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Standardization and Preliminary Phytochemical Evaluation of *Cucumis Melo* subsp. *Agrestis* var. *Agrestis* seeds

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ABSTRACT

Cucumis melo subsp. *agrestis* var. *agrestis* Naudin; belonging to Cucurbitaceae family is commonly known as Wild Melon (English), Kachari (Hindi), is an annual climber, probably indigenous to North India. In present study standardization of *C. melo* subsp. *agrestis* was performed as per WHO guidelines including macroscopy and microscopy, ash values, extractive values, loss on drying, fluorescence analysis, swelling index, foaming index, determination of volatile oil content. To complete the study methanol and water extract of powdered seeds was screened for various phytoconstituents. The qualitative chemical analysis of extracts was found positive for alkaloids, proteins, carbohydrates, flavonoids and sterols. These studies provide valuable information for the identification and standardization of this plant material.

Keywords: *Cucumis melo* subsp. *agrestis* var. *agrestis*, standardization, phytochemicals evaluation.

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INTRODUCTION

India having a rich heritage of traditional medicine constituting with its different components like *Ayurveda*, *Siddha* and *Unani*. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will helps not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare^{1,2}. Standardization of herbal formulations is an essential factor in order to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles³.

According to World Health Organization (WHO), herbal medicines serve the primary health needs of about 80% of the world's population, especially for people in the rural areas⁴. There are many plants which has been unexplored in the field of medicine or science. One of such plant is *Cucumis melo* var. *agrestis*. The fruit of which is mainly consumed as a vegetable. It is commonly called as wild melon, small gourd, and wild musk melon⁵.

Cucumis melo subsp. *agrestis* var. *agrestis* is an annual climber growing up to 1.5 m, Stem are generally covered with rough hairs, Leaves are triangular, ovate, 3-5-lobed, rough with rigid hairs, Leaf stalk is 1-6 cm long, Flowers are small, yellow, solitary or rarely in pairs or threes. Flower clusters are carried on 5-10 mm long stalk. Sepal is about 1.5 mm long. Fruits are ellipsoid, oval-round, smooth and hairless, 4 x 2.5 cm in size, generally with dark green stripes, looks like a miniature watermelon. Flowering takes place in period of July to November⁶.

The fruits can be used as a cooling light cleanser or moisturizer for the skin. They are also used as a first aid treatment for burns and abrasions. The flowers are expectorant and emetic while fruits are stomachic. The seed possess antitussive, digestive and vermifuge activity. Roots are used as diuretic and emetic. Plant seeds possess significant antioxidant, anti-inflammatory and analgesic activities⁷.

MATERIAL AND METHODS

Plant material collection and authentication

The fruits of plant were collected from local grain market of Piparcity, Jodhpur (Rajasthan) in month of Sep. 2011. The fruit was taxonomically identified and authenticated as. *Cucumis melo* subsp. *agrestis* var. *agrestis* Naudin; by Dr. H.B. Singh, Head, Raw Materials, Herbarium and Museum Division, National Institute of Science Communication and Information Resources (NISCI), New Delhi, vide reference no. NISCAIR/RHMD/Consult/-2011-12/1860/160. Seeds were isolated from fruits. Seeds were cleaned, washed, dried at room temperature for two days and coarsely powdered. The sample was kept in tight containers protected from light.

Extraction of plant seed material

The powdered seeds were extracted with two different solvents (Methanol and Water) for 72 h at room temperature. The extracted solvent was filtered off and residue macerated again with the fresh solvent. The extracted solvents were combined and concentrated under reduced pressure on a Rotary evaporator at 40⁰C. The concentrated extract was defatted with hexane. All the extracts were stored in refrigerator^{8,9}.

Physicochemical Investigations

The study of morphological characters helps to distinguish the plant from the similar species and adulterants. Microscopy of the seeds was carried out as the next step for identification. Healthy seeds of the plant were soaked in water overnight. Transverse sections were cut with a razor blade. The clear sections were mounted on a clean glass slide and covered with cover slip using glycerine. Routine double staining with saffranin and fast green was done. Further, the sections were also stained with phloroglucinol and hydrochloric acid to observe the presence of lignified sclerides. Loss on drying and ash value is particularly important in the evaluation of purity of drugs⁸.

Histochemical colour reactions and fluorescence characters of the plant seed powder

Histochemical colour reactions of powdered seeds serve as indicator of its chemical composition and observed under microscope. Fluorescence characters are useful for identification of the original plant material from their adulterants. The treatment of powdered drugs with different chemical reagents shows the presence of different chemical constituents with fluorescence character in UV light¹⁰. Fluorescent impurities can be easily identified if mixed in a non-fluorescent compound¹¹.

Preliminary phytochemicals screening

The preliminary phytochemical screening of the extract of powdered seeds of *Cucumis melo* subsp. *agrestis* var. *agrestis* was carried out to know the different constituents present in it as per standard procedures. The extracts were tested for alkaloids, sterols, triterpenes, flavonoids, tannins, anthroquinone, carbohydrates, protein and amino acids⁹.

RESULTS AND DISCUSSION

Preliminary physicochemical and phytochemical screening of *Cucumis melo* subsp. *agrestis* var. *agrestis* seeds was done. Results generated from the present study are represented in the respective tables. The methanolic extract showed the maximum presence of chemical constituents such as flavonoids, alkaloids and sterols.

Macroscopy of seeds

Seeds of *Cucumis melo* subsp. *agrestis* var. *agrestis* were buff or creamish yellow in colour, odourless, sweet and sour in taste Cone or triangle in shape, flat and smooth, 5 to 7 mm in length, 2 to 3 mm in width, 1 mm in thickness (Figure 1a, 1b)



Figure 1(a): Fruits with Seeds, 1(b): Dried Seeds

Microscopy of seeds

A transverse section of seed shows outer seed coat, epidermal layer with one or two cylindrical collenchymas layers and Inner seed coat-inner epidermis, elongated, lignified sclerides. Endosperm and Cotyledons- polyhedral parenchyma cell with fixed oil globules and aleurone grains. (Figure 2a and 2b)



Figure 2 (a): Transverse section of Seed (b): Transverse section of Seed (enlarged view)

Physicochemical Investigation (Ash value and Loss on drying)

The determination of various physicochemical parameters i.e. total ash, acid insoluble ash, water soluble ash and loss on drying were calculated. The results were given as below. (Table 1)

Table 1: Percentage ash value and Loss on drying of powdered seeds

S. No.	Parameters	(% w/w)
1.	Ash Value	
	(i) Total ash	3.98
	(ii) Acid insoluble ash	2.12
	(iii) Water soluble ash	1.86
2.	Loss on drying	7.59

Histo chemical colour reaction of powdered seeds

Histochemical colour reaction of the powdered seeds was observed under microscope and results were given in Table 2.

Table 2: Histochemical colour reaction of powdered seeds

Reagents for test	Test for constituents	Standard colour change in reaction	Degree of Change of colour	Inferer
Million;s Reagent	Proteins	Red	Brownish red	Present
KI & Iodine solution	Proteins	Light yellow	Yellowish brown	Present
Iodine solution	Starch	Blue black	Brownish black	Present
Aq. KOH solution (10%) + H ₂ SO ₄	Suberin	Brown	No colour change	Absent
FeCl ₃ solution	Tannins	Black	No colour change	Absent
Wagner's reagent	Alkaloids	Reddish brown	Reddish brown	Present
Dragendroff's reagent	Alkaloids	Orange	Orange	Present
Dilute H ₂ SO ₄	Sterols	Dark red	Reddish brown	Present
Chloroform + Dilute H ₂ SO ₄	Sterols	Dark red	Red colour	Present

Fluorescence characters of the powdered seeds:

Fluorescence characters of the seeds powder under ordinary light and UV light (254 & 366 nm) were determined and are tabulated in Table 3.

Table 3: Fluorescent nature of powdered seeds

S. No.	Treatment	Observations		
		Long UV (366nm)	Short UV (254nm)	Visible
1.	Powder as such	White	Bluish white	Pale yellow to white
2.	Powder + 1N HCl	Brown	Reddish brown	Pale yellow to white
3.	Powder + 1N NaOH	Brown	Reddish brown	Pale yellow to white
4.	Powder + 50% H ₂ SO ₄	Brown	Dark brown	Pale yellow to white
5.	Powder + Methanol	Pale yellow to white	Pale yellow to white	Pale yellow to white
6.	Powder + Acetic acid	Brown	Reddish brown	Brown

Determination of extractable matter (Extractive values)

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which no suitable chemical or biological assay exists as yet.

Table 4: Extractive value of powdered seeds

S.No	Extractive Solvents	Colour of extract	Extractive value (w/w)
1.	Water	Yellow	0.27
2.	Ethanol	Yellow	0.48
3.	Chloroform	Yellow	0.38

Extraction was performed by cold maceration method. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. Extractive value gives an idea about the nature of the chemical constituents present in crude drug. Results are tabulated in the Table 4.

Other parameters

Swelling index, Foaming index and Volatile oil content of the powdered seeds of *Cucumis melo* subsp. *agrestis* var. *agrestis* was determined and obtained results were given in Table 5.

Table 5: values of other parameters

S.No	Parameter	Value
1.	Swelling index	0.5
2.	Foaming index	< 100
3.	Volatile oil	NIL

Preliminary phytochemical screening

Results of Phytochemical screening of *Cucumis melo* subsp. *agrestis* var. *agrestis* seed extracts has been given in below Table 6.

Table 6: Phytochemical screening of *Cucumis melo* subsp. *agrestis* var. *agrestis* seed extracts.

Test	Observation		Inference
	Methanolic extract	Water extract	
1) Test for Alkaloids	(+)		Present
Mayer's test	(+)	(-)	
Dragendroff's test	(+)	(-)	
Wagner's test	(+)	(+)	
Hager's test	(+)	(+)	
2) Test for Anthroquinone Glycosides			Absent
Borntrager test	(-)	(-)	
3) Protein and Amino acid			Present
Millons reagent	(+)	(+)	
Ninihydrin test	(+)	(+)	
4) Tannins			Absent
Ferric chloride	(-)	(-)	
5) Saponins			Absent
Foam test	(-)	(-)	
6) Sterols			Present
Salkowski test	(+)	(+)	
Hersch_Sohn's Reaction	(+)	(+)	
8) Flavonoids			Present
Ammonia test	(+)	(+)	
Alkaline Reagent Test	(+)	(+)	
9) Carbohydrates			Present
Molish test	(+)	(+)	

Key: + = Present; - = Absent;

CONCLUSION

The present work on physicochemical investigation of powdered seed of *Cucumis melo* subsp. *agrestis* var. *agrestis* Naudin; provides valuable information regarding their identification, authentication and chemical constituents present in it, which may be useful for the standardization and preparation of monograph. The methanolic extract showed the maximum presence of chemical constituents such as flavonoids, triterpenoids, alkaloids and sterols. The chemical constituents present in *Cucumis melo* subsp. *agrestis* var. *agrestis* Naudin may have several pharmacological activities and can be utilized for the treatment of various diseases in future. Further research on this species may leads for the isolation of therapeutically potent compounds.

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