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Pharmacognostic and Phytochemical Studies on *Momordica charantia* L.

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Abstract

Context: Momordica *charantia* (Cucurbitaceae) fruit bark and leaves are used for various conditions of ailments in traditional systems of medicine since ancient times. **Objectives:**This study is designed to lay down the various pharmacognostic and phytochemical standards which will be helpful to ensure the purity, safety, and efficacy of this medicinal plant. **Methodology:**Various methods including macroscopic, microscopic, physicochemical, and phytochemical methods were applied to determine the diagnostic features for the identification and standardization of intact and powdered drug of *Momordica charantia* fruit. **Results:**The shape, size, color, odor, surface characteristics were determined for the intact drug and powdered materials of fruit of M. *Charantia*. Light and electron microscope images of cross-section of leaf and powdered microscopy revealed useful diagnostic features. Histochemical, phytochemical, physicochemical including fluorescence analysis of powdered drug proved useful to differentiate the powdered drug material. **Conclusion**:The data generated from this study would be of help in the authentication of various parts of M. *Charantia*, an important constituent of various herbal drug formulations. The qualitative and quantitative microscopic features would prove useful for laying down pharmacopoeial standards. Morphology as well as various pharmacognostic aspects of different parts of the plant were studied and have been described here along with phytochemical, physicochemical studies, which will help in authentication and quality control.

Keywords: Pharmacognostic, physicochemical, phytochemical, standardization, *Momordica charantia*

Introduction

Momordica charantiais a climbing shrub. It is widely consumed as a vegetable and bitter flavoring in cookery, especially in Asian countries such as China and India. Utilization of *Mordica charantia* has been linked to a variety of health-promoting benefits, including lowering blood glucose in hyperglycemic subjects. is a climber and bears oblong fruits resembling cucumber in shape(Figure 1); the young fruits are emerald green in color and turn to orange-yellow on ripening [1–3]. Bitter Melon has been cultivated as a vegetable and used traditionally as folk medicine. All parts of this plant have a bitter taste, including the leaves and fruits, which have a warty texture that resembles a small cucumber (Figure 2). Bitter melon is cultivated throughout the tropics and subtropics [4].

Material and method

Collection and Identification of Plant materials

*Momordica charantia*was newly gathered from Nursery and neighborhood market. The plants were distinguished and verified systematically at dept. of botany, Govt. Girl's degree college, Kurawali, Mainpuri.A voucher example (SOP-185) of the gathered example were stored in the departmental herbarium for future reference.

Extraction of Plant materials

Momordica charantia

The fruits of Momordica*charantia* was newly gathered and exposed to conceal dried. The dried fruits were processed to coarse powder. Progressive concentrates of *Momordica charantia* fruit powder 1 kg were readied utilizing soxhlet mechanical assembly 1-liter petrol ether (50-80°C), chloroform and half ethanol. The concentrates were sifted, pooled and thought through rotational evaporator.

Standardization of Momordica *charantia*

Macroscopic study

The external morphological character such as size, shape, colour, odor, taste, texture and odor

Colour: The untreated part of the fruit was taken and colour of the fruit was examined under sunlight.

Odour and Taste: A small portion of the fruit was taken slowly and repeatedly inhaled the air over the material and

examined the odour and taste, a small portion of fruit was taken on the tongue and find out the taste of the fruit.

Shape and Size: Width and length of fruit was measured with the help of scale. Shape of fruit was confirmed by comparing with literature.[5,6]

Physico-chemical parameters

Determination of foreign organic matter

Therapeutic plant materials were liberated from noticeable indication of defilement by molds or creepy crawlies, and other animal excreta. No strange smell, staining, sludge or indications of disintegration were distinguished. It is rarely conceivable to acquire such marketed plant materials that are altogether liberated from destructive unfamiliar matter or buildup[7].

Procedure: 100 g, of original sample was extended it out in a thin layer. Examined the sample with naked eye or with the utilization of 6 X focal point and isolated the unfamiliar natural matter physically. Percentage foreign organic matter was estimated from the weight[7].

Ash values

The sum amount of the inorganic salts present in the drug was estimated through ash value determination, which includes total ash and acid insoluble ash. Total ash and acid insoluble ash was measured after the ignition of the plant material.

Total ash

Total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological ash", which is residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. The total ash is particularly important in the evaluation of purity of crude drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. A larger value indicates that the plant material contains more of inorganic compounds.

Determination of total ash

Around 3 g of the powdered material was accurately weighed in a silica pot, which was already touch off and gauged. The powdered plant was spread as a fine even layer at the lower part of the pot. The crucible burning at a temperate not surpassing 450°C until liberated from carbon. The crucible was cool and gauged and the step was repeated to get the steady weight. The percentage of the total ash was calculated with reference to the air-dried sample .

Acid insoluble ash

Acid insoluble ash is the residue obtained after boiling the ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Concentrated acid, when added to ash, reacts with the calcium oxalate crystals. If the plant material contains a large number of calcium oxalate crystals, the amount of substance remaining after acid treatment wasquite less. Thus a lower value of acid insoluble ash content indicates the presence of large number of calcium oxalate crystals in the plant material, and the vice versa

Determination of acid insoluble ash

The ash was bubbled acquired by strategy for all out ash, with 25ml of 2M hydrochloric acid for 5 minutes, gather the insoluble matter on an ash less filter paper, wash with hot air, ignite and cool in a desiccator and gauged. the percentage of acid insoluble ash was calculated on the dried drug basis.

Water soluble ash Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash (both physiological ash and non-physiological ash) with water. Water soluble ash content gives the crude estimate of the water soluble extractable matter present in the ash.

Method To the crucible containing the total ash (both physiological ash and non-physiological ash), 25 ml of water was added and boiled for 5 min. The insoluble matter was collected in a sintered glass crucible or on ash-less filter paper, washed with hot water and ignited in a crucible for 15 min at a temperature not exceeding 450 ºC. The weight of this residue was subtracted from the weight of total ash. The percentage of ash with reference to air dried plant material was calculated.

Determination of Loss on drying

This parameter assesses the moisture content and volatile components present in a sample. 1.0 gm of powdered drug sample was kept on a charred evaporating dish for 6 hrs which was dried at 105 C and weighed. The drying was proceeded until two progressive readings coordinated with one another or the distinction between two progressive weighing was not over 0.25% of consistent weight.

Determination of pH

100 ml of distilled water was added in a 100 ml volumetric flask having 1g of powered sample. After about 10 minutes of sonication the solution was then filtered. The filtrate's pH was measured using the calibrated pH meter (Standard glass electrode)[8]

Determination of swelling index

Precisely weighed 1g of powdered sample was placed into 25 ml glass stoppered measuring cylinder. 25 ml water was then poured to it after which the mixture was stirred vigorously every 10 minutes for 1 hour. Measuring cylinder was then kept in standing position for 3 hours at room temperature. The occupied cubic capacity of the powdered sample was measured and the determinations were carried out in triplicate and mean value was calculated. [9,10]

Determination of foaming index

1 g of powdered sample was accurately weighed and transferred in to a 500 ml conical flask containing 100 ml water and boiled for 30 minutes, cooled and filtered into 100 ml volumetric flasks and made the volume with water. Each decoction was poured into 10 stoppered test tubes in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml and the volume of each test tube was adjusted with water up to 10 ml. Test tubes were stoppered and shaken in lengthwise motion for 15 seconds, two shakes per second. Test tubes were allowed to stand for 15 minutes and the height of the foam was measured. The results were assessed as follows: If height of foam in every tube was less than 1 cm, the foaming index was considered less than 100. If height of the foam was more than 1 cm in every tube, the foaming index was more than 1000. If height of foam in any test tube was 1 cm, the volume of the sample decoction in that tube was used for determination of index.

Formula used for calculation of

Foaming index = 1000/a

a = Volume of decoction used for preparing the dilution in tube where foaming height was 1 cm measured.

Extractive values

The idea about the nature of chemical constituents present and evaluation of crude drug is determined through extractive values

Method 1 Hot extraction

Alcohol soluble extractive value

The powder of selected dried plant material was accurately weighed (4.0 g) and placed in a previously weighed glass stopper conical flask. 100 ml of methanol was added and weighed to obtain the total weight including the flask. Then flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for another 1 h; cooled and weighed. The original total weight was adjusted with the methanol. Then flask was shaken well and filtered rapidly through a dry filter paper. 25 ml of the filtrate was transferred to a previously weighed flat bottom dish and evaporated to dryness on a water bath; dried at 105 ºC for 6 h, cooled in a desiccators for 30 min, it was then weighed without delay. The percentage of extractive values with reference to air dried plant material was calculated.

Water soluble extractive value

The powder of selected dried plant material was accurately weighed (4.0 g) and placed in a previously weighed glass stopper conical flask. 100 ml of water was added and weighed to obtain the total weight. Then flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for another 1 h; cooled and weighed. The original total weight was adjusted with the water. Then flask was shaken well and filtered rapidly through a dry filter paper. 25 ml of the filtrate was transferred to a previously weighed flat bottomed dish and evaporated to dryness on a water bath; dried at 105 ºC for 6 h, cooled in a desiccators for 30 min, it was than weighed without delay. The percentage of extractive values with reference to air dried plant material was calculated.

Method 2 Cold maceration

Alcohol soluble extractive value

About 4.0 gpowder of selected air dried material was accurately weighed and macerated with 100 ml of methanol in a glass stopper conical flask for 24 h, with frequent shaking during the first 6 hand allowed standing for another 18 h. It was filtered rapidly taking care not to lose any solvent and then 25 ml of filtrate was transferred on a previously weighed flat bottomed dish, content was evaporated and was dried at 105ºC for 6 h, it was cooled in a desiccator for 30 min then weighed without delay. The percentage extractable matter was calculated in mg/g of air dried material.

Water soluble extractive value

About 4.0 g of coarsely powdered air dried material was accurately weighed and macerated with 100 ml of water in a glass stopper conical flask for 24 h, with frequent shaking during the first 6 h and allowed to stand for another 18 h. It was filtered rapidly taking care not to lose any solvent and then 25 ml of filtrate was transferred on a previously weighed flat bottomed dish, content was evaporated and was dried at 105 ºC for 6 h, it was cooled in a desiccators for 30 min then weighed without delay. The percentage extractable matter was calculated in mg/g of air dried material.

Fluorescence test

The powders of plants were evaluated under visible and UV light. The powder of fruits of *Momordica charantia* was reacted with different reagents. 9,10

The fluorescent credits of the powder were found in the daylight under UV 366, UV 254 by keeping modest amount of drug powder on micro slide.

1. The fluorescent credits of the powder were found in the daylight under UV 366, UV 254 by keeping modest amount of drug powder on micro slide treated with distilled water.
2. The fluorescent credits of the powder were found in the daylight under UV 366, UV 254 by keeping modest amount of wet drug powder on micro slide treated with 1 N HCl.
3. The fluorescent credits of the powder were found in the daylight under UV 366UV 254 by keeping modest amount of drug powder on micro slide treated with 1 N NaOH.
4. The fluorescent credits of the powder were found in the daylight under UV 366UV 254 by keeping modest amount of wet drug powder on micro slide treated with 1N NaOH with methanol
5. The fluorescent credits of the powder were found in the daylight under UV 366UV 254 by keeping modest amount of drug powder on micro slide treated with 50% HNO3
6. The fluorescent credits of the powder were found in the daylight under UV 366UV 254 by keeping modest amount of drug powder on micro slide treated with concentrated HNO3.
7. The fluorescent credits of the powder were found in the daylight under UV 366UV 254 by keeping small modest amount drug powder on micro slide treated with 50% H2SO4 while still wet.

Phytochemical tests

Preliminary qualitative phytochemical screening of 50% ethanolic extract of fruits of *Momordica charantia* was performed for carbohydrates, glycosides, flavonoids, resins, saponins, triterpinoids, alkaloids, steroids and tannins.[11,12]

Alkaloids

(a) Dragendorff`s test: Appearance of orange red precipitate after adding 1 ml of 50% ethanolic extract to 1 ml Dragendorff's reagent, indicates the presence of alkaloids.

(b) Wagner's test: Appearance of reddish brown precipitate after adding 1 ml of 50% ethanolic extract to 2 ml Wagner's reagent, indicates the presence of alkaloids.

(c) Mayer's test: Appearance of dull white precipitate after adding 1 ml of 50% ethanolic extract to 2 ml Mayer's reagent, indicates the presence of alkaloids.

(d) Hager's test: Appearance of yellow precipitate after adding 1 ml of 50% ethanolic extract to 3 ml Hager's reagent, indicates the presence of alkaloids.

Carbohydrates

(a) Molisch test: Purple or reddish violet color at the junction of the two liquids shows the presence of carbohydrates when 2 ml of 50% ethanolic extract was mixed with 1ml α-naphthol solution and concentrated sulphuric acid carefully through the sides of test tube.

(b) Fehling's test: Development of a block red accelerate shows the presence of carbohydrate when after warming 1 ml of the half ethanolic remove was blended in with equivalent amounts of Fehling's answer A and B.

(c) Benedict's test: Development of a red accelerate affirms the presence of sugars when 5 ml of Benedict's reagent is added to 1 ml of half ethanolic separate arrangement bubbled for 2 minutes and cooled.

Flavonoids

Shinoda test: Formation of red color confirms the presence of flavonoids when 1 ml of the 50% ethanolic extract is added with magnesium turnings and 1-2 drops of concentrated hydrochloric acid.

Lead acetate test

Extracts were treated with few drops of lead acetate solution; development of yellow colored precipitateillustrated.

Glycosides

(a) Legal test: The concentrate was broken up in pyridine and added with sodium nitroprusside answer for make it basic. The presence of pink red-to-red shading shows the presence of glycosides.

(b) Baljet test: To 1 ml of the 5 % ethanolic extract remove added 1 ml sodium picrate arrangement and the change yellow to orange tone uncovers the presence of glycosides.

(c) Borntrager's test: Two or three ml of powerless sulphuric acid to 1 ml of the concentrate course of action followed by Boiling, filteration and extraction of the filtrate with chloroform. The chloroform layer was treated with 1 ml of alkali, which shows the plan of red tone exhibiting anthraquinone glycosides.

(d) Keller Killiani test: The extract was dissolved up in acidic corrosive containing hints of ferric chloride and transfered to a test tube containing sulfuric corrosive. arrangement of a ruddy earthy colored tone at the intersection happens which slowly becomes blue, affirming the glycosides test.

Triterpenoids

Dissolved two or three granules of tin metal in 2 ml thionyl chloride solution, after which added 1 ml of the extract into the test tube, which gives the formation of a pink color indicating the presence of triterpenoids.

Steroids

(a) Libermann-Burchard test: The concentrate was in 2 ml of chloroform in a dry test tube and mixed in with 10 drops of acidic anhydride and 2 drops of concentrated sulphuric destructive which shows red, blue ultimately light blue green game plan exhibiting the presence of steroids.

(b) Salkowski test: Dissolved the extract in chloroform and add equivalent volumes of concentrated sulfuric corrosive. Arrangement of pale blue red to cherry red tone in chloroform layer and green fluorescence in the corrosive layer addresses the steroid parts in the tried concentrate.

Detection of phenolic and tannins

Gelatin test

1% gelatin solution containing 10% sodium chloride was added to each extract. Development of white precipitate indicated the presence of tannins and phenolic compounds.

Vanillin hydrochloric acid test

Extracts were treated with a few drops of vanillin hydrochloric acid reagent. The formation of pinkish red colorillustrated.

Detection of proteins and amino acids

Ninhydrin test

To the extracts, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Development of blue color shows the presence of amino acids.

Xanthoproteic test

The extracts were treated with few drops of conc. HNO3. Formation of yellow colour indicates the presence of proteins. This is a colour reaction test for proteins. The yellow colour is due to the presence of xanthoprotein.

Detection of fixed oils and fats

Stain test

The extracts were constrained in between two filter papers. The stain on filter paper shows the presence of fixed oils.

Saponification test

The extracts were treated with a few drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein and heated on the water bath for 1-2 h. The soap formation or partial neutralization of alkali indicates the presence of fixed oils and fats.

RESULTS

Morphological evaluation of fruit of *Momordica charantia*

A pepo, fusiform, longitudinally grooved, ridged and warty,

Figure 1:*Momordica charantia* L. Fruit

Figure 2 :T.S. of fruit ***Momordica charantia***

Table 1:Preliminary macroscopical characters of *M. Charantia* (Fruit)

Color	greenish yellow
Length	3-25 cm
Diameter	3-9 cm
Pulp	Pithy
Compartments	3
Seeds	Numerous (enclosed in whitish aril which becomes bright red on maturity)
Odour	Characteristic
Taste	bitter.

Table 2: Physiochemical parameters of different plant extracts

Sr. no	Physical parameters	Drug Name & Obtained Value (%w/w)
		Momordica charinta Linn
1	Foreign Organic Matter	***Nil***
2	Ash Value (%w/w)	3.62 ± 0.18
3	Water Soluble Ash (%w/w)	1.46 ± 0.08
4	Acid insoluble Ash (%w/w)	1.21 ± 0.45
5	Extractive value	8.0
6	LOD (%w/w)	0.20 %w/w
7	pH	5.65
8	Swelling index	1ml/g
9	Foaming Index	<100

Table 3: Extractive values of *Momordica charantia* L. seeds

S. No.	Solvent	Colour of extract	Extractive value (% w/w)	
			Hot extraction	**Cold extraction**
1.	**Water**	Dark Brown	20.05±0.12	15.05±1.12
2.	**Alcohol**	Dark Green	35.45±0.42	25.51±0.47
3.	**Chloroform**	Dark Green	29.12±0.52	25.35±0.74

Value: % w/w mean + SEM: n=3

Table 4: Preliminary phytochemical screening of *Momordicacharantia* Linn. Fruits extracts

Test	Chloroform Extract	Ethanol Extract	Aqueous Extract
Carbohydrate			
Molish Test	+	+	+
Fehling Test	+	+	+
Benedict's Test	+	+	+
Glycosides			
Baljet test	+	+	+
Legal test	+	+	+
Borntrager's test	+	+	+
Keller Killiani test	+	+	+
Alkaloids			
Dragendorff`s test	+	+	+
Wagner's test	+	+	+
Mayer's test	+	+	+
Hager's test	+	+	+

(+= Present; - = Absent)

Flavanoids			
Shinoda test	+	+	+
Lead Acetate test	-	+	-
Protein and Amino Acid			
Ninhydrine test	+	+	+
Xanthoproteic test	+	+	-
Fixed oil & Fats			
Spot test	+	+	-
Saponification test	+	+	+
Phytosterol&triterpenoids			
Liberman-burchard's test	+	+	+
Salowaski test	+	+	+
Phenolic and tannins			
Gelatin test	+	+	+
Vanillin Hydrochloric acid test	+	+	+
Anthraquinone glycosides			
Modified Brontrager's test	+	+	+

Table 5: Florescence analysis of powdered extract of *M. Charantia*

Treatment	Visible	Long Wavelength UV Light UV 366	Long Wavelength UV Light UV 254
1 N NaOH (Aqueous)	Green	Brown	Dark Brown
1 N NaOH (Alcoholic)	Yellow-Green	Fluorescent Yellow	Fluorescent Dull Green
1N HCl	Orange	Light Brown	Greenish Yellow
50% HNO_3	Yellow	Fluorescent Light Green	Fluorescent Light Green
Conc. H_2SO_4	Dark green	Brown	Dark Brown
Conc. HNO_3	Yellow	Light Green	Greenish Yellow

DISCUSSION

Momordica charantia L. is a therapeutic plant from the Family Cucurbitaceae, used as a Indian traditional therapeutic agent. In light of the Phytochemical Investigation or Qualitative examination of *Momordica charantia* L. fruits the different physical parameters were evaluated.

The Fluorescence Analysis has given blue coloured fluorescence which was observed under the UV radiation lamp to gain more details about the Momordicacharantia L. fruits. Therefore, chemical tests were performed on 3 various extracts of the fruit to estimate the presence of different Phytoconstituents as aqueous extract shows, it contains carbohydrates (Reducing sugars, Monosaccharides, NonReducing Sugars), proteins (proteins containing sulphur), amino acids (tyrosine, tryptophan and cysteine). Alcoholic, chloroform and pet.ether extract of fruit proved the presence of sterols, triterpenes, glycosides (cardiac glycoside, saponins glycoside, anthraquinones glycoside), flavonoids alkaloids, tannins, phenolic compounds and lipids.

CONCLUSION

Pharmacognostic studies and phytochemical screening can serve as a basis for proper identification of a plant. Before any drug can be included in the pharmacopoeia, these standards must be established. A systematic approach is necessary in pharmacognostic study which helps in confirmation and determination of identity, purity and quality of a crude drug. The present findings provide the pharmacognostic, physicochemical, and phytochemical information about the *Momordica charantia* and this might be useful by providing additional support with regard to its identification and standardization parameters. The present findings are associated with standardization of parameters like macroscopic and microscopic characters, phytochemical screening, fluorescent analysis and physicochemical quantification of the plants. Ash values added more strength to crude drug standardization with prominent results indicating the involvement or non-involvement of irrelevant matter. Such study on the macro and microscopic anatomy, preliminary phytoconstituent screening and physicochemical parameters are important information which may be useful in verification and contamination for quality control of this therapeutic plant afterwards. This study will be helpful in the future pharmacognostic standardization of these important plants. Furthermore, information regarding physicochemical characteristics of trunk bark and nature of chemical constituents present in them would also be useful for standardization of such herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia. It would also help scientists to utilize such needful information regarding the plants identity and characteristics in building new polyherbal formulations.

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