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Physicochemical ,Phytochemical Evaluation and Cytotoxic Activity Analysis of the Fruit Extract of Cucumis *Callosus* and Cucumis *Prophetarum*

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Abstract

In the current research investigation on the plant of Cucurbitaceae family , the Physicochemical evaluation and Phytochemical analysis of the plants Cucumis *callosus* Cucumis *prophetarum* was conducted. Cucumis *callosus* and Cucumis *prophetarum* are well known Indian medicinal plants used in traditional system for various diseases. These comprises of various phytochemical constituents. The extracts of this plant have been pharmacologically evaluated for haematological and gynaecologic disorders anti-cancer activity, antioxidant activity, hepatoprotective activity etc. The therapeutic activity is due to the phytochemicals present in the plant. So, it is highly essential to standardize the medicinal plant on the basis of physicochemical and phytochemical aspects to identify the genuine plant part. The results demonstrated that the physico-chemical parameters such as ash value, extractive value, foreign matter are the suitable parameters in the standardization of the fruits of Cucumis *callosus* and Cucumis *prophetarum.* Thus, it can be assumed that these are the standard parameters in the standardization of fruits of Cucumis *callosus* and Cucumis *prophetarum.*

Key Words : Cucumis *callosus* , ash-value , foaming index , fruit pulp , purgative

INTRODUCTION

Herbal medicines are the oldest medicines known to mankind. Herbs had been used by cultures throughout history. It is an integral part of the development of modern civilization. The plants serve various purposes like food, clothing, shelter, and medicine:

Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base.

The World Health Organization encourages the use of medicinal plants especially in countries where conventional treatment of major diseases seems to be insufficient..

Cucumis callosus, family Cucurbitaceae, is a shrub with erect stem and pinkish white flowers. Seeds are aphrodisiac and useful in diarrhea, blood diseases and mouth sores. Leaves are useful in fever, inflammations, gonorrhea and strengthen the liver.

The herb is found across India in arid zones. The herb is much branched very common prostate, perennial herb, Leaves are cordate, suborbicular, deeply palmately 5-7 lobed.. Fruits are smooth, obovoid. ellipsoid, green variegated stripes..

Fruit is traditionally used to prevent insanity to strong memory and remove vertigo. The seeds are cooling and astringent and useful in bilious disorder. Cucumis prophetarum, also belongs to family Cucurbitaceae and is commonly known as cockscomb. The fluffy, light, airy texture of the plume types blow freely in a breeze and are shrubed more often by nature.

Cucumis prophetarum is used for the treatment of Constipation, atherosclerosis, leucorrhoea and osteoporosis. Its seeds have been used for removing "liver heat" improving eyesight, clearing wind-heat and as an anti-inflammatory agent, as recorded in Dian Nan's Herbal. The fruit pulp are being used in treatment of constipation as a purgative medicine.

Fig 1: Cucumis callosus fruit & plant

Fig 2 : Cucumis prophetarum fruit

MATERIALS AND METHODS

Plant material

Plant materials were procured from western arid zone of Rajasthan . Plants were identified and authenticated at Botany Department of the Apex University, Jaipur (Rajasthan)

PHYSICOCHEMICAL PARAMETERS (WHO, 2002)

Determination of Foreign organic matter

Collected plant material (fruits of Cucumis callosus & Cucumis prophetarum) was spread in a thin layer and sorted the foreign matter into groups either by visual inspection, using a magnifying lens (6x or 10x), or with the help of a suitable sieve, according to the requirements for the specific plant material. Remainder of the sample was sifted through a No.250 sieve, dust is regarded as mineral admixture. Weight of the portions of this sorted foreign matter was taken. It should be within the limit of 0.05g. The content of each group was calculated in grams per 100g of air-dried sample.

For some medicinal plant materials where the foreign matter may closely resemble the material itself, it may be necessary to take a pooled sample of the plant material and apply a critical test, either chemical, physical, or by microscopy. The proportion of foreign matter is calculated from the sum of the portions that fail to respond to the test.

Determination of Ash value

The residue remaining left after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. It may also include inorganic matter deliberately added for the purpose of adulteration. Hence, an ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash values are helpful in determining the quality and purity of drug

The ash remaining after complete ignition of the medicinal plant materials is determined by three different methods known as Total ash, Acid-insoluble ash and water-soluble ash.

Procedure for Total ash:

Accurately weighed 3 g of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to 500-600°C until it is white, indicating the absence of Carbon, Cool and weigh.

This process repeated till constant weight is obtained. Then the percentage of total ash was calculated with reference to the air dried drug.

Procedure for Acid insoluble ash:

The total ash was boiled with 25 ml of 2 N HCI for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot Water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

Determination of Water soluble Ash Value

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Moisture Content

The moisture content is determined by heating a drug at 105°C in oven to a constant weight. Some important physical or chemical methods used to determine the moisture present in drugs are as follows:

Loss on drying and

Azeotropic distillation method

Determination of moisture content by loss on drying

Moisture content determination is important, not only to know excess water, but also in conjunction with suitable temperature moisture will lead to the activation of enzymes and gives suitable conditions to the proliferation of living organism. Various methods for moisture determination are loss on drying, separation and measurement of moisture, chemical methods, electrometric methods, and spectroscopic methods as per IP.

- 10gm of powder was weighed and placed it in a moisture content apparatus.
- Temperature was adjusted to 100-110°c till weight get constant and collected in desiccators and weighed.
- The loss of weight was regarded as a measure of moisture content as per IP.

Determination of moisture content by aztotropic distillation method

10 g of powdered drug was taken in the flask. To it 100 ml of Toluene was added. It was connected to condenser. It was then boiled for about 01 Hr. Allowed to cool and

measured volume of water in the receiver. Procedure was repeated until there was no further increase in this volume. Moisture content of the sample was then calculated.

Determination of Foaming index (Vogel, 2002)

The foaming ability of an aqueous decoction of plant materials & their extracts is measured in terms of a foaming index.

Procedure Weighed accurately about 1 g of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water maintained at moderate boiling at 80-90°c for about 30 minutes. Then made it cold, filtered into a volumetric flask and added sufficient water through the filter to make the volume up to 100 ml (V1). Cleaned 10 stopper test tubes were taken and marked with 1 to 10. The successive portions of 1, 2 ml up to 10 ml drug was taken in separate tubes and adjusted remaining volume with the liquid up to 10 ml in each. After closing the tubes with stoppers, Shook them for 15 seconds and allowed to stand for 15 mins then measured the height. Foaming Index was calculated by using the formula

Foaming Index 1000/a in case of V 1

Foaming Index 1000 10/a in case of V2

Where, a Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

Determination of Swelling Index Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums containing an appreciable amount of mucilage, pectin or hemicellulose.

Procedure:

It was carried out simultaneously no fewer than three determinations for any given material. Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed I gm of plant material into a 25 ml glass- stopper measuring cylinder. The internal diameter of the cylinder was about 16 mm, the length of the graduated portion about 125 mm, marked in 0.2 ml divisions from 0-25 ml in an upwards direction. 25 ml of water was added and shake the mixture thoroughly every 10 minutes for 1 h. Allowed to stand for 3 h at room temperature. The volume was measured in ml occupied by the plant material, including any sticky mucilage.

Determination of Extractive value

1000g of course powder was subjected to Soxhlation with different solvents then the remained extract was weighed and calculated its percentage of extractive value using the formula

Extractive value X 100/1000

X = Amount of extract obtained after complete extract in grams.

Fluorescence analysis of powders (Chase et al., 1948, Kokoski, et al., 1958):

Plant material of fruits of Cucumis callosus & Cucumis prophetarum were shade dried, made into powder and observed under normal daylight, UV light.

A small quantity of dry plant powder is placed on grease free clean microscopic slide and 1-2 drops of freshly prepared reagent solution is added, mixed by gentle tilting the slide and wait for few minutes. Then the slide is placed inside the UV chamber and observe the colour in visible light, short (254 nm) and long (365 nm) ultra violet radiations. The colour observed by application of different reagents in different radiations was recorded.

Obtained results are given further in tabular form.

PRELIMINARY PHYTOCHEMICAL INVESTIGATION

All extracts of fruits of Cucumis callosus & Cucumis prophetarum were subjected to preliminary phytochemical investigation for the identification of various phytoconstituents,

Test solutions:

1 gm of powder extract was dissolved in 100 mL of distilled water.

1. TESTS FOR CARBOHYDRATES (Khandelwal, 2005; Harborne, 2008;)

a) Molisch's test:

To 2 ml. of test solution add few drops of a-naphthol solution in alcohol and add 2 mL. of concentrated H,SO, slowly from the sides of the test tube. A purple ring is observed at the junction of two liquids.

b) Fehling's test:

Mix 1 mL Fehling's solution A and Fehling's solution B, boil for 1 minute, add equal volume of test solution, heat in boiling water bath for 10 minutes. First yellow, then brick red precipitate is observed.

c) Benedict's test:

Test solution treated with Benedict's reagent and heating on a boiling water bath solution appears green, yellow red depending on amount of reducing sugar present in test solution.

d) Barford's test:

Test solution treated with Barfoed's reagent, on boiling on a water bath shows brick red precipitate at the bottom of the test tube.

Tests for Hexose sugars: (Tyler et al., 1977, Kokate et al., 2009):

a) Seliwanoff's Test (For ketohexose like fructose)

Heat 3 mL. Seliwanoff's reagent and I mL. test solution in boiling water bath for 1-2 min. Red colour is formed.

b) Tollen's Phloroglucinol Test for galactose:-

Mix 2.5 mL. concentrated HCI and 4 m. 0.5% phloroglucinol. Add 1-2 ml. test solution. Heat it --yellow to red colour appears.

c) Cobalt-chloride Test:-

Mix 3 ml. test solution with 2 mL cobalt chloride solution. Boil and cool. Add few drops of NaOH solution. Solution appears greenish blue (glucose) or purplish (fructose) or upper layer greenish blue and lower layer purplish (mixture of glucose and fructose)

2. TEST FOR GUMS:

Hydrolyse test solution using dilute HCI. Perform Fehling's or Benedict's test. Red color is developed.

3. TEST FOR MUCILAGE:

a) Powdered drug material shows red color with Ruthenium red.

b) Powdered drug swells in water or aqueous KOH.

4. TESTS FOR PROTEINS :-

a) Biuret test (General Test):

Test solution treated with 4% sodium hydroxide and dilute copper sulphate (1%) solution gives violet or pink colour.

b) Millon's test: Mix 3ml of test solution with 5 mL. Million's reagent. White precipitate is obtained which turns into brick red or red coloured solution on warming.

Millon's reagent- Dissolve 3 mL of mercury in 27 mL. of fuming nitric acid, keep the mixture to cool. Dilute the solution with an equal quantity of distilled water.

c) **Xanthoproteic test (proteins containing tyrosine or tryptophan):**

Add test solution, 2 mL to ImL of concentrated nitric acid boil, cool. Add 40% NaOH drop by drop. Orange coloured solution is obtained.

d) **Test for proteins containing sulphur**:

Mix 5 mL test solution with 2 mL 40% NaOH and two drops of 10% lead acetate solution. Solution turns black or brownish due to lead sulphide solution.

5. TESTS FOR AMINO ACIDS

a) **Ninhydrin test** (General test):

Heat 3 mL test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10 minutes. Purple or bluish color appears.

b) **Test for tyrosine:**

Heat 3 mL test solution and 3 drops Million's reagent. Solution shows dark red colour.

d) Test for tryptophan:

To 3 mL of test solution add few drops of glyoxylic acid and concentrated H_2SO_4 . Reddish violet ring appears at junction of the two layers.

e) Test for cysteine:

To 5 ml test solution add few drops of 40% NaOH and 10% lead acetate solution. Boil. Black precipitate of lead sulphate is formed.

6. TEST FOR FATS AND OILS

a) Solubility Test:

Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanol and in water. (Exception is castor oil, soluble in alcohol).

b) Saponification Test:

Evaporate extract to get 10 mL oil. To oil add 25 mL. 10% NaOH. Boil in water bath for 30 minutes. Cool. Add excess Na_2SO_4 solution. Soap forms and rise to the top. Filter, to filtrate add H_2SO_4 Evaporate, collect residue, dissolve in ethanol. With ethanolic solution perform following tests:

1) To ethanolic solution, add few crystals of KHSO, Heat vigorously. Pungent odour of acrylic aldehyde is produced.

2) To ethanolic solution, add few drops of $CuSO_4$, and NaOH solution. Clear blue solution is formed.

7. TEST FOR STEROLS AND TRITERPENOIDS:

a) Salkowaski test:

When a few drops of concentrated H_2SO, is added to the mixture of chloroform and test solution, shaken and allowed to stand, lower layer turns red indicating the presence of sterols and formation of yellow colour in the lower layer indicates the presence of Triterpenoids.

b) Liebermann's Test:

To a few mg of the residue in a test tube, add few ml acetic anhydride and heat gently, Cool the test tube. Add few drops of concentrated H_2SO_4 from the side of the test tube. A blue colour indicates presence of sterols.

c) Liebermann-Burchardt test: The test solution is treated with few drops of acetic anhydride and mixed. When concentrated H_2SO_4 is added from the sides of the test tube, it shows a brown ring at the junction of the two layers and the upper layer turns green.

d) Sulphur test:

Sulphur when added into the test solution, it sinks in it.

8. TESTS FOR CARDIAC GLYCOSIDES:

a) Legal's Test:

To the test residue add 1 mL pyridine and 2-3 drops of 0.5% aqueous sodium nitroprusside solution. The solution is made alkaline. Pink colour indicates presence of the cardiac glycosides.

b) Keller-Killiani Test:

To 2 mL. test solution add few drops of glacial acetic acid, ferric chloride solution and concentrated H_2SO_4. Through the sides of test tube which shows the separation between two layers, lower layer shows reddish brown and upper acetic acid layer turns bluish green.

9. TESTS FOR ANTHRAQUINONE GLYCOSIDES:

a) Borntrager's Test

To 3 mL extract , add dilute H_2SO_4. Boil and filter. To cold filtrate, add equal volume of benzene, chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns pink or red.

b) Modified Borntrager's Test for C-Glycosides:

To 5 ml. extract, add 5 mL. 5% $FeCl_3$, and 5 ml. of dilute HCL. Heat for 5 minutes in boiling water bath. Cool and add benzene or any other organic solvent. Shake well. Separate organic layer, add equal volume of dilute ammonia. Ammonical layer shows pinkish red colour.

10. TESTS FOR CYNOGENETIC GLYCOSIDES:

Grignard reaction or Sodium Picrate test:

Soak a filter paper strip first in 10% picric acid, then in 10% sodium picrate, dry. In a conical flask, place moistened powdered drugs. Cork it; place the above filter paper strip in the slit in cork. The filter paper turns brick red or maroon.

11. TESTS FOR COUMARIN GLYCOSIDES:

a) Coumarin Glycosides have aromatic odour.

b) Take a small amount of extractive in a test tube and moisten it. Cover the test tube with filter paper moistened with NaOH solution. Place the covered test tube in boiling water bath for several minutes, remove the paper and expose to ultraviolet light. The yellowish fluorescence indicates presence of coumarins.

12. TESTS FOR ALKALOIDS:

a) Mayer's test:

Test solution with Mayer's reagent (potassium mercuric iodide) gives cream coloured precipitate.

b) Wagner's test:

The acidic solution with Wagner's' reagent (iodine in potassium iodide) gives brown precipitate.

c) Hager's test:

The acidic solution with Hager's reagent (saturated picric acid solution) gives yellow precipitate.

d) Dragendorff's test:

The acidic solution with Dragendorff's reagent (potassium bismuth iodide) shows Orange brown precipitate.

13. **TESTS FOR SAPONINS:**

a) Foam test:

Saponins when mixed with water and shaken, shows the formation of foam which is stable at least for 15 minutes.

b) Haemolysis test:

2 ml. of 18% sodium chloride solution in two test tubes is taken. To one test tube added distilled water and to the other 2 mL. of filtrate. Few drops of blood are added to both the test tubes. Mixed, observed for haemolysis under microscope

14. TESTS FOR FLAVONOIDS:

a) Ferric-chloride test:

Test solution with few drops of ferric chloride solution shows blackish red color.

b) Shinoda test:

Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid, shows pink to magenta red colour.

c) Zinc-HCI reduction test:

Test solution with zinc dust and few drops of HCI, shows magenta red colour.

d) Alkaline reagent test:

Test solution when treated with NaOH solution, shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

e) Lead acetate solution test:

Test solution with few drops of lead acetate (10%) solution gives yellow precipitate.

15. TEST FOR TANNINS AND PHENOLIC COMPOUNDS

a) Ferric-chloride test:

Test solution treated with few drops of ferric chloride solution gives dark colour.

b) Gelatin test: Test solution treated with gelatin solution gives white precipitate.

c) Lead Acetate Test:

Add 10% w/v solution of lead acetate in distilled water to the test filtrate. Precipitate indicates presence of tannins.

d) Potassium Dichromate Test:

If on addition of 5% solution of potassium dichromate in a test filtrate, dark colour is developed, tannins are present.

e) Bromine Water Test:

2 mL of bromine water is added to the test filtrate. If decolourization of bromine occurs, tannins were present.

f) Acetic acid test: Red colour solution

g) Iodine test: Transient red colour

h) Nitric acid test: Reddish to yellow colour

i) NaOH & K-ferricyanide test: Red colour solution.

THIN LAYER CHROMATOGRAPHY

TLC Plate preparation

TLC plates are usually commercially available, with standard particle size ranges to rove reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water. This mixture is spread thick slury on an unreactive carrier sheet, usually glass, thick aluminium foil, or plastic. The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the adsorbent layer is typically around 0.1-0.25 mm for analytical purposes and around 0.5-2.0 mm for preparative TLC.

Procedure to run the TLC

A small spot of solution containing the sample is applied to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved. If a non-volatile solvent was used to apply the sample, the plate needs to be dried in a vacuum chamber. A small amount of an appropriate solvent (elutant) is poured in to a glass beaker or any other suitable transparent container (separation chamber) to a depth of less than 1 centimeter. A strip of filter paper is put into the chamber, so that its bottom touches the solvent, and the paper lies on the chamber wall and reaches almost to the top of the container. The container is closed with a cover glass or

any other lid and is left for a few minutes to let the solvent vapours ascend the filter paper and saturate the air in the chamber.

The TLC plate is then placed in the chamber so that the spot of the sample do not touch the surface of the elutant in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate (elutes the sample). When the solvent front reaches no higher than the top of the filter paper in the chamber, the plate should be removed and dried.

Cytotoxicity Activity : Brine Shrimp (In-Vivo) Lethality Assay (BSLA)

The brine shrimp lethality assay (BSLA) is a simple and inexpensive bioassay used for testing the efficacy of phytochemical present in the plant extracts.

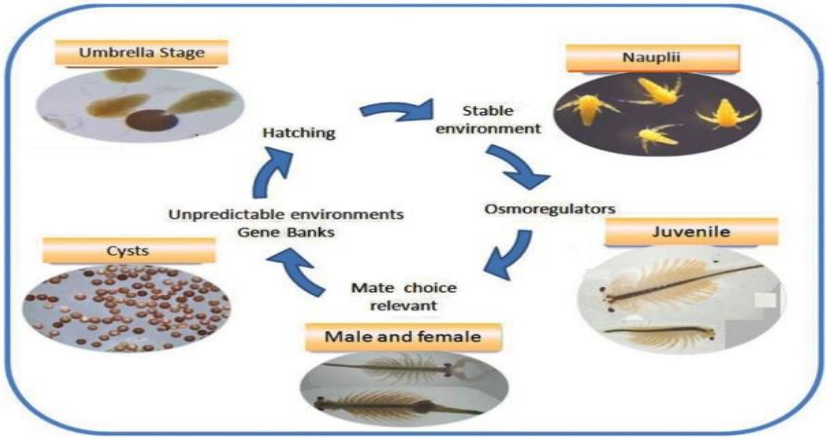


Figure 3.5: Life Cycle of Brine Shrimps

Procedure :

1. The Brine Shrimp eggs were procured from a laboratory.
2. The eggs were hatched at room temperature at approximately 30°C and in artificial sea water conditions (38 gms of NaCl in 1000 ml distilled water) for 48hrs for fresh eggs with continuous aeration under illumination. Eggs were allowed to hatch the shrimp and to be matured as nauplii.
3. The resulting nauplii (larvae) were attracted to the other side of the tank with a light source and collected
4. Samples for testing were prepared by initially dissolving 100 mg of aqueous and ethanolic extracts of both plants parts (fruits of Cucumis callosus & Cucumis prophetarum) in 10 ml of water.
5. By making appropriate preparations of concentrations [µg/ml, 2µg/ml, 3pgml, 4µg/ml and 5µg/ml of above prepared extract solutions in vials

6. Six brine shrimps were transferred to each sample vial, and boiled
7. The vials were maintained under illumination.
8. Survivors were counted after24 hr and the percentage mortality of each vial was determined using the equation: 6 mortality (no. of dead nauplii/ initial no. of live nauplli) x 100
9. The concentration at which lethality of brine shrimp represents 50% (LC50) LC50 values less than 100 ppm (or 100 µg/ml.) were considered significant (Nonita et al. 2010, Khan et al, 2008).

Breeding for Brine Shrimps

Figure 3.6: Brine Shrimp assay of Cucumis callosus & Cucumis prophetarum

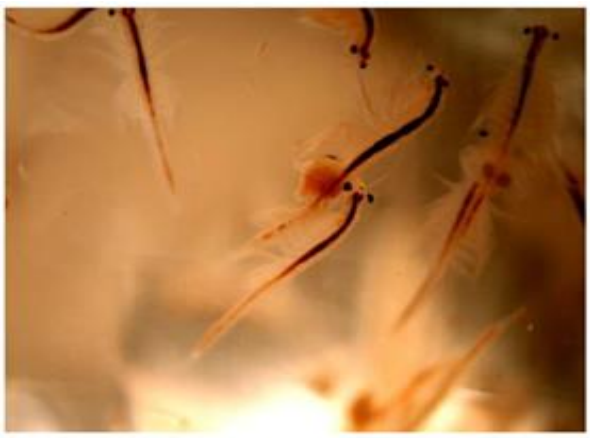


Figure 2. Fully grown Brine shrimps

RESULTS AND DISCUSSION

PHYSICOCHEMICAL PARAMETERS EVALUATION

The results of physicochemical analysis of crude powders of fruits of both plants are shown in Table 2. The average values of various parameters such as total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and moisture content are expressed as percentage of air-dried material. Each determination was carried out three times and then average values are reported.

Ash Values

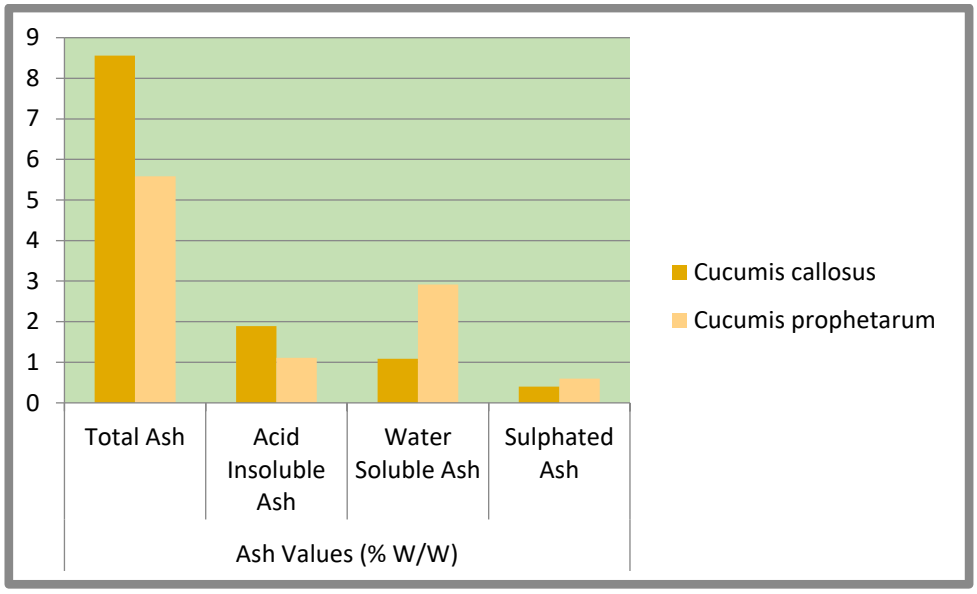
Ash values are indicative to some extent of care taken in collection and preparation of drug from market and of foreign matter content of natural drug.

The object of ashing is to remove all traces of organic material interfering in an analysis of inorganic elements. Total ash, acid insoluble ash and water soluble ash of fruits of Cucumis callosus and Cucumis prophetarum was obtained by reported methods.

Results of ash values for both the plants are given in table. Fruits of Cucumis callosus showed higher total ash value (8.58%) than that of fruits of Cucumis prophetarum (5.54%).

Table : Ash values of powdered fruits of Cucumis callosus and Cucumis prophetarum.

Name of the Plant	Ash Values (% W/W)			
	Total Ash	Acid Insoluble Ash	Water Soluble Ash	Sulphated Ash
Cucumis callosus	8.56	1.89	1.09	0.4
Cucumis prophetarum	5.58	1.11	2.92	0.6



Graphical presentation: Ash values of Cucumis callosus fruits and Cucumis prophetarum.

Extractive Values

It is employed for material to which as yet no suitable chemical or biological assays exist. Extractive values determine amount of active constituents present in given plant material in given solvent. Extracts were prepared with various solvents by soxhlation and maceration methods as described percentage of dry extract was calculated in terms of air-dried crude drug powder.

The results of extractive value are given in table of Cucumis callosus and Cucumis prophetarum both showed maximum extractive value in aqueous solvent than other polar and nonpolar solvents.

Table : Extractive values of powdered fruit Extract of Cucumis callosus and fruit Extract of Cucumis prophetarum .

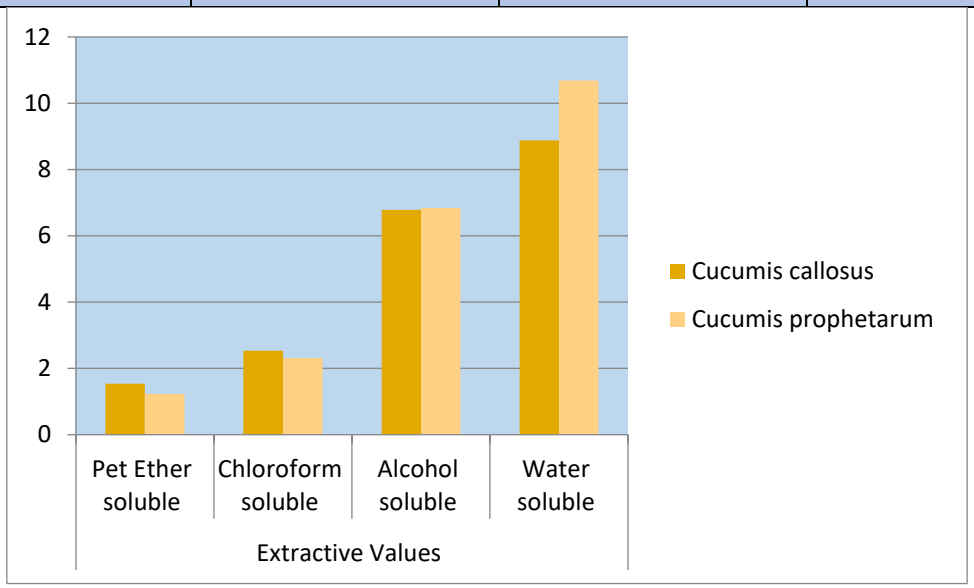
Name of the Plant	Extractive Values			
	Pet. Ether soluble	Chloroform soluble	Alcohol soluble	Water soluble
Cucumis *callosus*	1.54	2.53	6.78	8.88
Cucumis *prophetarum*	1.23	2.31	6.84	10.68

Table : Characteristics of Cucumis callosus fruit Extract

Cucumis callosus fruit Extract	**Colour**	**Odour**	**Consistency**
Petroleum Ether	Greenish Brown	Characteristic	Sticky
Chloroform	Brown	Characteristic	Sticky
Ethanol	Reddish Brown	Characteristic	Semisolid
Water	Blackish Borwn	None	Powder

Table : Characteristics of Cucumis prophetarum Fruit Extract

Cucumis callosus fruit Extract	**Colour**	**Odour**	**Consistency**
Petroleum Ether	**Greenish Brown**	**Characteristic**	**Sticky**
Chloroform	**Greenish Brown**	**Characteristic**	**Sticky**
Ethanol	**Brown**	**None**	**Solid**
Water	**Brown**	**None**	**Powder**



Graphical Figure : Extractive values of Cucumis callosus fruits and Cucumis prophetarum fruits

Moisture Content

Moisture content of Cucumis callosus fruits and Cucumis prophetarum fruits powder was determined by both the methods.

a. LOD method

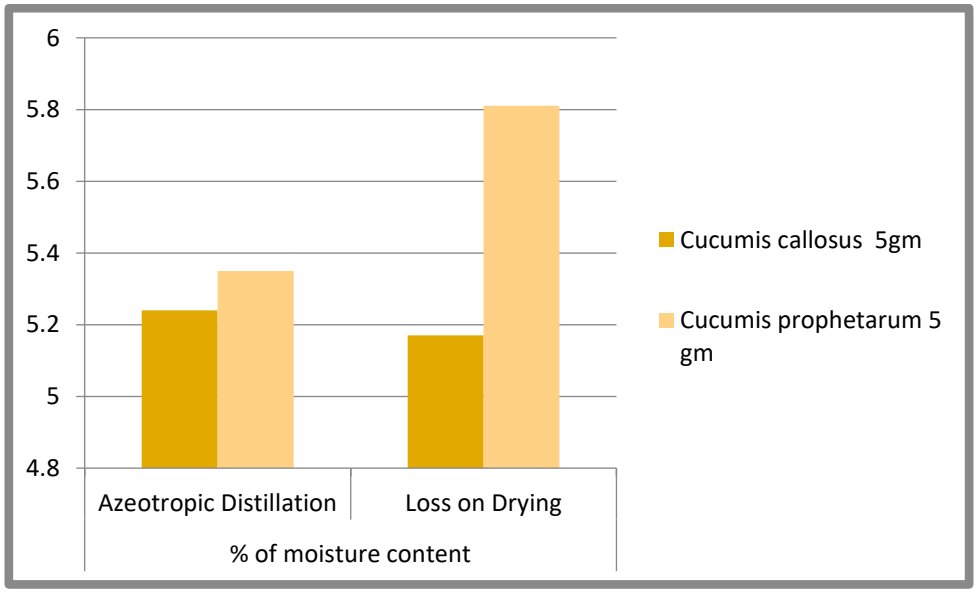
b. Azeotropic Distillation method

Loss on drying is the loss in weight in percent w/w resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions.

Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Results for moisture content are given in Table and represented in graphical format in figure. Results of lower values for moisture content indicate that drug is comparatively safe from microbial as well as chemical decomposition.

Table : Moisture Content of powdered fruits of Cucumis callosus and fruits of Cucumis prophetarum.

Name of the Plant	Weight of Powdered fruits	% of moisture content	
		Azeotropic Distillation	Loss on Drying
Cucumis callosus	5gm	5.24	5.17
Cucumis prophetarum	5 gm	5.35	5.81



Graphical Figure: Moisture content of Cucumis callosus fruits powder and Cucumis prophetarum fruits powder.

FLUORESCENCE ANALYSIS OF EXTRACTS

The fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colours and also to find out the fluorescent impurities. The study of fluorescence analysis can be used as a diagnostic tool for testing adulteration.

Plant powders were analysed under day light, short wavelength and in long wavelength region, results are given in below table :

Table : Fluorescence Analysis of Cucumis callosus fruits powder.

S.No.	Reagent	Visible	UV Light
1	Powder as such	Pale Yellow	Pale Green
2	1N NaOH (Aq)	Light Yellow	Green
3	1N NaOH (Alcohol)	Orange	Yellowish Green
4	1N HCl	Pale Yellow	Fluorescent Green
5	1%H_2SO_4	Blackish Yellow	Yellowish Green
6	1% Nitric Acid	Pale Orange	Green

Table: Fluorescence Analysis of Cucumis prophetarum fruits powder

S.No.	Reagent	Colour	UV Light
1	Powder as such	Brown	Yellowish Brown
2	1N NaOH (Aq)	Reddish Brown	Dark Brown
3	1N NaOH (Alcohol)	Reddish Brown	Dark Brown
4	1N HCl	Dark Brown	Greenish Brown
5	1%H_2SO_4	Dark Brown	Greenish Brown
6	1% Nitric Acid	Brown	Greenish Brown

RESULTS OF PHYTOCHEMICAL ANALYSIS

Extracts of Cucumis callosus fruits and Cucumis prophetarum fruits were subjected for phytochemical analysis by using various chemical reagents. Results were noted after observing and comparing results with standards.

Table : Phytochemical Analysis of Cucumis callosus fruits extracts

S.No.	Chemical Test For	Petroleum Ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
1	Carbohydrates	-	+	+	+
2	Proteins	-	-	+	+
3	Amino acids	+	+	+	+
4	Fats and oils	+	-	-	-
5	Alkaloids	-	+	+	-
6	Saponins	-	-	+	+
7	Flavonoids	-	-	+	+
8	Tannins and Phenolic comp.	-	-	+	+
9	Gums and mucilages	-	-	-	+
10	Steroids	-	-	+	+
11	Triterpenoids	+	+	-	-

Table : Phytochemical Analysis of Cucumis prophetarum fruits extracts.

S.No.	Chemical Test For	Pet Ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract
1	Carbohydrates	+	+	+	+
2	Proteins	-	-	+	+
3	Amino acids	-	-	+	+
4	Fats and oils	+	-	-	-
5	Alkaloids	-	+	+	-
6	Saponins	-	-	-	+
7	Flavonoids	-	-	+	+
8	Tannins and Phenolics	-	-	+	+
9	Gums and mucilages	-	-	-	+
10	Steroids	-	-	+	+
11	Triterpenoids	+	+	-	-

This phytochemical investigation can be used for isolation of pure components from plant. It can also be used to correlate nature of phytoconstituents with various pharmacological activities.

TLC PATTERN FOR EXTRACT

The stationary phase & mobile phase set for B-sitosterol as per Harborne, J.B

TLC for B-sitosterol:

Plate dimension: 5x15 cm.

Stationary phase: Silica gel G for TLC.

Sample preparation: 5mg methanolic extract was dissolved in 5ml of acetone.

Mobile phase: Various solvent systems have been tried for optimization of better resolution mainly using chloroform and ethyl acetate as the extracts contain sterols which are non- polar to semi polar in nature. **Visualisation** : Spraying by 20% Sulphuric acid in methanol. **Treatment after spraying**: Heated in oven at 105°C for 2-5 min.

The TLC profile has revealed the presence of spots after spraying with 20% methanolic sulphuric acid.

TLC for Stigmasterol (Khadbadi, 2011):

Plates were prepared using Silica gel G and spots of extract dissolved in chloroform were spotted. Mobile phase of Petroleum Ether: ethyl acetate was used in the ratio 7:3 respectively and was allowed to run. After sufficient distance travelled by the spots, the plate was exposed to the spray of Vanillin-sulphuric acid. Spots were compared with the standards. Prominent spots separated were identified for stigmasterol.

Colour of spot-Purple

Rf value - 0.62

TLC for Quercetin:

Glass plates were prepared with silica gel G 0.2-0.3 mm thick activated at 100 Celsius for 30 min in oven and then cooled at room temperature.

Mobile phase- n-butanol: acetic acid: water (4:1:5)

Developed plates were air dried and observed under UV.

Fluorescent spots were observed Blue in colour. Rf value: **0.83**

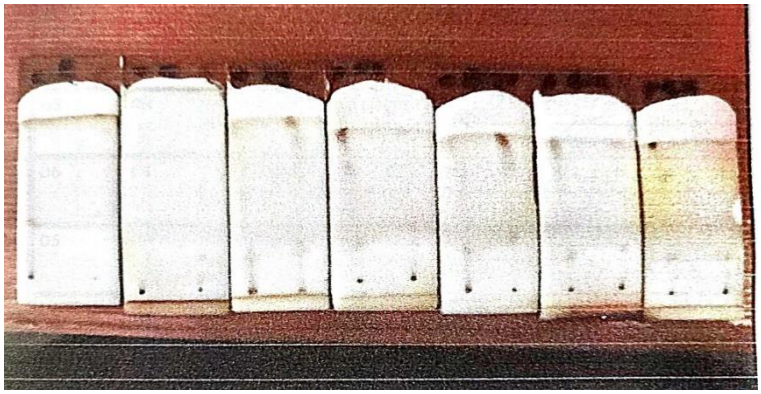


Figure : TLC of pet ether and Chloroform extract of Cucumis callosus fruits extract (Mobile Phase – Chloroform : Ethyl Acetate)

Table : TLC Profile of Cucumis callosus fruits extract

S.No.	Mobile Phase		R_f Values			
	Ethyl acetate	$CHCl_3$	PEE	CE	ME	AE
1	10	00	0.371, 0.883	0.351, 0.553	0.24, 0.347, 0.667, 0.711	0.253, 0.321, 0.445, 0.7
2	08	02	0.381, 0.974	0.275	0.091, 0.172, 0.252	0.26, 0.41, 0.681, 0.752
3	06	04	0.281, 0.9332	0.216, 0.957	0.5, 0.61	0.44, 0.68
4	05	05	0.268, 0.757	0.264, 0.931	0.330, 0.40	0.22, 0.36, 0.550, 0.82
5	04	06	0.3, 0.780, 0.868	0.3715	0.3, 0.53, 0.63, 0.761	0.34, 0.520
6	02	08	0.3	0.287	0.261, 0.36, 0.581	0.57, 0.7
7	00	10	0.167, 0.482	0.74	0.687, 0.74	0.2, 0.54, 0.73

Abbreviations :-

- R_f – Retention factor
- $CHCl_3$ – Chloroform
- PEE – Pet Ether Extract

- AE – Aqueous Extract

Table : TLC Profile of Cucumis prophetarum fruits extract

Extract	Mobile Phase (Proportion)			R_f values
	Toluene	Ethyl Acetate	Glacial Acetic Acid	
PEE	8	1	0.1	0.32, 0.54
CE	6	1	0.1	0.44, 0.48, 0.58, 0.62
ME	6	1.5	0.1	0.32, 0.49, 0.56, 0.60
AE	4	4	0.1	0.33, 0.52, 0.58, 0.60

Abbreviations :-

- R_f – Retention factor
- $CHcl_3$ – Chloroform
- PEE – Pet Ether Extract
- CE – Chloroform Extract
- ME – Methanol Extract
- AE – Aqueous Extract

BRINE SHRIMP LETHALITY ASSAY

A parallel series of tests with the standard potassium dichromate solution (LCs 20- 40 µg) were tested and the blank control was always included.

Table : Brine Shrimp Bioassay of Cucumis callosus Fruits Extract

Cucumis callosus Fruits Extract	% Mortality at different concentrations					LC_{50}, 24 hrs.
	1µg/ml	2µg/ml	3µg/ml	4µg/ml	5µg/ml	µg/ml
Aqueous	0	16.66	33.32	50	33.33	5.34
Ethanolic	50	33.32	33.34	16.67	83.33	4.36
Conc.	10	20	25	30	50	
$K_2Cr_2O_7$	1.33	8	22.66	45	72	38.16

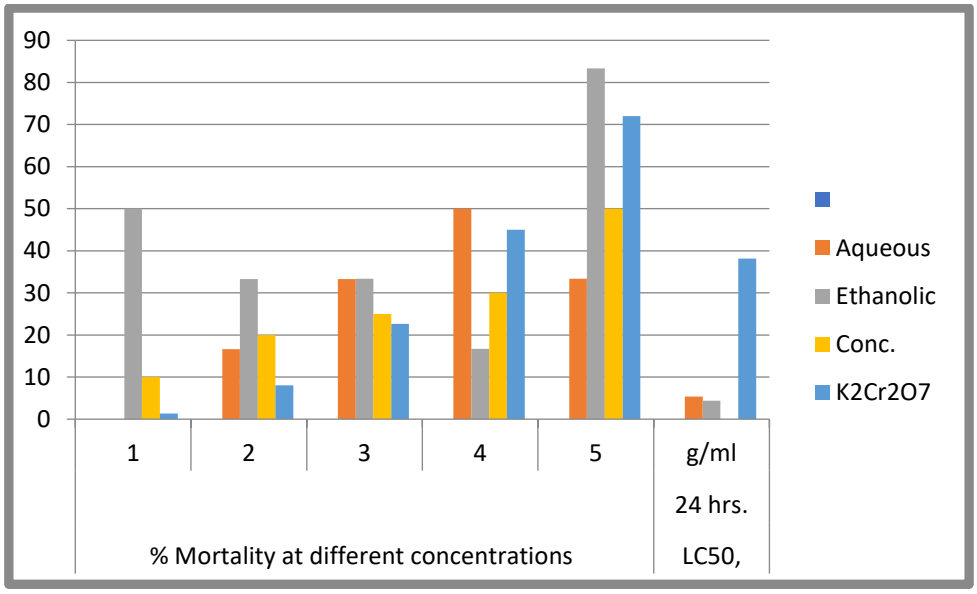


Figure : Brine Shrimp Bioassay of Cucumis callosus Fruits Extract (% Mortality at different concentrations)

Table : Brine Shrimp Bioassay of Cucumis prophetarum

Cucumis prophetarum Whole plant Extract	% Mortality at different concentrations					LC_{50}, 24 hrs.
	1µg/ml	2µg/ml	3µg/ml	4µg/ml	5µg/ml	µg/ml
Aqueous	16.66	32	48.31	81.66	100	3.84
Ethanolic	18.32	38.34	48.33	61.31	76.66	2.56
Conc.	10	20	25	30	50	
$K_2Cr_2O_7$	1.32	8	22.64	45	70	38.14

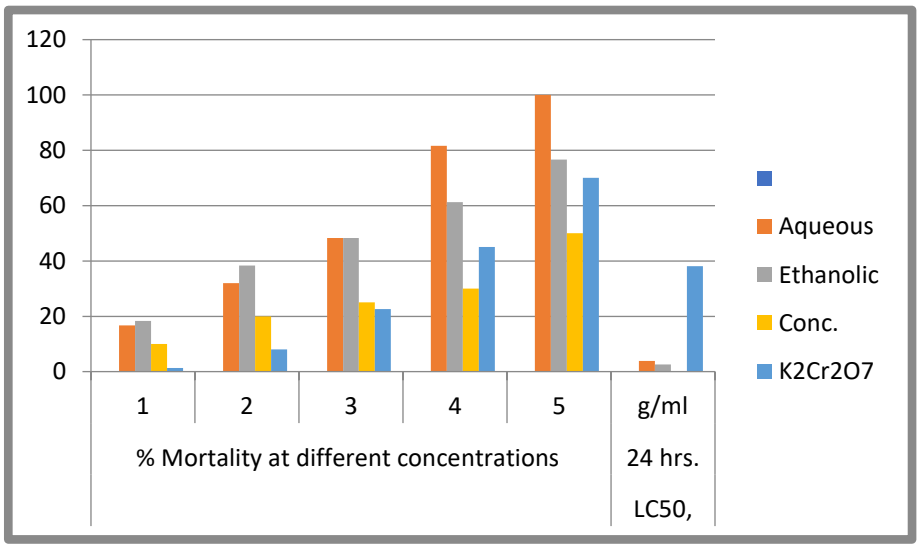


Figure : Brine Shrimp Bioassay of Cucumis prophetarum (% Mortality at different concentrations)

Survivors were counted after 24 h and the percentage mortality at each vial and control was determined using the equation :-

% mortality = (no. of dead nauplli / initial no. of live nauplli) x 100

Probit analysis by Finney (1971) was used to determine the concentration at which lethality to brine shrimp represents 50% (LC50). LC's values less than 100 ppm (or 100 µg /mL) were considered significant.

In cytotoxicity assay with brine shrimp nauplli, the LCs value of aqueous and ethanolic extracts of Cucumis callosus fruits extract were 5.33 µg/ml and 4.33 µg/ml respectively. The cytotoxicity of aqueous as well as ethanolic extracts is comparable to those of standard potassium dichromate solution. No mortality was found in the control group. An approximate linear correlation was observed when logarithm of concentrations versus percentages of mortality was plotted on graph paper.

In cytotoxicity assay with brine shrimp nauplli, the LCs value of aqueous and ethanolic extracts of Cucumis prophetarum fruits extract were 3.84µg/ml and 2.56 µg/ml respectively. The cytotoxicity of aqueous as well as ethanolic extracts is comparable to those of standard potassium dichromate solution.

Reference standard potassium dichromate showed LC50 (38µg/mL). Potassium dichromate served as the positive control for this brine shrimp lethality assay. The LC50 values for the positive control at 24 hours were 38µg/mL shown that it exhibits toxic expressions (LC50 was less than 1.0 mg/mL) against the brine shrimp.

CONCLUSION

In conclusion, the Physicochemical parameters reported in this study will be useful in the development of pharmacopeial standards for the future studies. The results obtained for ash values, which are of tremendous importance in quality control are used to detect foreign organic matter and detection of adulteration of sand or earth. The ash values obtained were adequate within the limits of experimental error since the total ash, acid-insoluble, water soluble ash and sulphated ash were determined were within the IP specifications.

Comparatively, the Fluorescence analysis results also very specific for both the species which can conclude the proper identification of the specie.

Fluorescence study of the powder helps in the qualitative evaluation which can he used as a reference data for the identification of adulterations. The fluorescence analysis of

the powder of Cucumis prophetarum in various solvents and chemical reagents under normal day light and ultraviolet light was done. Phytochemical evaluation of Cucumis callosus revealed presence of flavonoids, carbohydrates, tannins, phenolic compounds, saponins, glycosides and amino acids. Phytochemical screening of various extracts of Cucumis prophetarum revealed presence of carbohydrates, flavonoids, alkaloids, steroids, terpenoids, tannins and saponins.

In cytotoxicity assay with brine shrimp nauplii, the LC value of aqueous and ethanolic extracts of Cucumis prophetarum fruits extract were 3.84µg/ml and 2.56 µg/ml respectively. The cytotoxicity of aqueous as well as ethanolic extracts is comparable to those of standard potassium dichromate solution.

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