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Pharmacognostical Study and Development of Quality Control Parameters for *Cucumis melo* Linn

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ABSTRACT

Cucumis melo Linn. (family cucurbitaceae) is extensively cultivated throughout India particularly in the hot and dry North-Western areas. Its fruit pulp, root, seeds and seed oil are used for medicinal purposes. In Unani medicine, the seed kernel is commonly used to treat various disease conditions. Seeds are having diuretic, lithontriptic, laxative, demulcent and refrigerant properties. There are many varieties of melon available and they also show great diversity in foliage. Hence, adultration is very common with this plant. Standardization provides a more reliable, effective and high-quality product by confirming the presence of plant constituents qualitatively and quantitatively. Hence, efforts have been made to standardize the seed to provide scientific data for identification, authentication and distinguishing the plant from its adulterants. The morphological, microscopic, physicochemical and chromatographic studies of *Cucumis melo* seed will be useful for quality control of this drug in formulations and would serve as a standard reference for further studies.

Key words: Adultration, *Cucumis melo,* flavonoid, phenolic, standardization

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INTRODUCTION

Cucumis melo Linn. belongs to the family cucurbitaceae. It is commonly known as Musk melon. This herb is extensively cultivated throughout India particularly in the hot and dry North-Western areas. It is an annual climbing or creeping herb with angular, scabrous stem, simple soft hairy orbicular-reniform leaves and bears tendrils, by which it is readily trained over trellises. Flowers are unisexual and yellow.[1] *Cucumis melo* is extensively cultivated for its fruits, eaten as a vegetable in many tropical countries, including throughout India, where it is sometimes naturalized in open scrub forests.[2-3]

Fruit pulp, root, seeds and seed oil are used for medicinal purposes.[1-2] In Unani medicine, the seed kernel is commonly used to treat conditions such as kidney and bladder stones, painful and burning micturiation, ulcers in the urinary tract, oligouria and it has also been used for other ailments like jaundice, vitiligo, ascites, chronic fevers, inflammation of the liver and kidney, bile obstruction, eczema and used in general debility.[4-6]

The seeds are having diuretic, lithontriptic, laxative, demulcent and refrigerant properties. Phytochemical studies revealed that the seeds of *Cucumis melo* contain chromone derivatives, phenolic glycoside, arginine, aspartic and glutamic acids, alpha-galactosidases, dihydroxy triterpenes, sitosterol 2, and beta-sitosterol, etc. [7-11]

There are many varieties of melon available and they also show great diversity in foliage. [2-3] As such, adultration is very common with this drug. Therefor, there is a need to develop quality standards on pharmacognostical and physicochemical characteristics of this seed for authentication and to prevent adultration.

MATRIAL AND METHODS

Collection and authentication of drug

The seeds of *Cucumis melo* Linn. were purchased from Khari Baoli, local market of Delhi and authenticated by Dr. H. B. Singh, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen is submitted in the herbarium of NISCAIR, New Delhi (Ref. No. NISCAIR/RHMD/Consult/-2010-11/1657/255).

Macroscopical and microscopical study

Macroscopical and microscopical characters of the drugs were studied according to the WHO and pharmacopoeial guidelines.[12-13]

Physico-chemical studies

Extractive values(cold and hot extracts), ash values (total ash, acid-insoluble ash & water soluble

ash), loss on drying, swelling index and pH of 1% and 10% solution of *Cucumis melo* seed kernel were determined according to the standard methods.[12]

Preliminary phytochemical analysis

The individual extracts of *Cucumis melo* seed kernel such as petroleum, chloroform, methanol and aqueous were subjected to the preliminary phytochemical screening for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoid, protein and amino acids as per the method described by Trease and Evans.[14]

Fluorescence analysis

Fluorescence behavior of powder drug with different reagents was observed according to the reported method.[15]

Effect of different chemical reagent on crude drug powder

Chemical tests of powder drug with different reagents were performed according to method described by Sama et al. [15] Most of these tests were based on colour indication of powder drug with specific substance.

Determination of total phenolic contents by UV spectrophotometer

The phenol was determined in powdered crude drugs, extracts and beverages by Folin Ciocalteu method.[16] Standard stock solution was prepared by dissolving 25 mg of catechin standard in 100 ml distilled water. Different concentrations of the standard solutions were prepared for standard calibration curve starting from 4 to 24 µg/ml in water. The commercial Folin Ciocalteu (FC) reagent was diluted (1: 10) with distilled water on the day of use. 1M sodium acetate was prepared by dissolving 82 g of sodium acetate in 1000 ml distilled water

Sample preparation - 500 mg of the samples were taken in 50 ml volumetric flasks and added around 25 ml of distilled water and sonicated for 10 minutes then made up the volume with water.

Procedure - Take 3ml of each standard and sample solution in a 10 ml test tube and to this add 3 ml of FC reagent and 3 ml of sodium carbonate solution. A blank solution was prepared by adding 3 ml each of distilled water, sodium carbonate solution, and FC reagent in test tube. Keep the solution in dark for 30 minutes for colour development. Absorbance was taken at 415nm against blank solution. After taking the absorbance of standard dilutions calibration curve was plotted (Figure 1). Phenolic contents in drug were calculated by using standard calibration curve.

Determination of total flavonoid contents by UV spectrophotometer

The flavonoid content was determined in powdered crude drugs according to method described

by Pourmorad et al.[17] $AlCl_3$ (0.1 g/ml) and CH_3COONa (1M) were prepared, Prepared dilutions for Rutin (standard) from 10 µg /ml to 100 µg/ml.

Samples Preparation - 500 mg of the samples were taken in 50 ml volumetric flasks and added around 25 ml of methanol and sonicated for 30 minutes then made the volume with methanol.

Procedure - 0.5ml of each standard and sample solution was taken in a 10 ml test tube and added 1.5 ml methanol. To this added 0.1ml of $AlCl_3$ and 0.1 ml of CH_3COONa reagents and added 2.8 ml Distilled water and kept for 30 minutes. A blank solution was prepared by adding 2 ml of methanol + 0.1ml of $AlCl_3$ + 0.1ml of CH_3COONa reagents and then added 2.8 ml Distilled water. Absorbance was taken at 415nm against blank solution. After taking the absorbance of standard dilutions calibration curve was plotted (Figure. 2). Flavanoid contents in drug were calculated by using standard calibration curve.

Development of chromatographic HPTLC fingerprint profile of different extracts

The plant material was coarsely powered and extracted in Soxhlet apparatus for 6-24 h using methanol. The extract was evaporated to dryness in a rota-vapour and the solvents were recovered. The methanolic extract of drugs were treated with boiling chloroform for one hour and filtered. The process was repeated two times. All the chloroform soluble fractions were combined together.[18] Removal of chloroform by distillation method under reduced pressure gives chloroform soluble fraction of *Cucumis melo* seed. Gummy residues so obtained, were stored in deep freezer at -20 °C till further application. TLC and HPTLC samples were prepared by dissolving each extract in their respective solvent to get the concentration (40 mg/ml). The HPTLC finger printing was developed using TLC aluminum sheets silica gel. Tolune: Ethyl acetate (9: 1) solvent system was used for separation of constituents of extract and its fraction. The developed chromatograms were scanned at 254 nm and 450 nm (after spraying 10 % H_2SO_4) wavelengths for detection of visible spots.

RESULTS AND DISCUSSION

In the present scenario, the traditional system of medicine has gained more importance in the field of medicine. In most of the developing countries, a large number of populations depend on herbal medicine for their primary health care needs. As the usage of these herbal medicines has increased, issues and the motto regarding their quality, safety, and efficacy in industrialized and developing countries have cropped up.[19] Hence, more attention has forced on the scientific documentation of herbal drugs before their marketing and use in different illnesses.

Accounting to WHO standardization is the process involving the physicochemical evaluation of

crude drug covering the aspects, as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.[20] Effect of herbal drugs mainly based on the amount of medicinal principles or active constituents present within them which in turn depend on the quality of drug. But due to the absence of standards possibility of adulteration is increased and quality of drug is decreased. Therefore, in order to have high-quality raw materials, it has become essential to develop quality standards of samples available in the market. Thus good quality drugs could be utilized for medicinal preparations.

In present investigation, standardization was carried out under following parameters such as macroscopical and microscopical study, physicochemical analysis, phytochemical analysis and estimation of total phenolics and flavonoids content. HPTLC finger printings of methanolic extract of *Cucumis melo* seeds and its chloroform fractions were also developed.

Macroscopical and microscopical study is the first step towards establishing the identity and degree of purity of such materials.[13] By this the seed can be differentiated from other seeds of the same species. *Cucumis melo* seeds are exalbuminous smooth, elongated but laterally flattened, about 7 to 10 mm wide, tapering at one end and creamy colored. Taste is sweet but no specific odour. Under microscopical observation, the seed coat shows a layer of round to oval, sclerenchymatous cell, lignified with distinct lumen; followed by a narrow zone of endosperm, consisting of thin-walled, rounded and tangentially elongated, parenchymatous cells **(Figure. 3).** Cotyledons are two and straight covered by layer of tegment cells. Cotyledons showed single layered epidermal cells, radially elongated to squarish, parenchymatous cell and numerous oil globules and aleurone grains **(Figure. 4-6).** Powder is creamy yellow colored, sticky; consists of thin walled parenchyma cells, oil globules and aleurone grains.

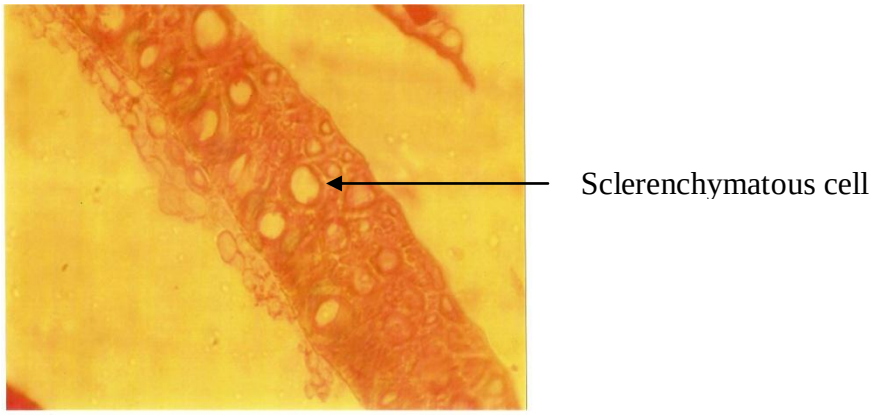


Figure 3: T. S. of testa of seed of *Cucumis melo* in 40X

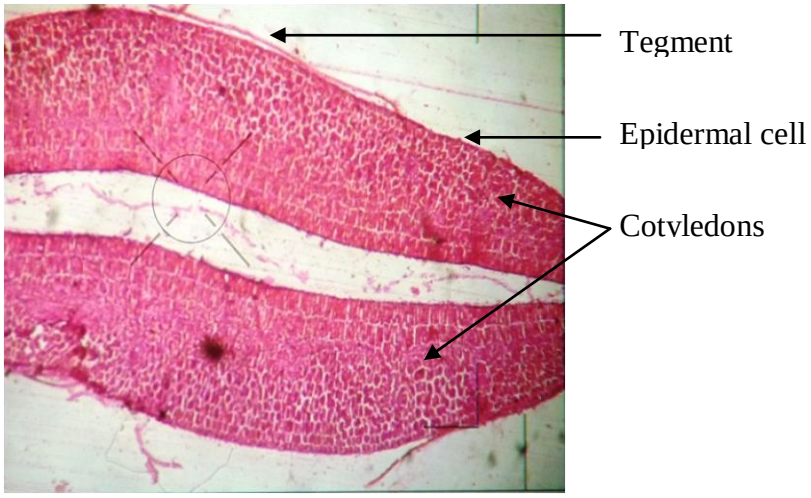


Figure 4: T. S. of seed of cotyledons of C*ucumis melo*10X

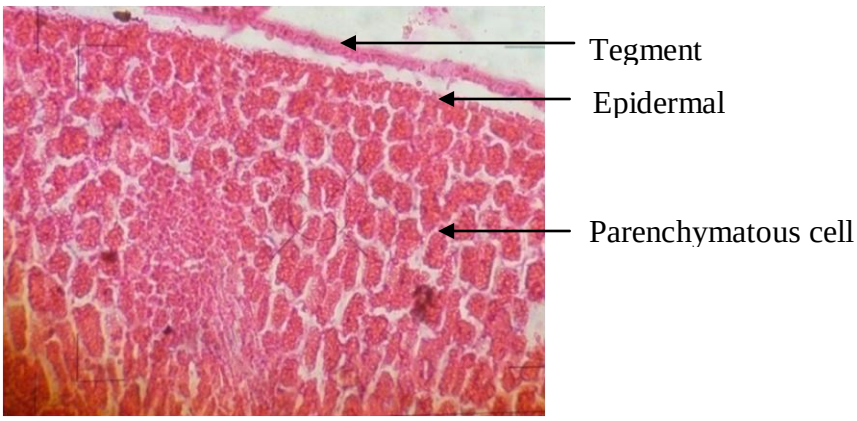


Figure 5: T. S. of cotyledon of seed of *Cucumis melo* in 40X

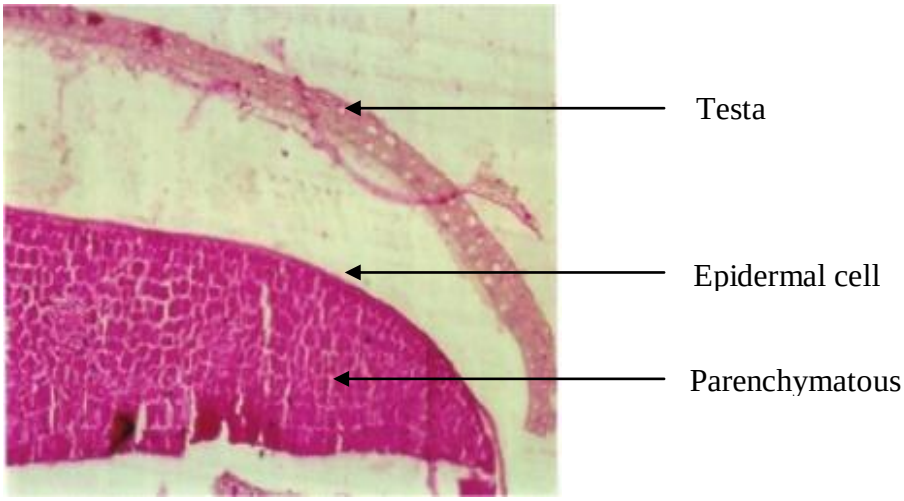


Figure 6: T. S. of seed of *Cucumis melo* with seed coat 10X

Physicochemical values such as extractive values (cold and hot extracts), ash values (total ash, acid-insoluble ash & water soluble ash), loss on drying, swelling index and pH of 1% and 10% solution of *Cucumis melo* seed kernel were evaluated. The results of physicochemical parameters

are summarized in **Table 1.** These data would be useful to determine the amount of active constituents present in the different extracts and help in identification and authentication of the drug. Swelling index is an important parameter as many herbal materials of specific therapeutic or pharmaceutical utility because of their swelling properties.[13]

Table 1: Summary of physicochemical and phytochemical results of seeds of *Cucumis melo*

A.	**Individual extractive values cold extract**	**Extractive value**
1.	Petroleum ether extract	20.36 ± 0.43 %
2.	Chloroform cold extract	31.96 ± 1.02 %
3.	Methanol cold extract	5.76 ± 0.37 %
4.	Aqueous extract	4.63 ± 0.39 %
B.	**Individual extractive values hot extract**	**Extractive value**
1.	Petroleum ether extract	37.65 ± 2.66 %
2.	Chloroform extract	39.85 ± 1.81 %
3.	Methanolic extract	10.84 ± 0.28 %
4.	Aqueous extract	8.61 ±0.38 %
C.	**Successive extraction**	**Extractive value**
1.	Petroleum ether extract	38.93 ± 0.64 %
2.	Chloroform extract	4.80 ± 0.17 %
3.	Methanol extract	3.88 ± 0.18 %
4.	Aqueous extract	3.08 ± 0.10 %
D.	**Ash value**	**value**
1.	Total ash	5.86 ± 0.13 %
2.	Acid insoluble ash	2.86 ± 0.13 %
3.	Water soluble ash	1.2 ± 0.24 %
E.	**Loss on drying in crude drug (%)**	6.5 ± 0.17 %
F.	**pH of the drug 1%**	7.21 ± 0.008
G.	**pH of the drug 10%**	7.24 ± 0.005
H.	**Swelling index**	3.16 ± 0.16 ml

Values represent mean values of 3 readings with standard error

Since the therapeutic effectiveness of the drug is mainly on the quality of secondary plant metabolized present in them, in present study the preliminary phytochemical screening was carried out using the extracts for presence of different types of chemical constituents like alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoid, protein and amino acids. The results of presence and absence of different phytoconstituents are presented in **Table 2**. This test may helpful to recognize the pharmacological activity and therapeutic efficacy of the *Cucumis melo* seed kernel.

Table 2: Phytochemical screening of individual extracts of *Cucumis melo*

Constituents	Petroleum ether	Chloroform	Methanolic	Aqueous
Alkaloids	-	-	+	+
Carbohydrates	+	-	+	+
Phenolic compounds	-	-	+	+
Flavonoids	-	-	+	+
Proteins and amino- acids	-	-	-	+
Glycosides	-	-	+	-

(-: Absent, +: Present)

Effect of drug with different reagent and its fluorescence behavior are important parameter of pharmacognostical evaluation to assess the quality of the drugs. Various chemical constituents present in plant material exhibit different colours when they react with different chemical reagents.

Fluorescence analysis of powdered drug with distilled water, Dil.HNO_3, Dil. H_2SO_4, Dil. HCl, ethyl acetate, 5% ferric chloride, ammonia, methanol, chloroform, petroleum either, 10% aq. NaOH and glacial acetic acid gave different characteristic colour under ultraviolet (254 and 366 nm) and under normal ordinary light. The observed results are produced in **Table 3.**

Table 3: Fluorescence behavior of crude drug powder with different chemical reagents

S. No.	Treatment	Day light	UV light 254 nm	UV light 366 nm
1.	Powder + distilled water	Light cream	White	White
2.	Powder + Dil.HNO_3	Cream	Dark orange	White
3.	Powder + Dil. H_2SO_4	Cream	Milky	White
4.	Powder + Dil. HCl	Cream	Milky	White
5.	Powder + Ethyl acetate	Cream	Milky	Light cream
6.	Powder + 5% Ferric chloride	Light orange	Dark orange	Yellow
7.	Powder + Ammonia	Light cream	Milky	White
8.	Powder + Methanol	Light cream	Transparent	Transparent
9.	Powder + chloroform	Light cream	Milky	Light cream
10.	Powder + petroleum either	White	Milky	Turbid
11.	Powder + 10% Aq. NaOH	Cream	Turbid	Light yellow
12.	Powder + Glacial acetic acid	Light cream	Cream	Turbid

Behaviour of powder drug of seed with different chemical reagents was also observed. It exhibited cream, orange, red, light green changed to purple, dark maroon, light cream and yellow with 10% Aq. NaOH, Con.HNO_3, Con. H_2SO_4, Con. HCl, Iodine, glacial acetic acid and picric acid (saturated) respectively.

It is an established fact that the phenolic and flavonoid present in the plants are responsible for the various pharmacological activities. Thus quantitative estimation of these plant metabolites in different spices of the drug will direct to identify the best quality drug with better therapeutic

efficacy. In this study the phenolic content was determined by Folin Ciocalteu method. The amount of phenolic content of 10 mg/ml of crude powder of *Cucumis melo* seed was calculated with the help of standard calibration curve (**figure. 1)** and was found to be 1.334 ± 0.015 % w/w. The flavonoid content was determined by aluminum chloride colorimetric method. The amount of flavonoid content of 10 mg/ml of crude powder of *Cucumis melo* seed was calculated with the help of standard calibration curve (**figure. 2)** and was found to be 0.756 ± 0.013 % w/w.

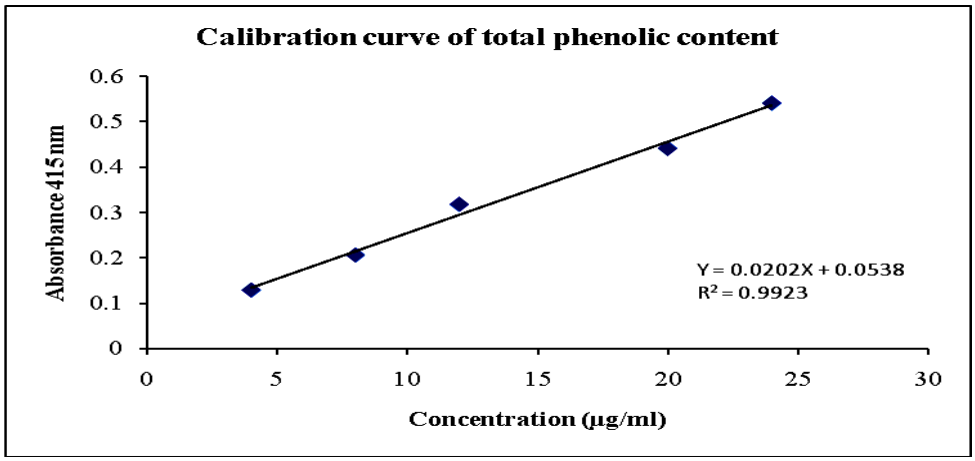


Figure 1: Calibration curve of standard catechin for total phenolic contents

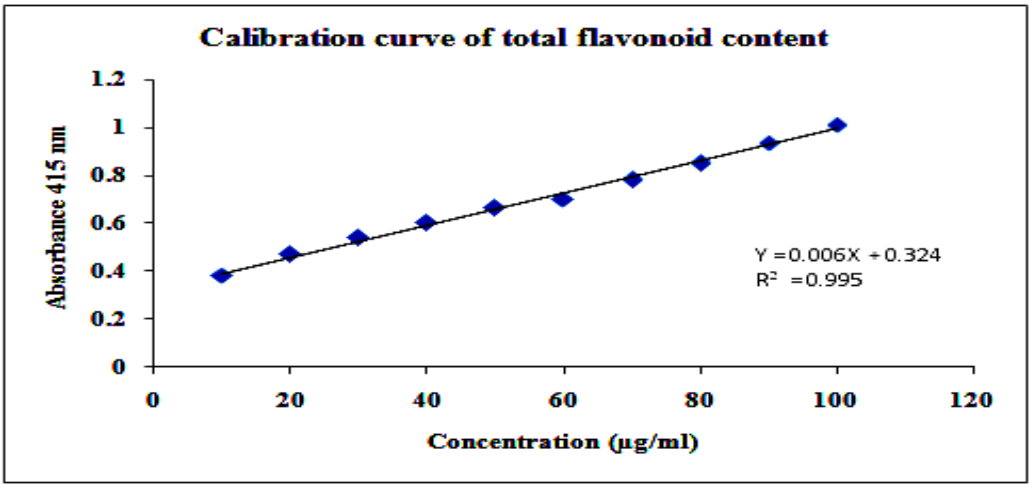


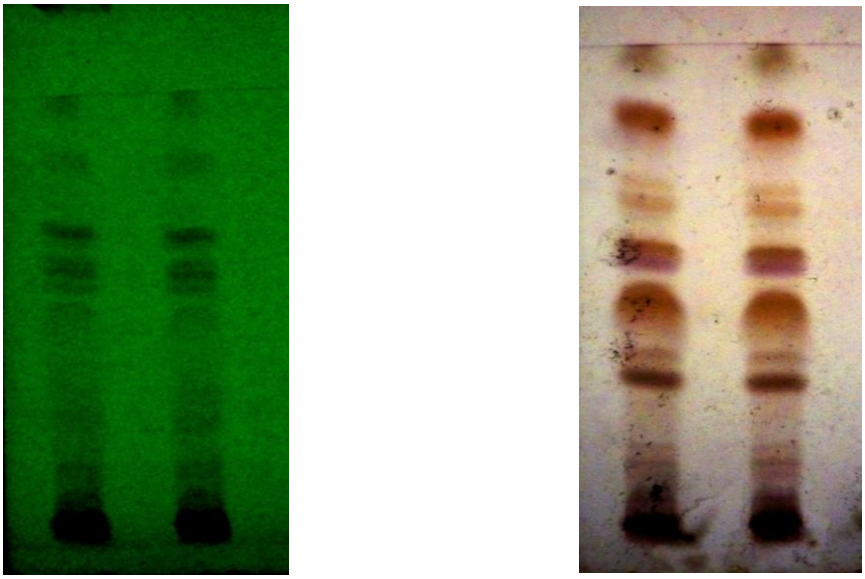
Figure 2: Calibration curve of standard rutin for total flavonoid content

HPTLC analysis will facilitate to detection of number of constituents present and quantitative and qualitative separation of pharmacologically active components. In present study, HPTLC finger prints of methanolic extract of *Cucumis melo* and its chloroform fraction were developed in Toluene: Ethyl acetate (9: 1) solvent system. HPTLC fingerprint of methanolic extract of *Cucumis melo* showed 11 spots in Tolune: Ethyl acetate (9: 1) solvent system at 254 nm and after spraying 10% H_2SO_4 it showed 10 spots at 450 nm. The Rf values of these spots were presented in **Table 4** and the developed chromotogram and 3D view presented in **Figure 7-9**.

HPTLC fingerprint of chloroform fraction of methanolic extract of *Cucumis melo* showed 12 spots in Toluene: Ethyl acetate (9: 1) at 254 nm and after spraying 10% H_2SO_4 it showed 17 spots at 450 nm. The Rf values of these spots were given **in table 5** and the developed chromotogram and 3D view presented in **Figure 10-12.**

Table 4: Results of HPTLC fingerprint analysis of methanolic extract of *Cucumis melo* at 254 and 450 nm.

Solvent system	No. of peak observed (Rf values)	
	254 nm	450 nm after spraying 10 % H_2SO_4
Toluene : Ethyl acetate (9 : 1)	0.14, 0.17, 0.24, 0.29, 0.45, 0.52, 0.60, 0.70, 0.77, 0.85, 0.92	0.16, 0.22, 0.27, 0.31, 0.34, 0.41, 0.49, 0.59, 0.68, 0.87



(a) Chromatogram at 254 nm (b) Chromatogram at 450 nm after 10% H_2SO_4 spray

Figure 7: Chromatogram of methanolic extract (40mg/ml) of *Cucumis melo*

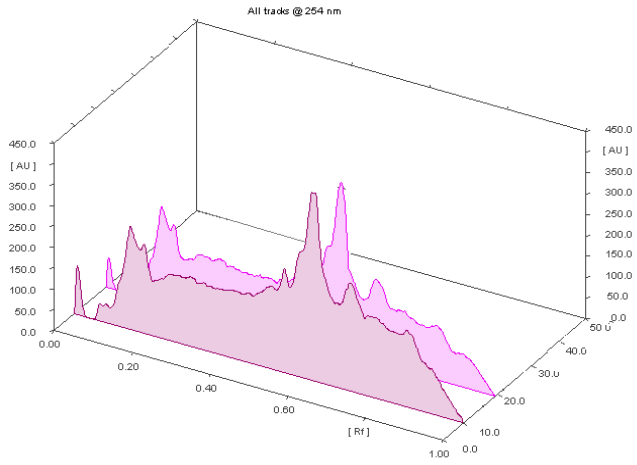


Figure 8: 3D view of methanolic extract of *Cucumis melo* at 254 nm

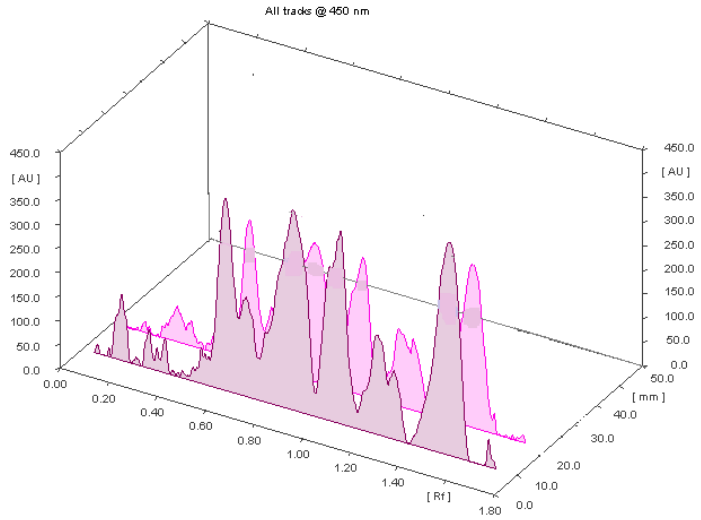
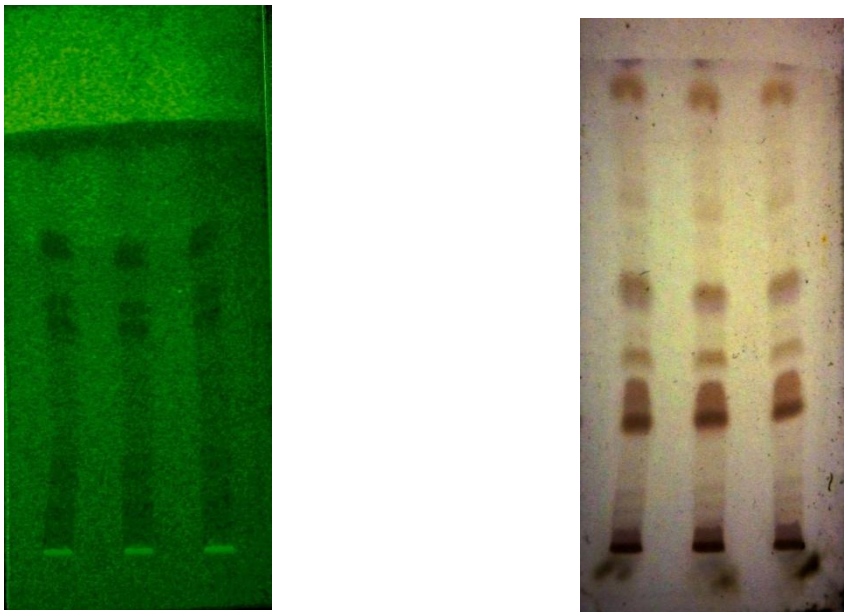


Figure 9: 3D view of methanolic extract of *Cucumis melo* at 450 nm after spraying 10 % H_2SO_4.

Table 5: HPTLC fingerprint of chloroform fraction of methanolic extract of *Cucumis melo* at 254 and 450 nm.

Solvent system	No. of peak observed (Rf values)	
	254 nm	450 nm after spraying 10 % H_2SO_4
Toluene : Ethyl acetate (9 : 1)	0.11, 0.14, 0.21, 0.24, 0.30, 0.38, 0.44, 0.57, 0.61, 0.67, 0.74, 0.91	0.10, 0.14, 0.18, 0.19, 0.21, 0.23, 0.28, 0.40, 0.48, 0.54, 0.60, 0.65, 0.70, 0.77, 0.85, 0.87, 0.92



(a)Chromatogram at 254 nm (b) Chromatogram at 450 nm after 10% H_2SO_4 spray

Figure 10: Chromatograms of chloroform fraction of methanolic extract (40 mg/ml) of *Cucumis melo*

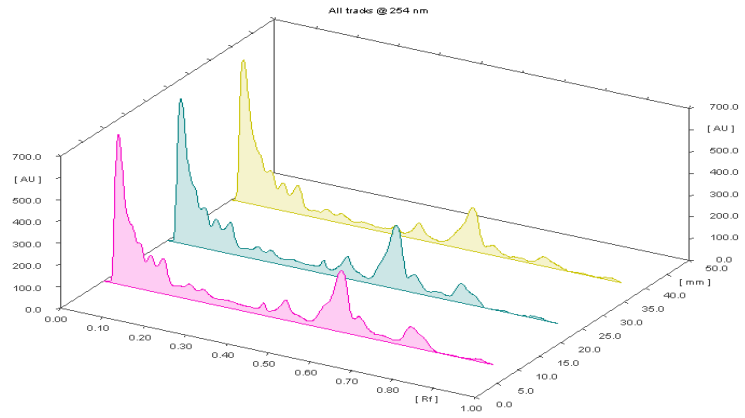


Figure 11: 3D view of chloroform fraction of methanolic extract of *Cucumis melo* at 254 nm.

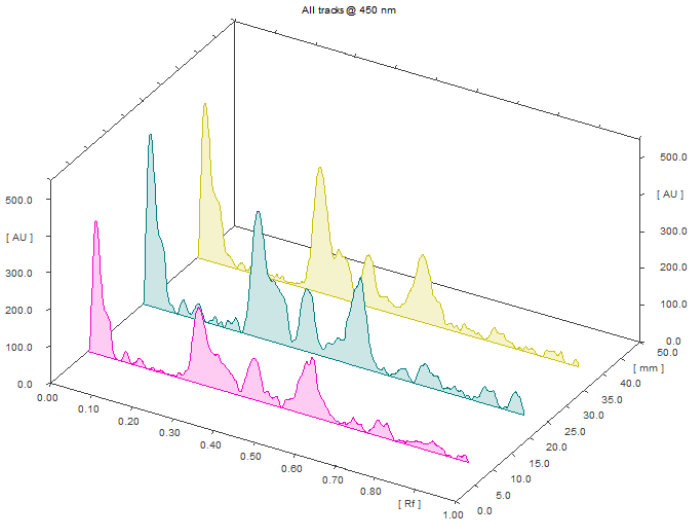


Figure 12: 3D view of chloroform fraction of methanolic extract of *Cucumis melo* at 450 nm after spraying 10 % H_2SO_4.

But more work on safety parameters and phytochemical research on isolation, purification and characterization of pharmacologically active components are necessary towards establishing authoritative standard data.

CONCLUSION

Authentication, identification, detection of adulteration and determination of quality and purity are crucial parts in drug evaluation. The purpose of standardization of medicinal plant products is obviously to ensure therapeutic efficacy. In order to have a high quality plant, it is necessary to develop definitive, specific and precise standard quality control methods using modern analytical methods. The *Cucumis melo* seed is an important drug used in Unani medicine and has been proved to have various important pharmacological effects. Therefore efforts have been made to

standardize the seed to provide scientific data for further studies. The morphological, microscopic, physico-chemical and chromatographic studies would serve as a standard reference for identification, authentication and distinguishing the plants from its adulterants and also useful for quality control of these drugs in formulations.

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