Formulation and evaluation of analgesic tablets from leaves of moringa oleifera

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Abstract

Aim :-

The aim of this study is to determine the standard water content of Moringa oleifera leaves and determine the appropriate concentration.

Methodology :-

Aqueous extract of Moringa oleifera leaves was extracted and formulated using different binders which included Maize Starch, Gelatin and Micro-crystalline Cellulose (MCC) to find out which one produce better tablets of aqueous extract of Moringa oleifera leaves. Formulations were characterized using various parameters such as physicochemical properties (bulk density, tapped density, moisture content, Hausner's ratio, Carr's index, ash value), strength (friability and crushing strength) and release properties (disintegration and dissolution times tests). The result showed that tablets formulated with Gelatin as a binder has lowest friability and disintegration time compared to those formulated with either MCC or maize starch. The crushing strengths were all within the acceptable limit (3 – 6 KgF) except maize starch which was higher.

***Conclusion**:-*

Moringa oleifera tablets were successfully formulated and based on experiments conducted, Gelatin is preferable in the formulation of Moringa oleifera tablets.

Keywords: -*Moringa oleifera; tablet; binder; maize starch; gelatin; MCC.*

Introduction-

Moringa oleifera is one of the most common and widespread species of the monogenic family Moringaceae (1). It includes 13 species of trees and shrubs distributed in sub-Himalayan ranges of India, Sri Lanka, Africa and Arabia (2). The flowers contain 9 amino acids, sucrose , D-glucose, traces of alkaloids, beeswax, quercetin and kaempferate. They also reported that it contains flavonoid pigments such as kaempherol, rhamnet, isoquercetin and kaempferitin (3). The leaves are the most nutritious part of the plant and are a great source of vitamin B6, C and vitamin A, as well as beta carotene, magnesium and protein, among other nutrients (4). It is used to treat several diseases such as infections, urinary tract infections, Epstein-Barr virus (EBV), herpes simplex virus (HSV-1), HIV-AIDS, hepatitis, helminths, trypanosomes, bronchitis, external sores/ulcers, and fever(2). The long-term medicinal benefits of this herb are well documented. Bark, root bark, seeds, flowers, leaves, seeds and seeds are widely used in Indian medicine. Its leaves and fruits are delicious when young and before they darken. In Malaysia, little squirrels were added to the basket (5).

M. oleifera is reported to contain many phytoconstituents such as flavonoids, alkaloids, saponins, and phenolic acids (6). Flavonoids will protect many things from oxidative stress. Additionally, anti-inflammatory and analgesic flavonoids have protective properties (7). Flavonoids contribute to the protection of hepatoma cells by reducing glutathione and increasing antioxidant enzymes associated with hepatoprotection. A study by Ekundina showed that M. oleifera leave extract could act as hepatoprotection at a dose of 400 mg/kg (8). All dosage forms of Moringa oleifera, except one (from Genius Nature Herbs Private Ltd) 13, are available as powder or thick, divided into hand-filled capsules (9).

Granulation can be defined as a grain expansion process that transforms small or large particles into solid physical and large agglomerates with good properties, good compression properties and compaction. There are many other reasons for stockpiling, such as: increasing the quantity of goods; facilitate measurement or volumetric application; controlling the rate of drug release; reducing dust and thus reducing worker exposure to drugs; Improve appearance (10). Powder dispersion increases consistency and consistency, improves use as a mass control solution for many products (even many drugs), reduces the amount of dispersion produced and provides a more efficient drug control system. Equity for fewer drugs (11).

The aim of this study is to formulate a standardized amount of aqueous extract of Moringa oleifera leaves to tablets and to determine suitable binder for the formulation. The objectives of the present study were to formulate M. oleifera leaves extract in suspension. The suspensions were characterized, and the in vivo hepatoprotection activity was performed to ensure the pharmacological properties of the extract.

Materials and methods:-

Materials:-

Moringa oleifera extract from Moringa oleifera leaves, Magnesium stearate , Talc , , Lactose , Micro crystalline Cellulose, PVP-30 . The reagents were purchase from a commercial source in Kano, Nigeria.

Collection and identification of Moringa oleifera:-

Moringa oleifera leaves were found at the Shivajirao pawar ayurvedic medical college and research centre, medicinal garden, pachegaon tq. Newasa Dist. Ahemdnagar .

Extraction of Moringa oleifera leaves:-

Fresh leaves are sorted and washed under tap water to remove unwanted substances and then washed again with aquadest. The leaves are dried for 3 days and turned into powder. Approximately 500 g of dry powder was added to 3.0 liters of 70% ethanol in a shearer and kept in the shade at room temperature for 3 days with occasional stirring. The ethanol fraction was filtered and concentrated in vacuum through a rotary evaporator , and the residue was dried in a desiccator on silica. The resulting powders were stored in air tight containers .

Determination of average moisture loss on drying :-

The method described In BP 2009, was adopted with slight modification. One gram of the Moringa oleifera leave powder was weighed in tarred petri dish. The petri dish with its content was placed in an oven and dried at 105°C for 3 h. Thereafter, the petri dish with content was cooled. The moisture content was then determined as the ratio of weight of moisture loss to weight of sample expressed as a percentage .

Preparation of granules :-

Eighteen (18) servings of Moringa oleifera leaf powder (15 g) and BP Corn BP. The ingredients (spice powder and 10.0% or 12.5% w/w binder as required) are dry mixed in a blender for 10 minutes, with the appropriate amount of binder solution (Gelatin) is mixed. , PVP or slime (cricket corn BP) was prepared according to the method suggested by previous researchers, except that the amount of solution or slime was kept at 7.5 ml . % w/w (Gelatin) or 5.0, 7.5, 10.0% w/w (corn starch BP) in final granules .The collection of sludge was carried out by minister and pest for 10 minutes. The volume of homogeneous water was then measured using a 1400 µm

sieve and the wet granules were dried in a hot oven for 2 hours at 50°C. The dried granules were then sieved using a 600 μm filter to determine the size of the granules and stored in airtight containers on silica gel before further testing was performed.

Particle size analysis of granules :-

Each metal was weighed to the nearest 0.001 g. Then, 10 g of Moringa oleifera leaf powder or granules were carefully packed onto a mesh sieve (1000 μm to 150 μm) and the lid was replaced. The flask was shaken for 25 min with 5-min shaking intervals using a shaker (AS 400 Retsch, Germany). The batteries were then carefully separated and the content of each metal carefully measured. The powder weight was recorded for each iron and the collection box was determined according to this difference. These values were used to calculate the percentage of samples retained for each metal and the average diameter of the particles (dav)using the formula:

dav = €(% retained× mean apature size) /100

Determination of powder / granule particle density :-

Xylene was used as the transfer fluid. Pycnometer , very clean and dry, weighed. It was filled with xylene, replaced with concrete and excess water, and cleaned thoroughly. The bottle and its contents are weighed and measured. The pycnometer was then cleaned, washed thoroughly with soapy water, rinsed with acetone and dried thoroughly in a hot oven (Lab. The model number is DHG-9101. 1HR, Ceword Medical Devices, United Kingdom) at 40oC. The dry pycnometer is weighed again to see if there is any difference between the new dry weight and the original weight. The amount of leaf powder/granules was then analyzed by carefully placing them in a dry pycnometer, the suspension was replaced and the vial was filled with the contents. Therefore, the difference was determined to be % of the powder/granule weight. A small amount of xylene is added to the pycnometer and the flask is shaken gently to remove vapor from the granular powder.

Finally, the flask is filled with xylene, the container is replaced and excess water is thoroughly washed off. The bottle and its contents were weighed and records were read. This procedure was performed three times for each batch of powder/granules and the values were compared by calculating the particle density (ρs) .

Ps = [W/ € + W-b] S€

Characterization of Moringa oleifera powder:-

Moisture content:

The moisture content of Moringa oleifera powder was determined using water analysis as described by Madu. Sugar weighing 3 g was poured into the mixed water and divided into strips. The machine was set at 130 ± 1°C. Measurements were made when the machine was stopped. The test was repeated twice and the moisture content was taken as the average of three measurements content (12)

Angle of repose:-

$$\text{Tan } \theta = h/r$$

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

The experiment was repeated twice and the average of the three readings was taken as the angle of repose.

Bulk density:-

The bulk density of each powder / granule sample was determined by pouring 10 g (M) of the powder into a 50 ml glass measuring cylinder and the bulk volume (Vo)

determined. The bulk density (Db) was then calculated from the relationship:

Db = M/Vo

Triplicate determinations were made and the mean values reported.

Tapped density :-

The tapped density of each powder was determined using Stampf Volumeter (model STAV 2003, JEF Germany). The ten grams (M) of each powder/granules sample after the bulk density determination was subjected to 250 taps mechanically and the volume V250 of the powder column determined and applied to evaluate tapped density (Dt) using the relationship:

$$Dt = M / V250$$

Triplicate determinations were made and the mean values reported (13).

Relative density :

Relative density and porosity of powder /granules bed after 250 taps were determined respectively:

$$RD = TD250/ ps$$

$$€ = 1- RD$$

Where RD = relative density, ρs = particle density, ε = porosity (14).

Determination of Carr's Index :-

Carr's index CI, was calculated from the results obtained from bulk and tapped densities above using the relation;

$$CI = (Td – Bd)x\ 100/Td$$

Determination of Hausner's Ratio:-

Hausner's ratio HR, was determined using the results obtained from both bulk and tapped densities. It was calculated using the formula

$$HR = Td / Bd$$

Ash value:-

Momin and Kadam's method was used with less chemicals. A 2 g sample of powder was cast into solid nickel, preheated to 105°C to constant weight, and then cooled. The solid and its contents are slowly heated until they become moisture-free and saturated. The temperature is gradually increased until most of the carbon is burned to °C. The sample was then superheated to 600°C until residue no longer contained any carbon (i.e., was almost white). Its essence and ingredients are cold and wet approved. The heating and cooling steps were then repeated until residue (ash) was formed always.

The weight of the ash was then determined and the percentage ash value calculated

$$\%\ Ash\ value = Wax\ 100/ Wsp$$

Where Wa and Wsp are weight of ash formed and initial weight of Moringa powder respectively (15).

Preparation of the Moringa granules :-

The Moringa granules were prepared by the wet granulation method according to the Working formula in (Table 1).

Table 1. Working formula for Moringa oleifera tablets

Ingredients	Maize starch (F1)	MCC (F2)	Gelatin (F4)	Control (F5)
Moringa extract (mg)	50.0	50.0	50.0	50.0
Lactose (mg)	86.5	86.5	89.7	94.5
Maize starch (mg)	12.0	12.0	12.0	12.0
Binder (mg)	8.0	8.0	4.8	0
Talc (mg)	3.2	3.2	3.2	3.2
Mg Stearate (mg)	0.3	0.3	0.3	0.3
Theoretical Weight (mg)	160.0	160.0	160.0	160.0

The formulations (F1 to F4) contain 12 mg maize starch each as disintegrant. The disintegrants were incorporated intra-granularly. In addition, maize starch was also used as binder in F1.

Weighing: 50 g of Moringa oleifera powder, 86.5g of lactose and 12 g of maize starch were weighed.

Mixing: The batches were small, mixing was done for 10 min, the extract and other exipients were mixed thoroughly.

Preparation of binder solution: 5% w/w of starch paste was prepared by weighing 5 g of binder maize starch powder and dispersed into 30 ml of distilled water. It was then added to a boiling distilled water placed on a hot plate with continuous stirring until translucent paste was formed. The final 100 ml mark was made with distilled water and allowed to cool.

Addition of binder: Small quantity of the paste was added gradually to the powder mixture until moistened mass was formed.

Wet screening: The moistened mass was passed through a 1.7 mm sieve.

Drying: The wet granules were dried in a hot air oven at 40°C

Dry screening: The granules were then passed through 1.4 mm sieve and oversize granules were size reduced. Same was done for F3 but for F2, MCC was added in dry form. For F4 distilled water was used instead of binder solution. The granules were then characterized.

Granules Characterization :-

The following tests (Angle of repose, Bulk density, Tapped density and Moisture content) were carried out as earlier described for Moringa powder on the granules produced prior to compression into tablets.

Compression of Granules into Tablets :-

The granules were then mixed with talc and magnesium stearate prior to compression. The granules were compressed into tablets on single punch tablet press using die and flat punch set of diameter 8 mm at

compressional force of 6 metric tons to produce circular tablets. The tablets were kept in air tight containers for 48 hr prior to quality control tests.

Pharmacological Activity:-

The plant Moringa oleifera posses broad pharmacological activities. Some of them are discussed below.

Antioxidant activity :-

Aqueous and alcoholic extracts (methanolic and ethanolic) of leaves and roots of Moringa oleifera exhibit potent antioxidant and radical scavenging activities in vitro. Leaves are a rich source of antioxidants; They can protect animals against diseases caused by oxidative stress. Administration of moringa oleifera leaf extract appears to protect against oxidative damage caused by a high-fat diet(16)..

Anti-diabetic activity:-

Aqueous extract of Moringa oleifera leaves shows anti-diabetic activity and controls diabetes and thus exhibit glycemic control (17)). Moringa oleifera pods in streptozotocin (STZ)-induced diabetic rats. Diabetic rats were treated with 150 or 300 mg/kg intravenously for 21 days, and the antidiabetic effect was evaluated by measuring biochemical changes in serum and pancreatic tissue. The development of diabetes was significantly reduced after treatment with the extract. In treated mice, both doses of the extract caused a decrease in serum glucose and nitric oxide and a -fold increase in serum insulin and protein (18).

Cardiovascular activity :-

Ethanol extract of Moringa oleifera leaves showed significant antihypertensive or hypotensive activity. In vivo activity was performed in the heart of animals and thiocarbamate and isothiocyanate glycosides were found to be responsible for this potent hypotensive activityactivity (19).

Anti-fertility activity :-

Moringa oleifera root extract has been shown to be effective as an antifertility agent in the presence of estradiol dipropionate or progesterone. In vivo antivertebral activity and histopathological studies were performed using liquid extracts to investigate the effects on uterine histoarchitecture before and after implantation (20).

Anti-inflammatory activity:-

The methanolic juice and bark, methanolic leaves and flowers, and ethanolic seeds of Moringa oleifera have anti-inflammatory effects. In vitro anti-inflammatory activity and pharmacological evaluation of hot water infusion of flowers, leaves, roots, seeds and stem or bark of Moringa oleifera using carrageenan derivatives (21).

Anti-microbial activity :-

The leaves, roots, bark and seeds of Moringa oleifera exhibit antimicrobial activity against bacteria and fungi. The plant shows in vitro activity against bacteria, yeast, dermatophytes and worms using the propagation method. Water extracts from fresh leaves and seeds inhibit the growth of Pseudomonas aeruginosa and Staphylococcus aureus (22).

Quality control on the formulated tablets :-

Uniformity of Thickness and Diameter :-

A caliper was used to measure the diameter and width of in each case, the mean value of five determinations was recorded. The test was repeated twice and the average of the three measurements was taken as width.

Uniformity of Weight Test :-

Twenty tablets were randomly selected and weighed individually. The mean weight of the tablets was then calculated and the standard deviation determined.

Crushing Strength :-

The Erweka test was used to measure the hardness of the tablets. Six tablets were randomly selected and each panel was placed between the anvil and spindle of a high-strength testing machine and exposed with a speed rotary hammer directed clockwise at a constant speed until the tablets were crushed. The value of the applied pressure was taken as the breaking force of the tablets. A six-stage decision-making process was followed.

Friability Test :-

20 tablets are selected and carefully measured. They were then placed in a Friabilator drum and rotated at a speed of 25 rpm for four minutes. Unremoved pellets are removed from the barrel, dusted and weighed. The weight percentage is calculated and recorded as a simple value.

Disintegration Time test :-

Six tablets were randomly selected and placed on their handles in six channels on the shelf of the folding machine. The metal is raised and lowered at a constant rate in deionized water in a glass beaker suspended in a water bath whose temperature is maintained at 37 ± 1 °C. The time required for the final mass or part of it to pass through a 2 mm mesh in water (depleted water) is recorded as the settling time.

Dissolution Time Test :-

Dissolution testing equipment was used to determine the % dissolution rate of Moringa tablets. The solvent used was 750 ml of 0.1 M HCL kept at 37 ± 0.5 °C. The wing was rotated at 50 rpm. A plate is placed in each glass. Dissolved samples (10 ml) were removed from over a specified period of 15, 30, 45, 60 min and analyzed at 205.1 nm at using a UV spectrophotometer. After removing the sample, a volume of fresh solution was added in its place.

Statistical Analysis :-

Statistical analysis was carried out using a statistical software SPSS version 16 and $P<0.05$ was considered significant (23).

Results and discussion :-

Characterization of Powder :-

The percentage yield of Moringa powder obtained from fresh leaves of Moringa oleifera (Table 2) shows the relative yield. This is good, because the tree is found both in the wild and in culture in and can produce leaves all year round .

The moisture content of Moringa oleifera is low as shown in (Table 2),indicating that it has a low risk of microbial contamination and inhibits tumor growth.

The angle of repose used to measure the properties of powders has a value less than 23°at good pressure, while values between 23-25° have a good pressure . Moringa extract showed good flow properties .

Moringa powder also has a bulk and tapped densities as shown in (Table 2) which is suggestive of good flow property.

Hausner's ratio also measures the flow property of powder and values less than 1.25 indicates a good flow property and as shown in (Table 2), Moringa extract has a value of 1.23 which indicates good flow ability.

For Carr's index, values below 16 indicate good flow property . Moringa extract has slightly higher value.

Table 2. Physicochemical properties of Moringa oleifera powder

Sr. No.	Parameters	Moringa oleifera powder
1.	Moisture content (%)	2.84±0.64
2.	Angle of repose (°)	23.90±1.37
3.	Bulk density (g/ml)	0.99±0.04
4.	Tapped density (g/ml)	1.22±0.12
5.	Carr's index (%)	18.90±0.93
6.	Hausner's ratio	1.23±0.08
7.	Ash value	0.23±0.23
8.	Percentage yeild (%)	13.25±1.09

As shown in (Table 2), the ash value of Moringa extract indicates the presence of mineral salts such as calcium oxalate naturally occurring in medicines and organic substances from foreign sources. Ash value test is one of the most important tests in measuring chemical powder.

Characterisation of Granules :-

(Table 3) shows the results of various tests carried out on the granules produced using different binders.

Table 3. Physicochemical properties of Moringa granules

Formulation	Angle of repose (°) ±SD	Bulk Density (g/ml) ±SD	Tapped density (g/ml) ±SD	Carr's index ±SD	Hausner's ratio ±SD	Moisture content ±SD

F1	15.06±0.28	1.08±0.023	1.23±0.03	12.2±0.06	1.14±0.01	1.77±0.01
F2	22.52±1.10	0.59±0.014	0.66±0.01	9.2±0.01	1.10±0.01	2.00±0.01
F3	22.10±0.58	0.61±0.022	0.65±0.01	6.2±0.01	1.07±0.01	1.58±0.01
F4	18.24±0.90	0.61±0.004	0.65±0.01	6.15±0.01	1.07±0.01	1.65±0.02

Key F1=maize starch, F2=MCC, F3=Gelatin and F4=control; MCC = Microcrystalline cellulose

The fluidity of the granules was generally better than that of Moringa powder. This can be explained by the presence of a binder that tends to produce larger grains than powder. It's a powder size, high quality , so it works well. Although all binders fall within the standard range of less than 23°, it is best to use corncob as it has a lower value than and has better flowability than other binders .

Quality Control of Formulated Tablets :-

(Table 4) shows the results of quality control tests performed on Moringa tablets prepared with various binders. Tablets with the same diameter and size meet the specification that the tablet size must be within ±5% .The weight balance results shown (Table 4) showed that tablets had a standard deviation of less than 0.1; this is consistent with the standards established by USP ;this states that the limits for tablets weighing should not exceed 7.5%. 130-324mg .

Table 4. Physicochemical properties of the Moringa oleifera tablets

Parameter	**MCC**	**Maize starch**	**Gelatin**	**Control**
Thickness (mm)	5.12±0.15	5.09±0.06	5.04±0.11	4.98±1.27
Diameter (mm)	8.03±0.18	8.17±0.58	8.10±0.03	8.09±0.43
Weight (g)	0.163±0.03	0.154±0.01	0.155±0.01	0.157±0.01
Crushing strength (Kg/F)	4.06±0.43	7.20±0.19	4.5±0.15	3.64±0.02
Friability test (%)	0.38±0.01	0.39±0.04	0.24±0.05	0.25±0.18

Disintegration time (min)	21.96±0.40	17.59±0.40	11.64±0.80	11.63±0.80
CSFR	10.68	18.46	18.75	14.56
CSFR-DT	0.49	1.05	1.61	1.25

Key: CSFR = Crushing Strength Friability Ratio, DT = Disintegration Time

All the formulations fall within the acceptable crushing strength range of 3-6 KgF except F1. There was significant difference between F4 (control) and either F1, F2 or F3 ($p<0.05$). From the friability test as shown in the (Table 4), all the tablets fall within acceptable compendial range. There was no significant difference between F4 and F3 ($p>0.05$), but there was a significant difference between F4 and F1 or F2 ($p<0.05$). Therefore F3 is the best formula (i.e. the preferred combination is PVP-30) because it has the best property of being harmless and not shrinkable. It is worth noting that the binder-free formula F4 passed the strength and tensile strength tests. This was as a result of the amount of starch added, removed, and replaced during granulation, causing to act as a binder.

As shown in (Table 4), gelatin has a longer degradation time, probably because it has a lower binder content. As a result, Moringa is ideal for making tablets as it has a good disintegration profile, which is the standard for taking the drug. There was significant difference between F4 and either F1 and F2 ($p<0.05$) but there was no significant difference between F4 and F3 ($p>0.05$).

Breaking strength and grinding strength values provide a measure of tablet strength and weakness . Therefore, CSFR can be used as a measure of the mechanical strength of Moringa tablets; The larger the CSFR, the stronger the tablet. CSFR results showed that tablets were made with gelatin as the binder with the highest mechanical strength. The order is gelatin > starch > MCC.

The effect of CSFR on tablet dissolution rate followed the same pattern. The CSFR-DT value for moringa seeds is gelatin > starch > MCC. This is definitive proof that gelatin is the best compound that can be used in the preparation of Moringa pills.

Disintegration is the time it takes for the tablet to move into solution, and the tablet must dissolve before it moves into solution. However, it is worth noting that the tablet can break quickly, but instead of being slow it actually has a delayed profile due to which it can break into small pieces .

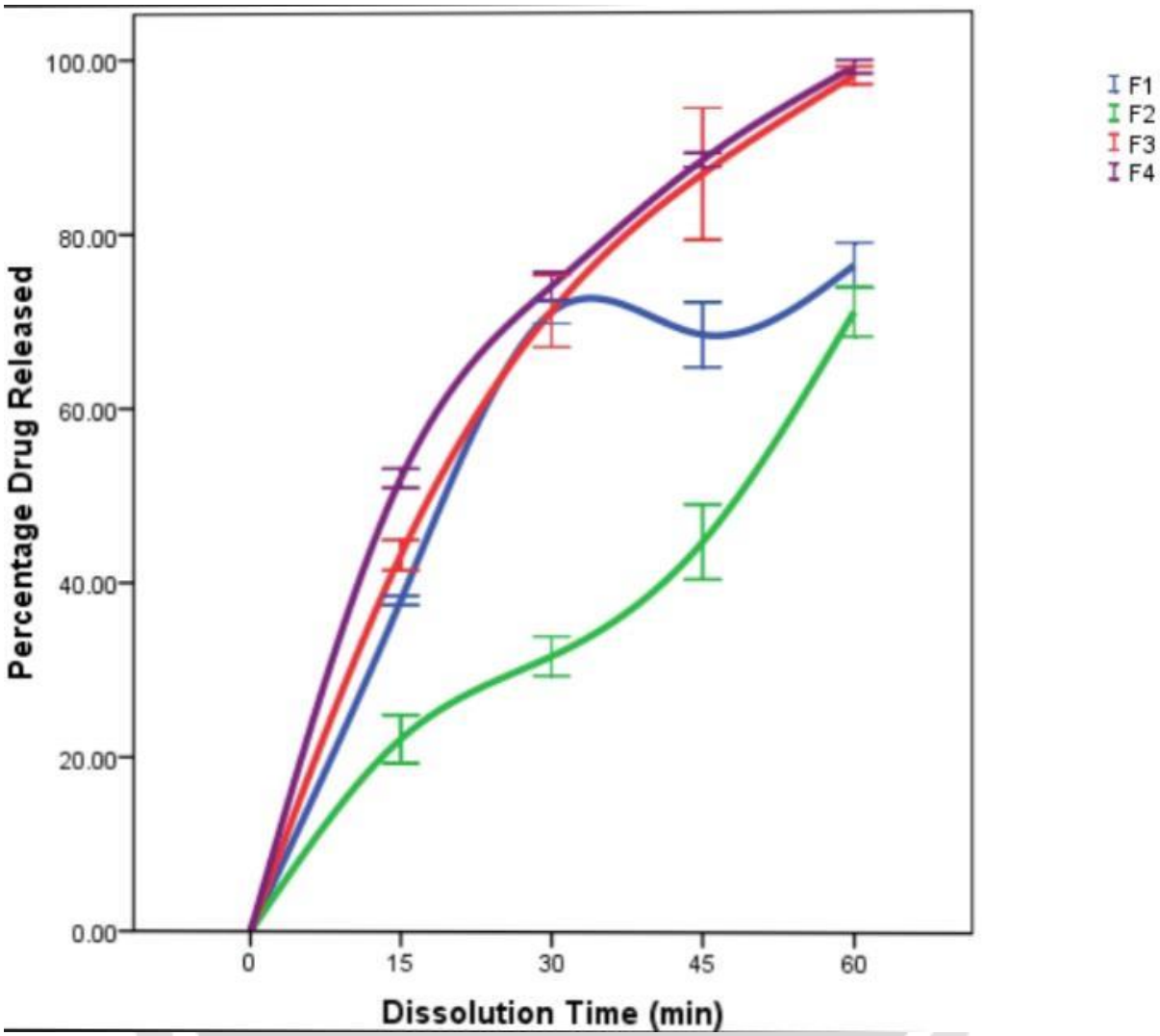

Fig. 1. Dissolution profile of various binders used in tableting Moringa oleifera

Key: F1 = Maize starch, F2 = Microcrystalline cellulose, F3 = Gelatin and F4 = Control

Conclusion :-

A 50 mg Moringa oleifera tablet was successfully formulated from aqueous extract of Moringa oleifera leaves. It can therefore be concluded that Moringa oleifera can be tableted using different binders and still get promising results. Based on experiments conducted, the binder of choice for producing Moringa oleifera tablets is Gelatin as it has passed all the tests required. Further studies should be carried out on Mechanical Strength and Lamination tendencies of Moringa tablets.

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