International Journal of Chemical Science
Online ISSN: 2523-2843, Print ISSN: 2523-6075; Impact Factor: RJIF 5.22
Received: 05-10-2019; Accepted: 06-11-2019
www.chemicaljournals.com
Volume 3; Issue 6; November 2019; Page No. 23-28

Pharmaceutical evaluation of *Malva verticillata* (Linnaeus) mucilage extracts as suspending agent

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Abstract
The study aimed at evaluating the potentials of mucilage obtained from the root bark of *Malva verticillata* as a suspending agent. The effect of concentration and time on the suspending capability of mucilage were determined. Physical and chemical properties were used to characterize the extracted mucilage. The suspending potentials of mucilage was evaluated by rate of sedimentation, degree of flocculation and redispersibility. The extracted mucilage is pseudo plastic, hydrophilic, consisting of protein, fats, and carbohydrates. The result showed a significant negative correlation between the rate of sedimentation and concentration of mucilage. The formulated suspension showed good sedimentation profile, redispersibility, and flow rates. Further, time and the number of particles settling did not affect the suspending properties. *Malva verticillata* mucilage has potential to suspend paracetamol particles.

Keywords: malva verticillata, mucilage, suspending agent, paracetamol

1. Introduction
Suspensions remains as one of the most important dosage forms of presenting insoluble drugs (10 to 1000µm) in liquid form [1]. Intermolecular interaction due to interfacial tension or residual charges, sedimentation due to density difference and formation of cake due to unprotected sediment leads to the loss of pharmacological activity, instability and separation of phases [2, 3]. Thus calling for the exploitation of additives, which would neutralize free charges, form a film around the suspended particle, increase viscosity and minimizes density difference.
Suspending agents impart viscosity, entrapped particle, decrease interparticle interaction and interfacial tension [4]. Suspension having high viscosity with lighter particles become difficult to redisperse. Deflocculated suspension form compacted mass and loose sediments are formed by flocculated suspension. Partial flocculation, low viscosity, minimum density difference, and high zeta potential enable adequate redispersion, controlled sedimentation and improved consistency [5]. Some suspension are stabilized by adjusting pH and ionic strength [3].
Mucilages from different plants have been evaluated as suspending agent. However, these effort yielded few potential mucilage with some being comparable to the commercial agent [6, 7], while others being superior [8, 9]. Mucilages are high molecular weight, sticky and gummy substances that form viscous dispersions in water. They are physiological products of metabolism, hydrophilic compounds composed of heteropolysaccharides, proteinaceous and mineral elements [10, 11]. Its viscous nature is due to arabinose, which is disposed to interact with water [12].
Malva verticillata L is a species of genus Malva in the family Malvaceae. Its roots when peeled or soaked in water releases a sticky slippery fluid that can be precipitated by organic solvent as an amorphous mass. The objective of this study was to extract mucilage present in the roots of *Malva verticillata* and evaluate its potentials as a suspending agent in both flocculated and deflocculated pharmaceutical Suspension.

2. Materials and Methods
2.1. Materials
M. verticillata roots were collected from Eastland in Nairobi (1.2953° S, 36.8721° E), Kenya. Paracetamol powder and Tragacanth gum was obtained from pharmaceutical manufacturing company. Chemicals was purchase from Pyrex limited and were of analytical grade. Equipments used include Brookfield Viscometer LVT, Homogenizer (HG-150, planetary commercial blender among others.

2.2. Extraction and Characterisation of *Malva verticillata* mucilage (MVM)
Extraction and physicochemical characterisation of the MVM was done according to the methods reported elsewhere [13].

2.3 Viscosity
2.3.1. Effect of mucilage concentration
Concentration of MVM (0.1, 0.25, 0.5, 0.75 and 1 % g/ml) was allowed to equilibrate for 30 minutes at 25 ^{O}C. Readings were taken in Brookfield LVT Viscosimeter at a speed of 12 rpm using spindle No.2, S-62 and viscosity calculated as.
Viscosity = dial-reading X 25 (conversion factor).

2.3.2. Effect of shear rates
0.5 % g/mL of MVM were brought into incubation temperature of 25 ^{0}C for 30 minutes. Readings taken at a speed of 6, 12 and 30 rpm using spindle No.2 S-62. Viscosity values calculated by using above formuler [14].

2.3.3. Effect of electrolytes
A solution of salt (NaCl and $CaCl_2$) at 0%, 0.2%, 0.4%, 0.6%, 0.8% w/v was added to 0.5 % g/mL of MVM solution and allowed to equilibrate at room temperature for 30

minutes before determining viscosity.

2.4. Phytochemical Test
The presence of carbohydrates, polysaccharides**,** phenols, tannins, saponins, proteins and glycosides was determined using standard protocols [15].

2.5. Proximate composition
Was expressed by proteins, ash, fats, moisture, and carbohydrates content [16, 17].

2.6. Preparation of suspension
Paracetamol was lavigated with glycerine and dispersed in distilled water to form dispersion A. Sucrose (bulking agent) and sodium benzoate (preservative) was dissolved in deionized water to form solution B. Dispersion A was then homogenize with solution B to form dispersion C. Dispersion C was used to prepare deflocculated and flocculated suspension by adding sodium citrate and magnesium aluminium silicate respectively. Hydrated MVM at 0.5, 0.75 and 1.0 % w/v as a suspending agent was then added to the deflocculated and flocculated dispersion and homogenize at low speed to form respective suspension (Table 1). The procedure was repeated using TCG as the standard suspending agent.

Table 1: Formulation of suspension with different amount of suspending agent.

Formulation	S-1	S-2	S-3	S-4	S-5	S-6
Suspending agent % w/v	0.5	0.75	1	0.5	0.75	1
Paracetamo (g) Drug model	1	1	1	1	1	1
Deflocculant (g)	0.1	0.1	0.1	-	-	-
Flocculant (g)	-	-	-	0.1	0.1	0.1
Glycerine (mL) Wetting agent	1	1	1	1	1	1
Sucrose (g) bulking	5	4.75	4.5	5	4.75	4.5
Sodium benzoate (g)	0.1	0.1	0.1	0.1	0.1	0.1
water (ML) qsv	100	100	100	100	100	100

2.7. Evaluation of Suspension
2.7.1. Apparent viscosity
100 mL of each formulation was allowed to equilibrate for 30 minutes at 25 ^{O}C. Viscosity determined by Brookfield viscometer at 12 rpm using Spindle no.2 and viscosity calculated as described in section 2.3.1 above.

2.7.2. Volumetric Flow rate
A stopwatch was used to measured time taken by 10 mL suspension to flow through a pipette and the flow rate calculated by the equation

Flow rate (mLs^{-1}) = Volume of suspension (mL) / time (Seconds).

2.7.3. Rate of Sedimentation
A portion of each of deflocculated and the flocculated suspension was transferred into a stoppered measuring cylinder and kept at room temperature for four weeks.

The volume of separated clear liquid was recorded at day one of preparation and at intervals of 7 days for 28 days.

The sedimentation volume calculated as the ratio of the ultimate volume of the sediment to that of the original volume of suspension. The degree of flocculation (β) was calculated as the ratio of ultimate sedimentation volume of flocculated suspension (S_F) to that of a deflocculated suspension (S_D) [2].

2.7.4. Redispersibility
10 mL of each of the formulation was kept in calibrated tubes and stored at room temperature for 30 days. At regular time intervals of 10 days of storage, suspension were removed and shaken to redistribute the sediment. The number of shakes to redisperse the sedimented particles was recorded.

2.8. Stability test
The stability study was carried out using 0.75 mg/ml suspensions stored at room temperature and at accelerated conditions of 40°C. Viscosity, redispersion, rate of settling, dissolution and pH were evaluated at 1, 30, 90 and 180 days [18].

3. Results & Discussion
3.1. Characterization of *M. verticillata* Mucilage
3.1.1. Viscosity
Malva verticillata mucilage (MVM) is pseudoplastic fluid with a tendency to spread and form macromolecular networks. The significantly ($p<0.01$) increase in the viscosities of mucilage with concentration (Figure 1a) is associated with the strengthening of intra-molecular interaction. The low viscosity at low concentrations indicates the capability of MVM to form solutions with a wide range of viscosities [19].

The decrease in viscosity (Figure 1b) with an increase in rpm was due to structural changes, interference of chain entanglements, bond enlargement and weaken protein-carbohydrate interaction [20, 21]. Newtonian behavior observed at 1.0% w/v indicates the resistance of MVM to the above factors. The insignificant negative difference between mucilage concentration and rpm on viscosity ($t(17) = -0.424$, $p = 0.683$) highlights the counter- influence of both variables. However, rpm had more influence as shown by a high regression coefficient of -0.327.

The intermolecular repulsion in water makes MVM tp be more viscous. The addition of positive ions (Na^{+}) to mucilage reduces repulsion resulting in contraction and formation of a solution with low rheological activity. The binding of calcium ions on the carboxyl groups result in neutralization, condensation, and formation of less viscous solutions (Figure 1c). MVM consist of negative charge entities that condense with acidic radical to form polyelectrolyte of low viscosity [12, 22].

3.1.2. Phytochemical results
Phytochemical result (Table 2) confirmed the presence of proteins, carbohydrates, polysaccharides, glycosides and amino acids. Contrary, most mucilage's contain tannins and flavonoids. Absence of tannins in MVM suggests that it's safe for pharmaceutical application as an additive.

Table 2: Phytochemical Constituents *M. verticillata* mucilage

Parameter	Remarks	Parameter	Remarks
Carbohydrates	+	Tannins	-
Polysaccharides	+	Proteins	+
Saponins	-	Amino Acids	+
Glycosides	+	Mucilage	+

(+) indicates that it's present and (-) indicates that it's absent

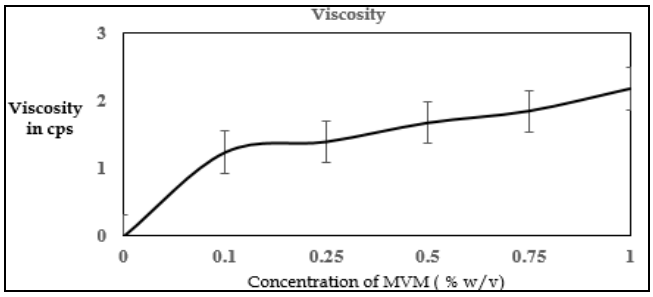

Fig 1a: Effect of concentration on viscosity of of *M. Verticillata* mucilage

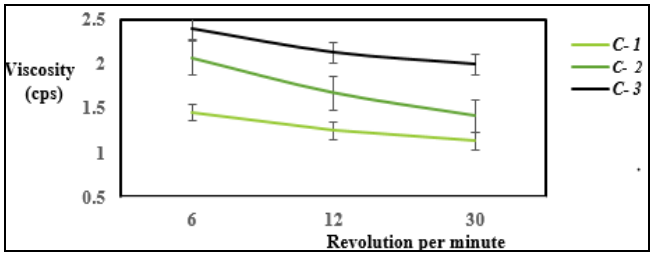

Fig 1b: Effect of shear rate (rpm) on viscosity of *M. Verticillata* mucilage.

C-1, C-2 and C-3 solution containing 0.1, 0.5 and 1.0 %w/v MVM

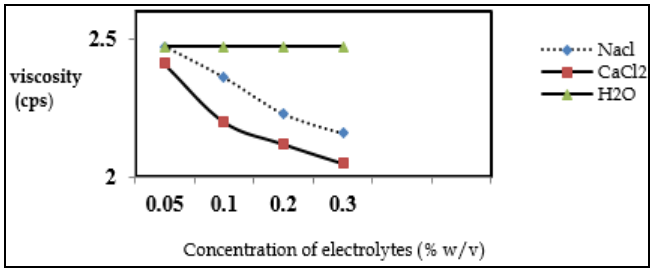

Fig 1c: Effect of electrolytes on viscosity of *M. verticillata* mucilage.

3.1.3. Proximate composition

MVM contains >13.63% g/g ash with significant amount of water-soluble ash (Table 3). This indicates the presence of water soluble salts and negligible contamination by siliceous matter. MVM contains significant amount of proteins which infer the existence of interaction between protein and other components of mucilage. This interactions occur through the hydrophilic functional groups and it explain its pharmaceutical potential [23]. The mucilage contained low percentage of fats < 2.0 % which is responsible for a lipophilic end, While the swelling capacity (>1.39 v/v) demonstrate that it's hydrophilic in nature [24] (Table 3). The moisture in hydrated form is above the pharmacopeial limit (≤ 7 %) and exist in bondage with glycoside. The swelling capacity along with anionic and viscous character contribute to drug retention and absorption.

Table 3: Proximate composition of *M. verticillata* mucilage.

Ash (% g/g)			Total Proteins (% g/g)	Fat (% g/g)	Moisture content % g/g	Sugars (% g/g)
Total	Acid Insoluble	Water soluble				
14.02±0.14	1.04±0.05	9.03±0.19	3.42±0.14	1.68±0.22	13.21±1.12	67.7±1.31

*All values represent mean ± SD; (n=3). SD: Standard deviation.

3.2. Evaluation of Suspension

3.2.1. Viscosity and Flow rate

The observed correlation between the viscosity of suspension and concentration of MVM (Figure 2) revealed pseudo-plastic behaviour which is an essential requirement in pharmaceutical suspension. The viscosity-enhancing effects of mucilage is due to the swelling of the insoluble fraction, reduction of surface tension at the interface by proteins [25]. Martin, 2006 attributed this to strong collision resulting in formation of interparticle bonds over time.

The flow rates decreased with an increase in the concentration-suspending agent (Figure 2). In deflocculated system particles exist as discrete entities exerting less resistance to the flow. The decrease in the flow rate and increase in the viscosity is a positive effect in lowering the rate of settling, enhancing stability and in the administration of the medicament. There is no significant difference in viscosity between deflocculated mucilage and trangacenth suspension. However, the low viscosity of mucilage suspension infer its lesser suspending ability.

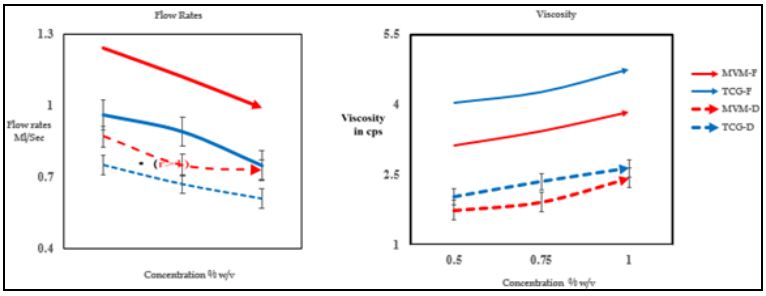

Fig 2: Flow rates and Viscosity of formulated paracetamol suspension containing 1% w/v suspending agent (S-3).

3.2.2. Redispersibility

Prolong storage and high concentration of mucilage resulted in the formation of strong sediment which requires more number of shaking (Table 4). This was attributed to strong binding and formation of the mucilaginous film. Suspended particles tend to settle slowly to form sediment which may

interact leading to the formation of a compact cake. The faster rate of particle settling in flocculated suspension tends to occlude liquid and formed a loose network structure.
While the exclusion of liquid in deflocculated suspension results in the formation of a compact mass. Both mucilage and trangacenth suspension did not form a caked system, though mucilage suspension showed a significant poor redispersibility. Acceptability of a suspension is where a few numbers of shakes are required to redisperse sedimented mass [26].

Table 4a: Redispersibility of deflocculated paracetamol suspension.

Formulation		Rate of redispersibility (cycles)					
		S-1		S-2		S-3	
		MVM-1	TCG -1	MVM-2	TCG -2	MVM-3	TCG -3
Time in days	10	1.3±0.85	0.83± 0.24	3.33±0.236	1.33±0.236	3.5±0.408	1.667±0.624
	20	2±0.408	1.67±0.624	3.66±0.236	2.83±0.236	4.5±0.816	3.0±0.408
	30	5±0.408	3.17±0.236	6.67±0.236	3.67±0.236	7.5±0.408	3.6±0.236

Table 4b: Redispersibility of flocculated paracetamol suspension.

Formulation		Rate of redispersibility (cycles)					
		S-4		S-5		S-6	
		MVM-1	TCG -1	MVM-2	TCG -2	MVM-3	TCG -3
Time in days	10	4.0±0.4	1.67± 0.57	4.66±1.2	2.33±1.1	8.0±0.5	3.66±0.57
	20	4.33±2.1	2.66±0.57	5.0±2.64	2.33±0.58	14±3.61	5.0±1.0
	30	6.1±1.73	4.0±1.0	6.0±1.73	4.0±1,0	20.0±1	6.0±1.0

MVM-1 MVM-2 and MVM-3 are suspension formulated with *M.verticillata* mucilage as suspending agent.
TCG-1, TCG-2 and TCG-3 suspension formulated with Tragacanth gum as suspending agent.
*All values represent mean ± SD; (n=3). SD: Standard deviation.

3.2.3. Rate of Sedimentation

The significant difference ($p<0.01$) between S-1 (0.5% w/v) and S-3 (1 % w/v) indicates that suspending ability is concentration dependent (Figure 3a and 3b). Insolubility of disperse system, presence of charged species and density difference may have contributed to phase separation [27].
The rate of settling was linear during the first 21 days and then remains almost constant, parallel to the time axis (Figure 3a). The weak and negative correlations between sedimentation volume and time (Figure 3) suggest that the suspending properties of mucilage were less affected by time and number of particles settling. MVM hinders the settling of dispersed particles by increasing the viscosity and minimizing density differences between disperse particles and vehicle. The small change in sedimentation volume, sedimentation profile and flow rates indicates good suspending properties of MVM.

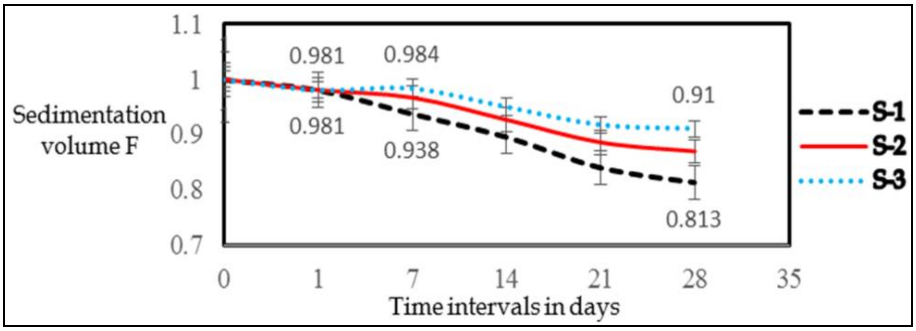

Fig 3a: Rate of sedimentation in defloculated suspension.

S-1, S-2 and S-3 are deflocculated suspension formulated with 0.5, 0.75 and 1 % w/v MVM as suspending agent respectively.

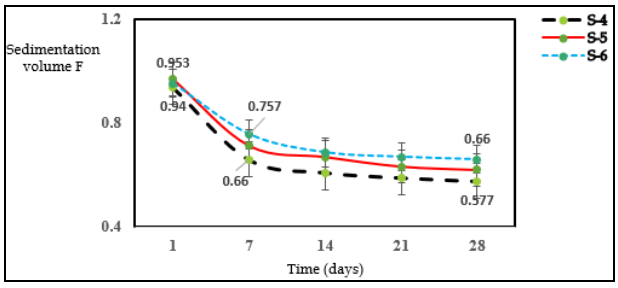

Fig 3b: Rate of sedimentation in flocculated suspension
S-4, S-5 and S-6 are flocculated suspension formulated with 0.5, 0.75 and 1 % w/v MVM as suspending agent, respectively.

The degree of flocculation compare sedimentation volume between flocculated and deflocculated system. Where the higher the value the better the suspending ability. The degree of flocculation increase with an increase in the concentration of MVM (Figure 4). Viscosity was more effective in deflocculated suspension ($S_F<S_D$) reduces sedimentation volume and hence resulting in high degree of flocculation.
MVM at a concentration of 0.5 % w/v (0.5 g/100 mL) was unable to suspend flocculated particles resulting in a high rate of settling, low sedimentation volume and low degree of flocculation. The low sedimentation values infer poor suspending properties of MVM as compared with that of TCG in both deflocculated and flocculated systems (Figure 4).

The accelerated stability test of malva formulations at 40 ^{0}C showed decrease in viscosity over time which affect the behaviour of settling and redispersion as shown in Table 5.

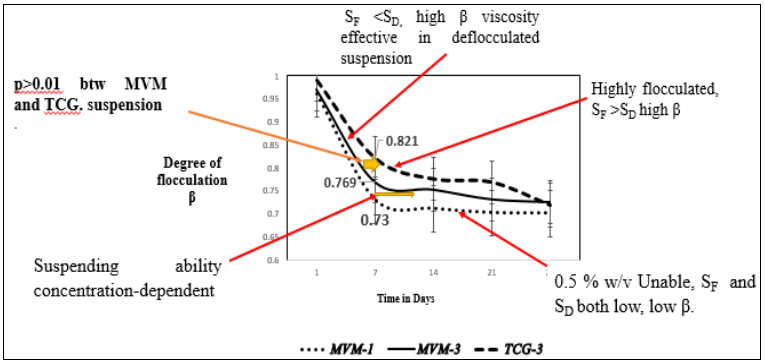

Fig 4: Degree of flocculation (β)

S_F - ultimate sedimentation volume of flocculated suspension

S_D - ultimate sedimentation volume of a deflocculated suspension

MVM-1 and MVM-3 suspension formulated with 0.5 and 1 % w/v with *M.verticillata* mucilage as suspending agent.

TCG-3 suspension formulated with Tragacanth gum as suspending agent.

Table 5: Stability test of 0.75mg/ml MVM suspension.

	25 ◦C			40 ◦C		
	day 1	Day 90	Day 180	day 1	Day 90	Day 180
pH	6.81±0.02	7.01±0.12	6.91±0.11	6.89±0.01	6.11±0.13	6.09±0.08
Viscosity	1.93±0.1	1.23±0.25	1.06±0.24	1.81±0.08	1.23±0.32	1.16±0.31
Redispersion	3.33±0.236	7.0±1.73	9.38±0.46	3.28±0.43	4.18±1.44	5.38±0.67
Rate of settling	0.981±0.07	0.81±0.06	0.67±1.11	0.98±0.81	0.77±0.36	0.57±0.27
Dissolution tests	97.08±1.2	97.13±2.05	97.11±1.32	97.07±2.2	95.33±2.05	94.14±3.37

4. Conclusions

Malva verticillata mucilage is a mixture, consisting of proteins, fats, and carbohydrates with glycosides and peptide linkages. The high moisture content and formation of mucilaginous film limit its applicability. Despite these setbacks, it exhibits functional properties such as shear-thinning, pseudoplastic, hydrophilic and the swell characteristic that contributes to its potential as suspending agent. The capability of *Malva verticillata* mucilage to suspend paracetamol particles and & imparts thixotropic properties was established.

5. Acknowledgments

The authors would like to acknowledge the financial support for the research work from the National Council for Science, Technology and Innovation (NACOSTI-Kenya) through Patrick C. Korir.

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