaf extract and pluronic F-127 as a result of good granular porosity with mesoporous pore sizes of 2.00 nm ~ 5.80 nm with an average of 15.6 nm particles size clustered with fainted thin layers of neighboring materials. BET results indicated 821.431 m^2/g surface area, 0.65400 cc/g pore volume and 27.4210 nm pore diameter. EDX results gave C, (52.16%, 5.90%), Na, (3.01%, 0.29%), K, (0.26%, 0.35%), Ca, (4.00%, 2.40%), and Si, (1.00%, 47.90%) respectively for encapsulated G. latifolium leaf extract and metformin. Fe, (21.09%) and N, (0.70%) were seen in the encapsulated G. latifolium leaf extract while Ba, (7.90%), P, (1.10%) and Al, (11.70%) respectively were seen in the metformin. The significance of the results is that pluronic F-127 encapsulated G. latifolium leaf extract has good surface activity, effects, adsorption capacity and surface area for drug adsorption and delivers anti-diabetic component at same rate with the conventional drug.

Keywords: Diabetes, Nano-encapsulation, *Gongronema latifolium* (Utazi) Leaf, Pluronic F-127,

Introduction

Diabetes mellitus is a health problem associated with high level of glucose in the blood prompting metabolic issues, and this can lead to health complications when the glucose level is not properly managed. There is an alarming increase of diabetes disease in Nigeria, (Ponnusamy *et al.*, 2011; Sy *et al.*, 2005; Papathodorou *et al.*, 2018). Types 1 and 2 diabetes, (Nall, 2021), are the major types of diabetes. In Nigeria, in 2018, diabetes mellitus prevalence was 3.7%, (Diabetes in Nigeria, 2016). When grouped into the geopolitical zones of Nigeria, it provides that 3.0%, 5.9%, 3.8%, 5.5%, 4.6%, and 9.8% of the people are suffering from diabetes in north-west, north-east, north-central, south-west, south-east and south-south respectively, (Uloko *et al.*, 2018), amounting to about 4.7 million Nigerians. It is projected that by 2045, 55 million people would be suffering from diabetes mellitus in Africa, (Ajikobi, 2018). The management of this disease is usually done by the use of synthetically produced drugs. Saxena, and Vikram, (2004), identified insulin and oral anti-diabetic drugs such as sulfonylurea, biguanides and glinides as some of the therapies to manage diabetes. But most of these drugs have damaging effects, (Lee, 2002). And the toxicity, severe side effects, cost, and poor delivery of these drugs raises serious concerns in the treatment regime, (Tiwari *et al.*, 2012; Patra *et al.*, 2018). A range of drugs of plant origin are effective due to their potency as established by scientific materials, (Petrovska, 2012). Nigeria has about 10,000 species of traditional medicinal plants due to good climatic conditions and arable land. One benefit about medicinal plants is that their metabolites and active constituents complements each other's effectiveness for treatment of a particular disease, (WHO, 1996). In this regard, a common medicinal plant used to manage diabetes in Nigeria is *Gongronema latifolium* (*G. latifolium*). *G. latifolium* belong to the family of *Asclepiadaceae.* It is called 'Utazi' in Igbo language, 'Utasi' in Efik and 'Arokeke' in Yoruba languages, (Olufunke, 2021). The phytochemical contents of the leaf include; essential oils, saponins, amino acids, alkaloids, flavonoids etc, (Schneider *et al.*, 1993; Morebise, and Fafunso, 1989; Morebise *et al.*, 2002; Morebise, 2015). *G. latifolium* has anti-diabetic, antimicrobial, antioxidant, anticancer, and anti-inflammatory properties, (Ugochukwu *et al.*, 2003; Ogundipe *et al.*, 2003; Imo and Uhegbu, 2015; Chauhan, 2010). However, the effectiveness of *G. latifolium* is hindered by its bioavailability, controlled release to targeted sites and stability of the bioactive constituents. Nanoparticles gives an excellent approach to these setbacks by encapsulating the leaf extract of *G. latifolium* to improve the therapeutic efficiency of the bioactive contents in the plant leaf. The application of pluronic F-127, a biodegradable and biocompatible polymer as a delivery system provides various benefits including; protecting the active components from degradation, and ensuring a targeted, safe and efficient therapeutic time release in the body, (Sundar *et al.*, 2010; Ottenbrite, and Javan, 2005; Cai *et al.*, 2022; Salama, 2021).

Experimental

One (1) kg of the fresh leaves of *G. Latifolium* were collected at the botanical garden of the department of Science Laboratory Technology, Federal Polytechnic, Oko. Orumba North L.G.A. Anambra State, Nigeria. The plant leaves were identified by Dr. U. A. Afugbem, a botanist in the department of Biology, Federal Polytechnic, Oko. The leaves were rinsed thoroughly using clean water, air dried at room temperature and ground into powder using electric blender.

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Quantitative Phytochemical Analysis of *Gongronema latifolium* Leaf

Alkaloid: Five (5g) gram of the sample was weighed and 200mL of 20% acetic acid in ethanol was added, and filtered using filter paper. Conc. ammonium hydroxide was added drop wise to the extract until it completely precipitated. The solution was allowed to settle, and the precipitate collected and filtered using a pre-weighed filter paper. The residue on the filter paper was dried in the oven at 80^0C and weighed.

$$\% \text{ weight of Alkaloid} = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper x } 100}{Weight\ of\ Sample\ Analyzed}$$

Flavoniods: Five (5) gram of the plant sample was extracted repeatedly with 100mL of 80% aqueous methanol at room temperature. The solution was filtered using filter paper, and the filterate transferred to a crucible and further evaporated to dryness with hot plate and weighed to a constant weight.

Saponin: Five (5g) gram of the sample was placed in 20% acetic acid in ethanol and allowed to stand for 24hours. This was filtered and the extract concentrated in a water bath to one quarter of the original volume. Conc. NH_4OH was added drop-wise to the extract until the precipitate was complete. The solution was allowed to settle and the precipitate collected using filtration and weighed.

$$\% \text{ weight of Saponin} = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper x } 100}{Weight\ of\ Sample\ Analyzed}$$

Tannin: Five **(**5) gram of the ground sample was added 100mL of petroleum either and covered for 24hours. The sample was filtered and allowed to stand for 15mins allowing petroleum ether to evaporate. It was re-extracted by soaking in 100mL of 10% acetic acid in ethanol for 4 hours. The sample was filtered and the filtered collected. 2mL of NH_4OH was added to the filtrate to precipitate alkaloid. The alkaloid was concentrated using water bath to remove NH_4OH in the solution. 5mL of the precipitated alkaloid was taken and 29mL of ethanol added to it. It was titrated with 0.1M NaOH using phenolphthalein.

$$\% \text{ weight of Tannin} = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper x } 100}{Weight\ of\ Sample\ Analyzed}$$

Cardiac Glycoside: Five **(**5) g of the sample was weighed and added 100mL of distilled water. It was allowed to stand for 3 hours and filtered. 2mL of the filtrate was added 1mL of 2% solution of dinitro-salicyclic acid in methanol and 1mL of 5% aq. NaOH. It was boiled for 2minutes to obtain brick-red precipitate, the weight of the filter paper noted and filtered. The filter paper with absorbed residue was dried in an oven at 105^0C till dryness and the weight residue was noted.

$$\% \text{ Cardiac Glycoside} = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper x } 100}{Weight\ of\ Sample\ Analyzed}$$

Terpenoids: Five (5) gram of sample was taken into a beaker and added 100mL of ethanol for 24 hours. It was filtered, added 10mL of petroleum ether and separated with separating funnel. The

extract was further separated in pre-weighed glass vials and dried (wf). Ether was evaporated and the yield (%) of the terpenoids noted.

Cyanogenic Glycoside: One (1) mL of methanol extract of the sample was placed in a 15mL test tube, and 0.5mL of linamarase and phosphate buffer at pH 7.0 were added. The mixtures was kept on a water bath at temperature of 37^0C for 30 minutes for incubation. 0.6mL of 0.2M NaOH was added into the mixture and kept for 5 minutes, and diluted with 2.8mL of phosphate buffer at pH 6.0. 0.6mL of barbituric acid was inserted, and kept for 20 minutes for colour development. Absorbance was taken at 600nm using UV-spectrophotometer.

Oxalate: Two (2) gram of the sample was placed in a beaker and added 190mL of distilled water, and 10mL of 6M HCl. The suspension was digested at 100^0C for 1 hour, cooled, and made up to 250mL, and filtered. 125mL of the filtrate were duplicated and 4 drops of methyl red was added. This was followed by adding NH_4OH solution (drop-wise) till the colour of the test solution changed from salmon pink to a faint yellow. The duplicate mixture was heated upto 90ºC, cooled and filtered. The filtrate was further heated to 90ºC and added 10mL of 5% $CaCl_2$ solution with continuous stirring, cooled and kept overnight at 25ºC, and the solution was centrifuged at 2500rpm for 5minutes. The filtrate was decanted, the precipitate completely dissolved in 10mL of 20% H_2SO_4 solution, and was made up to 300mL. Aliquots of 125mL of the filtrate was boiled and titrated against 0.05M standard $KMNO_4$ solution to obtain a faint pink colour which last for 30 seconds.

Oxalate = $\frac{(T)\times(Vme)(Df)(mg/100)}{(ME)\times Mf}$, Where T = titre value, Vme = volume-mass equivalent (i.e 1ml of 0.05M $KMN0_4$ solution is equivalent to 0.00225g anhydrous oxalic acid), Df = Dilution factor (2.4), ME = Molar equivalent of $KMNO_4$, Mf = Mass of sample used.

Extraction of *G. latifolium* Leaf Components for Nano-particles Synthesis

Maceration Method: Five hundred milliliter of 95% ethanol was poured into a flat bottom flask. 20g of the ground *G. latifolium* leaf was inserted into the flask, then stirred. The mixture was placed on magnetic stirrer, and inserted four glass beads and stirred for 5 minutes. The mixture was stirred for 5 minutes daily for 3days, and filtered with filter paper. The filtrate was placed into a conical flask and kept on a water bath at below 50^0C, while the ethanol evaporated.

Preparation of Nanoparticles Using *G. latifolium* Leaf Extract: Pluronic F-127 (1g) was dissolved in 50mL 1% (v/v) acetic acid solution. Leaf extract (0.25g) dissolved in 20mL of ethanol was added to the pluronic F-127 solution, and was stirred overnight. A small amount of sodium sulphate was added to the mixture to prevent aggregation of nanoparticles. The mixture was sonicated for 30 minutes to form uniform nanoparticles. The mixture was slowly added to a large volume of deionized water under constant stirring, this caused the nanoparticles to precipitate. The pH of the mixture was adjusted to neutral using a dilute NaOH and HCl solution. Centrifugation of the mixture was done at 10,000 rpm for 30 minutes afterwards the nanoparticles was collected. The nanoparticles were washed with deionized water to remove any unreacted materials and

surfactants. The centrifugation and washing steps were repeated for about 2-3 times. The purified nanoparticles were resuspended in a small volume of deionized water and the suspension was freezed using deep freezer.

Preparation of Nanoparticles Sample: Pluronic F-127 *G. latifolium* leaf nanoparticles (10mg) were dispersed in 10 mL of phosphate buffered saline; PBS (pH 7.4), placed in an incubator set at 37°C to simulate physiological conditions. After an initial sample temperature (T_0) was taking from the suspension for analysis to serve as a baseline. At predetermined intervals (0.5, 1, 2, 4, hours), a small aliquot (0.5 mL) was withdrawn from the suspension, centrifuged at a speed of 10,000 rpm for 10 minutes to separate the nanoparticles from the release medium. The filtrate containing the released flavonoids was collected. The absorbance of each collected filtrate was measured using a UV-Vis spectrophotometer at 295 nm.

Fourier Transform Infrared (FTIR) Analysis of Pluronic F-127 *G. latifolium* Leaf Extract
Two milligrams of pluronic F-127 *G. latifolium* leaf nanoparticles was mixed thoroughly with 100mg of dried potassium bromide (KBr) powder. The mixture was ground and compressed to form a transparent pellet. A drop of the pluronic F-127-*G. latifolium* leaf suspension was placed onto a KBr window and was allowed to form a thin film by evaporation. And measured in FTIR spectrometer at 1200 cm^{-1} wavenumber.

Scanning Electron Microscope (SEM) Analysis of Pluronic F-127 *G. latifolium* Leaf Extract
The pluronic F-127 *G. latifolium* leaf nanoparticles was fixed with glutaraldehyde, and coated with a thin layer of a conductive material, carbon, using a sputter coater. The coated sample was placed on the SEM stub using conductive adhesive, carbon tape.

Transmission Electron Microscope (TEM) Analysis of Pluronic F-127 *G. latifolium* Leaf Extract
Two milligrams of the pluronic F-127 *G. latifolium* leaf extract was dispersed in water. Ultrasonication was used to break up agglomerates and a uniform suspension of the nanoparticles was obtained. A small drop of the dispersed sample was placed onto a carbon-coated copper TEM grid using a fine-tipped pipette. The droplet was allowed to dry at room temperature. This ensured the nano-particle were deposited on the grid surface. The sample was stained with a heavy metal stain to enhance contrast, excess stain removed by wicking it off with filter paper, and the grid dried completely. The prepared TEM grid was placed into the sample holder and into the TEM column. A 180 kV accelerating voltage was set, and focus fine-tuned using the objective lens controls. The focus was adjusted until the nano-particles were clearly visible. A low magnification was used first to locate the area of interest on the grid and later increased the magnification to observe the fine details.

Brunauer-Emmett-Teller (BET) Analysis of Pluronic F-127 *G. Latifolium* Leaf Extract
One (1) gram of the dried pluronic F-127-*G. latifolium* leaf particle was placed into a BET sample tube which fits the BET analyzer, and degassed under a vacuum at a controlled temperature. The

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sample tube was carefully transferred to the analysis port of the BET instrument. The BET equation is used to plot the isotherm in the linear range of $0.05 < P/P_0 < 0.3$ relative pressures. From the BET plot, the slope and intercept were determined, which were used to calculate the monolayer adsorption capacity (Vm).

The BET equation: $\frac{1}{\left[\left(\frac{V}{VM}\right)\times\left(\frac{P}{P_0}\right)\right]} = \frac{(C-1)\times[(P/P0)]}{[C\times(1-(P/P0))] + {}^{1}/_{(Vm\times C)}}$, where V = adsorbed gas volume (mL/g), Vm = monolayer capacity (mL/g), P = equilibrium pressure (Pa), P_0 = saturation pressure (Pa), C = BET constant (related to enthalpy of adsorption).

Energy Dispersive X-Ray (EDX) Analysis of Pluronic F-127 *G. latifolium* Leaf Extract

The dried pluronic F-127-*G. latifolium* leaf particle was deposited onto a carbon sample holder. The EDX instrument was calibrated using a standard material with known elemental composition. The system detected and measured the characteristic X-rays emitted by the elements present in the sample, and identified corresponded elements present in the nano-particles.

Results and Discussion

The Results of the Quantitative Analysis *G. latifolium* Leaf

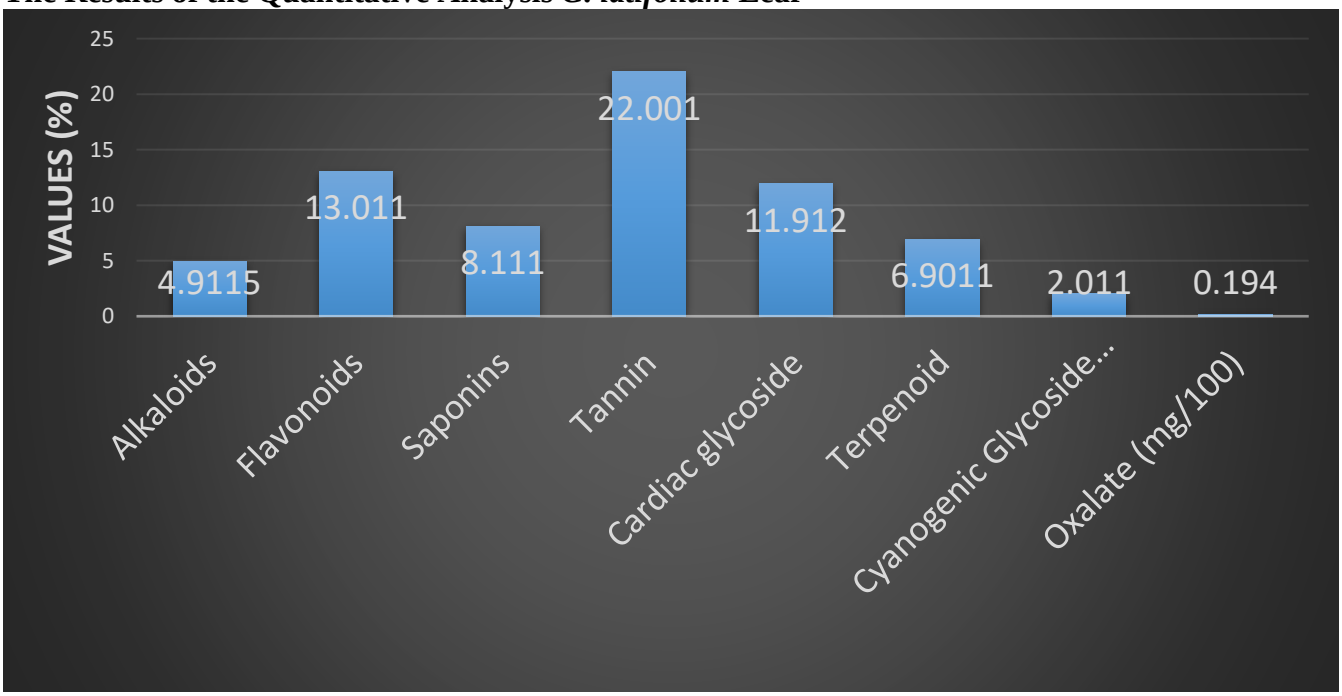

Figure 1: Shows the results of the Quantitative Analysis *G. latifolium* Leaf

The results of the quantitative phytochemical analysis of *G. latifolium* leaf showed in Figure 1, that the saponnin content of *G. latifolium* was 8.111% ±1.701. Saponins exhibit medicinal properties that includes anti-inflammatory, antibacterial, antifungal, insecticidal, and anticancer properties, (Elaziz *et al.*, 2019). The amount of saponin obtained in this work was in agreement

with 9.66 Mg/100mg, obtained by Anameze *et al.*, (2023). The flavonoid (13.011%±0.611), was above 6.83 mg/100mg±6.25, obtained by Anameze *et al.*, (2023). Flavonoids are polyphenolic antioxidant compounds that have broad biological and pharmaceutical properties. The alkaloid (4.9115%±2.315), content was the least contained in the plant leaf. The value was higher than 1.66%, obtained by Ilo *et al.*, (2015). Alkaloids are used as purgatives, sedatives, anti-malarial, anti-diabetic, pain reliever etc. medicine, (Gutierrez-Grojalva *et al.*, 2020). The cardiac glycoside shown in Figure 1 was 11.912%±0.911. This value was far higher than 1.4% obtained by Ilo *et al.*, (2015). Cardiac glycosides are a class of organic compounds that are important in treatments for congestive heart failure. However, their toxicity prevent them from readily being used, (Patel, 2016). The tannin content of *G. latifolium* leaf was very high with 22.001%±4.712. This value was higher than 2.4%, 4.54mgl100g±6.70 and 11.32mgl100±0.02 obtained by Ilo *et al.*, (2015); Anameze *et al.*, (2023); and Omogbai *et al.*, (2019), respectively. However, the high value of tannin content may be as a result of the soil content. Tannin exerts physiological effects in the body, but their types and dosages are important, (Chung, 1998). The substantial percentage of terpenoids, (6.9011%± 2.000) presented in Figure 1, showcases the plant's pharmacological versatility. Terpenoids, are good antioxidants, (Ezekwe *et al*., 2014). However, the cautious use of the plant leaf is imperative, especially considering the concentrations of potentially toxic compounds. The presence of cyanogenic glycosides (2.011 mg/100 ± 0.465), as presented in Figure 1, raises health concerns about its potential toxicity. While cyanogenic glycosides can serve as defense mechanisms in plants, their levels must be carefully managed to avoid adverse effects in human (Marinho *et al*., 2017). Also, the low levels of oxalates (0.194 mg/100 ± 0.202), indicates a potential balance, as moderate oxalate intake is associated with antioxidant benefits, but excessive consumption can bring about kidney stone formation.

Percentage Cumulative Time-Release Profile of Pluronic F-127 Nano-Encapsulated *G. latifolium* Leaf Extract and Metformin (Standard) at 295nm

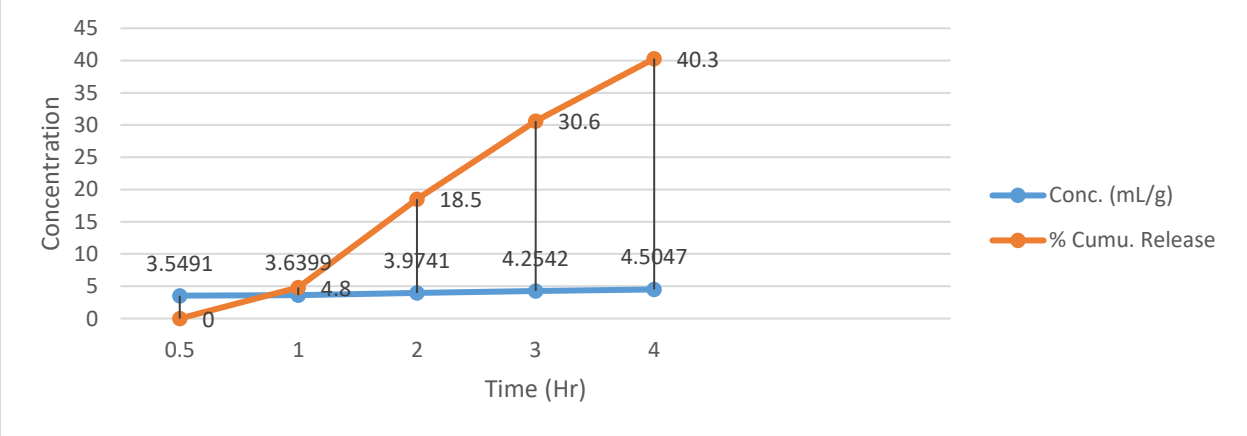

Figure 2: % Cumulative Time-Release Profile of Pluronic F-127 Nano-Encapsulated *G. latifolium* Leaf Extract at 295nm

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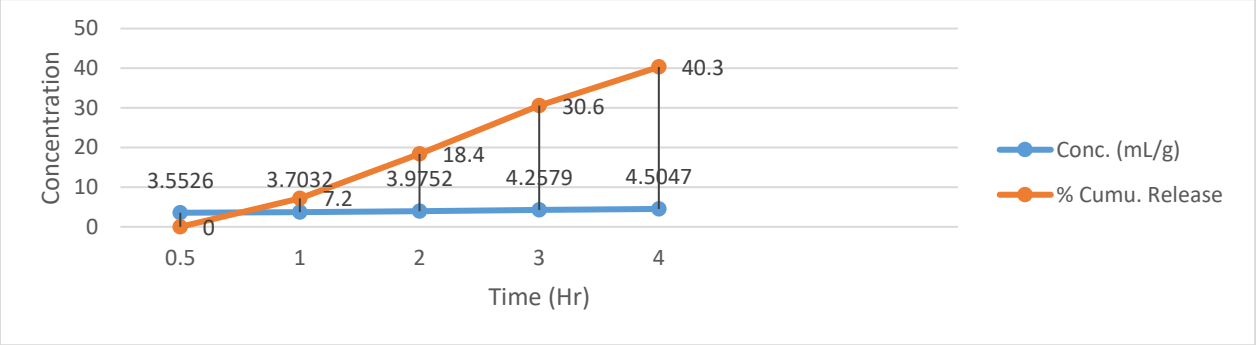

Figure 3: % Cumulative Time-Release Profile of Metformin at 295nm

The percent cumulative time-release profiles of the nano-encapsulated *G. latifolium* leaf extract and a standard anti-diabetic drug shown in Figures 2 and 3 respectively, gave similar trends of releases of the components at equal amounts. At 0.5-hour, 1 hour, 2 hours, 3 hours and 4 hours respectively, the percentages of releases for the encapsulated *G. latifolium* leaf extract and metformin were 0%, 4.80%, 18.50%, 30.60%, 40.30%, and 0%, 7.20%, 18.40%, 30.60%, 40.30% respectively. This shows that 40.30% of both the encapsulated *G. latifolium* leaf extract and metformin were released within the 4 hours of ingestion into the body. Therefore, at about 8 hours, about 80.60% of the components would be released. Kim *et al.*, (2018), opined that the peak plasma concentration for quercetin-chitosan nanoparticles was reached within 6 hours, with a sustained release over 24 hours. Li, *(*2019) stated that the 70% of catechins was released within 12 hours, with a slow-release phase up to 48 hours in a catechin-chitosan release microparticles. The peak plasma concentration for immediate release for metformin was 2-3 hours after ingestion, (Graham, 2011). The peak plasma concentration for the extended time release was 7-10 hours after ingestion, (Graham, 2011), and duration of action was 12-24 hours (Bailey *et al.*, 2010). The study of time release profiles is important as a result of the enhancing effects of controlled release bioavailability and efficacy, and thereby reduces side effects and provide targeted delivery to cells in the body.

FTIR Spectra of Encapsulated *G. latifolium* leaf Extract and Metformin (Standard)

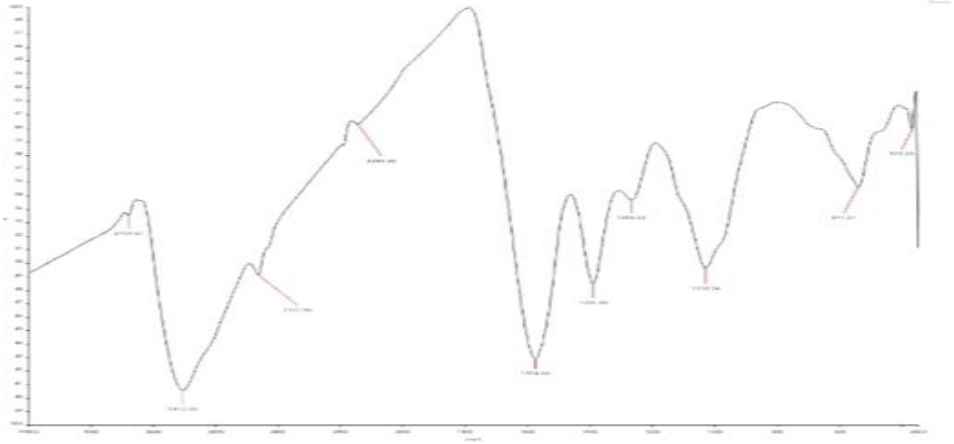

Figure 4: **FTIR Spectra of Encapsulated *G. latifolium* leaf Extract**

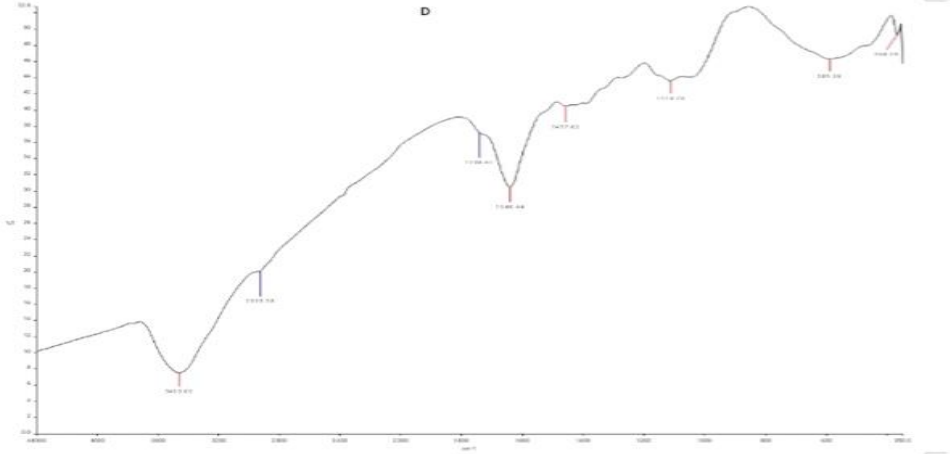

Figure 5: FTIR Spectra of Metformin (Standard Drug)

FTIR identifies functional groups present in samples by quantifying the absorption of infrared light at different wavelengths, which corresponds to specific molecular vibrations. Peak around 2920.65cm^{-1} are indicative of the presence of C-H stretching vibrations of aliphatic chains. The peak around 2362.08cm^{-1} might correspond with C≡C or C≡N triple bond stretching, though such bonds are not common in pluronic F-127 of flavonoids. This may be as a result of specific interactions between the *G. latifolium* leaf extract and nanoparticles. The peak within 1647.77 cm^{-1} correspond to C=O stretching, and it is characteristic of carbonyl groups. It could be as a result of the presence amide I band in the metformin. The peak around 1540.06cm^{-1} is corresponded to N-H bending and C-N stretching vibrations, for amides in alkaloids. The peak at 875.13cm^{-1} could represent C-H out of plane bending, usually found in aromatic rings, showing the presence of aromatic compounds within the flavonoid structure of the plant leaf extract. The presence of these

peaks confirms the successful encapsulation of the leaf extract components with in the pluronic F-127 matrix. The corresponding peaks for both the *G. latifolium* and pluronic F-127 are indicative that the encapsulation did not affect the chemical structure of the composite materials of the pluronic F-127 and *G. latifolium* leaf extract.

In Figure 5, the FTIR spectra for the metformin presented peaks of alcohols (OH), amino acids (NH) and sulfone (SO_2). The presence of alcohol (OH stretch at 3666.99cm^{-1}), amines and amides (NH symmetric stretch at 3126.00cm^{-1}), sulfonamide (SO_2 symmetric stretch at 1189.54cm^{-1}), sulfoxide (S=O stretch at 1057.41cm^{-1}), aldehydes (CHO bending at 2738.18cm^{-1}), confirms that the extracted *G. latifolium* leaf extract is a medicinal plant, due to the presence of these functional groups other anti-diabetic drugs such as insulin. NH is present in acarbose. Acarbose is an alpha-glucosidase. Metformin contains alcohols (OH stretch at 3603.29cm^{-1}), amino acids (NH_2 symmetric stretch at 1598.09cm^{-1}) and sulfone (SO_2 stretch at 1150.34cm^{-1}). The chemical formular of metformin is $C_4H_{11}N_5$.HCl (Metformin Hydrochloride, 2016; Maruthur *et al.*, 2016). Roy *et al.*, (2013), stated that metformin has strong absorption at 1634cm^{-1}, 1573cm^{-1} and 1562cm^{-1} due to the presence of C=N stretching vibration.

Scanning Electron Micrograph of Encapsulated *G. latifolium* Leaf Extract

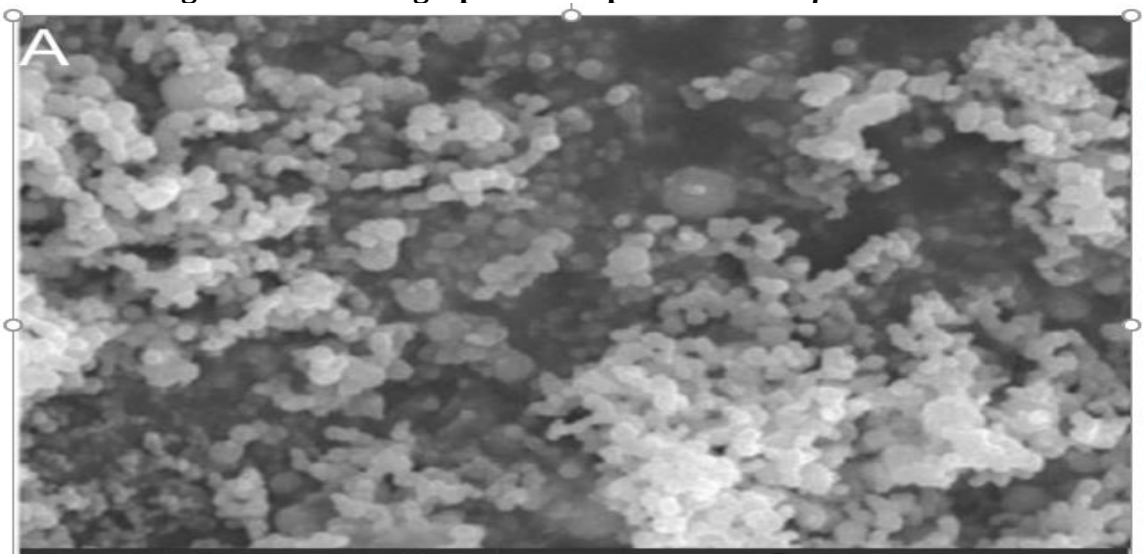

Figure 6: **SEM Micrograph of Encapsulated *G. latifolium* Leaf Extract**

Scanning electron microscopy technique is a form of high-resolution surface imaging that uses the principle of light microscope to scan the sample that is being tested with a focused electron beam to produce an image. In figure 6, showed the scanning electron micrographs of the pluronic F-127-*G. latifolium* leaf particle. The micrographs disclose the structure of the surface and the actual morphology of the nano-particle. Pluronic F-127 acted as adsorbent in the encapsulation process of the *G. latifolium* leaf extract, and this showed that there was interconnected granular microporosity between the pluronic F-127, the *G. latifolium* leaf extract and the cross-linkers to give the drug product. This is an indication of the compatibility between the *G. latifolium* leaf

extract, pluronic F-127 and all other materials mixed together to form the product. This however, established irregular shape, porosity and surface structure which has confirmed the applicability of chitosan in the adsorption of flavonoids for encapsulation for drug delivery. Importantly, the micrograph gave a high degree of micro-particles agglomeration, with smaller spherical particles on the surface of the larger ones, and providing stronger interactions with each other. The agglomerated structure will provide additional stability to the encapsulated plant leaf because of the protections the inner particles will enjoy from the outer ones. There were no reasonable surface imperfections. These submissions were in tandem with that of Lourenco *et al.*, (2021), who conducted the optimization of natural antioxidants extraction from pineapple peel. And further collaborated by Banerjee *et al.*, (2012).

Transmission Electron Micrograph of Encapsulated *G. latifolium* Leaf Extract

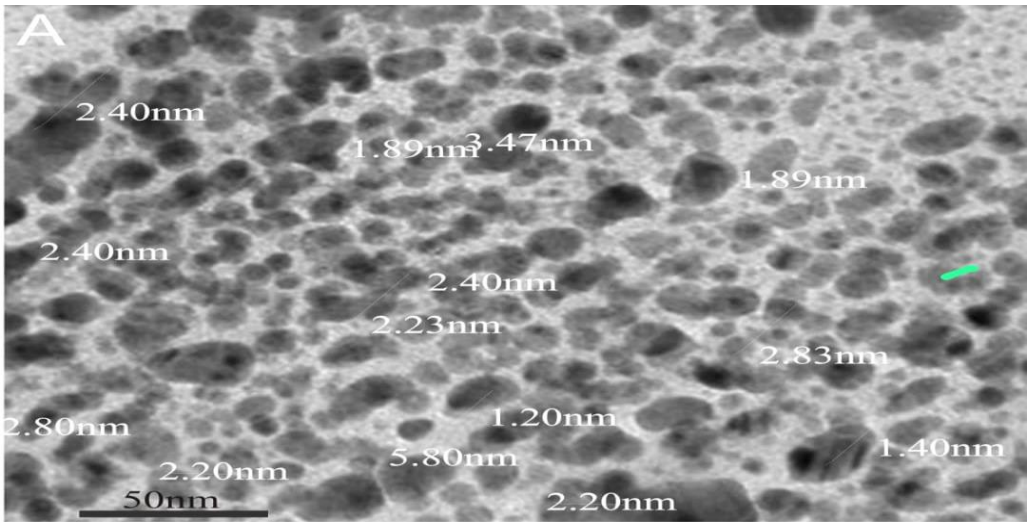

Figure 7: TEM of Encapsulated *G. latifolium* Leaf Extract

The image of the encapsulated *G. latifolium* leaf extract obtained using transmission emission microscope showed the pore sizes of the nanoparticles as indicated in Figure 7. From the magnified pore sizes which were visible in the TEM instrument, presented mesoporous particles which are between 2~5.80 nm pore sizes. The shapes of the particles were spherical, with agglomerated nanoparticles. The nanoparticles have an average of 15.6 nm particles size, and these particles were clustered with fainted thin layers of neighboring materials. The TEM images obtained in this analysis was in agreement with the submission made by Mallikarjuna *et al.*, (2012). According to Akpeji *et al.*, (2024), high surface area of nanoparticles makes its applicability in drug delivery useful, and this was in tandem with the results of the TEM images of the pluronic F127 *G. Latifolium* leaf extract particles.

Table 1: The BET Results of the Encapsulated *G. latifolium* Leaf Extract

Sample	Surface area (m^2/g)	Pore Volume(cc/g)	Pore diameter(nm)
A	821.431	0.65400	27.4210 A

In Brunaur-Emmett-Teller analysis, it is assumed that on the surfaces of samples, gas molecules form monolayers. The technique provide useful information about samples because molecules of gas formed is used to determine the size distribution and surface area of porous materials at low temperatures by using N_2 . From the results of the BET analysis of the nanoparticle obtained from the encapsulation of *G. latifolium* leaf extract using pluronic F 127, shown in Table 1, gave surface area of 821.431 m^2/g, pore volume of 0.65400 cc/g and pore diameter of 27.4210 nm. The encapsulated particle is mesoporousic in nature because the pore diameter was 27.4210 nm, which fall within 2~50 nm. The general classification of pore diameters is based on: micropore (< 2 nm), mesoporous (2~50 nm), macropore (>50 nm). The results obtained in BET analysis were in agreement with the TEM and SEM images, and this has confirmed the significant surface area of the nanoparticles. And as noted, the larger the surface area, the stronger the surface effect, surface activity, catalytic activity, and surface adsorption ability.

Table 2: The Percentage Element Composition of Pluronic F-127 *G. latifolium* Leaf Extract and Metformin.

Elements	Pluronic F-127 *G. latifolium* Leaf Extract (%)	Metformin (%)
Oxygen	10.30	21.20
Carbon	52.16	5.90
Sodium	3.01	0.29
Iron	21.09	-----
Nitrogen	0.70	-----
Potassium	0.26	0.35
Calcium	4.00	2.40
Silicon	1.00	47.9
Barium	----	7.90
Phosphorus	----	1.10
Aliminium	----	11.70
Magnesium	----	0.98
Gold	----	0.78

Energy dispersive x-ray spectroscopy (EDXS), is used to determine the elemental components and the chemical characterization of a sample. In Table 2, the elements found in the pluronic F-127 *G. latifolium* leaf extract and metformin which serve as standard are presented in Table 2. It gives the the weight percentage (Wt %) of the elements detected in the nano-encapsulated *G. latifolium* leaf extract and metformin respectively using EDXS. Nano-encapsulated *G. latifolium* leaf extract had the highest concentration of carbon (52.16 %), iron (21.09 %) and calcium (4.00%) respectively. Silicon (47.90 %), concentration was highest in metformin, followed by oxygen (21.2 %),

aluminium (11.70 %), barium (7.90 %), and carbon (5.90 %), respectively. Barium, phosphorous, aluminium, magnesium, and gold, respectively were not detected in the nano-encapsulated *G. latifolium* leaf extract, while iron and nitrogen were not found in metformin. It is evident from the EDXS analysis that nano-encapsulated *G. latifolium* leaf extract can provide the body anti-diabetic properties but also essential mineral nutrients especially iron, but care should be taken in its consumption to avoid elevated blood pressure due to the presence of sodium.

Conclusion

Gongronema latifolium plant leaf contains very important bioactive phytochemicals with anti-diabetic properties, but its effectiveness and potency is hindered by the mode of delivery inside the body. Pluronic F-127 serves as a bridge in this regard, when used to encapsulate the plant leaf extract. The nano-encapsulated plant leaf extract had excellent release profile, adsorption potential and surface area when compared with a standard anti-diabetic drug (metformin) sold in the market without having an observable alteration to the chemical composition of the plant.

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