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Research Article

PHARMACOGNOSTICAL STUDIES AND ESTABLISHMENT OF QUALITY PARAMETERS OF *CUCUMIS MELO* L. CV. NAMDHARI

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ABSTRACT

Cucumis melo Linn belonging to Cucurbitaceae family, is a commercially important fruit crop, commonly known as Muskmelon or sweet melon (in English), Kharbuja (in Hindi), Ervaru (in Sanskrit), an annual creeping, angular, scabrous, extensively cultivated throughout India and said to be truly wild in India, Baluchistan and tropical Africa. Establishment of its quality parameters were done as per WHO guidelines including macroscopy and microscopy, ash values, extractive values, loss on drying, phytochemical screening, fluorescence analysis, bitterness value, foaming index, determination of pH, determination of fat content and resin content, determination of microbial load, pesticide residue, heavy metal analysis. These observation will enable to standardize the botanical identify of the drug in its crude form. Data's evolved in this investigation could be used in laying down Pharmacopoeial standards for the drug studied, as standardization of herbal medicines is absolutely essential and is need of the hour.

Keywords: *Cucumis melo*, Cucurbitaceae, Quality standards, WHO guidelines.

INTRODUCTION

Cucumis melo Linn belonging to Cucurbitaceae family, commonly known as Muskmelon or sweet melon (in English), Kharbuja (in Hindi), Ervaru (in Sanskrit). Leaves about 7.5 cm diameter, orbicular-reniform in outline, 5-angled or lobed, scabrous on both surfaces and also often with soft hairs; lobes not deep nor acute; petiole 5 cm; petals 1.6 cm. Female peduncle sometimes 5 cm. Fruit spherical ovoid elongated or contorted, glabrous or somewhat hairy, not spinous nor tuberculate[1].

The unripe fruit is bitter, sour; may cause skin eruptions and strangury. The ripe fruit is sweet, oily, wholesome; cooling, fattening; tonic, laxative, aphrodisiac, diuretic; cures "vata", biliousness, insanity, ascites; allays fatigue; causes "kapha" (Ayurveda). The fruit is of different kinds: sweet, acrid, sour:tonic, laxative, galactagogue, diuretic, strengthens the heart, the brain, and the body in general cures ophthalmia, urinary discharges; causes congestion of the eyes in plethoric people gives headache; may cause indigestion. The oil from the seeds is said to be very nourishing. Not only the seeds but the pulp of the fruit is a powerful diuretic, very beneficial in chronic, and also in acute, eczema. In China and Japan, the stalks of the fruit are considered cooling and demulcent. General anasarca and indigestion are said to yield to its use[1]. It is widly used as cosmetics such as skin lotions containing melon juice[2]. Skin-protecting cosmetic liposomes comprise Ca ion and/or Mg ion and superoxide dismutase from melon concentrates[3].

The previous phytochemical works suggest that isomultiflorenol is the major component, accompanied by its Δ7-isomer, multiflorenol, in the triterpene alcoholic fractions of the unsaponifiable matter of *C. sativus* and *C. melo* seed lipids. The other triterpene alcohols identified in the seeds were α- and β-amyrins, taraxerol, lupeol, cycloartenol, 24-methylenecycloartanol, 24-methyl-25(27)-dehydrocycloartanol (most probably cyclolaudenol), 24-methylene-24-dihydrolanosterol, 24-methylene-24-dihydroparkeol, euphol, and tirucallol[4,5]; amino acids[6], ascorbic acid[7,8], sugars fructose, glucose, sucrose, raffinose, and stachyose[9], Phenolic constituents[10], Carbohydrate compounds[11]. Sulfur containing compounds are responsible for aroma in melons (*Cucumis melo*). The incidence of six thioether esters, methyl (methyl-thio) acetate, ethyl (methylthio) acetate, 2-(methylthio) ethyl acetate, methyl 3-(methylthio) propanoate, ethyl 3-(methylthio) propanoate, and 3-(methylthio) propyl acetate, considered to be of importance to the aroma profiles of *C. melo* fruit[12]. Present study deals with the evaluation of its pharmacognostical characteristics and establishment of its quality parameters, including physicochemical and phytochemical evaluation of *C. melo* cv. Namdhari

MATERIAL AND METHODS

Crude drug

Cucumber fruits (Kharbuja) were bought from a local market (Sangam Vihar) in New Delhi. Fruits were identified as *Cucumis melo* L. cv Namdhari by Dr. H. B. Singh, and the voucher specimens (NISCAIR/RHMD/Consult/-2007-08/824/08) are deposited in the Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi. All the chemicals and reagents used were of analytical grade.

Morphological studies

Proper examination of the untreated sample of fruits of *Cucumis melo* cv. Namdhari was carried out under diffused sunlight and artificial source similar to day light (Fig 1)[13].

Powder microscopy

The microscopic examination of powdered fruit material was performed to detect and established various identifying microscopic characters which will be help full in differentiation of the substitute of the drug supplied in the form of dried powder. Slides of powdered fruit material were prepared and studied. Microphotography on different magnifications was carried out with Motic microscopic unit. Polarized light was used for the study of crystals, starch granules and lignified cell (Figure 2.1-2.4)[13].

Physicochemical Standardization

The various physico-chemical values of fruits such as ash values[14], extractive values[15] (Table 1), loss on drying were determined according to the Pharmacopoeial method.

Powdered drug reaction with different reagents

The powdered drug was treated separately with different reagents and acids like, picric acid, hydrochloric acid, nitric acid, iodine, ferric chloride, and sodium hydroxide the colour shown by that treatment is noted as such and under the microscope[16] (Table 2).

Fluorescence Analysis

Many herbs fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (254 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc (Table 3)[17].

Phytochemical screening

The phytochemical evaluation of drug was carried out as per the method described. Previously dried powdered fruits (5 gm) were extracted in a Soxhlet apparatus with petroleum ether, chloroform, methanol and water successively. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents *viz*. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc (Table 4)[18, 19].

Loss on drying

The powdered drug sample (10 gm) without preliminary drying was placed on a tarred evaporating dish and dried at 105 ºC for 6 hours and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 30 minutes in a desiccator, showed not more than 0.01 gm difference[20].

Bitterness value

Bitters are the medicinal plant materials that have a strong bitter taste and are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastro intestinal tract, especially of gastric juice[13].

Foaming index

About 1 g of plant material was reduced to a coarse powder, weighed accurately and transferred at moderate boiling for 30 minutes. Cooled and filtered into 100 ml volumetric flask. The detection was poured into 10 ml and adjusted the volume of liquid in each tube with water to 10 ml. Stoppered the tubes and was shaken them in a lengthwise motion for 15 sec.; two shakes per second. Allowed to stand for 15 minutes and the height of foam were measured[13].

Determination of pH

pH 1% solution

Dissolved an accurately weighed 1 g of the drug in accurately measured 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode[21].

pH 10% solution

Dissolved an accurately weighed 10 g of the drug in accurately measured 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode[21].

Determination of fat content

A weighed quantity of sample (3g) is extracted with anhydrous ether in a continuous extraction apparatus for six hours the extract is filtered into a clean dry weighed flask. The extraction flask is rinsed with small quantity of ether, filtered and added to the weighed flask. The solvent is evaporated and dried to constant weight at 105ºC[22].

Determination of resin content

The accurately weighed drug sample (5 g) was rapidly refluxed with acetone (3 X 200 ml) for 6 hours to exhaust the drug for the resin content. The excess solvent was removed by distillation on a water bath. The residue so obtained was suspended in water and transferred to a separating funnel, repeatedly extracted the suspension with solvent ether (2 X 200ml) to extract all the resin contents. The ether extracts were cooled out dried over anhydrous sodium sulphate and excess ether removed over a water bath. It was transferred to a weighed beaker and the final weight is noted[22].

Determination of microbial counts (Table 5, fig 3.1-3.4), heavy metal analysis (Table 6) and pesticide residue contents (Table 7) was also done as per WHO guidelines[13].

RESULTS AND DISCUSSION

Morphological characters

Proper examination of the untreated sample of fruit of the *Cucumis melo* cv. Namdhari was carried out under diffused sunlight and artificial source similar to day light (Fig. 1).

Fig. 1: *Cucumis melo* cv. Namdhari fruits (Muskmelon)

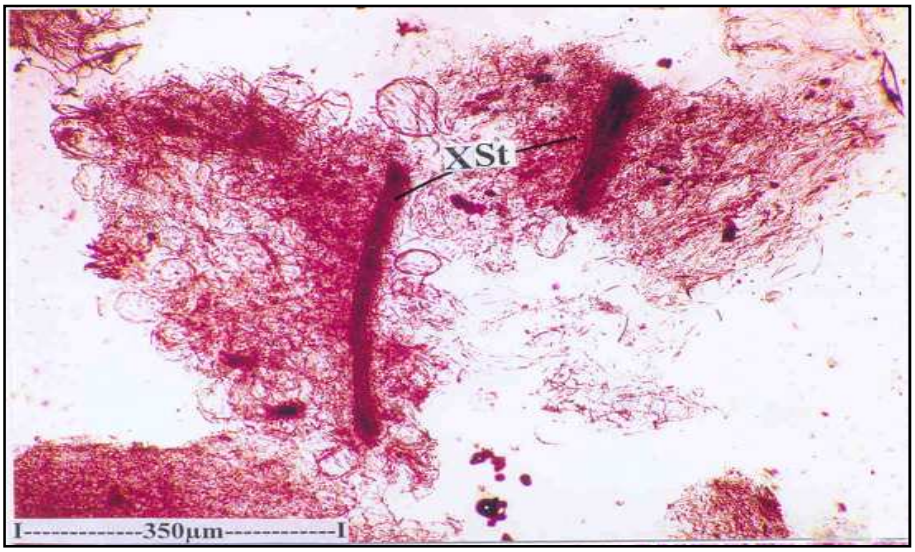

Fig. 2.1: Spherical parenchyma cells and broken pieces of xylem strands.

(XSt: Xylem strand)

Fig. 2.2: Xylem strands enlarged showing annular and spiral thickening. (BX: Bundle of xylem elements showing wall thickenings)

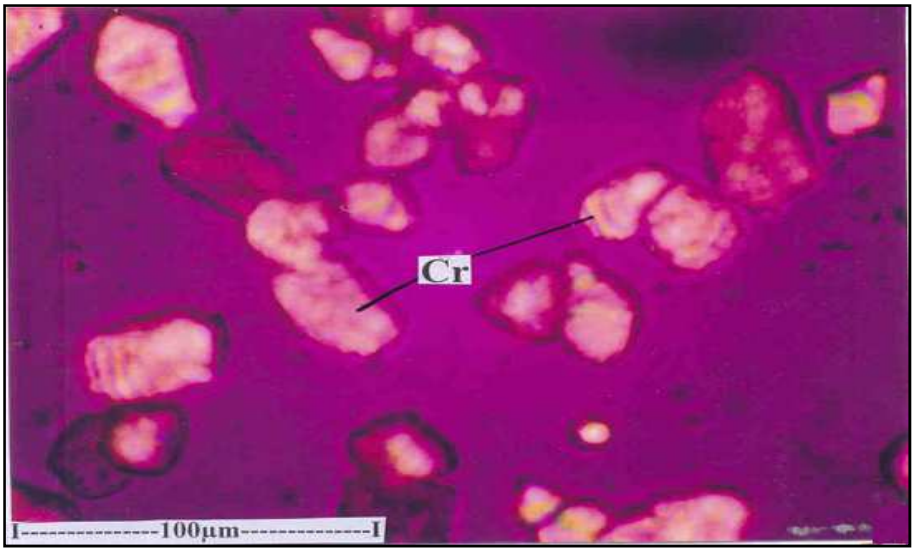

Fig. 2.3: Prismatic calcium oxalate crystals as seen under the polarized light microscope. (Cr: Crystals**)**

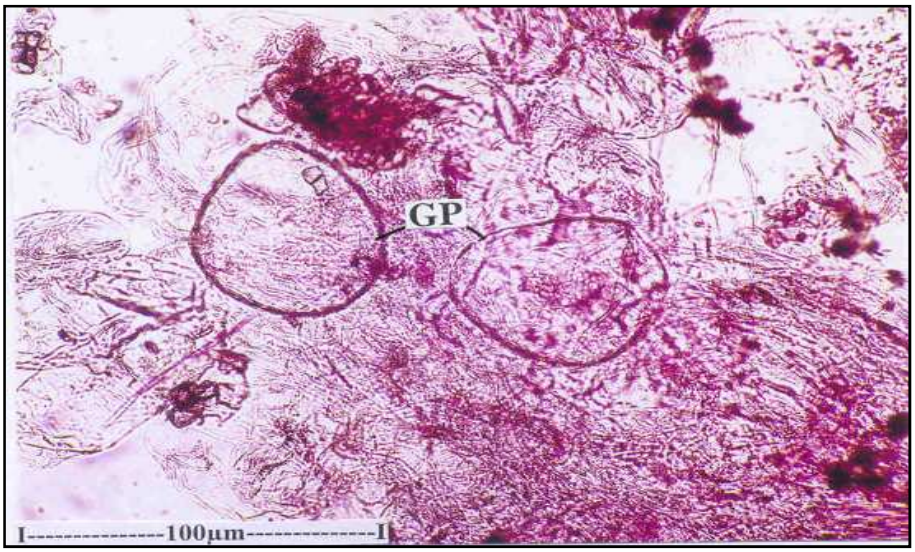

Fig. 2.4: Spherical thin walled ground parenchyma cells. (GP: Ground parenchyma)

External colour: Silver

Size: 10x 10 cm

Shape: Round

Apex: Blunt

Surface: Rough with ridges on whole surface

Odour: Characteristics

Taste: Sweet

Microscopical characters of powdered drug: (Fig. 2.1, 2.2, 2.3, 2.4)

Powder microscopy

The powder consists of 1. Xylem strands 2. Prismatic crystals and 3. Spherical ground parenchyma cells.

1. Xylem strands (Fig. 2.1, 2.2)

Thick masses of broken xylem bundles are scattered in the powder. The elements are only primary xylem and they have annular and loose spiral or close spiral lateral wall thickenings (Fig 2.2). The elements are 50 µm. thick. They are seen embedded in the parenchymatous ground tissue.

2. Prismatic crystals (Fig. 2.3)

Large, prismatic crystals of calcium oxalate are abundant in the powder (crystals are absent in *Cucumis melo* Cv. Madhu). The crystals vary in shape and size. Some of them are cuboidal, some are pyramidal and rectangular. The crystals are 30- 40 µm. long and 20-30 µm. wide.

3. The powder also contains spherical and ovoid thin walled parenchyma cells. The cells do not possess any specific inclusions. The cells are 60 x 50 µm. in size. (Fig. 2.4)

Extractive values

The extractive values the dried fruits of drug were done as per procedure described above. All the procedure was repeated in triplicate (Table 1). The mean values are given below:

Table 1:

Extraction	Extractive value, Mean (%)					
	Petroleum ether	Chloroform	Acetone	Alcoholic	Hydro alcoholic	Aqueous
Hot	0.387	1.058	2.186	31.673	53.449	57.646
Cold	1.240	1.935	2.568	27.954	48.09	38.737
Successive	0.511	0.614	2.138	51.431	9.259	12.374

Ash Values

The total ash value, acid insoluble ash value and water soluble ash value were found to be 5.068 %, 0.364 % and 2.675 % w/w respectively. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards.

Table 2: Powdered drug reaction with different reagents

S. No.	Chemical treatment	Observation	S. No.	Chemical treatment	Observation
1.	Iodine	Dark brown	6.	Sodium hydroxide (5%)	Light brown
2.	50% HNO_3	Brown	7.	Glacial acetic acid	Brown
3.	1N H_2SO_4	Reddish black	8.	Ferric chloride (5%)	Greenish black
4.	Lead acetate	Light brown	9.	1N HCL	Dark brown
5.	Picric acid	Yellow	10.	KOH (1%)	Orange

The powder of the fruit of *Cucumis melo* cv. Namdhari (mesh size 40) was examined under daylight and UV light. The observation was recorded as under:

Table 3: Fluorescence analysis

S. No.	Treatment	Day light	UV light 254 nm	UV 366 nm
1.	Powder as such	Dark brown	Dark brown	Dark brown
2.	Powder treated with dist. Water	Dark brown	Dark brown	Dark brown
3.	Powder treated with 1N NaOH in water	Light brown	Greenish black	Black
4.	Powder treated with 1 N NaOH in methanol	Dark brown	Light green	Black
5.	Powder treated with 50% HNO_3	Light brown	Greenish black	Light black
6.	Powder treated with 50% HCl	Light brown	Greenish black	Light black
7.	Powder treated with Conc. HCl	Light brown	Greenish black	Light black
8.	Powder treated with H_2SO_4	Light brown	Greenish black	Light black
9.	Powder treated with Acetone	Brown	Light brown	Light brown
10.	Powder treated with $CHCl_3$	Brown	Light brown	Light brown

Table 4: Phytochemical Screening

Extract constituents	Pet. ethr	Chloroform	Acetone	Alcoholic	Hydro-alcoholic	Aqueous
Alkaloids	-	-	-	-	-	-
Carbohydrates	-	-	++	++	++	++
Glycosides	-	-	+	++	++	++
Phenolics	-	-	++	++	++	++
Flavonoids	-	-	-	++	+	+
Proteins & amino –acids	-	-	-	+	+	+
Saponins	-	-	-	-	-	-
Acidic comp.	-	-	-	-	-	-
Mucilage	-	-	-	-	-	-
Resins	-	-	-	+	+	+
Lipids/fats	-	-	-	-	-	-
Sterols	-	+	++	++	++	++

(++) Strongly Positive, (+) Positive test, (-) Negative test

Foaming index

No foaming index was found of the drug with respect to *Glycyrrhiza glabra* whose foaming index was calculated to be as100.

Loss on drying

The mean loss on drying was found to be 12.175 %.

Resin content

The mean resinous matter was found to be 0.403 %.

pH values

The mean pH value of 1 % solution and 10 % solution was found to be 5.67 and 5.36, respectively.

Fat content

The mean per cent of fat content in the sample was found to be 0.360.

TLC/ HPTLC Fingerprinting

The weighed quantity of fruit was extracted in a Soxhlet apparatus for 6 h using twice the amount of solvent (methanol) at a controlled temperature. The extract was dissolved in the respective solvent (2 mg/ml). The spots were applied with the help of Linomat syringe using Linomat applicator (Table 4).

Table 4: TLC/ HPTLC fingerprinting analysis

Extract	Solvent system	No. of spots (with R_f value) at 254 nm	No. of spots (with R_f value) at 366 nm
Methanolic	n-butanol: Acetic acid: Water (4: 1: 5)	**10** (0.12, 0.15, 0.23,0.30, 0.33, 0.45, 0.48,0.50, 0.53, 0.55)	**05** (0.20, 0.26, 0.32, 0.33, 0.55)

Colony forming units on nutrient agar medium.

Table 5: Microbial load

Dilution of stock	No. Of colonies		Colony characteristics		
	Drug	Control	Shape	Colour	Observation aid
1:1	Not Countable	Nil	Round	White	Naked eye
1:10	06	Nil	Round	White	Naked eye
1:100	04	Nil	Round	White	Naked eye

Microbial load determination in *Cucumis melo* Cv. Namdhari. (Fruit powder)

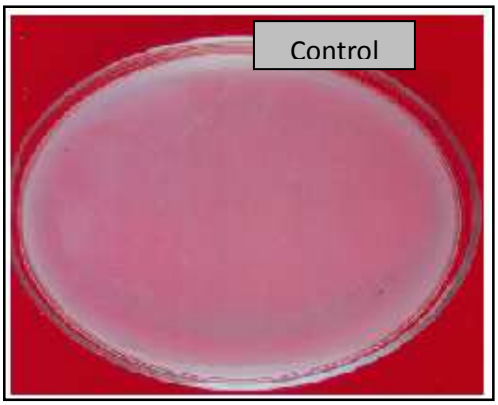

Fig. 3.1: Control

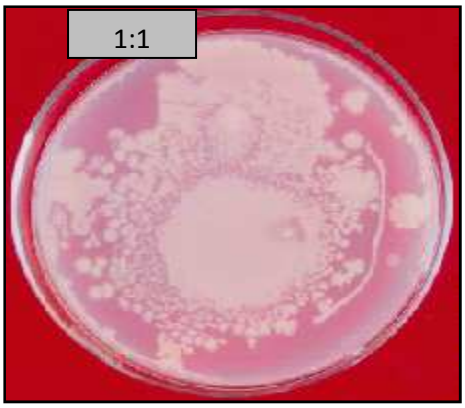

Fig. 3.2: 1: 1 Microbial Load

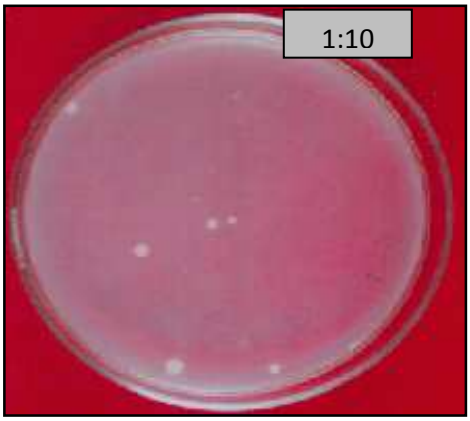

Fig. 3.3: 1: 10 Microbial Load

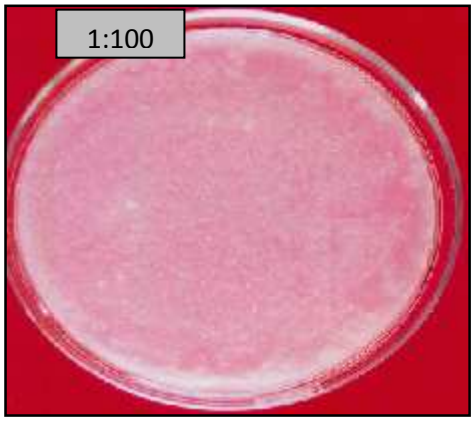

Fig. 3.4: 1: 100 Microbial Load

Table 6: Heavy metal content

S. No.	Heavy metal	Heavy metal in percentage of drug	Pharmacopoeial percentage limit of heavy metals in crude drugs
1.	Lead	1.32 ppm (Within limits)	Not more than 10.0ppm
2.	Cadmium	Less than 0.07 ppm (Within limits)	Not more than 0.3ppm
3.	Mercury	Less than 0.3 ppm (Within limits)	Not more than 1.0 ppm
4.	Arsenic	0.23 ppm (Within limits)	Not more than 10.0ppm

Table 7: Pesticidal residues

S. No.	Pesticide	Pesticide residues in percentage of drug	Pharmacopoeial limits
1.	Dichloro diphenyl Trichloroethane (in mg/kg)	Less than 0.02 ppm	Not specified
2.	2,4-dichlorophenoxyacetic acid (in mg/kg)	Less than 0.1 ppm	Not specified
3.	γ-Benzene hexachloride (in mg/kg)	Less than 0.05 ppm	Not specified
4.	Malathion (in mg/kg)	Less than 0.04 ppm	Not specified

CONCLUSION

The present study is related to pharmacognostical, physiochemical and preliminary phytochemical screening of *Cucumis melo* Linn. Fruits provided useful information about its correct identity and evaluation. It helps to differentiate from the closely related other species of *Cucumis melo* Linn. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug. The other parameters observed are also useful for the future identification of the plant and serves as a standard monograph for identification and evaluation of plant[23,24].

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