

# Optimization of Extraction Condition and Characterization of *Malva Verticillata* Root Bark Mucilage

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Received: January 9, 2018 Accepted: February 8, 2018 Online Published: March 10, 2018

doi:10.5539/ijc.v10n2p1

URL: <https://doi.org/10.5539/ijc.v10n2p1>

## Abstract

The study aimed at optimizing the extraction conditions and establishing the physicochemical properties of the mucilage obtained from root bark of *Malva verticillata*. Effects of temperature, time, particle size and amount of water as a solvent on the yield and composition of mucilage were established. Micrometric, physical, chemical and structural properties were used to characterise the extracted mucilage. Result showed significant correlation between extraction conditions and mucilage yield. Low temperature and short extraction time results into pharmaceutically valuable mucilage with low protein content, high viscosity and good dispersion in aqueous solvent. Large and small size particles of the plant material results into low mucilage yield without significant difference ( $p=0.054$ ). The mucilage shows mixed cohesiveness with less tendencies to flow. It exhibits vibration bands in FTIR that are associated with the presence of polysaccharides and proteins. The mucilage contains 2.65% protein, 13.83% inorganics, 1.85% fats, and the rest carbohydrates. High extractive value  $>80\%$  and moisture content  $>12\%$  implies that mucilage has high tendency to microbial attack. Optimized extraction conditions results into high yield of the mucilage with the same physicochemical properties and mineral composition regardless of the plant material sampling sites.

**Keywords:** *malva verticillata*, mucilage, pharmaceuticals, extraction condition

## 1. Introduction

Application of plant mucilages as pharmaceutical adjuvant requires characterization and optimization of extraction conditions as the physico-chemical properties vary with the type of plant, method of extraction and purification. There is need to optimize mucilage extraction conditions in order to enhance yield and achieve desired physical and chemical properties for pharmaceutical applications. Mucilage's are widely applied in the manufacture of drugs as tablet binders and both gelling and suspending agent. Plants have been established to be viable source of mucilage that can be exploited for pharmaceutical applications with a number of plant species being targeted for the coveted pharmaceutical adjuvant (Senthil, Gopalkrishnan, Ramajayam, & Rahul, 2010; Alalor, Avbunudiogba, & Augustine, 2014; Chaudhary, Kulkarni, Rajendra, & Kumar, 2016).

Yield and physicochemical properties of mucilage obtained from different plant species varies with extraction conditions (Amin, Shamsuddin, Yinyin, Yahya, & Ibrahim 2007). Mucilage yield increases with temperature and time of extraction. Thanatcha and Pranee, (2011) reported the significant effect of solvent on yield of mucilage. No thermal method of hydrating and agitation results in high yield than non-agitation method (Monrroy, Garc ía, R ós, & Ren á, 2017). Mucilage precipitated by acetone is more soluble and swell totally in water than using ethanol (Patel, Shah, Mahajan, & Dushyant, 2011). High temperatures and extended time of extraction yield mucilage with high viscosity and proteins content (Singthong, Ningsanond, & Cui, 2009). Other researchers have reported the negative effect of high extraction temperature on proteins and viscosity of mucilage.

Mucilage's are high-molecular-weight, sticky and gummy substances which form dispersions in water. The Mucilage dispersion can be precipitated as amorphous or granular mass by organic solvents (Chaudhary *et al.*, 2016). According to Goycoolea and Cardenas, (2003), mucilage consists of arabinose, galactose, mannose, xylose, uronic acids and acid resistant nucleus that can be hydrolysed enzymatically. Bruneton, (1999) classified mucilage as heterogeneous

polysaccharide consisting of protein, starch and sugar. Alalor *et al.*, (2014) reported mucilage as an ester of sulphuric acid with glycoside linkages with high-molecular-weight, sticky and gummy substances.

*Malva verticillata* L. (Cluster mallow, Malvaceae) is often used as a vegetable as well as an herb in traditional medicine. Its leaves are alternate with five to seven lobes (Laurence, 2007). The roots of *M. verticillata* when peeled or soaked in water releases a sticky slippery fluid which can be precipitated by organic solvent as amorphous mass. The objective of this study was to optimise extraction condition and to determine composition of mucilage obtain from *M. verticillata* roots bark.

## 2. Methods

### 2.1 Materials

All reagents used were of analytical grade. *Malva verticillata* roots was collected from Nairobi Eastland (Sample A), Nairobi county and Kericho (Sample B), Kericho county, Kenya, after authentication by the Taxonomist. Sample specimen were preserved at Nairobi University Herbarium, Voucher number, PCK2015/01.

### 2.2 Optimization of Extraction Method

#### 2.2.1 Extraction of Mucilage

Air dried root bark of *M. verticillata* were grounded into 149-250, 250-500 and 750-1000  $\mu\text{m}$  fractions to open up mucilage cells. Each fraction was pre- treated with 95% ethanol in a boiling-water bath for 1 hr to leach out organic compounds as slurry. Distilled water containing 1% sodium metasilphite was added to the pre-treated mass at specified extraction conditions as shown in Table 1 below to extract the mucilage. The whole mass was strained through a fourfold muslin cloth to separate filtrate from the marc. Filtrate was then centrifuge at 3000 rpm for 10 mins to precipitate out insoluble fibre. 95% ethanol at equal volume was added to the supernatant to separate out mucilage as crude solid mass. The crude mucilage was washed with acetone and dried in an oven. Crude mucilage was then purified by back extracting with water and deproteinating with chloroform/n-butanol (v/v = 4:1) in boiling water bath for 10 mins. Mucilage was then precipitated out by adding 95% ethanol before drying in an oven at 45 °C for 2 hrs. Dry *Malva verticillata* mucilage (MVM) was passed through BSS no. 60 (250  $\mu\text{m}$ ) before storing in air tight bags at room temperature.

Table 1. Mucilage extraction conditions

Coded level	Independent Variables				Dependent Variable
	Amount of solvent per g of MV Powder mL/g	Time in hours	Temperature in °C	Particle size in $\mu\text{m}$	Yield of Mucilage g/g
-1	10	1	25	149-250	
0	15	3	50	250-500	
+1	20	6	75	500- 750	
+2	25	12	100	750- 1000	

#### 2.2.2 Effect of Extraction Temperature on Composition Yield of Mucilage

This was determined by percent yield of ash, proteins and viscosity. Viscosity was determined by hydrating MVM (1% g/mL) and equilibrate at 25°C before determining viscosity by Brookfield RVT Viscosimeter at 1.5 rpm using spindle No.2. Ash content was determined by UK Essays, (2015) method using 3.0 g of unpurified mucilage powder <105 microns. Protein content was determine by Gebresamuel and Tsige, (2012) method.

### 2.3 Physical Properties of Mucilage

#### 2.3.1 Micrometric Properties of Mucilage

Mucilage powder was grounded and fractionated into the size range of 149-250, 105-149 and 74- 105  $\mu\text{m}$ . 10 g from each fraction was transferred into tarred 50 mL graduated cylinder and tapped at a height of 5 cm from the bench once and till equilibrium (constant volume). Bulk density ( $D_b$ ) and tapped density ( $D_t$ ) was calculated as mass of powder/tap volume, based on one and equilibrium tapings respectively. Hausnrs index ( $D_t/ D_b$ ) and Compressibility index ( $1- D_b / D_t$ )  $\times 100$  was then calculated (USP, 2012).

#### 2.3.2 Solubility Index

The solubility index was determined by dispersing 0.1 g (<74 $\mu\text{m}$ ) MVM in 50 mL distilled water and incubated at temperatures 25, 50 and 75 °C for 30 minutes. It was then centrifuged at 800 rpm for 15 mins and supernatant was dried at 125 °C until constant weight. Solubility was calculated by the following equation (Sciarini, Maldonado, Ribotta, Perez, & Leon, 2009).

$$\text{Solubility} = (\text{weight of dried supernatant} / \text{sample weight}) \times 100\%$$

### 2.3.3 Water Holding Capacity

This was determined by suspending 1 g of MVM in 50 mL distilled water, mixed and centrifuged at 3000 g for 10 min. The supernatant decanted and water holding capacity was expressed as grams of water absorbed per 100 g of mucilage (Adebowale, Adeyemi, & Oshodi, 2005).

### 2.3.4 Moisture Sorption

1 g of MVM was placed in Pyrex desiccators containing distilled water. Weight gained after 5 days as percentage of initial weight was used to express moisture Sorption (Medina, Brito-De, Torrestiana, & Kathain, 2000).

### 2.3.5 Swelling Capacity

1 g of MVM was transferred into 25 mL graduated cylinder and its tap volume determined. It was then dispersed in 50 mL distilled water and kept standing for 24 hrs. Volume of hydrated mucilage was noted. Swelling capacity was calculated as the ratio of the hydrated volume to tap volume of dry mucilage (Satheesh, Gayathri, Pradeep, & Saravanan, (2012).

### 2.3.6 Extractive Value

1 g of MVM was grounded with chloroform – water mixture to form a dispersion which was incubated for 24 hrs with regular shaking. It was then centrifuged at 500 rpm for 5 mins. Extractive value determined by the amount of dissolved solid in the supernatant dispersion (UK Essays, 2015).

### 2.3.7 Conductivity and pH

MVM was homogenised in 100 mL distilled water to get concentration in the range of 0.02 to 1% g/mL and centrifuged for 5 mins at 500 rpm. Conductivity and pH of supernatant dispersion was then determined by conductometer and pH meter, respectively.

## 2.4 Chemical Characterisation of Mucilage

### 2.4.1 Elemental Composition

X-ray Fluorescence (XRF) was used to identify and quantify different elements present in *M. verticillata* mucilage.

### 2.4.2 Functional Group Characterization

Functional groups were determined by Fourier transform infrared (FTIR) spectroscopy. 100 mg of the dried MVM was mixed with KBr and compressed into discs by applying a pressure of 5 tons in a hydraulic press. The pellet was placed in the light path and infrared spectra recorded between 4000 and 500  $\text{cm}^{-1}$  (Verma, Bansal, Kumar, Malviya, Sharma, & Kumar, 2014).

## 3. Results and Discussions

### 3.1 Optimization of Extraction Methods

Optimization of extraction condition for aqueous extraction of *M. verticillata* mucilage was studied by varying temperature, time, particle size and amount of solvent. Yield was expressed as gram of dry MVM per gram of air dry roots bark (% g/g). Mucilage extracted from *M. verticillata* root bark was brown off, amorphous and sticky when moist. Figure 1 below shows the solid mass of MVM during its isolation with ethanol.

Effect of increasing extraction condition (temperature, time and amount of solvent per gram of powder) on yield of mucilage are as displayed in Figure 2. There was statistically significant correlation ( $r > 1$ ,  $P < 0.01$ ) between temperature and yield of mucilage. This indicates that heat is required during mucilage extraction to reduce viscosity and enhance its release. The insignificant effect ( $p = 0.634$ ) of temperatures above 50°C, suggest that there is an optimum amount of heat required to break mucilage cells so as to drive out mucilage.

Release of mucilage was observed to be time dependant (Figure 2) as solvent had to accumulate enough thermal energy so as to penetrate mucilaginous cell, enhance dissolution and transfer of mucilage out of the cell. However, the insignificant effect of time after 3hrs could be related to degradation of mucilage cell. Decrease in mucilage yield at prolonged extraction time may be due to presence insoluble components which retard solubility of the mucilage (Sepulveda, Saenz, Aliaga, & Aceituno, 2007).

Result showed a linear correlation ( $r = 0.353$ ,  $p = 0.033$ ) between yield of mucilage and amount of solvent added. This was due to an increase in concentration gradient which enhanced mucilage extraction. High absorption capacity and viscosity of the marc at low ratio tend to trap non polar impurities retarding mucilage solubility and its yield (Najmuldeen, Hariitaraan, Ghasak, & Zulkafli, 2017). The insignificant effect ( $p = 0.284$ ) at high ratio was due to saturation of mucilage cell which hindered mass transfer.

Figure 2 illustrates that yield of mucilage increase with increase in extraction condition (process parameters). There was no significant difference ( $t_8 = 1.130, p > 0.05$ ) on obtained yield when time and temperature were increased at constant amount of solvent. Positive value of  $t$  suggests that increase in both conditions resulted in high yield. Increase in time and volume of solvent at constant temperature resulted in insignificant difference ( $t_8 = -0.341, p > 0.05$ ) on yield. Negative value of  $t$  suggests that one of the extraction condition contributed strongly to yield more than the other variable. Extraction model, therefore was proposed as shown by the following formula.

$$Y = 8.35 + 4.39X_3 + 2.66X_2 + 1.29X_1 \quad R^2 = 88.2\% \quad \text{Equation 1}$$

Where  $X_1$  is time of extraction,  $X_2$  is temperature,  $X_3$  is solvent volume and  $Y$  is yield of mucilage. High coefficient of time (4.39) indicates that it has more influence on yield than other extraction variables (temperature and volume of solvent).



Figure 1. *M. verticillata* mucilage

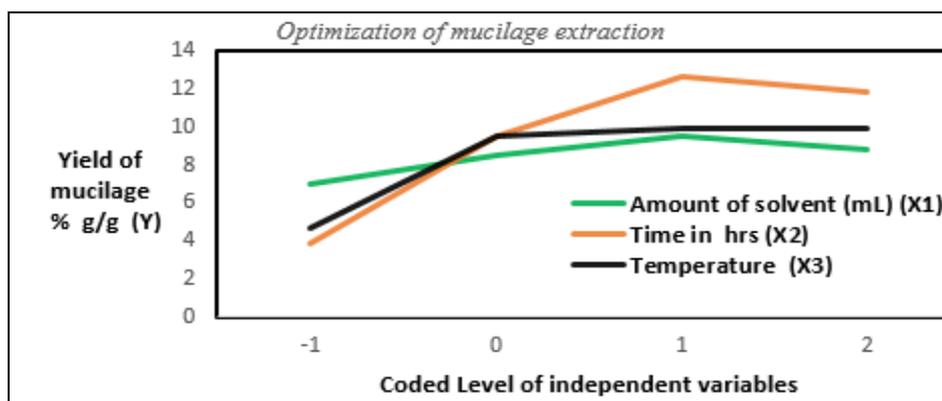


Figure 2. Plots of yield of MVM as a function of Temperature, Time and Amount of solvent used

Figure 3 below shows the effect of particle size on mucilage yield. Result shows that yield obtained from particles  $< 250 \mu\text{m}$  and  $> 750 \mu\text{m}$  were low and insignificant ( $p=0.054$ ). Lowest yield obtained from particles  $> 750 \mu\text{m}$  was due to closed mucilage cell which retarded penetration and consequent release of mucilage. While development of impervious film and high viscosity of the marc contributed to low yield as was observed in small Particles less than  $250 \mu\text{m}$ . Factors like open mucilage cell, even distribution of solvent, efficient mixing, decrease in viscosity of the marc and easy separation of extracted mucilage contributed to the increase in the yield as was observed in the particle of size range of  $250\text{-}500 \mu\text{m}$ .

Regression analysis of result in Figure 3 prove that there was no correlation ( $r = -0.224, p=0.492$ )  $R^2 = 70\%$ ) between yield and particle size. This indicates that yield does not depend on particle size. Negative correlation and coefficient implies that yield increases with decrease in particle size.

It was observed that yield obtain from sample A was higher than the one obtained from sample B as shown in Figure 3. The variation was significant ( $p < 0.05$ ) and therefore it was considered has a hint of components present (Woolfe, Martin, Chaplid, & Otchere, 1977). High stress due to dry condition, make *M. verticillata* to synthesis and store more mucilage than in fertile soil, where it store less amount of mucilage.

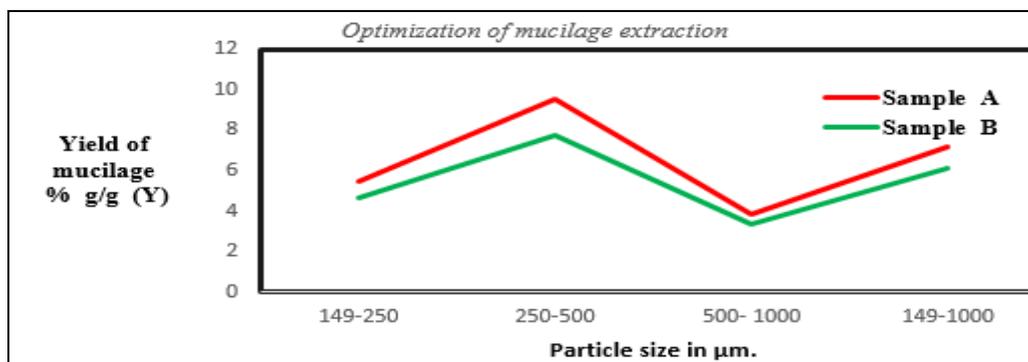


Figure 3. Plots of yield of MVM as a function of Particle size and Sampling sites

Here [A] and [B] denote samples collected from Nairobi and Kericho region respectively.

### 3.2 Effect of Extraction Temperature on Composition Yield of Mucilage

Effect of temperature on viscosity, percent yield of ash and proteins are given in Table 2. Increase in temperature had no significant effect ( $P = 0.142$ ) on yield of ash with the positive correlation ( $r > 1$ ) being associated with the increase in solubility of inorganic components in the plant material. Amount of water soluble ash were significant as compared to acid insoluble ash. This indicates that most of the salts present in MVM are soluble in water and that there was low contamination by siliceous materials.

The significant ( $P < 0.01$ ) increase in proteins with temperature was due to the use of excess solvent at high temperature, co-precipitation by ethanol and presence of fat which create lipophilic environment. Despite purification, high percentage of proteins was retained by mucilage (Table 2). This may infer existence of a strong interaction between protein and components of mucilage. The negative correlation ( $r < 1$ ,  $p = 0.015$ ) between viscosity and temperature indicates that high extraction temperature yield mucilages which are of low viscosity. This observation can be associated with irreversible weakening of intermolecular forces and denaturing of proteins (Gebresamuel and Tsige, 2012).

Table 2. Effect of extraction temperature on composition of *M. verticillata* mucilage

Temperature in $^{\circ}\text{C}$	Ash in % g/g			Total Proteins in % g/g		Viscosity in cps	
	Total Ash	Insoluble Ash	Water soluble Ash	Crude MVM	Purified MVM	Crude MVM	Purified MVM
25	14.41 $\pm$ 0.49	1.04 $\pm$ 0.05	9.03 $\pm$ 0.19	4.12 $\pm$ 0.22	2.12 $\pm$ 0.37	2.47 $\pm$ 0.16	2.17 $\pm$ 0.42
50	14.63 $\pm$ 0.37	0.93 $\pm$ 0.04	12.27 $\pm$ 0.05	5.66 $\pm$ 0.45	3.25 $\pm$ 0.41	2.48 $\pm$ 0.19	2.15 $\pm$ 0.32
75	15.09 $\pm$ 0.08	0.88 $\pm$ 0.07	12.78 $\pm$ 0.06	6.43 $\pm$ 0.09	2.03 $\pm$ 0.35	1.81 $\pm$ 0.25	1.08 $\pm$ 0.43

Therefore, the optimum condition for extraction of mucilage from *M. verticillata* root bark were powder to volume ratio of 1:20 for 3 hrs at  $50^{\circ}\text{C}$  using particle size in the range of 250- 500  $\mu\text{m}$ . This condition resulted in high yield, pervious mucilage, and of high viscosity with low protein content. High temperature yielded mucilage which was of low viscosity, poor solubility and high percentage of proteins. Longer time of extraction compromises microbial contamination while use of excess solvent results in high yield of mucilage due to occlusion of impurities and consumes a lot of dissolving solvent.

### 3.3 Micrometric Properties of Mucilage

Micrometric properties of MVM as a function of particle size are as shown in Table 3. Difference in tapped and bulk density depicts mucilage particles to be surface active with better packing rearrangement. tapped density was higher than bulk density to suggests that tapping causes interparticle attraction and interlocking. Obtained values of  $\text{CI} < 30 > 11$  and  $\text{HI} < 1.45 > 1.12$  shows that MVM exhibits mixed cohesiveness and free-flowing behaviour. These properties are influenced by processes such as grounding which increases surface area, moisture content which bridge by capillary force and amorphous nature of particles which contribute to interlocking (Fitzpatrick and Ahrne, 2005).

Table 3. Effect of particle size on Micrometric properties of MVM

Particle size in $\mu\text{m}$	Bulk density g/ml	Tap density g/ml	Hausner's index HI	Compressibility index CI in %
74-105	0.822 $\pm$ 0.010	1.146 $\pm$ 0.08	1.39 $\pm$ 0.009	28.22 $\pm$ 0.44
105-149	0.726 $\pm$ 0.005	0.923 $\pm$ 0.06	1.27 $\pm$ 0.001	21.17 $\pm$ 0.32
149-250	0.723 $\pm$ 0.004	0.818 $\pm$ 0.006	1.13 $\pm$ 0.01	11.66 $\pm$ 0.714

### 3.4 Physical Properties of Mucilage

Results displayed in Table 4 shows that temperature does not influence solubility of MVM in water. The insignificant correlation ( $r > 1$ ,  $p = 0.067$ ) between solubility and temperature suggests that the intermolecular interaction become weak as temperature increases (Doublrier and Cuvelier, 2006). Low solubility of MVM in water was due to presence of proteins, fats and formation of mucilaginous and hydrated magnesium film. Ability of mucilage to form dispersion was found to decrease with increase in concentration, temperature and force of centrifugation. Solubility of MVM was observed to be independent of sampling areas.

Table 4. Effect of Temperature on Solubility of *M. Verticillata* mucilage in water

Temperature in $^{\circ}\text{C}$		25	50	75
Solubility in % g/g	Sample A	44.22 $\pm$ 1.09	50.39 $\pm$ 0.83	48.36 $\pm$ 0.76
	Sample B	45.59 $\pm$ 1.91	49.48 $\pm$ 1.13	49.77 $\pm$ 1.66

*M. verticillata* mucilage had ability to absorb and retain water when subjected to centrifugation stress and humid environment. This was shown by water holding, moisture sorption and swelling capacity (Table 5). The difference in swelling and water holding capacity explains the effect of centrifugation force on capability of mucilage to bind water. Absorption of water by mucilage resulted in swelling, conformational changes and interactions between permeating water and polar groups of mucilage (Diab, Biliaderis, Gerasopoulos, & Sfakiotakis, 2001). Hydroxyl groups enable mucilage to absorb, retain and interact with water, while presence of calcium and proteins block water binding sites by forming insoluble complexes (Fedeniuk and Biliaderis, 1994).

High extractive value of  $>80\%$  and moisture content of  $>13\%$  (Table 5) indicates that most of mucilage constituents are water extractable and residual water exist in hydrated form, in bondage with glycoside. *This characteristic* implies that MVM has high tendency to microbial attack (Dafam, Abu-Bakr, Nuhu, Ajima, & Okwori, 2013).

Table 5. Hydration properties of *M. verticillata* mucilage

	Swelling Capacity v/v	Water Holding % g/g	Moisture Sorption % g/g	Extractive % g/g	Moisture content % g/g
Sample A	1.45 $\pm$ 0.07	17.2 $\pm$ 1.26	34.53 $\pm$ 3.05	80.15 $\pm$ 3.64	13.21 $\pm$ 1.12
Sample B	1.39 $\pm$ 0.11	14.12 $\pm$ 1.76	28.51 $\pm$ 2.3	79.25 $\pm$ 2.48	14.17 $\pm$ 1.15

Result in Table 6 shows that pH of MVM increases significantly ( $p < 0.01$ ) with increase in concentration. MVM was found to be alkaline with the conductivity increasing significantly ( $p < 0.01$ ) with increase in mucilage concentration. This indicates that mucilage dissociates in water to yield mobile ions that can generate electrical stress. Insignificant increase in conductivity ( $p > 0.05$ ) at higher concentration and small deviation in pH  $\pm 0.3$  indicates that the mucilage is fairly stable in aqueous environment.

Table 6. pH and Conductivity of *M. verticillata* mucilage

Concentration g/100 mL	0.1	0.25	0.5	0.75	1
pH	7.55 $\pm$ 0.04	7.63 $\pm$ 0.03	7.71 $\pm$ 0.01	7.82 $\pm$ 0.04	7.91 $\pm$ 0.05
Conductivity $\mu\text{S/cm}$	352 $\pm$ 10.45	596 $\pm$ 10.03	860 $\pm$ 8.53	1140 $\pm$ 12.05	1151 $\pm$ 9.61

### 3.5 Chemical Properties of Mucilage

Composition and proportion of mineral present was found to be constant and independent of sampling areas as shown in Table 7. Presence of calcium and sulphur makes *M. verticillata* mucilage exist of complex compound entities (Nuhu and Abdullahi, 2009; Senthil *et al.*, 2010). The hard compact mass of the mucilage formed on drying may be attributed to presence of magnesium which interact with water and atmospheric oxygen leading to formation of knit-webbed

mucilage structure. Loss on ignition (LOI) indicates that MVM contain high proportion of carbon and bound water. Presence of Aluminium, chloride and sulphate may limit the mucilage application as pharmaceutical adjuvant (USP, 2012).

Table 7. Elemental composition of *M. verticillata* mucilage in %

Sampling Region	Elemental composition									
	K	Na	Ca	Mg	Al	P	Si	S	Cl	LOI
Sample A	15.07±1.99	6.17±1.14	22.08±1.38	4.34±0.5	3.69±0.31	7.34±0.51	5.72±0.43	2.19	0.57	85.65
Sample B	6.56±1.55	1.86±0.66	29.91±1.05	0.96±0.13	1.82±0.66	2.58±0.42	4.54±0.38	0.86	0.36	79.41

Figure 4 shows FTIR spectra bands of *M. verticillata* mucilage. The picks at 798.8 and 1148.2 $\text{cm}^{-1}$  represent C-O and C-O-C finger print region for carbohydrates. The presence of C-O and O-H entities in the carbohydrate unit is further corroborated with the absorption bands appearing at 1002.4  $\text{cm}^{-1}$  and 3471.22  $\text{cm}^{-1}$  (broad), respectively (Kemp, 1991). The weak signals at 3025  $\text{cm}^{-1}$  and 2907.6  $\text{cm}^{-1}$  represent stretching of N-H in proteins and C-H in the pyranose group, respectively (Han, Gao, Yin, Jin, & Chen, 2016). The absence of one of absorption bands associated with carboxylic group (1680  $\text{cm}^{-1}$ , s) indicates the absence of uronic acid which is usually present in a number of plant mucilages (Monroy *et al.*, 2017).

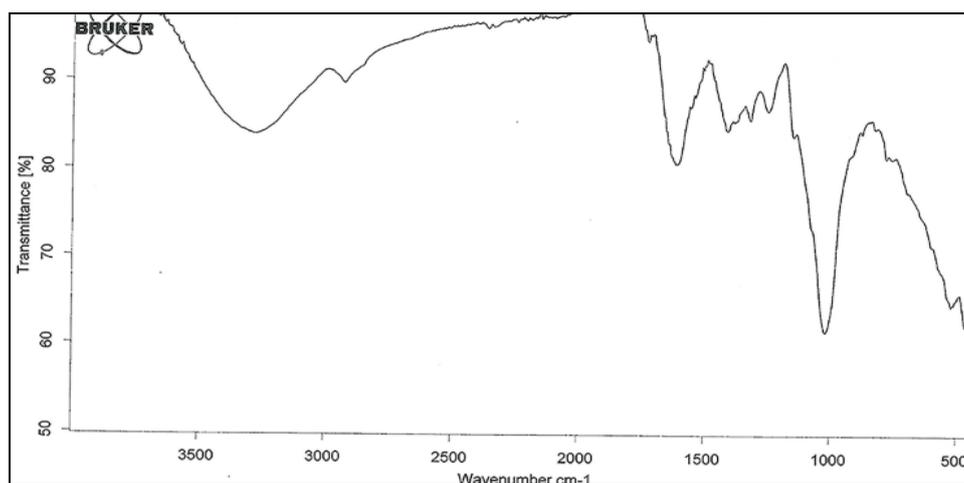


Figure 4. FTIR Spectrum of *M. verticillata* mucilage

#### 4. Conclusion

*Malva verticillata* mucilage is amorphous, cohesive and sticky when moist. Its solubility in water depends on the process of grounding and not temperature. Low temperature and short duration results in more dispersible mucilage with high viscosity. Yield and composition of mucilage depends on extraction conditions and not particle size. *M. verticillata* mucilage have ability to hold water, high tendency to microbial attack and exist as a polysaccharides complex of calcium. Absence of tannins and constant composition independent of sampling areas suggests the high potential of the mucilage as pharmaceutical adjuvant subject to biocompatibility evaluation.

#### Acknowledgements

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 PC Korir. We thank Mr P. Mutiso of University of Nairobi Herbarium for verification and identification of plant samples.

#### Conflicts of Interest

The authors declare no conflict of interest.

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