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Formulation and Evaluation of Oral Reconstitutable Suspension of Aqueous *Moringa oleifera* Lam Root Extract

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Moringa oleifera* Lam in crude form is employed for preventive or curative purposes in various ailments. Studies on the formulation of *Moringa oleifera* tablet and also the use of *Moringa oleifera* gum as binders have been reported.

Objective: This study is aimed at formulating and assessing the pharmaceutical properties of an oral reconstitutable suspension of aqueous root extract of *Moringa oleifera* Lam, a plant with proven diverse medicinal applications that has not been commercially formulated into liquid dosage form to the best of our knowledge.

Methods: Extract of *Moringa oleifera* was obtained from the dried roots by maceration and subsequently characterized for moisture content, angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index and ash value. The oral reconstitutable suspension was formulated with sodium carboxymethyl cellulose (SCMC) [0.0, 0.5, 1.0 and 1.5 %] as suspending agent and assessed for sedimentation volume, re-dispersion time, flow rate and dissolution profile.

Results: The results obtained demonstrates that the aqueous root extract of *Moringa oleifera* had moisture content within acceptable limit for crude drugs with a good flow property supported by the angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index while ash value demonstrates the likelihood of inorganic substances in the extract.

Pharmaceutical properties of the suspensions assessed revealed sedimentation volume were high. The suspensions had short re-dispersion time, good flow rate and more than 70 % of the active ingredient was released within the first 30 minutes.

Conclusion: The aqueous root extract of *Moringa oleifera* can therefore be formulated as an oral reconstitutable suspension using SCMC as a suspending agent at concentrations between 0.5 to 1.5 %.

Keywords: Formulation, Reconstitutable suspension, Aqueous extract and *Moringa oleifera* Lam root

INTRODUCTION

Suspensions are heterogeneous systems containing two phases. The external phase, which is also referred to as the continuous phase or dispersion medium, is generally a liquid (e.g., liquid suspensions) or semisolid (e.g., gels), and the internal or dispersed phase is made up of particulate matter, which is practically insoluble in the external phase. Most pharmaceutical suspensions consist of an aqueous dispersion medium, although organic or oily liquids are also used in some instances (Nutan *et al*., 2010).

The preparation of suspension requires a number of excipients or formulation additives so as to render it stable and present it in desired form with desired properties. The various excipients used in the formulation of suspension are: vehicles, wetting agents, suspending agents, flocculating agents, viscosity modifiers, formulation additives (Ancha *et al*., 2010).

Reconstitutable suspension is good choice for sustained release liquid oral dosage form and best choice when drug stability is a major concern (Damor *et al*., 2015). They also contribute to pharmaceutical dosage form development by supplying drugs that are insoluble and often distasteful in acceptable medium (Doye *et al*., 2017).

Moringa oleifera lam (Family: *Moringaceae*) is a small to medium-sized tree, found almost all over the plains of India. Although there is scarcity of liquid formulation of the root extract, it contains several phytochemicals, some of which are of high interest because of their medicinal value. In particular, this plant family is rich in unique group of glycoside compounds called glucosinolates and isothiocyanates which have chemotherapeutic effect, wound healing effect and can be employed in the repair of cartilage and bones among other medicinal applications of this phytochemicals (Villarreal-García, 2016; Sarin and Bafna, 2012). *Moringa oleifera* is one of the leading names recently in medicinal plant research and development. A large number of reports on the nutritional qualities of *Moringa oleifera* exist in both scientific and the popular literature (Garima *et al*., 2001). It has been described as having medical and health importance such as abortifacient (Nathi *et al*., 1992), aphrodisiac (Fauci *et al*., 1993), birth control (Shukla *et al*., 1988; Faizi *et al*., 1998). Audu and Arra, (2000) worked on Benzyl carbamonodethianate from the root bark of *Moringa oleifera* lam and it's toxicology of liver, kidney, heart and lungs did not reveal acute toxicity. Various parts of this plant such as the leaves, roots, seeds and barks, fruits, flowers, immaterial pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, anti-epileptic, anti-inflammatory, anticancer, antispasmodic, cholesterol lowering, antibacterial, anti-diabetic and antioxidant properties (Anwar *et al*., 2007). As a result of this varied health benefits of *Moringa oleifera*, and the non-existence of the preparation in liquid formulation to the best of our knowledge, this study attempts to formulate an oral reconstitutable suspension of aqueous *Moringa oleifera* root extract and evaluate its properties.

METHODOLOGY

Materials

All chemicals and reagents employed in the study [Methanol (Merck Germany) and Sodium hydroxide (Merck Germany)] are of analytical grade.

Plant Collection and Identification

Moringa oleifera lam root was obtained from Maiduguri Monday market, Maiduguri, Borno state, Nigeria. It was subsequently identified at the Department of Biological Sciences, University of Maiduguri.

Preparation of the Aqueous *Moringa oleifera* lam Root Extract

The method used by Kumar *et al*., (2011) was adopted with some modifications. Dried *Moringa oleifera* Lam root was washed with distilled water and air dried prior to size reduction using a pestle and mortar. The sample obtained was divided into three (3) groups of 7.5 g each and soaked in distilled water for 48 h with occasional shaking. Subsequently, the mixture was filtered and the filtrate concentrated using Rotary evaporator (R201D, U.S.A). The concentrate was dried using an incubator at 35 ºC for 24 h and the yield determined (9.95 %w/w). The extract was stored in air tight container refrigerated at 5 °C for further use.

Physicochemical Characterization of Aqueous *Moringa oleifera* Lam Root Extract

Angle of Repose

The angle of repose of *Moringa oleifera* Lam root extract was determined using a glass funnel clamped on a retort stand which was 10 cm away from the flat surface of the bench. A 30 g weight of the extract was placed into the funnel and allowed to flow freely forming a conical heap. The angle of repose was calculated from the heap of the *Moringa oleifera* Lam root extract using Equation 1;

Angle of repose, $\tan\theta = \frac{h}{r}$ …….. Equation 1

Where h = height and r = radius of the circular heap

Bulk and Tapped Density

A 30 g weight of aqueous *Moringa oleifera* Lam root extract was transferred into a graduated measuring cylinder. The measuring cylinder was then tapped 50 times on a stable wooden table from a height of 2 cm. The bulk and tapped density were calculated using equation 2 and 3 respectively.

$BD = \frac{WS}{VS}$…….. Equation 2

Where BD = bulk density WS = Weight of sample and VS= volume of sample

$TD = \frac{WS}{TVS}$…… Equation 3

Where TD = Tapped density WS = Weight of sample and TVS= Tapped volume of sample

Hausner's Ratio

Hausner's ratio was ascertained using the results/data obtained from bulk and tapped density as shown in equation 4.

$Hausner's\ ratio = \frac{TD}{BD}$…….. Equation 4

Where TD = tapped density and BD = bulk density

Carr's Index

The outcome of both bulk and tapped density was utilized in the determination of the Carr's index as shown in equation 5.

$CI = \frac{TD-BD}{TD} x100$..... Equation 5

Where CI is Carr's index, TD= tapped density and BD = bulk

Ash Value

A 2 g weight of aqueous *Moringa oleifera* Lam root extract was poured into a nickel crucible which was initially heated at 105 °C to a constant weight and allowed to cool. The crucible with its content was then gently heated until it was moisture free and completely charred. Subsequently, the heat was increased gradually until most of the carbon vaporized. The sample was finally heated strongly at 600 °C until the residue was free from carbon (i.e. almost white). The crucible with its content was allowed to cool and weighed. The heating and cooling step was then repeated until the residue (ash) was constant.

Enumeration of Microbial Count

Inoculation by pour plate method was carried out after serial dilutions of the extract were prepared ranging from 1 in 10 to 1 in 10,000 using 1 g sample. One milliliter of the diluted sample was then aseptically aspirated into the media (Nutrient agar). The media was poured aseptically into a sterile petri dish at 40 to 45 °C then swirled and allowed to solidify for incubation (Uniscope, England) at 37 °C for 24 h. Typical colonies of microbial growth on plates were counted with an electronic colony counter at the end of incubation and the result presented in colony forming unit per gram [cfu/g] (Esoje *et al*., 2016).

Formulation of Oral Reconstitutable Suspension

Powder blend of aqueous *Moringa oleifera* root extract was prepared using suspending agent, preservative and sweetener as per the formulae in Table 1. Adjuvants present in small amounts (SCMC [0.0, 0.5. 1.0, 1.5 %], Sodium benzoate and Sugar Pharm grade) were first mixed homogenously in a porcelain pestle and mortar using the doubling up technique (Langley and Belcher, 2008). Similarly, aqueous *Moringa oleifera* root extract was added by same technique and triturated until a uniform mixture was obtained.

Prior to quality assessment studies the powder blend was reconstituted with distilled water (to a volume of 60 mL) giving an equivalent concentration of aqueous *Moringa oleifera* root extract as 150 mg/mL.

Table 1: Formulation of Aqueous *Moringa oleifera* Root Extracts Reconstitutable Suspension (60 mL)

Batches	Extract (mg)	SCMC (%)	Sodium Benzoate (mg)	Sugar Pharm Grade (mg)
A	1800	0.00	11.64	2.32
B	1800	0.50	11.64	2.32
C	1800	1.00	11.64	2.32
D	1800	1.50	11.64	2.32

Evaluation of Aqueous *Moringa oleifera* Root Extracts Reconstitutable Suspension

Sedimentation Parameters

The following sedimentation parameters were evaluated:

Sedimentation Volume

A 50 mL volume of the reconstituted suspension was emptied into 100 mL measuring cylinder. The volume of the sediment formed at the 1st, 2nd, 3rd, and 7th hour was noted, and then, after 24 h for seven (7) days. Sedimentation volume was then computed using equation 6:

$$F = \frac{Vu}{Vo} \quad \text{..... Equation 6}$$

Where vu and vo are final height of sediment and original height of suspension respectively.

Re-dispersibility

Re-dispersibility was evaluated similar to the method used by Jain *et al.*, (2011). A volume of 30 mL of each reconstituted suspension were retained in calibrated tubes stored at room temperature for 3 days. Each test tube was removed and shaken to redistribute the sediment and the presence of deposit if any was recorded. Re-dispersion was recorded as the number of inversions required for re-suspension to occur.

Rheology

The time that taken for 10 mL reconstituted suspension to flow through the orifice of a 10 mL pipette was determined and the flow rate calculated using equation 7:

$$Flow\ rate = \frac{Volume\ of\ pipette\ (mL)}{Flow\ time\ (Sec)} \quad \text{.... Equation 7}$$

Drug Release Studies

Calibration Curve

The calibration curve was constructed using the aqueous extract of *Moringa oleifera* root and 0.1 N NaOH as dissolving medium. A 4 mg weight of aqueous extract of *Moringa oleifera* root was weighed and serially diluted to obtain a stock solution of 0.08 mg/mL (4.00 µg/mL). 0.50, 1.00, 1.50, 2.00, 2.50, 3.00 and 3.50 mL of the stock was then re-diluted in 10 mL volumetric flask to give 4.00, 8.00, 16.00, 20.00, 24.00 and 28.00 µg/mL respectively. The absorbance's of the different concentrations was spectrophotometrically determined at 230 nm and a graph of absorbance against concentration was plotted.

Procedure for Drug Release Studies

The Erweka dissolution test apparatus (Model DT 6R, Germany) was used to determine the drug release profile of the aqueous *Moringa oleifera* root extract suspension. The procedure used by Azam and Haider, (2008) was adopted with some modifications.

The dissolution medium used was 0.1 N NaOH. Dissolution medium (900 mL) was poured into a glass jar which was suspended in a water bath thermostatically maintained at 37 ± 0.5 °C. The paddle was set to rotate at 50 rpm and 25 mm away from the base of the glass jar. About 10 mL of the reconstituted suspension was introduced carefully into the bottom of the apparatus. Five (5) mL samples were withdrawn at specified time interval of 5, 15, 30, 45, and 60 minutes respectively and spectrophotometrically analyzed for aqueous *Moringa oleifera* root extract at 230 nm. After each withdrawal, same volume of the dissolution medium was replaced.

Statistical Analysis

The data were analyzed using statistical package for social sciences version 17.0 (SPSS Inc., Chicago, IL, USA). The results were presented in tables and graphs. Parametric data were presented as Mean ± SD. P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

As shown in Table 2, *Moringa oleifera* aqueous root extract had a moisture content of 7.78 %L. The powdered extract had a good flow as demonstrated by the findings of the angle of repose (28.04 °), bulk density (0.29 g/mL), tapped density (g/mL), Hausner's ratio (1.26 %) and Carr's index (20.53 %). The ash value of the extract was 25.00 %.

Table 2: Physicochemical properties of *Moringa oleifera* aqueous root extract

Parameter	Mean ± SD
Moisture content (%L)	7.78 ± 1.29
Angle of repose (°)	28.04 ± 2.27
Bulk density (g/mL)	0.29 ± 0.014
Tapped density (g/mL)	0.36 ± 0.021
Hausner's ratio	1.26 ±0.001
Carr's index (%)	20.53 ± 0.74
Ash value (%)	25.00 ± 0.07

All the data represented the average of triplicate analysis ± standard deviation (SD)

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The microbial load of *Moringa oleifera* aqueous root extract is presented in Table 3 which demonstrates an increase in contamination as the concentration of the extract was increased per milliliter.

Table 3: Microbial load of *Moringa oleifera* aqueous root extract

Concentration of Extract (mg/mL)	Microbial Load (cfu/mL)
100	1093.33 ± 34.49
10	792.33 ± 95.80
1	454. 33 ± 50.24
0.1	74.00 ± 14.53

All the data represented the average of triplicate analysis ± standard deviation (SD)

The sedimentation volume of the samples with different concentrations of suspending agents is comparable to the samples without suspending agent (figure 1).

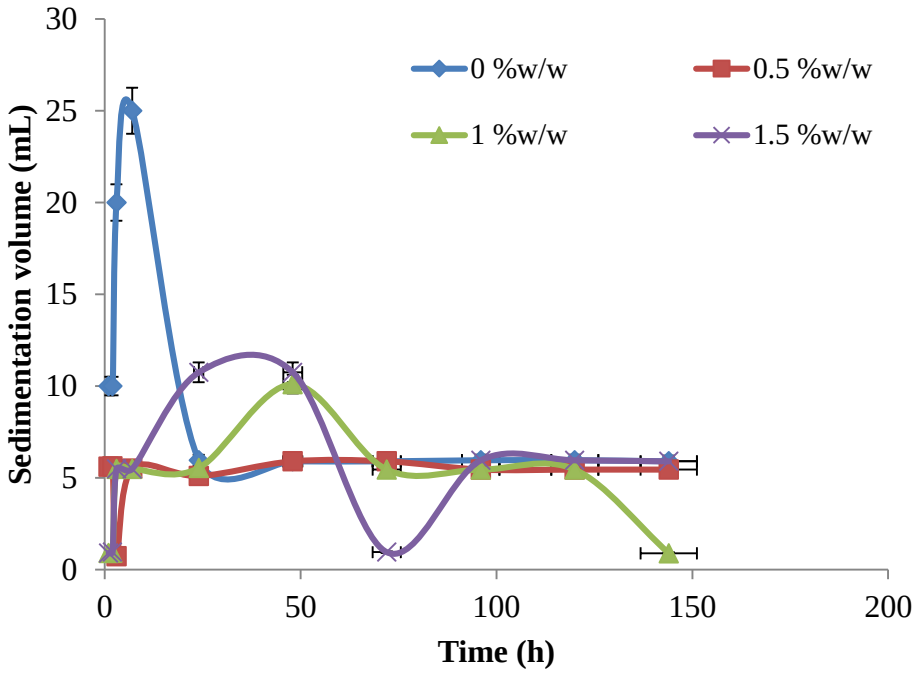

Figure 1: Sedimentation volume of oral reconstitutable suspensions formulated at various concentrations

The re-dispersion time as presented in Figure 2 shows that samples with different concentrations of suspending agent and the sample without the suspending agent were re-dispersed in less than 2.30 seconds for the period of 3 days studied.

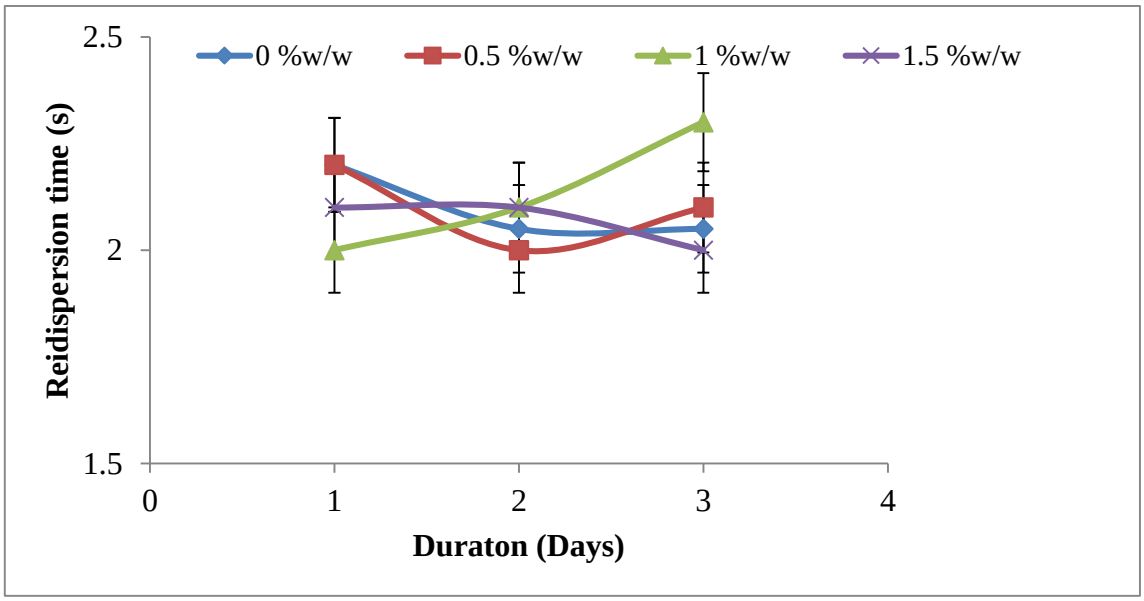

Figure 2: Graph of the effect of concentration of suspending agent on re-dispersion time

Figure 3 shows that with an increasing concentration of suspending agent, there was an insignificant decrease in the flow time (1.77, 1.66 and 1.48 s for 0.5, 1.0 and 1.5 % w/w respectively) while an insignificantly higher flow time (1.89 s) was recorded for the sample without suspending agent compared to the samples in which suspending agent was incorporated.

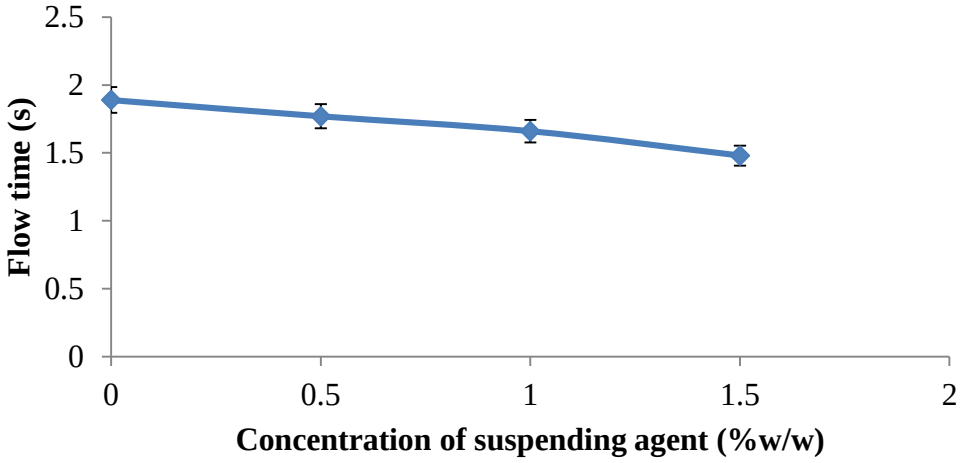

Figure 3: Graph of the effect of concentration of suspending agent on flow rate

The percentage concentration of drug dissolved at 30 minutes was between 91.18 to 133.38 % for samples formulated with suspending agents while the sample devoid of suspending agent released 70.18 % (Figure 4).

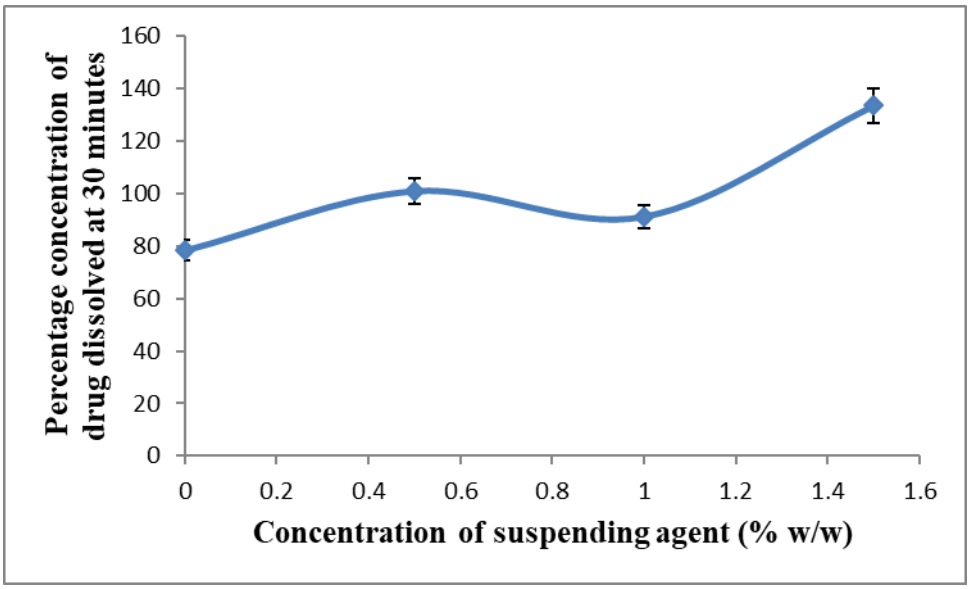

Figure 4: Effect of concentration of suspending agent on the percentage concentration of drug dissolved at 30 minutes

The yield of aqueous root extract of *Moringa oleifera* (9.95 %) was below the findings of Rathi *et al*., (2006) [32.40 %] from the aqueous leaf extract but higher than that of Omobowale *et al*., (2014) [3.33 %] for methanol leaf extract. Such variations in yield might be as a result of processing parameters, botanical source, biological source, time of collection, age of plant, plant part used and environmental conditions of cultivation which can affect or result in variation in chemical composition of plants.

The results of the physicochemical properties of aqueous root extract of *Moringa oleifera* are presented in Table 2. The moisture content of the aqueous root extract of *Moringa oleifera* (7.75 %L) was a suggestion of the powders stability to microbial degradation on storage as a result of its low moisture content (Urmila, 2012) compared to the findings of Samidha and Sasangan, (2017) for leave extract of *Moringa oleifera.*

The angle of repose of the powdered extract was excellent (28.04°) (Aultons, 2013) implying that the suspension might have a good flow when formulated. The results of bulk density, tapped density, Hausner's ratio and Carr's index are in concordance with that of angle of repose on the powder flow (Aultons, 2013).

Ash value usually gives an idea of the quality and purity of crude drugs (Urmila, 2012). As shown in Table 2 above, the ash value determined as total ash appears to be higher than those reported by other authors (Samidha and Sasangan, 2017; Urmila, 2012; Upreti *et al*., 2013) thus indicating the likelihood of the presence of inorganic substances.

Table 3 shows that the microbial contamination of the *Moringa oleifera* aqueous root extract was less than the upper limit (10^3 viable micro-organisms per gram or per milliliter) as specified by the United states Pharmacopoeia, (2009) and the European Pharmacopoeia which implies that the powdered extract is suitable for formulation for oral administration.

Sedimentation volume provides only a qualitative account of flocculation of a suspension (Martin, 2001). Contrary to the findings of Azubuike *et al*., (2017) who found out that as the concentration of suspending agent increases the sedimentation volume also increases, with increasing concentration of suspending agent (SCMC) it was observed that the sedimentation volume was fairly constant for both suspensions formulated with and without suspending agent (Figure 1). The sedimentation volume was also high thus indicating that the internal phase particles have settled but the inter particle attraction and

bonding was loose and not strong enough to form cake during the study period (Anyebe *et al*., 2017).

Figure 2 presents the re-dispersion time of the suspensions formulated. The redispersibility of the suspensions formulated at the various suspending agent concentration shows that all the samples were re-dispersed in a short time with little agitation at all the times recorded. This might imply that the formulation would be easy to remove from the container and transferred to the site of application. Also, it gives an idea as to the suitability of SCMC as a suspending agent in the formulation.

Flow time of the formulated suspension was found to decrease as the concentration of suspending agent was increased. This might be attributed to the type and concentration of the suspending agent employed (Figure 3). This result is similar to the findings of Azubuike *et al*., (2017) who found out that the concentration of suspending agent was inversely proportional to flow rate of a pharmaceutical suspension.

Figure 4 shows the concentration of drug dissolved in 30 minutes. More than 70 % of drug was released within 30 minutes with increasing concentration of drug released as the concentration of suspending agent was increased.

CONCLUSION

In conclusion, the oral reconstitutable suspension of *Moringa oleifera* aqueous root extract formulated had desired features of a good suspension at all the concentrations of the suspending agent employed based on the results obtained from the quality assessment.

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