OBJECTIVE: The aim of this research work was to comparatively study various proportions of a natural hydrocolloid ‘Raphia africana’, and polyvinylpyrrolidone (PVP) as release sustaining agents in diclofenac sodium tablet formulation.

METHODS: The purified hydrocolloid (R. africana) was characterized by evaluating its organoleptic, physicochemical and flow properties. Diclofenac-polymer ratios of 1:0, 1:0.2, 1:0.4, 1:0.6, and 1:0.8 were employed to produce different granule batches using wet granulation method (that is, the drug was formulated with 0.5, 1.0, 1.5 and 20 % w/w of either R. africana hydrocolloid or PVP, and coded DWB-00, DRA-05, DRA-10, DRA-15, DRA-20, DPP-05, DPP-10, DPP-15 and DPP-20, respectively). Flow properties of granules were studied by determining bulk density, tapped density, Carr’s index, and Hausner’s ratio for all the formulations. Compressed tablets were evaluated using various parameters as weight variation, friability, hardness, tablet thickness and diameter, content uniformity and in vitro dissolution evaluated in phosphate buffer (pH 7.3).

RESULTS: Flowability, mechanical and release parameters determined were within pharmacopoeial limits. Generally, the values of bulk and tapped densities increase as binder concentrations increase for both PVP and R. africana hydrocolloid. The values were significantly different across the batches (p<0.05). Hardness values obtained varied significantly (p<0.05) and were between 5 and 12 KgF which imply that most of the tablet batches are harder than normal depending on the proportion of the polymer used. All the batches exhibited friability within the standard limit without significant difference in values (p>0.05), indicating that tablet formulated with the experimental binders would not undergo surface abrasion. All the formulations exhibited zero order kinetics except batches DPP-10 and DPP-15 which showed Higuchi mechanism. Formulation batches DRA-05 and DRA-10 showed maximum drug release of 98% and 95% respectively after 6 h. A prolonged drug release was observed on increasing polymer ratio. Significantly higher release rates (p<0.05) were observed in the tablets formulated with PVP than those containing R. africana gum. All the batches followed non-fickian diffusion release mechanism.

CONCLUSION: From the study, purified R. africana hydrocolloid generally appeared to perform better than PVP as sustained release agent.

KEYWORDS: R. africana hydrocolloid, Diclofenac sodium, Sustained release, Zero order kinetics
powder, lactose, maize starch (BDH Chemicals Ltd, Poole, UK), magnesium stearate (Sigma-Aldrich, USA). Raffia gum was extracted and purified in the Pharmaceutical Technology Laboratory, University of Uyo, Nigeria. All other materials employed were of the Analar grade.

Methods

Gum extraction

Raphia gum was obtained from the incised trunk of *R. africana* palm at a village in Uyo Local Government Area of Akwa Ibom State, Nigeria. The plant was authenticated in the herbarium facility of the Faculty of Pharmacy, University of Uyo, Nigeria and the voucher number of authentication is UUPH8 (eii). The gum was purified using an established procedure [10, 15, 16]. The raw Raffia gum was hydrated in 0.5: 95.5 (v/v) chloroform water mixture for 5 d with intermittent stirring; extraneous materials were removed by straining through a piece of calico cloth. The gum was precipitated from solution using absolute ethanol. The precipitated gum was filtered washed with diethyl ether and then dried in hot air oven at 40 °C for 18 h. The dried gum was pulverized using a laboratory blender (Neelam Industries, India). The extracted gum was screened to obtain fines (<100 μm) and the percentage yield of the gum then computed.

Physicochemical properties

Moisture content and pH

The moisture content of the purified gum was determined by employing an Ohaus moisture balance (Ohaus Scale Corporation, USA). The pH of the supernatant liquid obtained after shaking a 2 g quantity of the gum powder with 100 ml of distilled water for 5 min was determined using a pH meter (Kent Industrial Measurements, England).

Swelling index

The swelling index of the gum sample was determined by the method employed by some workers [17, 18]. A quantity (1 g) of the powdered and dried sample was poured into 15 ml plastic centrifuge tubes and the volume occupied was recorded (V1). Ten milliliters (10 ml) of distilled water was added and stoppered. The dispersion was mixed on a vortex mixer (Vortex Gennie Scientific, USA) for 2 min and then allowed to stand for 10 min and thereafter immediately centrifuged at 1000 rpm for 10 min on a centrifuge (GallenKamp, England). The supernatant was decanted and the volume of sediment obtained (V2). The swelling index was then calculated as follows:

\[
\text{Swelling index} = \frac{V2}{V1} \quad \ldots \ldots \ldots (1)
\]

Water absorption capacity (WAC)

To 2.5 g of gum sample in a weighed 50 ml centrifuge tube, 30 ml of distilled water was added. This was then agitated on a vortex mixer for 2 min, centrifuged at 400 rpm for 20 min (Optima Centrifuge, Type BHG 500, Germany) and the supernatant carefully decanted. The residue was weighed (W1). The adsorbed drops of water were removed by drying the residue at 60 °C to constant weight (W2) in an oven. WAC was then expressed as the weight of water bound by 100 g dry gum powder [19].

\[
\text{WAC} = \left(\frac{V1-W2}{V1}\right)100 \quad \ldots \ldots \ldots (2)
\]

Evaluation of flow and density properties of purified gum

Angle of repose

The fixed funnel and free-standing cone method [20–22] was employed to determine the static angle of repose of the purified gum. A glass funnel was clamped on a retort stand with its tip 2 cm above a plain white sheet of paper, placed on a flat horizontal surface. A 30 g quantity of the powder was carefullly poured through the funnel until the apex of the cone-shaped heap thus formed by the powder just reached the tip of the funnel. The height of the sample (H) was obtained using a cathetometer. The diameter (D) of the static base was divided by two to obtain the radius R. Angle of repose (θ) for the gum was then calculated using the equation:

\[
\theta = \tan^{-1}\frac{H}{R} \quad \ldots \ldots \ldots (3)
\]

Flow rate

The flow rate was determined by clamping a clean dry funnel on a retort stand at a fixed position such that its lower tip was 8 cm above a piece of plain white sheet of paper placed on the horizontal bench surface directly under the funnel tip. The orifice of the funnel was blocked at the tip and 30 g of the gum powder was accurately weighed into the funnel. The bottom of the funnel was unblocked and the time taken for the entire sample to flow out of the funnel was taken. The flow rate was then determined as the mass of the sample per unit time (g/s).

Bulk and tapped densities

A 25 g quantity of the gum powder was placed in a 100 ml clean, dry measuring cylinder and the volume Vs occupied by the sample without tapping was determined. After some mechanical taps of the cylinder on a padded horizontal surface, the tapped volume Vt was recorded (when a constant volume was reached). The means of four determinations was taken. The bulk and tap densities, Db and Dt (g/cm³) were calculated as the ratio of sample weight to volume (Vs and Vt, respectively) [22].

Hauser’s index

The Hauser’s index was calculated as the ratio of the tapped density to the bulk density of the sample, that is,

\[
\text{Hauser’s index} = \frac{D_t}{D_b} \quad \ldots \ldots \ldots (4)
\]

Carr’s compressibility index (CI %)

The value of Cl was computed by employing the following equation:

\[
\text{Cl %} = \frac{D_t-D_b}{D_t} \times 100 \quad \ldots \ldots \ldots (5)
\]

Preparation of diclofenac granules

Diclofenac sodium granule preparation was carried out based on the following formula:

\[
\text{Diclofenac sodium-100 mg Polymer-x % Magnesium stearate-1 % Talc-1 % Corn starch-5 % Lactose-q s}
\]

NOTE: x ranges from 0–20 %

Each batch of 20 g of granule was prepared by incorporating either *Raphia* gum extract or PVP as a binder at 5%, 10%, 15% and 20% with other ingredients as presented in table (1). Appropriate quantities of diclofenac sodium, cornstarch and lactose were weighed out and mixed using mortar and pestle to obtain a homogenous mix. Then an appropriate amount of the binder (*Raphia* gum) was dispersed in a small quantity of hot water and was used to granulate the homogeneous powder mixture in the mortar to form a damp coherent mass. The wet mass was passed through a 2.0 mm stainless steel sieve with the aid of a spatula. The wet granules were dried in the hot air oven at a temperature of 50 °C for about an hour after which they were further passed through the 1.0 mm stainless steel sieve to obtain finer granules. This was reported for the different batches varying the ingredients based on the formula in the table (1).

Evaluation of the flow and density properties of diclofenac sodium granules

Various flow and density characteristics for all the batches of prepared granules: angle of repose, flow rate, bulk and tapped densities, Hauser’s ratio and Carr’s index—were evaluated using the same methods as earlier explained for purified gum powder.

Preparation of tablets

Carefully weighed magnesium stearate (0.2 g) and talc (0.2 g) were mixed with each batch of granule in a sample bottle and then transferred into a beaker. Each tablet of 400 mg was compressed using a single punch tabletting machine (SSF-3) (Cadmach machinery Co. PVT. Ltd; India) at a compression pressure of 35 kN. Each batch of tablets produced was then stored in an airtight container for further analysis.
maintained at 37 ±1°C and rotated at a speed of 50 rpm. The experiment was allowed to run for 6 h and 10 ml aliquot withdrawn at 30 min intervals filtered through Whatman filter paper No.2 and assayed spectrophotometrically using the UV 2100 spectrophotometer. The assayed was done at a wavelength of 276 nm where diclofenac experiences peak absorption. An equal volume of fresh medium was replaced into the dissolution medium after each sampling to maintain the constant volume throughout the test. The concentration of the active ingredient was then obtained from the standard Beer Lambert’s plot. The data obtained from the dissolution studies were fitted into the release kinetics of Zero order, First order, Higuchi and Korsmeyer equations using the model dependent methods [27, 28].

Content uniformity test
Ten tablets were selected randomly from each batch and powdered. A quantity of this powder corresponding to 100 mg of diclofenac sodium was dissolved in 100 ml of 7.3 pH phosphate buffer stirred for 60 min and filtered. A quantity of 1 ml of the filtrate was diluted to 100 ml with the phosphate buffer. The absorbance of this solution was measured at 276 nm and content of diclofenac sodium was estimated [26, 29].

All the determinations were made in quadruplicates and the values presented were the average values (±standard deviations).

Statistical evaluation
All determinations were carried out in quadruplicates (n = 4). Values of parameters were presented as mean±SD. Statistical evaluation was performed by employing a two-way Analysis of Variance (ANOVA) on a computer software GraphPad Prism® 4 (GraphPad Software Inc., San Diego, USA). Post-hoc (Turkey-Kramer multiple comparison) tests was used in comparing the individual differences between the samples. At 95% confidence interval, probability (p) values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION
The pure gum obtained from R. africana exudates was 30.4 %w/w. From the result obtained from the evaluation of gum powder flow (table 2), it was found that the flow rate, Carr’s index, Hausner’s ratio and angle of repose had values that comply with the official standard for good powder fluidity.

Table 2: Flow properties of purified R. africana hydrocolloid

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R. africana hydrocolloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angle of repose (°)</td>
<td>39.60 ± 1.90</td>
</tr>
<tr>
<td>Flow rate (g/sec)</td>
<td>1.90 ± 0.10</td>
</tr>
<tr>
<td>Bulk density (g/ml)</td>
<td>0.68 ± 0.02</td>
</tr>
<tr>
<td>Tapped density (g/ml)</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>Carr’s index (%)</td>
<td>22.72 ± 1.11</td>
</tr>
<tr>
<td>Hausner’s ratio</td>
<td>1.29 ± 0.06</td>
</tr>
</tbody>
</table>

Data presented are as mean values±SD, n = 4.

The values of physicochemical properties (moisture content, pH, swelling index, water absorption capacity and moisture sorption capacity) determined for the hydrocolloid are presented in Table 2. The values indicate not only that the performance of the experimental hydrocolloid as excipient would be enhanced; it is also suggestive of its compatibility with other components of the formulation [10, 14].

Table 1: The basic formula for the preparation of sustained release diclofenac sodium tablet containing 100 mg of the drug per unit

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>DWB-00+</th>
<th>DRA-05+</th>
<th>DRA-10+</th>
<th>DRA-15+</th>
<th>DRA-20+</th>
<th>DPP-05+</th>
<th>DPP-10+</th>
<th>DPP-15+</th>
<th>DPP-20+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac Na</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>Raffia gum</td>
<td>20 mg</td>
<td>40 mg</td>
<td>60 mg</td>
<td>80 mg</td>
<td>80 mg</td>
<td>80 mg</td>
<td>80 mg</td>
<td>80 mg</td>
<td>80 mg</td>
</tr>
<tr>
<td>PVP</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>Talc</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
<td>4 mg</td>
</tr>
<tr>
<td>Starch</td>
<td>20 mg</td>
<td>20 mg</td>
<td>20 mg</td>
<td>20 mg</td>
<td>20 mg</td>
<td>20 mg</td>
<td>20 mg</td>
<td>20 mg</td>
<td>20 mg</td>
</tr>
<tr>
<td>Lactose</td>
<td>272 mg</td>
<td>252 mg</td>
<td>252 mg</td>
<td>252 mg</td>
<td>252 mg</td>
<td>252 mg</td>
<td>252 mg</td>
<td>252 mg</td>
<td>252 mg</td>
</tr>
<tr>
<td>Total</td>
<td>400 mg</td>
<td>400 mg</td>
<td>400 mg</td>
<td>400 mg</td>
<td>400 mg</td>
<td>400 mg</td>
<td>400 mg</td>
<td>400 mg</td>
<td>400 mg</td>
</tr>
</tbody>
</table>

*DWB-00 is diclofenac tablet formulation without binder (that is, 0.0% binder), DRA are diclofenac tablet formulations containing R. africana gum binder at concentrations of 05, 10, 15 and 20 %w/w, DPP are diclofenac tablet formulations containing polyvinylpyrrolidone (PVP) binder at concentrations of 05, 10, 15 and 20 %w/w.

Evaluation of tablet physicomechanical properties

Tablet dimensions
The diameter and thickness of ten tablets from the different batches were determined using a micrometre screw gauge (0.25±0.01 mm). The average values were then computed for each batch [23].

Weight uniformity
Twenty tablets were obtained from each of the batches and weighed individually with an electronic analytical weighing balance (Ohaus Scale Corporation, USA). The mean weight and standard deviation were determined for each tablet batch to establish whether or not it conforms to the official weight uniformity standards [23, 24].

Hardness test
Tablet hardness was determined using the Monsanto hardness tester, India (MHT-20). Ten tablets from each batch were used. Each tablet was placed diametrically between the jaws of the tester and the force needed to just crush the tablet was noted. The mean of the hardness was also determined [23, 25].

Friability test
Ten tablets from each batch were obtained, de-dusted, weighed and placed in separate drums of a Roche friabulator (DT-2D). The tablets were then removed, de-dusted and weighed again. The friability of the tablets was expressed as a percentage using the formula below [23, 25].

\[ \text{Friability} = \frac{\text{weight loss}}{\text{original weight}} \times 100 \]  

Tablet dissolution and release kinetics studies

In vitro dissolution test for the tablets was carried out using the United States Pharmacopoeia basket method in the tablet dissolution test apparatus [26]. The tablet was placed in a wire mesh basket suspended in a dissolution medium of 900 ml of dissolution medium containing 2.1 %w/v of citric acid and phosphate buffer maintained at 37±1°C in a water bath. The wire mesh basket was rotated at a speed of 50 rpm. The experiment was allowed to run for 6 h and 10 ml aliquot withdrawn at 30 min intervals filtered through Whatman filter paper No.2 and assayed spectrophotometrically using the UV 2100 spectrophotometer. The assayed was done at a wavelength of 276 nm where diclofenac experiences peak absorption.
From the study, the hardness obtained ranges from 5 - 12 kgF which influenced by the different density of granules and the speed of compression. From the result, the tablets exhibited good uniformity of a drug [26]. The data obtained for weight uniformity of tablets contributes to the dose uniformity across the batches (p > 0.05). Thus, each tablet contains the same amount of drug, which ensures consistent therapeutic effect.

The uniformity of weight of tablets contributes to the dose uniformity of a drug [26]. The data obtained for weight uniformity test indicate that the tablets exhibit uniformity. Variations in tablet weight may be due to inconsistent powder or granule density and particle size distribution.

Official hardness values for conventional immediate release tablets range from 4.6 kgF to 5.8 kgF for sustained release tablets [7, 26, 30]. From the study, the hardness obtained ranges from 5 - 12 kgF which imply that most of the tablet batches are harder than normal. This difference in hardness may be due to the quantity of the polymer used. There was significant variation (p<0.05) observed between the hardness values between the tablets formulated with either of the two binders. Tablet hardness influences the rate of release of drug during dissolution test. If a tablet is too hard, it may be difficult to dissolve during dissolution test and soft tablet cannot withstand the pressure force applied to it. The nature of the polymer used as binder influences the hardness of tablets. Hardness influences compaction and tablets with high compaction have a high ability to retard solvent penetration into the tablet core.

All the formulated tablets had friability within the official limits of ≤ 1% [30], and the difference between the values was statistically insignificant (p>0.05). Indicating that tablet formulated with the experimental binders would exhibit better friability. Friability test measures the ability of tablets to withstand abrasion during packaging, handling, and shipping. The normal limit for friability is less or equal to Lower values observed implies that the tablet would not readily undergo surface abrasion upon handling. All the batches complied with the standard requirement for uniformity of active ingredient. That is, diclofenac content of the tablets was relatively uniform for most of the batches and statistically, there was generally no significant difference in the drug content across the batches (p>0.05). Thus, when such tablets are administered, no dose variation is expected [26].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R. africana hydrocolloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>7.2±0.35</td>
</tr>
<tr>
<td>pH</td>
<td>6.7±0.32</td>
</tr>
<tr>
<td>Swelling index</td>
<td>3.9±0.20</td>
</tr>
<tr>
<td>Water absorption capacity (g/100 g)</td>
<td>78.00±4.15</td>
</tr>
<tr>
<td>Moisture sorption capacity (%)</td>
<td>102.50±5.22</td>
</tr>
</tbody>
</table>

Data presented are as mean values±SD, n = 4

Table 4: Flow and density properties of sustained release diclofenac Na formulated with R. africana gum and PVP binders

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<tr>
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</tr>
</tbody>
</table>

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The pre-compression evaluation of flowability and densification of the formulated granules equally depict good flow (as shown in table 4). The higher the concentrations of either PVP or R. africana gum and PVP binders, the higher were the values of bulk and tapped densities. Generally, as the size of granules increases, the orifice would be blocked and the flow retarded.

<table>
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</tr>
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<tbody>
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</tr>
</tbody>
</table>

Data presented are as mean values±SD, n = 4

Mechanical properties of the compressed tablets are presented in table 5. The thickness and diameter of a tablet play a key part in dose uniformity. The diameter size and shape of tablet depend on the die and punches selected for making the tablet. From the values of tablet dimensions determined, the tablets generally exhibited uniform diameter and conform to the limits for tablets. Tablet thickness is an important quality control test for tablet packaging. Tablet thickness may influence ease of packaging either in blisters or plastic containers. Tablet thickness can vary without any change in its weight. This may depend on the die size of the tablet machine as well as the force applied to compress the powder. It can also be influenced by the different density of granules and the speed of compression. From the result, the tablets exhibited good uniformity of thickness and conform to the official standard limit.

The uniformity of weight of tablets contributes to the dose uniformity of a drug [26]. The data obtained for weight uniformity test indicate that the tablets possess significant dose uniformity. Variations in tablet weight may be due to inconsistent powder or granule density and particle size distribution.

Official hardness values for conventional immediate release tablets range from 4.6 kgF but 5.8 kgF for sustained release tablets [7, 26, 30]. From the study, the hardness obtained ranges from 5 - 12 kgF which imply that most of the tablet batches are harder than normal. This difference in hardness may be due to the quantity of the polymer used. There was significant variation (p<0.05) observed between the hardness values between the tablets formulated with either of the two binders. Tablet hardness influences the rate of release of drug during dissolution test. If a tablet is too hard, it may be difficult to dissolve during dissolution test and soft tablet cannot withstand the pressure force applied to it. The nature of the polymer used as binder influences the hardness of tablets. Hardness influences compaction and tablets with high compaction have a high ability to retard solvent penetration into the tablet core.

All the formulated tablets had friability within the official limits of ≤ 1% [30], and the difference between the values was statistically insignificant (p>0.05). Indicating that tablet formulated with the experimental binders would exhibit better friability. Friability test measures the ability of tablets to withstand abrasion during packaging, handling, and shipping. The normal limit for friability is less or equal to Lower values observed implies that the tablet would not readily undergo surface abrasion upon handling. All the batches complied with the standard requirement for uniformity of active ingredient. That is, diclofenac content of the tablets was relatively uniform for most of the batches and statistically, there was generally no significant difference in the drug content across the batches (p>0.05). Thus, when such tablets are administered, no dose variation is expected [26].
From the results obtained for dissolution test, plots were made of percentage diclofenac released against time for all the tablets containing *R. africana* hydrocolloid and PVP as binders. Typical plots for tablets formulated with 5% w/w and 20% w/w of the binders are presented in Fig. (1). Values obtained when data from dissolution studies were fitted into the equations of Zero order, First order, Higuchi and Korsmeyer are as shown in the table (6). Batches DWB-00, DRA-05, DRA-10, DRA-15, DRA-20, DPP-05, and DPP-20 showed zero order kinetics meaning that diffusion in these batches was independent of the initial concentration of drug in the dissolving medium. Two batches of the formulations containing synthetic polymer-DPP-10 and DPP-15 showed Higuchi kinetics. Therefore *R. africana* gum is a good choice for sustained release formulations, thus confirming the findings of Bhosale and co-workers that natural hydrocolloids are effective alternative binders to synthetic ones in the formulation of sustained release tablets [29]. The release rates shown by tablets containing PVP were significantly higher (p<0.05) than those exhibiting by those containing *R. africana* hydrocolloid. The mechanism of release for all the batches followed non-fickian diffusion.

Batches formulated with polyvinylpyrrolidone (PVP) showed higher release than those formulated with *R. africana* gum. This may be due to slow hydration of matrix and its property to form a gel layer which retards the drug release from the tablet. Highest release of the polymer (Raffia gum) was obtained in batch DRA-05 with 98% and DRA-10 with 95% release. The highest release for the synthetic binder (PVP) was obtained in batches DPP-05, at 110%, 103% and 96%, respectively.

The results obtained indicate that the batches complied with the standard requirements for uniformity of active ingredients. This implies that the tablets contain a relatively uniform amount of active ingredient (diclofenac). As a result, the tablets would exhibit uniform potency which characterizes a positive content uniformity test result.

**CONCLUSION**

From the study, the use of Raphia gum as a binder in formulating sustained release diclofenac tablet was satisfactory based on compliance with official standards. All the formulations containing the natural gum exhibit sustained release with formulation DRA-20 containing 20% of the gum found to release the drug in a slow, controlled manner with maximum drug release of 83% after 6 h. This implies that the percentage of drug release decreases with increasing polymer content. The rate of release for the synthetic polymer (PVP) was much higher than for the natural polymer (Raphia gum) thereby making Raphia gum a better candidate for sustained release formulation. The kinetics of drug release from the tablets followed zero order with non-fickian diffusion mechanism.

**ACKNOWLEDGEMENT**

The authors appreciate the technical staff of Departments of Pharmaceutics and Pharmaceutical Technology, and Pharmacognosy and Natural Medicine, University of Uyo, Nigeria.

**AUTHORS’ CONTRIBUTION**

The first author conceived and designed the work, supervised the laboratory aspect, assembled, analysed and interpreted the data and wrote the final manuscript. The second author carried out the laboratory work and collected the raw data.

**CONFLICTS OF INTERESTS**

Declared none.
REFERENCES


