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Phytochemistry, Fluorescence and Crystallization of Bitter Cucumber

Iqbal dar A, Ud Din Gagloo M, Untoo SA and Sarvar S*

Islamia College of Science and Commerce, India

Abstract

Our grandparents were consuming various herbs for combating various disorders and now herbal medicines are becoming popular in modern world as people resort to natural therapies. Novel isolations from the plants in the form of secondary metabolites is the basis of the formulation of natural therapies and the scientific justification of the traditional use of plants. Keeping this in view, one of the important medicinal plants *Citrullus colocynthis L.* (Bitter Cucumber) whose fruit has been explored to investigate its phytochemistry and fluorescence characters, purification and chromatographic separation of penultimate fractions and then the standardization of these fractions to form the crystals for further spectral analysis was carried out in the present study. The aim of this study is to evaluate these parameters of the Bitter Cucumber to prove its claim in folklore practice against various disorders.

Keywords: *Citrullus colocynthis L*; Bitter Cucumber; Phytochemistry; Ash content; TLC; Column chromatography; Fluorescence; Rf value

Introduction

The present study was undertaken after thorough consultation with the people of Vidisha District of Madhya Pradesh, India. As per their views, they are collecting the fruits of bitter Apple from fields, which are lemon sized, yellowish in color and extremely bitter in taste. They called it as bitter cucumber because of its bitter taste and after collecting the fruits, they dried the same and make a paste of it and then apply it against parasitic infections and inflamed breasts, they also consume the small pieces of bitter apple as a strong laxative and to avoid constipation and painful menstruation. Keeping their traditional practice in consideration we select the fruits of *Citrullus colocynthis L.* for scientific investigation. Previous reports suggest that refining and washing of bitter cucumber with citric acid removes its bitter taste as reported by Ramakrishna et al. [1]. Plants have potent complex substances called secondary metabolites as reported by Karthikeyan et al. [2]; Lozoya and Lozoya [3]. They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins; carbohydrates and essential oils, and any part of the plant may contain potent compound as reported by Gordon and David [4]. The present study was undertaken to investigate the phytochemistry and fluorescence characters of Bitter Cucumber and then the preparation and standardization of the purified crystals was done scientifically which will help us in justifying the pre-modern medicinal uses of the Bitter Cucumber.

Experimental Design

Plant collection

The fruits of *Citrullus colocynthis L.* used for the present study were collected from the Vidisha district of M.P in India and the details of the same is mentioned in Table 1. The fruit of the plant was identified, confirmed and authenticated by Prof. P.N. Srivastav, Department of Botany S.S.L Jain College, Vidisha. The herbarium specimen of the plant was kept in Herbarium of Pest Control and Ayurvedic Drug Research Laboratory, S.S.L Jain P.G College, Vidisha (M.P) under voucher specimen no. PCAADRL/CC/2010. The fruits were shade dried and pulverized.

Preparation of extract (Soxhlet extraction method)

The extraction procedure adopted is of Harborne, 1984 and the extraction of shade dried powder material of the fruits of *Citrullus colocynthis L.* (Bitter Cucumber) were carried out by soxhlation techniques by using different solvents according to increasing order of polarity *viz.* n-hexane, Pet-ether, Chloroform, Ethyl acetate, Ethanol and distilled water. Extraction of the plant material is needed for any phytochemical or phytopharmacological experimental work. The extraction of shade

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Correspondence:
Arshed Iqbal dar, Islamia College of Science and Commerce, Srinagar, India,
E-mail: drarshediqbal@gmail.com

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Table 1: Showing plant material and its part used.

Sr. No.	Plant species	Common name	Family	Part used	Month of collection	Season of collection
1	*Citrullus colocynthis L.*	Bitter Cucumber	Cucurbitaceae	Fruit	Aug-Oct.	Summer and Autumn

Table 2: Showing percentage of loss in weight of plant material on drying.

Sr. No.	Plant species	Wet weight of plant material	Dry weight of plant material	Loss in weight on drying	% loss in weight
1	*Citrullus colocynthis L.*	4 Kg	1 Kg	3 Kg	75%

Table 3: Showing ash content of Citrullus *colocynthis L.* with acid soluble and water soluble ash content.

Plant material	Weight of plant material before burning	Weight of ash content after burning of plant material	% yield of ash content after burning of plant material	% of acid soluble ash content	% of water soluble ash content
Citrullus colocynthis L.	5 gm.	2.76 gm.	55.2 gm.	19.6 gm.	35.6 gm.

Table 4: Showing percentage yield of Citrullus *colocynthis L.* in different solvents by soxhlet extraction method.

Plant species	Solvent used (ml)	Weight of powdered plant material (g)	Volume of solvent (ml)	Weight of extract obtained (gm)	% yield of extract (g)
Citrullus colocynthis L.	n-hexane	150 g	300 ml	6.79 g	4.52 g
	Petroleum-ether	150 g	400 ml	0.44 g	0.29 g
	Chloroform	150 g	350 ml	1.35 g	0.9 g
	Ethyl acetate	150 g	400 ml	0.3 g	0.2 g
	Ethanol	150 g	390 ml	2.73 g	1.82 g
	Water	150 g	400 ml	5.9 g	3.93 g

Table 5: Thin layer chromatography of 90% ethanolic extract of plant material with Rf value and color characteristics.

Crude extract of plant materials	Solvent systems	Rf values	Visual light	Iodine chamber	UV light
Citrullus colocynthis L.	n-butanol: HOAC: H_2O	.66,	Yellow green,	Brownish green,	Blackish green,
	(4:1:5)	0.73	Reddish yellow.	Brownish yellow.	Brightish blue.

Table 6: Showing column chromatography of the 90% ethanolic extract of the plant material.

Crude extract of plant materials	Solvent system (ml.)	Weight of fractions (gm.)	Chromatographic color of fractions.
Citrullus colocynthis L.	n-butanol: HOAC: H_2O (4:1:5)	13 gm.	Dark brown,
		17 gm.	Light brown,
		11 gm.	Reddish brown

dried powdered material (40-60 mesh size) of the selected material was carried out by Soxhlet apparatus in the laboratory using different solvents of increasing order of polarity. The different solvents used for the extraction purpose depends on the chemical nature of the active principles to be separated out from the particular plant material. The mode of action of plant material depends on the texture and water content of the plant material and substance to be extracted either in any solvent.

Determination of percentage yield

The percentage yield of extracts was calculated by using following formula as adopted by Francis T lynch [5].

Percentage yield = Weight of extract/Weight of powder drug × 100.

Standardization of crude extract

Determination of moisture content or loss on drying: The method used for the determination of moisture content adopted was of John Kenkel [6]. An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis as reported by WHO [7]. Limits for water content should therefore be set for every given material. This is especially important for materials that absorb moisture easily or deteriorate quickly in presence of water. Loss on drying is the loss of mass expressed as percent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible. An accurately weighed quantity of about 2g of powdered material was taken in a tared glass Petri-dish. The powder was distributed evenly. The petri-dish kept open in vacuum oven and the sample was dried at a temperature between 100°C to 150°C for 2 h until a constant weight was recorded. Then it was cooled in desiccators to room temperature, weighed and recorded.

% Loss on drying was calculated by using the following formula:

% Loss on drying = Loss in weight of the sample/Weight of the sample × 100.

Phytochemical screening

In order to determine the presence of alkaloids, glycosides, flavones, tannins, terpenes, sterols, saponins, phenolics, fats and sugars, a preliminary phytochemical study (color reactions) with various plant extracts were performed as per the methods adopted was of Khandelwal [8].

Purification and chromatography

Purification of crude extract: There are various secondary metabolites *viz.* alkaloids, flavonoids, glycosides, saponins, phenolics, tannins etc. present in the plant extracts, and further isolation and

Table 7: Phytochemical tests of the successive extracts of *Citrullus colocynthis L.*

Sr. No.	Tests	Reagents used	Results
AQUEOUS EXTRACTIVES			
1	Starch	12-KI	-ve
2	Tannins	Acidic Fecl3	+ve
3	Saponins	H_2SO_4 + Acetic anhydride	+ve
4	Proteins	Million's test	+ve
5	Reducing sugars	Benedict's	+ve
ALCOHOLIC EXTRACTIVES			
1	Alkaloids	Mayer's,	+ve
		Wagner's,	+ve
		Dragnendorff's	+ve
2	Flavonoids	HCl + Mg turnings	+ve
3	Glycosides	Benzene + hot ethanol	+ve

+ve = Present, -ve = Absent

Table 8: Fluorescence characters of the *Citrullus colocynthis L.* extracts were observed under UV (254 and 366 nm.) and visible light with different solvents.

Sr. No	Name of the extract	UV light		Visible light
		Short (254 nm)	Long (366 nm)	
1	Petroleum ether	Greenish brown	Blackish brown	Light brown
2	Chloroform	Brown	Dark brown	Light brown
3	Ethanol	Green	Dark green	Light green
4	Aqueous	Yellowish brown	Dark brown	Light brown

purification are required to find the pure active compound that is most effective in the treatment of various disorders. In the present study, the crude extracts obtained from vacuum evaporation of the selected botanical was subjected to isolation, purification, chemical examination by different chromatographic techniques.

Chromatographic purification of crude extract

Column chromatography: Column chromatography is a method used to purify individual chemical compounds from mixture of compounds as reported by Stock and Rice [9]. The main advantage of column chromatography is the relative low cost and disposable of stationary phase used in the process. It prevents cross contamination and stationary phase degradation due to recycling. Two methods are generally used to prepare a column, the dry method and the wet method. In column chromatography the stationary phase, a solid adsorbent is placed in a vertical glass column and the mobile phase, a liquid is added to the top which flows down through the column (by either gravity or external pressure). Column chromatography is advantageous over most other chromatographic techniques, because it can be used in both analytical and preparative applications. Not only can column chromatography be used to determine the number of components of a mixture, but it can also be used to separate and purify substantial quantities of those components for subsequent analysis.

Thin layer chromatography: The method used for TLC and the estimation of R_f value adopted was of Andrzej and Jan [10]. TLC was produced with the aim of identifying the individual substances in a mixture and also testifying for purity or for separation of mixtures. TLC is performed on a sheet of glass, plastic or aluminum foil which is coated with a thin layer of adsorbent material, usually with silica gel or cellulose. This layer of adsorbent is known as stationary phase. The plate or sheet is placed in a chamber containing a small amount of solvent which acts as mobile phase. The height of the solvent front and center of spots were measured in the form of R_f value. The R_f value indicates the position at which a substance was located in the chromatogram.

R_f = Compound distance from origin (midpoint)

Solvent front distance from origin

The R_f value is always <1.

Acid hydrolysis

Acid hydrolysis was done by adopting the method of John Whittall and Peter W Sutton [11]. 5 ml to 10 ml each fraction from column chromatography was mixed with 2 ml of methanolic HCL (10%) and was refluxed for four hours in evaporator at the temperature 35°C to 40°C. After 4 h, reaction mixture was diluted with 3 ml of distilled water and then again evaporated to remove methanol, it was performed two times then the aqueous solution was extracted with $CHCL_3$, after evaporation the aqueous layer was neutralized with 10% NaoH and concentrated under reduced pressure.

Methylation

Methylation was also done by adopting the method of John Whittall and Peter W Sutton, 2012 [11]. The acid hydrolyzed fractions were methylated. After the removal of methanol, the solution was extracted three times with ethyl acetate. The extract was washed with methanol. It was crystallized with methanol repeatedly till the crystals were obtained.

Discussion

Traditional practice of the folk of Vidisha District of Madhya Pradesh, India to use the fruits of Bitter cucumber for curing many disorders is clearly justified in this study as the presence of strong secondary metabolites in the aqueous and alcoholic extract of Bitter Cucumber. *Citrullus colocynthis L.* showed higher % of loss in weight i.e. (75%) on drying as mentioned in the Table 2. Total ash content, acid soluble and water-soluble ash content of the plant material has been portrayed in the Table 3. Percentage yield of the ash content after burning of plant material was found to be 55.2 gm in *Citrullus colocynthis L.* Acid soluble ash content was found to be 19.6 gm while as, water soluble component of the ash was found to be 35.6 gm in *Citrullus colocynthis L.* Ash content shows the moisture present in the powdered material as well as it indicates the nutrients and mineral composition. The mineral composition in the ash also gives an indication of the particular nutrient present in it. Different solvents were used according to increasing order of polarity i.e., n-hexane, petroleum ether, chloroform, ethyl acetate, ethanol and water. Table 4 shows the percentage yield of *Citrullus colocynthis L.* in different solvents by Soxhlet extraction method. *Citrullus colocynthis* yielded (4.52 gm.) in n-hexane, (0.29 gm.) in petroleum ether, (0.9 gm.) in chloroform, (0.2 gm.) in ethyl acetate, (1.82 gm.) in ethanol and (3.93 gm.) in water respectively. Sukumar et al. 1991 [12] have reported that percentage yield of the same species grown at different climatic conditions may be different. The yield also depends on the month of collection of the plant material as well as soil texture of the region. Therefore, the present study is in agreement with the previous workers in the field that percentage yield gives an indication of the availability of particular bioactive material in the plant. In the present study TLC of the crude extracts of the plant material have been worked out using different solvent systems. The TLC plates were visualized in iodine chamber, UV light and by naked eyes to visualize the color

pattern of each spot as depicted in Table 5. Two spots in *Citrullus colocynthis L.* were obtained with R_f (0.66, 0.73) respectively. Column chromatography is advantageous over most other chromatographic techniques, because it can be used in both analytical and preparative applications. Not only can column chromatography be used to determine the number of components of a mixture, but it can also be used to separate and purify substantial quantities of those components for subsequent analysis. *Citrullus colocynthis L.* yielded three fractions having dark brown, light brown and reddish brown chromatographic color characteristic by using n-butanol:HOAC:H_2O in the ratio of 4:1:5 as portrayed in Table 6. Preliminary phytochemical screening of the successive extracts of *Citrullus colocynthis L.* were assessed as reported in Table 7 which depicts the strong positive results for starch, tannins, saponins, proteins and reducing sugars in aqueous extract. Alkaloids, flavonoids and glycosides were present in alcoholic extract. Fluorescence characteristic of the *Citrullus colocynthis L.* extract revealed the different color characteristics when observed under UV (254 and 366 nm) and visible light with different extracts as mentioned in Table 8. Purification and chromatographic separation of penultimate fractions and then the standardization of these fractions to form the crystals for further spectral analysis was carried out in the present study [13]. Hence forth, the results observed in the present study and the claim of folklore practice of making use of Bitter Cucumber is scientifically justified and demands further research for the formulation of strong natural therapy.

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References

1. Ramakrishna G, Azeemoddin G, Lakshminarayana T. Processing of tuba seeds & oil. Oil Technol Assoc. 1993;25:3-5.
2. Karthikeyan A, Shanthi V, Nagasathaya A. Preliminary phytochemical & antibacterial screening of crude extract of the leaf of *Adhatoda vasica L.* Int J Green Pharm. 2009;3(1):78-80.
3. Lozoya M, Lozoya X, González JL. Pharmacological properties *in vitro* of various extracts of *Mimosa pudica* Linn. Tepescohuite. Arch invest Mex. 1990;21(2):163-9.
4. Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm Biol. 2001;39(Suppl 1):8-17.
5. Lynch FT. The book of yields: Accuracy in food costing and purchasing. John Wiley & Sons, INC. 2007.
6. Kenkel J. Analytical chemistry for technician's, 3rd Ed. CRC Press LLC, Lewis Publishers. 2003;43.
7. WHO. Determination of water and volatile matter. Quality control methods for medicinal plant materials, World Health Organization Geneva. 1998;9.
8. Khandelwal KR. Practical pharmacognosy technique and experiments, 23rd Ed. 2005;15-29:149-56.
9. Stock and Rice. Chromatographic methods. Chapman & Hall, London. 1974;376.
10. Waksmundzki A, Rozylo J. R_F values and structure of the adsorbent in thin layer chromatography using mixed developing solvents. J Chromatography A. 1968;33:96-102.
11. Whittall J, Sutton PW. Practical methods for biocatalysis and biotransformations 2. John Wiley and Sons. 2012:388.
12. Sukumar SK, Micheal JP, Boohan LR. Botanical derivatives in mosquito control. J Am Mosq Control Assoc. 1991;7(2):210-37.
13. Harborne JB. Phytochemical methods. A guide of modern techniques of plant analysis second edition. Chapman & Hall, London, New York. 1984;282.