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RESEARCH ARTICLE

ISOLATION AND EVALUATION OF SEED COAT CONSTITUENTS OF MORINGA OLEIFERA

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ABSTRACT

Traditionally, *Moringa oleifera* seed powder has been used as eco-friendly clarifying agent for drinking water. Natural polysaccharides are widely used as excipients in pharmaceutical industry. In the present *In vitro* study, starch and protein-mucilage fractions were isolated from the seed coats of *Moringa oleifera*. The isolated fractions were evaluated as binder and disintegrant in the preparation of paracetamol tablets. The prepared tablets were assessed for comparative *In vitro* quality control parameters such as weight variation, hardness, friability and disintegration time. The parameters were compared with paracetamol tablets prepared using potato starch as binder and disintegrant. Significant variation was observed in hardness, friability, and disintegration time among three formulations. Paracetamol tablets with protein-mucilage fraction were found to be relatively harder, less friable, and taking more time to disintegrate than the tablets made with potato starch. The tablets with isolated starch fraction were found to be almost similar to tablets prepared with potato starch with respect to hardness, friability and disintegration time. The isolated starch fraction and protein-mucilage fractions exhibited good binding and disintegrating properties and were natural in origin, nontoxic, biodegradable and bio compatible. Hence, they could be employed as binding and disintegrating agents in the formulation of paracetamol immediate release dosage forms. Since the protein fraction showed relatively higher values of hardness and disintegration time with less friability and could be explored for designing sustained release paracetamol tablets.

Key words: *Moringa* seeds, starch fraction, protein-mucilage fraction, Paracetamol tablets, binder and disintegrant

INTRODUCTION

Plants have played a significant role in maintaining human health and in improving the quality of life for thousands of years. In recent times, focus on plant research is increased throughout the world due to their extensive applications. *Moringa oleifera* is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. English name of *Moringa*, is drumstick tree. The fruit is a hanging, three-sided brown capsule of 20–45 cm size which contains dark brown, seeds with a diameter of approximately 1 cm. The seeds have three whitish papery wings and are dispersed by wind and water [1].

The seeds of *Moringa oleifera* are used in traditional system of medicine to treat arthritis, rheumatism, gout, sexually transmitted diseases and boils. Roasted seeds are used in the treatment of epilepsy, skin infections and as a diuretic [2]. The chemical constituents reported in the seeds are crude protein, crude fat, carbohydrates [3], methionine, cysteine, benzylglucosinolate [4], moringyne, mono palmitic and di-oleic triglycerides. The mucilage from the pods is called drum stick polysaccharide, consists of Galactose, Dextrose, Xylose and potassium, sodium, magnesium, calcium salts of Glucoronic acid. The mature seeds contain 332.5g crude protein, 412g crude fat, 211.2g carbohydrate and 44.3g ash per kg dry matter. The content of methionine + cysteine (43.6g/kg protein) was exceptionally high and close to that of human milk, chicken egg and cow's milk [5]. Protein, fibre and ash contents were 26.50-32.00, 5.80-9.29 and 5.60-7.50%, respectively [6].

The plant is widely distributed throughout the world. Traditionally, the seed extract has been used as eco-friendly clarifying agent for drinking water [7]. Natural polysaccharides are widely used as excipients in pharmaceutical industry as they are easily available, biocompatible and biodegradable. Binders enable compressibility of a drug and make it convenient to administer. They can also be used for modifying drug release.

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Literature survey revealed that the seed coats of drum stick pods were not explored for pharmaceutical applications. The present *In vitro* study was designed to isolate, identify and evaluate the constituents from the seed coats of *Moringa oleifera* for pharmaceutical applications. Paracetamol is a widely used over-the-counter analgesic and antipyretic drug [8]. It is also used in the management of more severe pain like pain in cancer in combination with other drugs [9]. Paracetamol being a popular antipyretic was selected for the evaluation of binder and disintegrant properties of isolated seed coat constituents.

Moringa oleifera pods

Moringa oleifera seed

MATERIALS AND METHODS

Beakers, Glass rod, china dish, muslin cloth, Funnel, homogeniser (Remi motors), microscope, stage micrometer, eye piece micrometer, electronic balance, tablet machine (Rinek Mini press-1), hardness tester (Monsanto Hardness Tester (Shreeji Tablet hardness tester), friabilator (Roche friabilator) and disintegrator (REMI).

Chemicals: HCl analytical grade, NaCl, Ammonium sulphate, Molisch's reagent, Ninhydrin reagent, N/50 Iodine solution, Ruthenium red reagent, distilled water, Acetone and Rectified spirit. Paracetamol, potato starch, Talc, Magnesium stearate (Sd Fine chemicals limited) and Lactose (Merck Specialists private limited).

Isolation of seed coat constituents:

For the present study, fresh drum sticks of good quality were procured from the plant at Padmavathinagar, Tirupati. The fresh fruits were washed thoroughly under tap water and the seeds were separated. The seed coats were peeled from the seeds and 25g of wet weight of seed coats were minced coarsely using a mortar and a pestle. The minced material was soaked overnight in 500 ml of 0.15 M NaCl solution in a glass beaker at room temperature.

The next day supernatant from the beaker was decanted and tested for proteins and the marc was tested for carbohydrates. To the supernatant ammonium sulphate (5%w/v) was added and stirred for 1 hr at 400 rpm using a REMI homogeniser and refrigerated for 18 hrs [10]. The fraction was decanted after 18 hrs and to the sediment 5 times the volume of rectified spirit was added kept aside to precipitate the sediment. The supernatant was decanted and the precipitate was washed repeatedly with rectified spirit and dried in sun light. After drying the fraction-1 was weighed (1.08g) and packed in a container.

The marc with carbohydrates was extracted using 5 times the volume of distilled water. The marc was heated on a water bath with stirring for about 15minutes and filtered through a muslin cloth while hot. To the filtrate 5 times the volume of acetone was added with constant stirring using a glass rod resulted in precipitation of white colour material. The material was left undisturbed for 30minutes and decanted. The carbohydrate fraction was dried in sunlight for 4hrs after complete drying the fraction-2 was weighed (0.44g) and packed in a container.

Test for isolated fraction-1 and fraction-2:

The fraction-1 was soluble in water and formed a jelly on boiling with water. The fraction was treated with Molisch's, Ninhydrin and Ruthenium red reagents [11]. The fraction-2 was insoluble in cold water and formed a jelly on boiling with water. Fraction-2 was tested with Molisch's and N/50 Iodine reagents. A pinch of fraction-2 was taken on a slide a drop of N/50 Iodine was added, the sample was spread with a brush and observed under microscope under 10 x 45 magnification. The sample contained bluish black coloured starch grains. Measurement of starch grains present in the isolated fraction-2 was done using stage micrometer and eye piece micrometer [12].

Formulation of Paracetamol tablets using wet granulation method:

In the present study, three batches each of 25 Paracetamol tablets were formulated using potato starch, isolated fraction-1and fraction-2 as binder to assess their effect on *In vitro* quality control parameters i.e. weight variation, hardness, friability & disintegration time.

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Table 1: Composition of paracetamol tablets with different binders

S.no	Ingredients	Formula-1 Potato starch as binder	Formula-2 Isolated fraction-1 as binder	Formula-3 Isolated fraction-2 as binder
1	Paracetamol	59.17	59.17	59.17
2	Lactose	35.51	37.88	37.88
3	Binder	3.55	1.18	1.18
4	Talc	1.18	1.18	1.18
5	Magnesium stearate	0.59	0.59	0.59

Manufacture of tablets employing wet granulation method:

Wet granulation method of tablet manufacturing was employed for the preparation of paracetamol tablets. Three batches of tablets were prepared using potato starch, isolated fraction-1 and isolated fraction-2 as binder. The composition of the three formulations is given in table No. 1. Paracetamol, lactose, binder, talc, Magnesium stearate were weighed and taken in a clean, dry mortar. They were blended with respective binders in the form of a gel. Then the blend was passed through sieve No 12 to obtain granules. The granules were allowed to dry at 60^{o}C for 1hour in an oven. Then the granules were milled through sieve No12. Talc and magnesium stearate were added to the dried granules. The blend was compressed into tablets using a single punch tableting machine (Rinek Mini press-1), with a punch dimensions of (1.5x 0.8x 0.3cm) at 6 ton compression pressure. The die volume was adjusted to the weight of the tablet to ensure that 500mg paracetamol is obtained [13].

Evaluation of the prepared Paracetamol Tablets:

The prepared 3 batches of tablets were evaluated by subjecting them to *In-vitro* quality control parameters such as weight variation, hardness, friability and disintegration time [13].

Weight variation test:

Twenty tablets from each of the batch were weighed individually on an electronic balance. The mean weight and standard deviation were calculated (Table No.2).

Hardness Test:

Hardness of the tablets was determined to assess the ability of the prepared tablets to withstand pressure during handling, packaging and transportation. Five tablets from each batch were taken and subjected to hardness test using Monsanto Hardness Tester (Shreeji Tablet hardness tester). The average force required crushing the tablets from each batch was calculated (Table No.2).

Friability test:

Friability test is essential to evaluate the ability of a tablet to withstand abrasion in packing, handling and transporting. The loss in tablet weight due to abrasion or fracture was the measure of tablet friability. Friability of less than 1% is considered acceptable. Ten tablets from each batch were weighed and placed into the Roche friabilator and the friabilator was operated at 25 rpm for 4 minutes. The tablets were weighed again to determine the percentage loss of weight (Table No.2).

$$F = \frac{W\ (initial) - W\ (final)}{W\ (initial)} \times 100$$

Disintegration time:

The method specified in the USP/NF (1995) [14] was used. The volume of disintegration medium used was 100ml of 0.1N HCl and the temperature was maintained at 37°C throughout the experiment for each tablet of all the batches. Six tablets from each batch were selected and placed in each of the cylindrical tubes of the basket in a disintegrator (REMI equipments). The time taken to break each tablet into small particles and pass out completely through the mesh was recorded. The average disintegration time required for each of the batches was calculated (Table No.2).

Data Analysis:

In vitro quality control parameters were analysed by subjecting the results to one way analysis of variance (ANOVA) using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Fraction-1 and fraction-2 isolated from the seed coats of Moringa oleifera were identified as protein-mucilage and starch fractions respectively. The fraction-1 was soluble in water and formed a jelly on boiling with

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water. The fraction produced purple ring with Molisch's reagent, deep violet colour on treatment with Ninhydrin reagent and pink colour with Ruthenium red reagent [11] indicating that the isolated compound as protein-mucilage in nature. The fraction-2 was insoluble in cold water and formed a jelly on boiling with water. The fraction gave purple colored ring with Molisch's reagent and produced bluish black colour with N/50 Iodine [11] and showed simple oval shaped starch grains on microscopy indicating that the isolated fraction was starch. The range of starch grains observed in the isolated starch fraction was 67.60- 75.30- 202.8µm in length and 50.70- 86.19- 148.5µm in width.

Lectins are proteins or glycoproteins with ability to bind to specific carbohydrates expressed on different cell surfaces [15]. The rationale behind lectin mediated drug targeting is very simple. Most cell surface proteins and many lipids in cell membranes are glycosylated and these glycans are binding sites for lectins[16]. Lectins function as haemoagglutinins[17]. Santos et al., 2009 isolated, purified and characterised a new lectin from seeds of Moringa oleifera. They reported that the isolated seed lectin exhibited good coagulating activity in water purification and haemoagglutinating activity on rabbit erythrocytes [10]. In the present study, the isolated protein-mucilage fraction may be a lectin as the isolated fraction gave deep violet color with Ninhydrin and pink color with Ruthenium red reagents [11].

Natural polysaccharides are widely used in the pharmaceutical industry as excipients[18] Okra gum is reported as a binder, control release[19] film coating[20] and bioadhesive material[21]. As the isolated two fractions produced jelly on boiling with water we tried them as binder and disintegrant in the formulation of paracetamol tablets. The prepared tablets were compared with the paracetamol tablets prepared by using potato starch as binder.

Paracetamol is a popular over the counter analgesic and antipyretic drugs[8] generally used to treat headache and minor body aches. The drug is also present in cold and flu remedies [9]. The therapeutic response of any formulation is based on its quality parameters. In the present study, two fractions were isolated from the seed coats of Moringa seeds and were evaluated as gelling agent, binder and disintegrant in the preparation of Paracetamol tablets. Three batches of paracetamol tablets were prepared using potato starch/isolated protein-mucilage fraction/isolated starch from moringa seeds as binder and disintegrant. The prepared tablets were assessed for comparative *In vitro* quality control parameters such as weight variation, hardness, friability and disintegration time [table 2].

Table 2: Quality control parameters of the prepared paracetamol tablets

S.no	Binder & disintegrant used	Weight variation* (mean ± S.D)	Hardness (kgf) A	Friability (%) a	Disintegration (Time) a
1.	Potato starch	0.845±0.015	4.32	0.95%	5 min 15 sec
2.	Isolated protein-mucilage fraction	0.839±0.013	6.42	0.44%	10 min 8 sec
3.	Isolated starch fraction	0.835±0.013	5.20	0.87%	7min 4 sec

*p≤*0.5; p≤a 0.01*

In the present study, the granules were prepared by wet granulation method. Wet granulation improves flow, handling, appearance and reduces variation in tablet dissolution. The type of binder used in granulation influences the properties of granules as well as the quality of the tablets produced. Weight variation influences hardness, friability and disintegration of tablets. The variation of the weight of individual tablet is a valid indication of the corresponding variation in the drug content. Controlling tablet weights within limits will help in maitaining tablet hardness and friability [22]. Uniformity of weight of tablets indicates uniformity of active pharmaceutical ingredient. The compendial specification for uniformity of weight states that for tablets weighing more than 324mg, weights of not more than two tablets should deviate from the average weight by more than 5% [14]. In the present study, there was no significant difference in the weights of the prepared three formulations of paracetamol tablets and all the three formulation tablets passed the weight variation uniformity test [14].

Hardness is an important physical feature for assessing a tablet [22]. The test shows the ability of tablets to withstand pressure or stress during handling, packaging and transportation [13]. The acceptable range of hardness is 4 to 7 kgf [23]. Hardness of a tablet determines the disintegration time and the rate of dissolution. In the present study, there was a significant difference in the hardness of tablets of three formulations prepared. The protein-mucilage fraction showed hardness of about 6.42 kgf, while isolated starch and potato starch formulations showed 5.20 and 4.32 kgf respectively. The study results revealed that all the three batches of paracetamol tablet formulations passed the hardness test. The tablets with protein-mucilage as binder were harder than the other batches of paracetamol tablets.

Friability is another mechanical property of tablets with compendial specificity of not more than 1% [14]. Friability test is essential to evaluate the ability of a tablet to withstand abrasion in packing, handling and transportation. Hardness test indicates bulk deformation of a tablet while friability indicates surface deformation and which may be enhanced by the morphology of the tablet. The rougher the surface of a tablet the more friable it will be. In the present study, there was a significant difference in the friability of the tablets prepared. The friability value for protein-mucilage paracetamol tablets was 0.44(%), whereas the friability value for isolated starch formulation and potato starch were 0.87(%) and 0.95(%) respectively. In all the formulations the percent (%) friability was less than 1% which ensures that all the tablets of the three formulations were mechanically stable [14].

Disintegration is the breakdown process of tablet into smaller particles and is the first step towards dissolution. The standard disintegration time for USP uncoated tablet must be as low as 5 minutes. But majority of the tablets have a maximum disintegration time of 30minutes [13]. Disintegration is a crucial step in release of drugs from immediate release dosage forms. The rate of disintegration is directly proportional to the rate of dissolution. The rate of disintegration is influenced by the rate of influx of water into the tablet which is also dependent on the porosity of the tablets. In the present study, there was a significant difference in the disintegration time of the tablets prepared. The tablets with potato starch formulation had a disintegration time of 5 minutes 15 seconds, whereas the tablets with protein –mucilage and starch formulations have a disintegration time of 10minutes 8 seconds and 7 minutes 4seconds respectively. The results obtained in the study showed that the hardness of the tablets directly related to the disintegration time. More hardness of protein-mucilage fraction formulation increased its disintegration time than the tablets of paracetamol with the isolated starch or potato starch [22].

The isolated starch and protein-mucilage fractions from the seed coats are natural in origin, nontoxic, biodegradable and bio compatible. The granules prepared with isolated protein-mucilage fraction, starch fraction showed good flow properties and good compressibility, less variation in tablet uniformity of weight compared to the tablets prepared with potato starch as binder. Significant variation was observed in hardness, friability, and disintegration time among the three formulations.

Paracetamol tablets with protein-mucilage fraction were found to be harder than tablets made using isolated starch fraction followed by potato starch. The friability of tablets with protein-mucilage fraction was less than isolated starch and potato starch. The tablets with protein-mucilage fraction consumed more time to disintegrate when compared to tablets prepared with isolated starch and potato starch. The tablets were found to be relatively harder, less friable, and taking more time to disintegrate than the tablets made with potato starch but all the values were within the specified limits. The tablets with isolated starch fraction were found to be almost similar to tablets prepared with potato starch in hardness, friability and disintegration. Therefore the protein-mucilage fraction and starch fraction could be used as substitutes for potato starch as binder in the formulation of paracetamol tablets. Since the protein-mucilage fraction showed relatively higher values of hardness and disintegration with less friability it could be explored for designing sustained release tablets.

CONCLUSION

Paracetamol tablets prepared using isolated starch fraction and protein-mucilage fraction exhibited good binding and disintegrating properties compared to the tablets prepared with potato starch as binder and disintegrant. Therefore, they could be used as binder and disintegrant in the formulation of paracetamol immediate release tablets. As the tablets prepared with the protein fraction were found to be relatively hard, less friable and exhibited more disintegration time it could be explored for designing sustained release paracetamol tablets. However, dissolution studies have to be done for these formulations before trying for sustained release tablets.

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