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Research Article

Further, Physical Characterization of Biopolymer Isolated from Seeds of *Manilkara Zapota* (Linn.) P. Royen Syn. with Potential Pharmaceutical Use

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ABSTRACT

Manilkara zapota (Linn.) P. Royen syn. a shrub belonging to family sapotaceae. The whole plant have been reported as a source of biomedicine. In our previous study we reported mucoadhesive charaterization of isolated gum from the seeds of *Manilkara zapota*. Here in, we further investigated the physical property of tablets including wetting time, water absorption ratio, mass degree of swelling, water uptake, mass loss, and *in-vitro* swelling developed using seed mucilage of *Manilkara zapota* fruit. The results of physical characterization indicated that the biopolymer possess similar bioadhesive property as compared to synthetic polymer used in study. The result showed that physical property of seeds mucilage is comparable to synthetic under the experimental conditions. Briefly, it could be concluded that the seed mucilage of *M. zapota* can be used as a pharmaceutical excipient in oral mucoadhesive drug delivery systems. Moreover, it has the potential to also replace existing synthetic mucoadhesive polymers upon further polymerization or chemical modifications.

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INTRODUCTION

Functional biopolymers have been successfully employed in the design and development of several pharmaceutical dosage forms including controlled release, extended release, and modified release with specific use in formulation

of mucoadhesive drug delivery systems. Both synthetic and natural biodegradable polymers have been extensively investigated, however the use of natural polymers for pharmaceutical applications is gaining attention towards researcher due to non-toxic, economical, readily available, and capable of chemical modifications, potentially biodegradable and biocompatible (Singh et al., 2021). Recent trends towards use of plant based natural products demand the replacement of synthetic additives with natural gum or excipients (Sudarshan and Sunil B, 2015, Singh and Bothara, 2014, Singh and Bothara, 2013, Singh et al., 2020b).

Gums and mucilages are water-soluble, viscous, and highly fermentable by the microorganisms of the intestinal tract (Huffman, 2003). The most important natural gums used in the pharmaceuticals are flax seed gum (Liu et al., 2018), guar gum (Sharma et al., 2018), and tragacanth (Nazarzadeh Zare et al., 2019), which are obtained from natural resources. *M. zapota* (Linn.) P. Royen syn. is a large, evergreen, forest tree more than 30 m in height a tree belonging to family from Sapotaceae. *Manilkara zapota* was used since Vedic age for various biomedical and in our previous study attempt have been made to evaluate for physiochemical property, micromeritics characterization, and mucoadhesive characterization (Sudarshan and Sunil B, 2015, Singh and Bothara, 2014, Singh and Bothara, 2013). However, we yet not reported the physical characterization of seed mucilage tablets. Moreover, earlier study reported that non-toxic (Singh and Bothara) polysaccharides from the seed consist of arabinose, fructose, mannose, rhamnose and xylose (Singh and Bothara, 2014) and also showed controlled release of active pharmaceuticals with excellent mucoadhesive strength (Singh and Bothara, 2013).

Bioadhesion is a state in which two materials, at least one biological in nature, are held together for an extended period of time by interfacial forces (Selvaraj et al., 2020). The mucins/polymer interactions are strongly influenced by polymer-related and intestinal environmental factors, both of which can modulate the degree of adhesion and the residence time of the mucoadhesive dosage form (Russo et al., 2016). Considering the complexity of the mucoadhesion process, present paper reports physical evaluation of *M. zapota* seed mucilage as mucoadhesive agent by various reported method (Singh et al., 2021, Singh et al., 2020a) in compressed tablet form.

MATERIALS AND METHODS

Fruits of *Manilkara zapota* (Linn.) P. Royen syn. were collected from the forest of Chhattisgarh, and authenticated by Prof. H. B. Singh, NISCAIR, New Delhi, India. Mucilage from seeds of *M. zapota* (MZM) was isolated as per previous report (Bothara and Singh, 2012) and stored in airtight polypropylene jars till further use. Guar gum (GG) was procured from Loba Chemie, Mumbai. Methocel E5 premium (HPMC E5) was purchased from Sigma-Adrich, Singapore. De-ionized water was used for all experiments. All other chemicals used were of analytical reagent grade. The mucilage tablets prepared (Table 1) as previously reported (Sudarshan and Sunil B, 2015) and characterized for physical property.

Table 1: Composition of Tablets for Mucoadhesion and Physical Characterization

Excipients	F1	F2	F3	F4	F5	F6	F7
Seed mucilage (mg)	50	-	-	-	100	-	-
Guar gum (mg)	-	50	-	-	-	100	-
HPMC E5 (mg)	-	-	50	-	-	-	100
Binding agent (w/v %)	qs	qs	qs	qs	qs	qs	qs
Barium sulfate (mg)	50	50	50	50	-	-	-
Diluent (mg)	20	20	20	70	20	20	20
Talc (mg)	2	2	2	2	2	2	2
Magnesium stearate (mg)	3	3	3	3	3	3	3

Physical Characterization of Tablets

Wetting time and water absorption ratio

A piece of folded tissue paper (12 cm x 10.75 cm) was placed on petri pate (ID = 6.5 cm) containing 6 ml of phosphate buffer pH 6.8. Amaranth red indicator (1 mg) placed over tablets was transferred on the paper, and the time for complete wetting was measured. The wetted tablet was weighed and water absorption ratio 'R' determined using the formula,

$R = [(W_b-W_a)/Wa] \times 100$ ---------- [1]

Where, W_a and W_b are weight of tablet before and after water absorption.

Mass degree of swelling

The swelling properties and erosion characteristic of test and standard mucoadhesive tablets were evaluated by determining percent hydration and percent matrix erosion. Each tablet was weighed (W_1) and immersed in a phosphate buffer pH 6.8 for predetermined time (5 h). After immersion for specified time, tablet was wiped off to remove excess of surface water using filter paper and weighed (W_2). The swollen tablet was dried at 60 °C for 24 h in an oven and kept in desiccator for 48 h and reweighed (W_3). Percent hydration and matrix erosion were calculated using following formula.

$$\text{Percentage hydration} = \frac{W_2 - W_1}{W_1} \times 100 \quad \text{------[2]}$$

$$\text{Percentage matrix erosion} = \frac{W_1 - W_3}{W_3} \times 100 \quad \text{---[3]}$$

Moisture absorption

Moisture absorption study was performed according to the previous report (Singh et al., 2020a, Singh et al., 2021). Briefly, agar (5 % m/v) was dissolved in hot water and inducted to solidify in petri plates. Previously, vacuum dried test and standard mucoadhesive tablets (n=6) were taken, weighed individually and laminated with plastic tape (impermeable backing membrane) on one side. The tablets were placed individually to petri plates in manner that other side had contact with the agar medium followed by incubation at 37 °C for 1 h. Post incubation, mucoadhesive tablets were re-weighed, and the percentage of moisture absorption was calculated using the following formula.

$$\text{Percentage Moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100 \quad \text{-----[4]}$$

***In-vitro* swelling studies**

In vitro swelling study of bio-adhesive polymer was performed according to the previous report (Singh et al., 2021, Singh et al., 2020a). In brief, mucilage tablet was weighed and separately placed in a series of pre-weighed glass tubes closed at the bottom by stainless steel mesh 120 #. Each tube was vertically placed for predetermined times (5 h) in a beaker containing 50 ml salivary fluid pH 6.8 such that the tablet remained in contact. Tablets were removed and re-weighed, (wet weight) dried at 40 °C for 24 h and again reweighed (dry weight). The percent wet weight recovered was calculated by using the following formula.

$$\text{Percentage wet recovered} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100 \quad \text{-------[5]}$$

Water uptake and mass loss

The water uptake and mass loss study was performed according to the previous report (Singh et al., 2020a, Singh et al., 2021). In brief, water uptake study was carried out using USP-XXIII paddle type dissolution test apparatus at 37 ± 0.5 °C at 0.833 rps and 1.66 rps using 900 ml of phosphate buffer (pH 6.8) as dissolution medium for test and standard mucoadhesive tablets. At predetermined time (5 h) the ring mesh assemblies supporting the partially hydrated tablets were lightly blotted with tissue paper to remove extra amount of water, the tablets were dried at 70 °C for 1 day, and remaining dry wet was determined gravimetrically using equation:

$$\text{Percentage Water Uptake} = \frac{\text{Wet weight} - \text{Remaining dry weight}}{\text{Remaining dry weight}} \times 100 \quad \text{-------[6]}$$

$$\text{Percentage Mass Loss} = \frac{\text{Remaining dry weight}}{\text{Original dry weight}} \times 100 \quad \text{----------[7]}$$

STATISTICAL ANALYSIS

Results obtained for physical assessment of tablets are expressed as mean ± Standard Error Mean (SEM) and subjected to one-way analysis of variance (ANOVA) followed by Dunnett's test and values with $p < 0.05$ were considered to be statistically significant.

Physical characterization tablets

The swelling degree of bio-adhesive polymer is an important factor affecting bioadhesion (Singh et al., 2020b). Water absorption ratio and wetting time, are important criteria for understanding the capacity of mucilage to disintegrate/swell in presence of small amount of water (Singh et al., 2020c). The result of physical characterization of mucoadhesive tablet is tabulated in Table 2 and 3.

RESULTS AND DISCUSSION

Table 2: Data Showing Changes in Radial and Axial Swelling, Percentage Hydration and Matrix Erosion of *Manilkara Zapota* Seed Mucilage, Guar Gum, and HPMC E5 Tablets

Formulation	Q	R (%)	Wetting time (min)	Hydration (%)	Matrix erosion (%)	Moisture absorption
F1	3.20 ± 0.033**	87.28 ± 0.039**	072 ± 0.007**	52.38 ± 0.090**	32.83 ± 0.086**	57.69 ± 0.106**
F2	4.13 ± 0.020**	61.11 ± 0.012**	108 ± 0.037**	63.68 ± 0.086**	16.00 ± 0.051**	84.28 ± 0.042**
F3	1.06 ± 0.038**	07.69 ± 0.020**	027 ± 0.024**	18.50 ± 0.064**	13.59 ± 0.097**	64.70 ± 0.127**
F4	00.0 ± 00.0	87.79 ± 0.006**	014 ± 0.016**	00.00 ± 00.00	00.00 ± 00.00	00.00 ± 00.00
F5	3.45 ± 0.010**	89.08 ± 0.038**	100 ± 0.033**	55.00 ± 0.068**	22.22 ± 0.065**	37.50 ± 0.046**
F6	8.84 ± 0.007	95.02 ± 0.005	109 ± 0.033	77.99 ± 0.040	12.50 ± 0.045	77.50 ± 0.085
F7	1.20 ± 0.013**	10.00 ± 0.039**	034 ± 0.025**	36.00 ± 0.089**	01.26 ± 0.085**	10.00 ± 0.079**

Q: radial and axial swelling; **R**: water absorption ratio; Values are expressed as mean ± SEM (n = 6); Data was analyzed by one way ANOVA followed by Dunnett test, $^{(ns)}p > 0.05$; $*p < 0.05$; $**p < 0.01$

Table 3: Data Showing Changes in Percent Water Uptake, Mass Uptake and Wet Recovered of *Manilkara Zapota* Seed Mucilage, Guar Gum, and HPMC E5 Tablets

Formulation	Percentage water uptake		Percentage mass loss		Percentage wet recovered
	0.833 rps	1.66 rps	0.833 rps	1.66 rps	
F1	298.41 ± 0.008**	250.90 ± 0.038**	82.60 ± 0.073**	93.44 ± 0.030**	33.39 ± 0.031**
F2	364.28 ± 0.015**	310.73 ± 0.167**	88.18 ± 0.017**	98.40 ± 0.006**	54.06 ± 0.006**
F3	024.03 ± 0.037**	011.11 ± 0.006**	97.72 ± 0.038**	99.73 ± 0.019**	19.49 ± 0.024**
F4	011.11 ± 0.014**	018.33 ± 0.041**	48.50 ± 0.004**	58.09 ± 0.041**	10.62 ± 0.007**
F5	427.94 ± 0.029**	365.30 ± 0.020**	33.91 ± 0.035**	41.52 ± 0.012**	78.26 ± 0.034**
F6	469.54 ± 0.006	389.26 ± 0.035	94.56 ± 0.012	96.44 ± 0.015	89.41 ± 0.011
F7	104.80 ± 0.018**	100.00 ± 0.005**	91.20 ± 0.030**	91.26 ± 0.044**	19.74 ± 0.014**

Values are expressed as mean ± SEM (n = 6); Data was analyzed by one way ANOVA followed by Dunnett test, $^{(ns)}p > 0.05$; $* p < 0.05$; $** p < 0.01$

Barium sulfate labeled mucoadhesive tablets prepared for x-ray analysis with plain mucilage tablets were subjected to physical characterization. The observation indicated that

the guar gum tablet presented high degree of radial and axial swelling (figure 1). Equilibrium degree of swelling indicated that the tablet swelled more axially than radially as the proportion of mucilage changes (Singh et al., 2021, Singh et al., 2020a). The swelling index and wetting time of seed mucilage tablets prepared was significant higher than HPMC E5, lower than guar gum ($p < 0.05$). The result indicated that the percent hydration and matrix erosion are inversely proportional to each other. Moreover, the wetting time (min) of guar gum tablets was significantly high, compared to alone lactose tablets ($p < 0.05$). The results of wetting time, water absorption, and percentage hydration are presented in figure 1. The wetting time study indicated that due to slow water absorption capacity of guar gum and seed mucilage, tablets showed longer wetting time, compared to HPMC E5 and lactose tablet. The control lactose tablets showed immediate wetting which indicated that the isolated biopolymer contain property to absorb water and swell (Fig. 1).

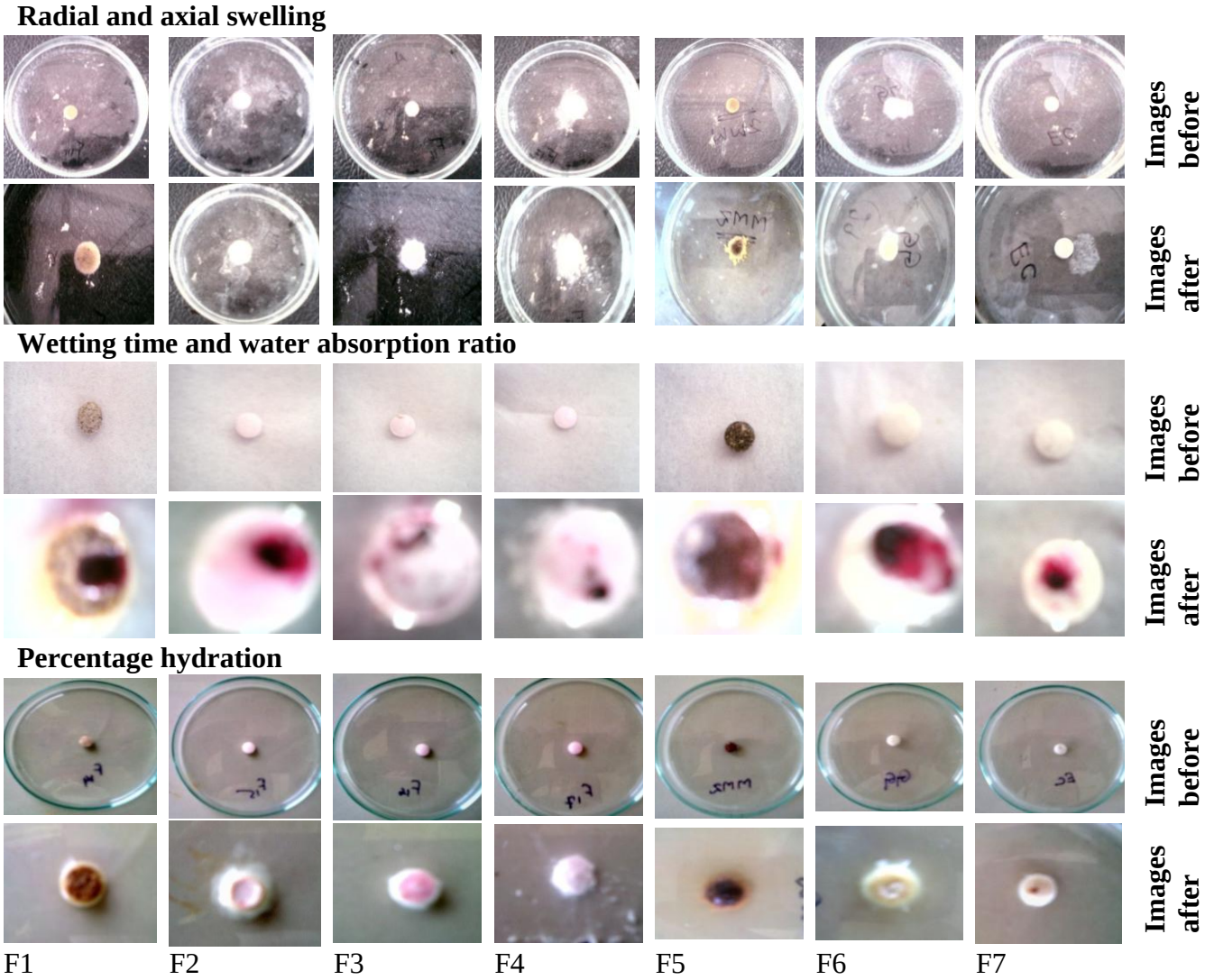

Fig. 1: Radial and Axial Swelling, Wetting Time and Water Absorption Ratio, and Percentage Hydration of Tablets

A high degree of moisture absorption was observed for guar gum as compared to lactose tablet (Fig. 2). The percent hydration and matrix erosion for tablets fortified with seed mucilage was significantly higher than HPMC, and lower than guar gum tablets. Moreover, the results

indicated that percentage water uptake and mass loss were inversely proportional to each other. In addition, the guar gum tablet showed reduction in mass loss and enhancement in water uptake on change in polymer concentration, compared to lactose and HPMC E5 tablet. The percent water uptake, mass loss and wet recovered for tablets prepared seed mucilage was significantly ($p < 0.05$) higher than HPMC E5 but lower than guar gum tablets (Fig. 3).

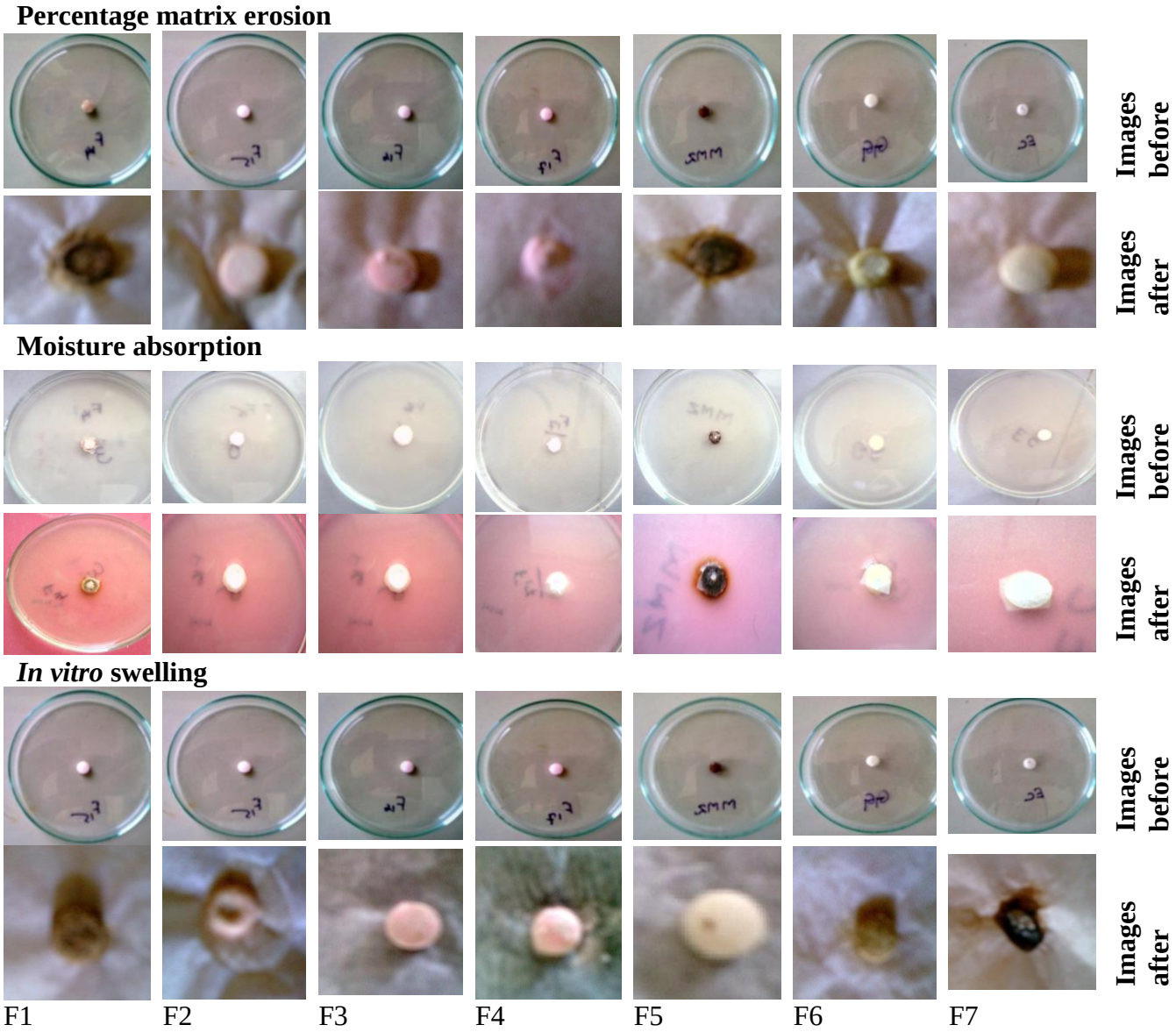

Fig. 2: Percentage matrix erosion, moisture absorption, and *in vitro* swelling of tablets

The water uptake study indicated that the rate at which the formulation swells was proportional to absorption of water (Fig. 3). The changes in weight, characteristic of water uptake and swelling, started from the beginning and continued till the end of experiment. Visual observation denoted that the matrices appeared swollen almost from the beginning, a viscous gel mass was bent when tablet came into contact with the liquid. The matrix erosion measured the weight loss from matrix tablet immersed in dissolution media as function of time (Singh et al., 2021, Russo et al., 2016). The moisture absorption study gives an indication of the relative moisture absorption capacities of test and standard polymer, and whether the formulation maintains their integrity after moisture absorption.

The suitability of polymer regarding bioadhesives property was determined by mass

degree of swelling, result indicated that isolated seed mucilage can used as mucoadhesive polymer due hydration and low degree of erosion during the study. But in case of control were lactose was used as diluents showed immediate matrix erosion.

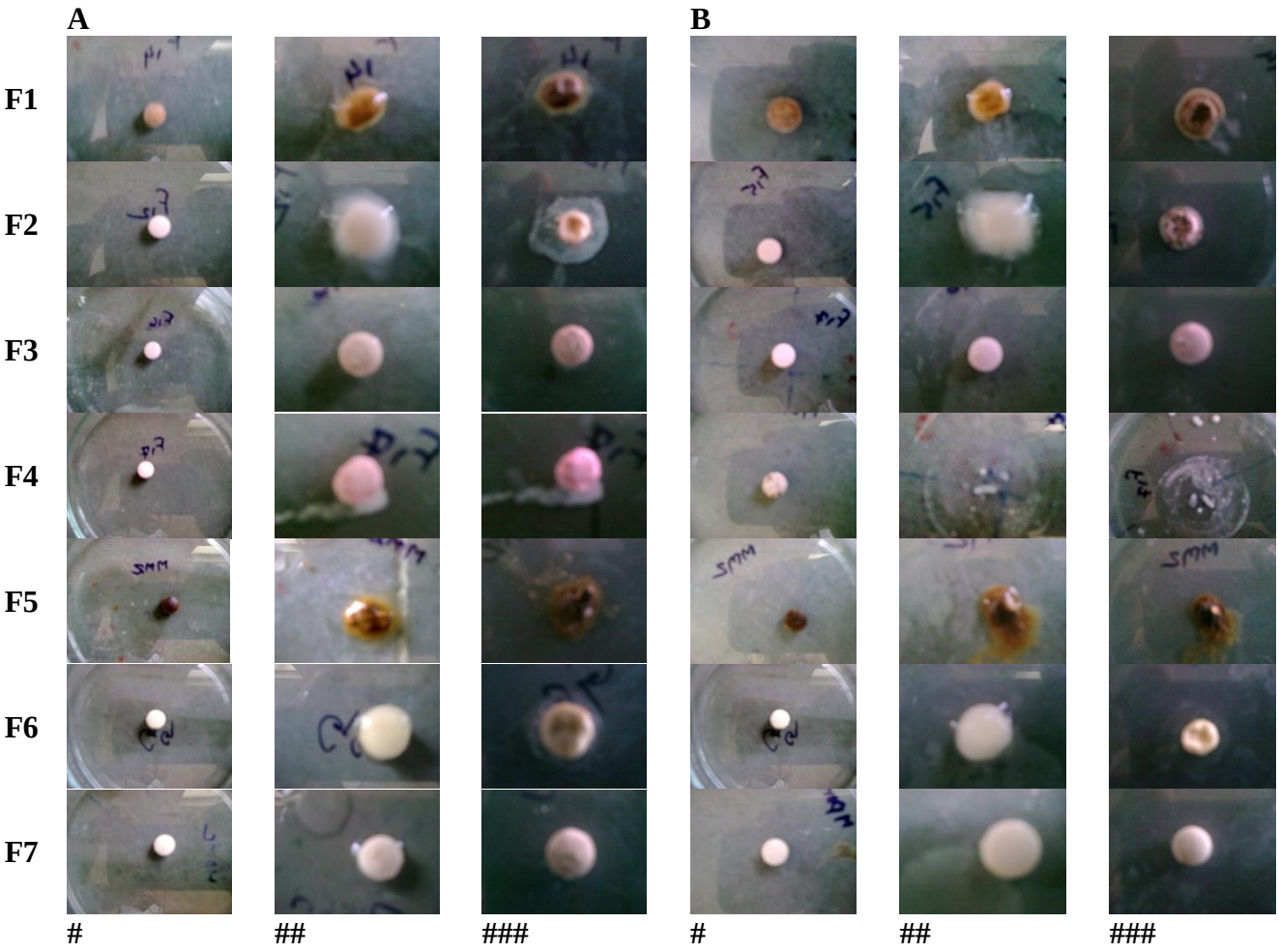

Fig. 3: Percent water uptake and mass loss of tablets at 0.833rps (A) and 0.833rps (B), (# image before, ## image after 3h, ### images after 24h)

CONCLUSION

The isolated seed mucilage from seeds of *Manilkara zapota* was successfully characterized for physiochemical test of tablets. The results were comparable to that of same synthetic polymer. Physical characterization results showed that natural polymer possess similar bioadhesive property compared to standard polymer used in study. So, it can be concluded that more or less the test material have good bio-adhesion property. The result also suggested that isolated seed mucilage may replace the synthetic non-ideal mucoadhesive polymer on possible chemical and structure modification.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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