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Evaluation and Extraction of Crude Drugs from Moringa Oleifera Leaves and Preparation of an Antibacterial Tablet

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Abstract

Moringa oleifera is a medium-sized tree that stands 10-12 metres tall. It is also known as the “Drumstick” tree, the horseradish tree, and the mother’s closest friend. The leaves of Moringa oleifera have an abundance of minerals like calcium, potassium, zinc, magnesium, iron, and copper. When pathogens enter the body via food, the condition is often called "food poisoning." Hydro-alcoholic extraction, simple distillation was subjected to powdered Moringa oleifera leaf. The extract was again heated using a water bath, and the crude drug was obtained. In vitro, anti-bacterial screening is performed by the disc diffusion method. Escherichia coli-Gram-negative bacteria were grown on nutrient agar in the refrigerator. Similarly, streptomycin was prepared as a standard antibacterial drug. The test drug and standard drug of streptomycin were compared for their zone of inhibition. Parameters such as organoleptic, microscopic, physical and chemical were evaluated. The presence of an alkaloid confirms the antibacterial activity. The 500 mg of orally disintegrating tablet was prepared by the direct compression method using a 10 mm diameter stainless steel die. Along with the crude drug powder, microcrystalline cellulose (MCC) was used as a diluent/binder and mannitol as a sweetener. The tablet was further evaluated.

Keywords: oleifera, hydro-alcoholic, anti-bacterial, compressed tablet, evaluation parameters

1. INTRODUCTION

Moringa oleifera belongs to the Moringaceae family. It is also known as the “Drumstick” tree, the horseradish tree, and the mother’s closest friend. It is a medium-sized tree that stands 10–12 meters tall. Every part of Moringa oleifera is a storehouse of important nutrients. The leaves of M. oleifera have an abundance of minerals like calcium, potassium, zinc, magnesium, iron, and copper. Proteins, vitamins, folic acid, and alpha-carotene are also present. Every plant portion, including leaves, roots, fruits, flowers, and bark, is used as a high-nutrient diet. Moringa Oleifera has anti-thyroid, anti-bacterial, anti-fungal, anti-diabetic, anti-epileptic, anti-inflammatory, anti-oxidant, anti-diuretic, and anti-hypertensive properties. As stated by WHO, about 80% of the world’s population depends on traditional medicine for primary health care (Jaya Gupta et al, 2014).

The digestive tract appears to be an appealing habitat for bacteria seeking a spot to colonize a person or an animal. It gives them protection, supplies a wide range of nutrients, and is less likely to induce an immunological response. It is hardly a surprise that they reside inside the stomach and are harmless. However, some of them can be harmful. These enteric bacterial infections commonly include gastroenteritis, diarrhea,

and other life-threatening consequences (Gurrent et al, 1991). When pathogens enter the body via food, the condition is often called "food poisoning" (Archer et al, 1988). Common symptoms include nausea, vomiting, and diarrhea. This can be treated by using marketed, herbal, or traditional medical preparations (Hatheway et al, 1990).

Thus, this study was carried out to evaluate the various phytochemical constituents, properties, and antibacterial activity of the Moringa oleifera leaf. The leaves were selected to formulate and evaluate a pharmaceutical dosage form, such as oral disintegrating tablets.

2. MATERIAL AND METHODS

2.1 For extraction of crude drug from leaf:-

Source and preparation of plant material

The plant material used for the study was obtained from the local area of Narhe, Pune. The plant was authenticated by the Agharkar Research Institute, G.G. Agharkar Road, Pune. SIOP/AUTH/2021-22/2983. The leaves were cleaned in sterile distilled water, and dried in the open air. After drying, the leaves were ground into a fine powder using a mixer. The powder was then sieved to insure a consistent particle size.

Extraction of plant material

Hydro-alcoholic extraction was subjected to powdered Moringa oleifera leaf. The water to ethanol ratio was taken at 40:60%. Briefly, 30 grams of powdered plant leaf were weighed and percolated with 300 ml of hydro-alcoholic solution (180 ml of ethanol and 120 ml of water) in a Soxhlet apparatus. The temperature was maintained at 70-80 ^{o}C for eight hours, and the extract recovered was dark green. The ethanol from the extract was further separated by the simple distillation method. The temperature was kept at 38 ^{o}C. The extract was again heated using a water bath, and the crude drug was obtained. Before using, all the extracts were kept in the refrigerator.

2.2 For Antibacterial assay:-

The crude drug was obtained by the hydro-alcoholic Soxhlet extraction technique. The test bacteria chosen were Escherichia coli-Gram-negative bacteria. These isolates were maintained on nutrient agar in the refrigerator. In vitro, anti-bacterial screening is performed by the disc diffusion method (Vander et al, 1999).

The disc diffusion method is highly effective, and the activities of therapeutic crude drugs are measured by the diameter of the zone of inhibition (Cheesbrough et al, 2006).

Muller-Hinton Agar plates were used, and the E. coli bacteria were spread on them. The extract was simultaneously added to the disc hole made with the sterilized cork borer (Cheesbrough et al, 2006). The extract was diluted with DMSO (Dimethyl-sulfoxide) and a 10, 20, 40, and mg/ml concentration of the stock solution was prepared. Similarly, the other agar plate was prepared as a standard antibiotic, and streptomycin was used as a standard antibacterial drug. The agar plates were incubated in an incubator at 37^{o} C temperature for 24 hours. This was further followed by marking up the zone of inhibition and comparing both the standard and sample agar plates (Akinyemi et al, 2006).

Micro-broth dilution test for minimum inhibitory concentration (MIC)

The plant extract MIC was obtained by serial doubling dilutions in distilled water. Concentrations of 16 g/ml, 32 g/ml, and 64 g/ml were made. Initially, in a sterile test tube, an equal volume of extracts and Mueller-Hinton broth were dispensed. Each test tube received 0.1 ml of standardized inocula and was further incubated at 37 ^{o}C for 24 hours. Tubes containing broth and plant extract were used as a negative control, and tubes containing broth without extract were incubated as a positive control. After incubation, the tubes were evaluated for MIC (Hugo et al, 2007).

2.3 For evaluation of crude drug:-
Evaluation Parameters
2.3.1 Organoleptic evaluation

Organoleptic parameter refers to the study of a drug or plant using sense organs. It includes an analysis of color, odor, taste, size, shape, and texture.

2.3.2 Microscopic evaluation

This evaluation method allows for a detailed examination of plant material and is used to confirm the structural details. The determination of leaf constant includes stomatal number, stomatal index, vein islet, vein termination, and palisade ratio.

2.3.3 Chemical evaluation

Chemical evaluation includes qualitative chemical tests, quantitative chemical tests, and chemical assays.

Test for alkaloid

To a small amount of extract, add a few drops of dilute hydrochloric acid and then filter. The filtrate is treated with 2 ml of Wagner's reagent, which was prepared freshly with iodine, potassium iodide, and water to make up the volume. The formation of a yellowish-brown precipitate confirms the presence of the alkaloid. Due to the presence of alkaloids, the leaves of Moringa oleifera have antibacterial properties (Jaya Gupta *et al*, 2014).

Test for Flavonoids

To the extract filtrate, add 5 ml of dilute ammonia solution and then add a few drops of concentrated sulphuric acid. The presence of flavonoids is indicated by the yellow color formation (Jaya Gupta *et al*, 2014).

Test for Glycosides

To 1 ml. of extract, add 5 ml. of dilute sulphuric acid and boil it for 15 minutes. Cool and neutralize with 10% sodium hydroxide before adding 5 ml of Fehling's solution. The brick-red precipitate indicated the presence of glycosides (Jaya Gupta *et al*, 2014).

Test for Terpenoids

To 5 ml. of extract, 2 ml. of chloroform were added, followed by the careful addition of concentrated sulphuric acid. The formation of a reddish-brown color at the layer junction confirms the presence of terpenoids (Jaya Gupta *et al*, 2014).

Test for Tannins

A small amount of extract was diluted with water and then 4-5 drops of 10% ferric chloride were added. The presence of tannins is indicated by the formation of the green color (Jaya Gupta *et al*, 2014).

Test for Saponins

Take 2 mL of alcohol and dilute it with water. Add 2 ml of the plant extract and shake well for 15 minutes. The formation of foam shows the presence of saponins in the crude drug (Jaya Gupta *et al*, 2014).

Test for Steroids

Leaf extract was added to concentrated sulphuric acid and chloroform. The presence of steroids is indicated by the appearance of red color at the top layer of the test tube (Jaya Gupta *et al*, 2014).

2.3.4 Physical evaluation
Moisture content-

The moisture content of the drug is responsible for the decomposition of the crude drugs, either producing chemical change or microbial growth. So, the moisture content is determined by heating a drug at 105 ^{o}C in an oven at a constant weight.

Ash value-

The ash value determination is useful for detecting the quality and purity of crude drugs, especially when they are present in powdered form.

Determination of Total Ash-

Accurately weigh 2 gm of the powdered drug in a silica crucible. Place the crucible in the incinerator and keep increasing the heat gradually to 450-600 ^{o}C and then cool it. For cooling, place the crucible in desiccators. Weigh the ash and calculate the total ash percentage (Abdul et al, 2016).

Total Ash value percentage =100x (W2-W)/W1

Where W2= weight in grams of the crucible with ash

W1= weight in grams of a sample taken

W= weight in grams of empty crucible

2.4 For formulation and evaluation of antibacterial tablets:-

2.4.1 Materials and methods

Along with the crude drug powder, microcrystalline cellulose (MCC) was used as a diluent/binder; mannitol was used as a sweetener, and a flavoring agent in the Moringa Oleifera orally disintegrating tablet formulation (MODT).

The 500 mg of orally disintegrating tablet was prepared by the direct compression method using a 10 mm diameter stainless steel dies. Two formulations were prepared-

a) Crude drug-40%, MCC-24%, Mannitol-36%
b) Crude drug-40%, MCC-35%, Mannitol-25%

2.4.2 Physical properties of powders

Physical properties such as tapped density, bulk density, true density, and mean size particle of pure formulated powder were determined.

2.4.3 Compressed tablets were analyzed based on standard parameters.

Size and shape

It can be dimensionally described and controlled. The thickness of the tablet is only variable. Thickness is measured by a sliding caliper scale (pharmastate blog).

Organoleptic properties

Colour distribution must be uniform (pharmastate blog).

Hardness and friability

To withstand mechanical shakes during manufacturing, packaging, and dispensing of a tablet requires a certain amount of strength or hardness. The hardness of the tablet is measured by the Monsanto Tester. The hardness of compressed tablets is between 5 to 8 kg (pharmastate blog).

The friability of the tablet was determined by the Roche friabilator. The tablets were placed in a plastic chamber that rotated at 25rpm for 100 revolutions. The tablets were dropped from a distance of 6 inches. The tablets are reweighed. The test passes if less than 0.5 to 1.0% of the tablet weight is lost (pharmastate blog).

Weight variation test

Take 10 tablets and weigh them individually. Calculate the average weight and compare it with the individual weight of the tablet. The tablet passes the test if no more than 2 tablets are outside the percentage limit and if no more than 2 tablets differ by more than 2 times the percentage limit (pharmastate blog). For standard parameters refer table no 1.

In-vitro disintegration time

In-vitro disintegration time was determined by placing 500 ml of distilled water into the dissolution chamber tester. The paddle speed and temperature of the apparatus were set at 100 rpm and 37°C ± 2°C, respectively. A tablet was dipped in a water chamber and a stopwatch was used to record the time when the tablet disintegrated completely (pharmastate blog).

3. Result and Discussion

The crude drug obtained after hydro-alcoholic Soxhlet extraction, simple distillation, and water bath heating is 112.5 ml.

The antibacterial activity of a hydro-alcoholic extract of Moringa Oleifera leaf was investigated using the disc diffusion method against the enteric human pathogen Escherichia coli. The extract results were then compared

to those of the standard streptomycin disc. All bacterial isolates showed the most susceptibility at a 20 µg concentration. Escherichia coli had a minimum inhibitory concentration (MIC) of 20µg/ml.

Organoleptic evaluation- The crude drug is dark green, has a papery smell like green tea, a bitter taste, and the leaves are twice or thrice pinnate.

Microscopic evaluation- The epidermis was single-layered, present on both sides, and interrupted my stomata on both sides. The stomata were anomocytic and were scattered more on the lower surface. The stomatal index was 1.48. The vein termination was recorded at 30.39 per mm^2 and the vein islet number was 47.18 per mm^2 (Monika et al, 2020).

Chemical evaluation- The results are mentioned in Table no 2.

Physical evaluation- The ash value was determined to be 7%.

Precompression analysis of MODT mixtures

Both the formulations were evaluated and, based on formulation one (containing 40% drug, 24% MCC, and 36%Mannitol), was used for tablet preparation. The bulk density was 0.36g/ml and the tapped density was 0.58g/ml. The true density was calculated at 21.55g/ml.

Evaluation of MODT

For result refer table no 3.

4. Tables

Table 1: Weight variation tolerance for uncoated tablets

Sr. No	Average weight of tablet (mg)	Maximum % difference allowed
1	130 or less	10%
2	130-324	7.5%
3	324 or more	5%

Table 2: Result for Chemical evaluation

Sr. No	Plant constituents	Hydro-alcoholic extract test
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Glycosides	Positive
4	Terpenoids	Positive
5	Tannins	Positive
6	Saponins	Negative
7	Steroids	Positive

Table 3: Evaluation of MODT

Sr. No	Parameters	Observation	Result
1	Size and shape	10 mm in diameter and round-shaped	-
2	Colour	Green color	-
3	Hardness	Less than 30 N	Pass
4	Friability	5%	Fail
5	Weight variation	2% difference	Pass
6	In-vitro Disintegration test	3 mins	Pass

5. CONCLUSION

This research was conducted to study various evaluation parameters of Moringa oleifera leaf. Phytochemical screening showed the presence of an alkaloid in the crude drug extracted, which confirms that it possesses antibacterial activity. The study of antibacterial activity through the disc diffusion method and MIC shows the most resistance at 20 ug/ml of concentration. The making of orally disintegrating tablets by extracting crude drugs from moringa oleifera leaves could potentially serve as a treatment for various food-borne diseases as it is more resistant to bacterial infections when compared to common antibiotics used for this treatment. The tablets were prepared at 500 mg using standard pharmacopeial parameters by direct compression. To mask the bitter taste, a mannitol sweetening agent was added.

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