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Pharmacognostic & Phytochemical Evaluation Of Selected Seeds Of '*Cicer arietinum*' Linn. Seeds From Roopnagar Punab

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ABSTRACT: *Seed samples of Cicer arietinum were purchased from two retail stores of local market of Roopnagar Punjab India .This species is of interest to researchers due to its medicinal value. Pharmacognstic ,Phsico and Phytohemical evaluation of the seeds of Cicer arietinum were carried out..Total ash values and alcohol and extractive values were determined for physic chemical evaluation. Phytochemical analysis indicated the presence of various phytoconstituents viz. phytosterols, flavonoids, phenolic compounds, tannins, carbohydrates, proteins, amino acids, fixed oils and fats etc. Thin layer chromatography studies on extracts revealed the presence of a number of compounds. These findings can be useful to supplement information with regard to its identification parameters, which are supposed to be noteworthy in the way of satisfactoriness of herbal drugs, in the present scenario, which lacks regulatory laws to manage the quality of herbal drugs.*

KEYWORDS:*Cicer arietinum ,Pharmacognostic, Physico-chemical, Phytochemical, Thin Layer Chromatography*

I. INTRODUCTION

Plants are rich source of healthy and important nutrients, as well as disease fighting constituents. A number of phytonutrients are known to treat various diseases. These properties are attributed to the phytochemicals present in high concentration in vegetables and fruits. [1][9]These have precious effects and are commonly known as Nutraceuticals. Nutraceuticals are food product that provides health as well as medicinal benefits including the prevention and treatment of disease. Few nutraceuticals are being used as pharmaceutical and a number of other being used and purchased by general public as self medication. Such products may range from dietary supplements to genetically engineered foods, herbal products and processed foods. The term Nutraceutical was defined as a product isolated or purified from foods. Phytochemicals and antioxidants are two specific types of nutraceuticals. Research has proved that foods with Phytochemicals may help to provide protection from diseases such as cancer, diabetes, heart disease and hypertension, chronic diseases. Over the last 20 years, numbers of nutraceuticals are available for self medication [7][8] [9]Chickpea (Cicer arietinum L.) is one of the most important grain-legume crops in the world used as nutraceutical.Chickpeas have high nutritional value due to high protein and dietary fibre. It has high antioxidant activity (AoxA) due to this it is a well known and potent nutraceutical [2][3]The seeds of plant Cicer arietinum Linn. (Fabaceae) has been traditionally used for cooking purposes in kitchen for hundreds of years .This plant has also been traditionally used for treating number of diseases as abortifacient, tonic to hair, useful in cold pains. It is used as anti infertility, anti leukemic, immunostimulant. Used in indigestion, diarrhoea and dysentry. The seed is sweet when raw , refrigerant, tonic, anthelmintic. Seeds are diuretic and antifungal. Gram is given as preventive diet to atherosclerosis patients because of its phosphorus content. The seeds are useful in vitiated condition of pitta, bronchitis, inflammations and skin diseases. The seeds are used in enlargement of liver and spleen, complaints of chest, throat troubles foul mouth and fever. It is is also used as Uighur herb. Due to estrogenic activities of the isoflavones .[4][5][6].The potential of this plant is due to phytoconstituents present in this plant.In the present paper work has been done to evaluate the same.

II MATERIAL AND METHODS

The plant consists of dried seeds of Cicer arietinum Linn.samples (S1,S2) belonging to family Fabaceae. The seed part of the plant cicer arietinum Linn., and family Fabaceae was purchased from two local retail stores of market named as S1 and S2 and methanolic and hydroalcoholic extracts by cold maceration named as(MES1,HES1,MES2,HES2) in the month of January and seeds were stored in air tight container.Chemicals: Chloral hydrate, ferric chloride, iodine, phloroglucinol, nitric acid, silica gel G (Merck), picric acid, potassium iodide, sodium hydroxide, ammonium hydroxide, aluminium chloride, sodium chloride, sulphuric acid were used for phytochemical screening of the plant extracts, ascorbic acid, sodium carbonate, butylated hydroxyl toluene for total flavonoid and phenolic content. Solvents: Acetone, acetic acid, acetic

anhydride, chloroform, butanol, ethanol, ethyl acetate, methanol, haxane, glacial acetic acid, cyclohexane, diethylamine, dioxane, formic acid and toluene (S.D. Fine chemical) were used for extraction of plant material, subsequent testing and for chromatographic studies.Equipments: Mechanical shaker, centrifuge, incubator, rotary evaporator, sterile centrifuge tubes, lyophilizer, Sphectophotometer,UV cabinet Soxhlet apparatus, glass bottles, glass pipettes.

II. PHARMACOGNOSTIC STUDIES

Macroscopic Evaluation: The macroscopic evaluation of a seed samples includes its visual appearance to the naked eye. For each particular morphological group, a particular systemic examination was carried out. It refers to the included evaluation of seed samples by colour, odour, size, shape, taste and special features including touch and texture etc. They are of primary importance before any further testing can be carried out. The following macroscopic investigations were performed ([16] (a) Colour: The untreated sample was properly examined under sunlight or artificial light source with wavelengths similar to that of daylight. (b) Shape and size: The length, breadth and thickness of the seed samples are of great importance while evaluating a crude samples. A graduating ruler in millimetre is adequate for the measurement. Seeds were measured by aligning ten of them on a sheet of a calibrated paper 1 mm apart between the lines and the result was divided by 10. Average length, breadth and thickness were determined. (c) Odour and taste: The odour and taste of a crude seed samples are extremely sensitive criteria based on individual's perception. Therefore the description of this feature may sometimes cause some difficulties. The sample was crushed by applying pressure and the strength of the odour like weak, distinct, strong were first noted and then the odour sensation like rancid, fruit, aromatic etc. were determined. (d) Surface characteristics and texture: The texture is best examined by taking a small quantity of material and rubbing it between the thumb and forefingers, it is usually described as smooth, rough and gritty. The touch of the material determined the softness or hardness. The study of morphology of seeds were done by taking ten samples and observed for various qualitative and quantitative macroscopic characters, length and width of the same [13]

Extractive Values: Extractive values obtained for crude seed powder in a particular solvent are indicative of approximate measure of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of seeds, water, alcohol and other solvents were used for determination of extractives. Extractive values was determined as per the procedure given in Indian Pharmacopoeia and the World Health Organization standards for quality control of herbal drugs. Alcohol soluble extractives give an indication of the amount of resins, tannins and such other constituents if present in the seed samples . Water-soluble extractives are applied to seedss which contain water-soluble constituents like tannins, sugar, plant acids, mucilage, glycosides etc. Extractive values were determined by following the procedure give in WHO guidelines.

Cold maceration: Alcohol soluble extractive value: About 4.0 gm of coarsely powdered air dried material accurately weighed and macerated with 100 ml of the methanol in a glass stopper closed flask for 24 h with frequent shaking during the first 6 h and allowed to stand for 18 h. It was filtered rapidly taking care not to lose any solvent and then 25 ml of the filtrate was transferred in to a tarred flat bottom dish and evaporated to dryness on a water bath. Dried at 105 °C for 6 h and cooled in a vaccum dessicator for 30 min. then weighed without delay. Calculated the content of extractable matter in mg/g of air dried drug.Water soluble extractive value: About 4.0 gm of coarsely powdered air dried material accurately weighed and macerated with 100 ml of the distilled water in a glass stopper closed flask for 24 h with frequent shaking during the first 6 h and allowed to stand for 18 h. It was filtered rapidly taking care not to lose any solvent and then 25 ml of the filtrate was transferred in to a tarred flat bottom dish and evaporated to dryness on a water bath. Dried at 105°C for 6 h and cooled in a vaccum dessicator for 30 min. then weighed without delay. Calculated the content of extractable matter in mg/g of air dried drug[12].

Ash Values: The ash values, following ignition of medicinal plant materials is determined by the different methods, which measures total ash, acid insoluble ash and water-soluble ash. Total ash: It is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself and "non-physiological ash", which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant material. The finely ground and dried plant material was accurately weighed (2-4 gm) and placed in a previously ignited and tarred crucible (silica). The material was spread in an even layer and ignited by gradually increasing the heat to 500-600°C until it is white, indicating absence of carbon. It was then cooled in a desicator and weighed. If carbon-free ash cannot be obtained in this manner, the crucible was cooled and moistened the residue with about 2 ml of water or a saturated solution of ammonium nitrate. Dried on a water bath, then on a hot plate and ignited to constant weighed. The residue was allowed to cool in a suitable desicators for 30 min., and weighed without delay.

The percentage of ash with reference to air dried drug was calculated [16] Acid-insoluble ash: It is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. To the crucible containing the total ash, add 25 ml of hydrochloric acid was added.It was covered with a watch glass and boiling gently for 5 min. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in vacuum desiccators for 30 min., and weighed without delay. The percentage of ash with reference to air dried drug was calculated [16]. Water-

soluble ash: It is the difference in weight between total ash and the residue left after treatment of total ash with water. To the crucible containing the total ash, 25 ml of water was added and boiled for 5 min. The insoluble matter was collected in a sintered glass crucible or on an ashless filter paper. Washed with hot water and ignited in a crucible for 15 min. at a temperature not exceeding450 ̊C, and the ash obtained was weighed. The weight of this residue was subtracted from the weight of total ash. The percentage of ash with reference to air dried drug was calculated (WHO, 1998).

Florescence Analysis: Many herbs florescence when cut surface or powder is exposed to UV light and this can help in their identification method. The florescence character of the plant powders (40 mesh) was studied both in daylight and UV light (254 and 366 nm) after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc [14].

Loss on Drying: The powdered seed sample (3 gm) without preliminary drying was placed on a tarred evaporating dish and dried at 105 ̊C for 6 h and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 30 min. in a desiccators, showed not more than 0.01 g difference [16]

Swelling Index: Specified quantity of plant material (3 gm) concerned previously reduced to the required fineness and accurately weighed taken into 25 ml glass stoppered measuring cylinder. 25 ml of water was added and the mixture was shaken thoroughly every 10 min. for 1 h. It was allowed to stand for 3 h at room temperature. The mean value of the individual determinations was calculated related to 1 g of plant material [16]

Foaming Index: About 1 gm of plant material was reduced to a coarse powder, weighed accurately and transferred at moderate boiling for 30 min. Cooled and filtered into 100 ml volumetric flask. The detection was poured into 10 ml and adjusted the volume of the liquid in each tube with water to 10 ml. Stoppered the tubes and was shaken them in a lengthwise motion for 15 sec.; two shakes per second. Allowed to stand for 15 min. and the height of foam were measured. [16]

Determination of pH:

pH 1% solution:

Dissolved an accurately weighed 1 gm of the seed powder in accurately measured 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

pH 10% solution:

Dissolved an accurately weighed 10 gm of the seed powder in accurately measured 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode [11]

Stabilization And Preparation Of Extract:

1 Stabilization of extract: Seed powder from the two samples (S1,S2) was obtained by milling seeds in a local grinding mill, followed by sieving to separate powder in a local grinding mill, followed by sieving to separate powder from seeds. Stabilization of seed powder was done by heating the powder in microwave oven. The microwave chamber was preheated to 100% power for 3 min. The moisture content of the seed powder was adjusted to temperature of the sample after heating in the microwave was 107 ± 2ºC. The sample was allowed to cool to room temperature and stored in an uralow freezer (-80ºC) until further analysis [17]. 2 Preparation of extract: Cicer arietinum Linn. Seed powders (8g) of each was extracted trice with 48 ml of methanol for 3 hour in an electrical shaker or in sonicator at 40ºC. The extracts were filtered through Whatman No.2 filter paper and evaporated under vaccum using rotary evaporated. The residual crude methanolic powdered extract was weighed (Rao et.al., 2010). 4 Successive extraction method: After collection of seed samples they were powdered separately. Powder materials were passed through sieve no. 40 and used for extraction. A weighed quantity of powder was extracted using hexane, chloroform, ethyl acetate, ethanol, and aqueous solution in

Soxhlet apparatus for 16 h using twice the amount of solvent. The extract was evaporated to dryness at 40°C in rotary vacuum evaporator (Harborne, 1998).

Phytochemical Screenings: The extracts obtained from cold maceration i.e Methanol extract (ME) and extracts obtained from successive extraction i.e Hexane extract (HE), Chloroform extract (CE), ethyl acetate extract (EAE), and residual aqueous extract (AAE) were subjected to preliminary phytochemicaning for the detection of various phytocontituents such as alkaloids, steroids, flavonoids, glycosides, tannins, phenolic compounds, carbohydrates, proteins, amino acids and fats. The following tests were carried out to identify the various phytoconstituents present in hexane, ethyl acetate, chloroform, methanol and aqueous extracts .[10]

1. Test for alkaloids: A small quantity of each crude extract was taken separately in 5 ml of 1.5% v/v hydrochloric acid and filtered. These filtrates were then used for testing alkaloids with following reagents:
a) Mayer's test:
The Mayer's reagent was prepared as follow: 1.36 gm of mercuric chloride was dissolved in 60 ml of distilled water. The solution were mixed and diluted to 100 ml with distilled water.
To a little of the test filtrate, taken in a watch glass, a few drops of the above reagent were added. Formation of cream coloured precipitate showed the presence of alkaloids.
b) Dragendorff's test:
It was prepared by mixing two solutions. Solution A (17 gm of bismuth subnitrate + 200 gm
tartaric acid + 800 ml distilled water) and solution B (160 gm potassium iodide + 4 ml distilled water) were mixed in 1:1 v/v proportion. From this solution working standard was prepared by taking 50 ml of this solution and adding 100 gm of tartaric acid and making upto 500 ml with distilled water.
The above dragendorff's reagent was sprayed on whatmann No. 1 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with dragendorff's reagent, with the help of a capillary tube. Development of an orange red colour on the paper indicated the presence of alkaloids.
c) Wagner's test:
1.27 gm of iodine and 2 gm of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. When few drops of this reagent were added to the test filtrate, a brown flocculent precipitate was formed indicating the presence of alkaloids.
d) Hager's reagent:
A saturated aqueous solution of picric acids was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained, indicating the presence of alkaloids.
2. Test for saponins:
a) Froth test:
A few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. It is a stable, characteristic honeycomb like froth is obtained, saponins are present.
3. Test for sterols:
a) Salkowaski reaction:
Few mg of the residue of each extract was taken in 2 ml of chloroform and 2 ml of conc. sulphuric acid was added from the side of the test-tube. The test-tube was shaken for few minutes. The development of red colour in the chloroform layer indicated the presence of sterols.
b) Liebermann's test:
To a few mg of the residue in a test-tube, few ml of acetic anhydride was added and gently heated. The contents of the test-tube were cooled. Few drops of conc. sulphuric acid were added from the side of the test-tube. A blue colour gave the evidence of presence of sterols.
c) Liebermann-Burchard's reaction:
Few mg of residue was dissolved in chloroform and few drops of acetic anhydride were added to it, followed by concentrated sulphuric acid from the side of the test tube. A transient
colour development from red to blue and finally green indicated the presence of sterols.

III. TEST FOR CARBOHYDRATES:

a) Molisch's test:
To 2-3 ml of extract, few drops of 95% α-naphthol solution in alcohol was added; shaken and conc. H_2SO_4 was added from the side of the test tube. Appearance of a red brown ring at the junction of the liquids revealed the presence of carbohydrates.
b) Fehling's solution test:

The 1 ml of fehling's A and fehling's B solution were mixed and boiled for one minute. Equal volume of extract was added and heated on boiling water bath for 5-10 min. Appearance of a yellow coloured first and then brick red coloured precipitates revealed the presence of reducing sugars.

c) Benedict's solution test:

Equal volume of benedict's reagent and extract were mixed in test tube and heated on boiling water bath for 5 min. Appearance of red solution revealed the presence of reducing sugars.

5. Test for tannins:

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents.

a) Ferric chloride reagent:

A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. If dark green or deep blue colour is obtained, tannins are present.

b) Lead acetate test:

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, tannins are present.

6. Tests for proteins and amino acid:

a) Ninhydrin test:

The Ninhydrin reagent is 0.1% w/v solution of ninhydrin in n-butanol. A little of this reagent was added to the test extract. A violet or purple colour is obtained if amino acids are present.

b) Millon's test:

Millon's reagent was prepared by dissolving 3 ml of mercury in 27 ml of fuming nitric acid, keeping the mixture well cooled; this solution was then diluted with equal quantity of distilled water. Aqueous solution of the residue was taken and to it, 2 to 3 ml of Millon's

reagent was added. The white precipitate, which slowly turns to pink, is obtained if proteins are present.

7. Tests for flavonoids:

a) Shinoda test:

A small quantity to test residue was dissolved in 5 ml ethanol (95% v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. The pink, crimson or magenta colour is developed within a minute or two, if flavonoids are present.

b) Ammonia test:

Dissolve a few mg of extract residue in 1 ml of 95% v/v ethanol and apply a drop of this solution on filter paper, then expose this paper to ammonia vapors. If yellow colour is appeared then flavonoids are present.

c) Lead acetate solution test:

To small quantity of extract lead acetate solution was added. Appearance of yellow coloured precipitates revealed the presence of flavonoids.

8) Triterpenoids:

a) Libermann-Burchard test:

Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from side of tubes, A brown ring at the junction of two layers and the upper layer turns green indicates the presence of sterols and formation of deep red colour indicates the presence of sterols and formation of deep red colour indicates the presence of triterpenoids.

b) Salkowski's test:

Treat extract in chloroform with few drops of concentrated sulphuric acids, shake well and allow to stand for some time, red colour appears in the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicating the presence of triterpenoids [13].

Thin Layer Chromatography

1 Preparation of the plates:

The adsorbent used for thin layer chromatography was silica gel G. About 25 gm of silica gel G was taken in a glass mortar and required volume of distilled water was added to it. The mixture was stirred with glass rod until it became homogeneous. The mixture was then allowed to swell for about 15 min. Then an additional 15 ml of distilled water was added to it with stirring. The suspension was then transferred to a 150 ml flask fitted with a plastic stopper, and was shaken vigorously for about 2 min. This suspension was then spread immediately on thin layer chromatographic plates with the help of a thin layer chromatography (TLC) applicator.

2 Drying and storage of the plates:

The freshly coated plates were then air dried until the transparency of the layer had disappeared. The plates were then stacked in a drying rack and were heated in an oven for 30 min. at 110° C. The activated plates were kept in a desiccators, till required for further use.

3 Application of the sample:

For applying test samples on TLC plate, glass capillaries were used. The spots were applied with the help of a transparent template, keeping a minimum distance of 1 cm between the
two adjacent spots. The spots of the samples were marked on the top of the plate to know their identity.
4 Chromatographic chamber, conditions of saturation and the development of TLC plates:
Chromatographic rectangular glass chamber (16.5 cm × 29.5 cm) was used in the experiments. To avoid insufficient chamber saturation and the undesirable edge effect, a smooth sheet of filter paper approximately of 15 × 40 cm size was placed in the chromatographic chamber in a 'U' shape and was allowed to be soaked in the developing solvent. After being thus moistened, the paper was then pressed against the walls of the chamber, so that it adhered to the walls. The chamber was allowed to saturate with occasional shaking. The experiments were carried out at room temperature in diffused daylight.
5 Developing solvent system:
A number of developing solvent systems was tried, for each residue, and the satisfactory resolution system were noted down.
6 Detector/spraying equipment:
Compressed air sprayer with a fine nozzle was used to detect the different constituents
present on TLC plates. Air compressor was attached to a glass sprayer. The sprayer was filled with about 50 ml of the detecting reagent and then used. After each spray, the sprayer was washed separately with water, chromic acid, distilled water and then with acetone. Other, UV chamber can be used for the substance exhibiting fluorescence nature. Maximally, the results were observed at 254 nm and 366 nm of UV light and at day light [10].

IV. RESULTS AND DISCUSSION

PHARMACOGNOSTIC STUDIES

1 Macroscopic evaluation:
The macroscopic studies indicated important characteristics which are useful diagnostic characters.
Macroscopic features of the seed powder of Cicer arietinum Linn. samples (S1, S2) was observed.
The details of result has been shown in Table I

Table I: Macroscopic evaluation of seed powder of two samples:

S.No.	Parameters	Observation	
		S1	S2
1.	Colour	Yellowish brown	Yellowish brown
2.	Odour	Characteristic	Characteristic
3.	Taste	Characteristic	Characteristic
4.	Nature	Coarse powder	Coarse powder

2 Extractive values:
Cold maceration: Extractive values of air dried seed powders of two samples of Cicer arietinum L. by cold maceration are shown below:

Table II: Extractive Values

Type	Weight of extractives (% w/w)	
	S1	S2
Alcohol soluble extractive	4.5	3.7
Water soluble extractive	5.5	6.2

3 Ash values:
Results of total ash, acid insoluble ash and water soluble ash are shown below:

Table III: Ash values of Cicer arietinum Linn. seed samples (S1,S2)

Plant name	Part used	Ash values	S1	S2
Cicer arietinum	Seed powder	Total ash	6.9	6.8
		Acid-insoluble ash	1.9	1.8
		Water-soluble ash	1.9	1.5

4 .Foreign matter analysis:
Foreign matter was found to be nil (absent) due to proper cleaning of the material by the miller.

5 .Fluorescence analysis:

Fluorescence is an important phenomenon exhibited by various chemical present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products, which do not fluorescence in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescence derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is important parameter of pharmacognostic evaluation.

Table IV: Fluorescence analysis of seed powders of Cicer arietinum Linn. (S1)

S.No.	Treatment	Day light	Short UV light	Long UV light
1.	Powder as such	Cream	Brown	Dark brown
2.	Powder + 5% $Fecl_3$	Yellowish brown	Dark brown	Dark brrown
3.	Powder + 1 M NaOH	Cream	Creamish brown	Brown
4.	Powder + Picric acid	Yellow	Cream	Green
5.	Powder + Acetic acid	Cream	White	Creamish brown
6.	Powder + H_2SO_4	Dark brown	Creamish Black	Brown
7.	Powder + Water	Cream	Cream	Brown
8.	Powder + Nitric acid	Creamish brown	Cream	Brown
9.	Powder + I_2 solution	Greyish	Dark green	Green
10.	Powder + Hcl	Light brown	Brown	Dark brown

Table V: Fluorescence analysis of seed powders of Cicer arietinum Linn. (S2)

S.No.	Treatment	Day light	Short UV light	Long UV light
1.	Powder as such	Cream	Brown	Dark brown
2.	Powder + 5% $Fecl_3$	Brown	Dark browns	Black
3.	Powder + 1 M NaOH	Cream	Cream	Light green
4.	Powder + Picric acid	Yellow	Cream	Green
5.	Powder + Acetic acid	Grey	Light grey	Light brown
6.	Powder + H_2SO_4	Dark brown	Black	Brown
7.	Powder + Water	Cream	Cream	Light green
8.	Powder + Nitric acid	Creamish brown	Yellow	Green
9.	Powder + I_2 solution	Black	Black	Black
10.	Powder + HCl	Light brown	Black	Dark brown

Result of loss on drying of powder of Cicer arietinum Linn. seed samples (S1,S2) shown below:
Table VI: Loss on drying:

Plant name	Part used	LOD (%w/w) S1	LOD (%w/w) S2
Cicer arietinum Linn.	Seed powder	8	9.5

Histochemical study:

Table VII: Histochemical study for Cicer arietinum Linn. (S1, S2)

Chemical	Presence	Colour	Results	
			S1	S2
Phloroglucinol-Hcl	Lignin	Reddish brown	+ve	+ve
Iodine solution + H_2SO_4	Cellulose	Black	+ve	+ve
Sudan-III	Fixed oils and fats	Pink	+ve	+ve
Caustic alkali + Hcl	Calcium oxalate	Yellow crystal	-ve	-ve
Weak Iodine solution	Starch	Blue	+ve	+ve
Lugol's solution	Protein	Black	+ve	+ve
Millon's reagent	Protein	Yellow to brown	+ve	+ve

Swelling index:
Result of swelling index of seed powder of Cicer arietinum Linn. samples (S1, S2) shown below:

Table VIII: Swelling index

Plant name	Part used	Swelling index	
Cicer arietinum L.	Seed powder	S1	S2
		3ml	2ml

Foaming Index of Cicer arietinum L. Seed Powder:
Foaming index of seed powder of Cicer arietinum Linn. samples (S1,S2) are shown below:

Table IX: Foaming index

Plant name	Part used	Foaming Index		
Cicer aretinum Linn.		Seed powder	S1	S2
			100	100

pH analysis of Cicer arietinum Linn. seed powders:
pH of air dried seed powder of Cicer arietinum Linn. samples (S1, S2) is shown below:

Table X: pH analysis

Plant name	Part used	Samples	pH 1% Solution	pH 10% Solution
Cicer arietinum Linn.	Seed powder	S1	7	<4
		S2	7	<4

PREPARATION OF EXTRACTS
Methanol extracts by cold maceration:
Percentage yield and physical characteristic of methanol extracts of Cicer arietinum Linn. seed samples (S1, S2) are shown in table

Table XI: Percentage extractive and characteristics of Cicer arietinum Linn. seed samples

Sr. No.	Extract	%age Yield (w/w)	Colour	Odour	Consistency
1.	HES1	4.50	Yellow	Characteristic	Sticky
2.	MES1	3.81	Yellow	Characteristic	Sticky
3.	HES2	6.03	Light brown	Characteristic	Sticky
4.	MES2	4.32	Yellowishgolden	Characteristic	Sticky

ME- Cicer arietinum Linn. methanol extract and HE-Cicer arietinum Linn. Hydroalcoholic extract of samples 1 and 2.
6.2.2. Successive solvent extraction with hexane, choroform, ethyl acetate, ethanol and water:Percentage yield and physical characteristic of various extracts of Cicer arietinum Linn. seed samples (S1, S2) are shown in table

Table XII: Percentage extractives and Characteristics of Cicer Arietinum Linn. seed samples

Sr. No.	Extract	%age Yield (w/w)	Colour	Odour	Consistency
1	HES1 HES2	6.90 6.64	Light yellow	Characteristic	Sticky
2	CES1 CES2	7.74 6.94	Light yellow	Characteristic	Sticky
3	EAES1 EAES2	6.86 6.48	Dark brown	Characteristic	Sticky
4	EES1 EES2	9.02 8.36	Dark brown	Characteristic	Sticky
5	AES1 AES2	9.12 9.01	Dark brown	Characteristic	Sticky

HE- Cicer arietinum L. hexane extract, CE- Cicer arietinum L. chloroform extract, EAE- Cicer arietinum L. ethyl acetate extract, EE- Cicer arietinum L. ethanol extract AE- Cicer arietinum L. aqueous extract. (S1 , S2)

Phytochemical Screening:
Results of qualitative tests for extracts of two samples (S1, S2) obtained by successive extraction are shown below:

Table XIII: Phytochemical screening of various extracts of two samples of Cicer arietinum Linn.

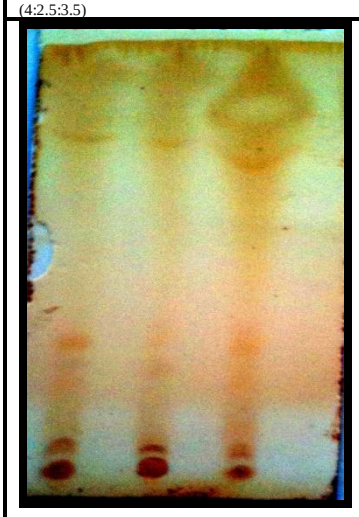
Phytochemical tests	Extracts				
	HE (S1,S2,)	CE (S1,S2,)	EAE (S1,S2,)	EE (S1,S2,)	AE (S1,S2,)
Phytosterols					
Leibermann Burchard's test	+	-	+	-	-
Leibermann's reagent	+	-	+	-	-
Salkowaski test	+	-	+	-	-
Fixed oils and fats					
Strain test	+	-	-	-	-
Alkaloids					
Mayer's reagent	+	-	-	+	+
Dragendorff's reagent	-	-	-	+	-
Wagner's reagent	-	-	-	+	-
Hager's reagent	+	-	-	+	+
Carbohydrates					
Molish’s test	-	+	-	+	+
Fehling’s test	-	+	-	+	-
Benedict test	-	-	-	+	-
Legal test	-	-	-	+	-
Proteins and amino acids					
Millon’s test	-	-	-	-	+
Biuret test	-	-	-	-	+
Phenolic compounds and Tannins					
Lead acetate solution	+	-	+	+	+
Dilute iodine solution test	+	-	+	+	+
$Fecl_3$ solution	+	-	+	+	+
Flavonoids					
Lead acetate test	+	+	+	+	+
Shinoda test	+	+	+	+	+
Glycosides					
Legal’s test	-	-	-	-	-
Liebermann-Burchard test	-	+	-	-	-

-: absent, +: present; HE: Hexane; CE-Chloroform; EAE-Ethyl acetate; EE-Ethanol; AE-Aqueous
Phytochemical study shows the presence of phenolic, flavonoids and tannins in greater amount mainly in EAE and EE. Other types of phytoconstituents are also present like phytosterols, glycosides, carbohydrates, alkaloids, protein and aminoacids.

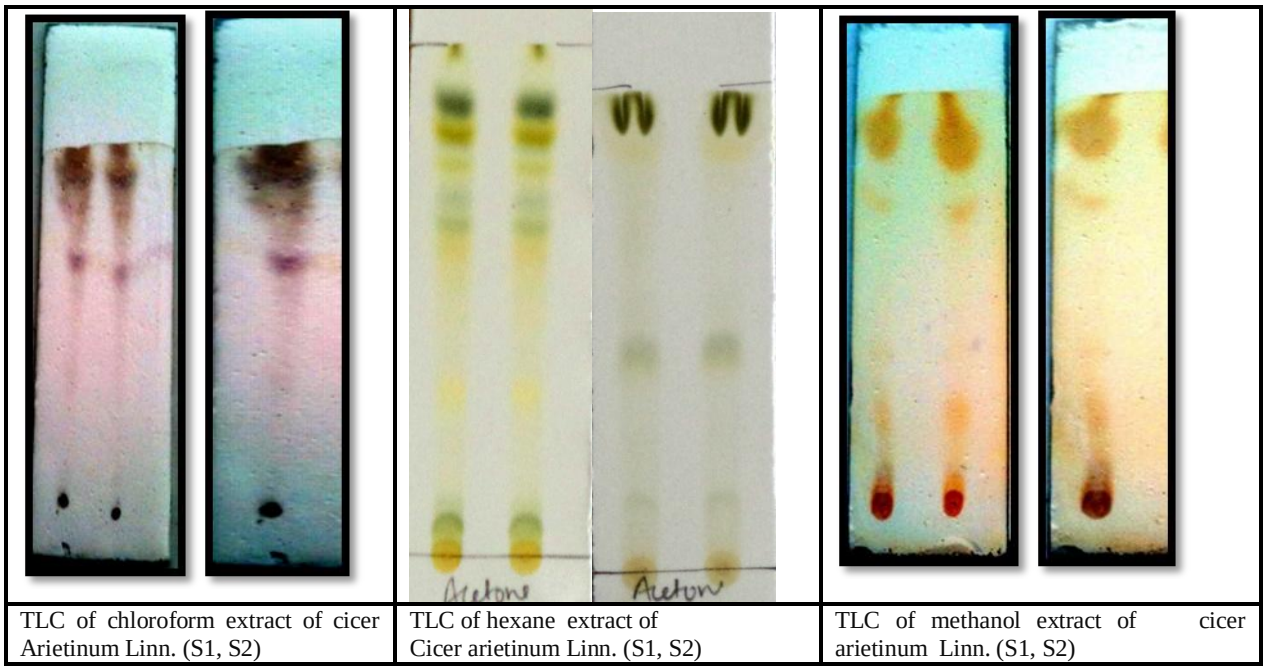
Thin Layer Chromatography: The table represents the various coloured spots observed with their R_f values in respective extracts using different solvent systems and detecting agents.

Table XIV: TLC profile of various extracts of Cicer arietinum L. seed samples
CE- Cicer arietinum L. chloroform extract, EAE- Cicer arietinum L. ethyl acetate extract, EE- Cicer arietinum L. ethanol extract, (S1, S2). AS- Anisaldehyde sulphuric acid

Extracts	Solvent system	Ratio	No. of spots	Detecting agent	R_f
ME	Toluene: Acetone: $CHCl_3$	4: 2.5: 3.5	S1 = 5	A.S.	0.05,0.23,0.28,0.74, 0.95
			S2 = 5		0.05,0.15,0.56,0.75, 0.81
CE	Chloroform : Methanol	9.7: 3	S1=4	A.S.	0.330.37, 0.41, 0.75
			S2=4		0.24,0.41,0.47, 0.75
EAE	Chloroform : Methanol	9.7: 3	S1=3	A.S.	0.06, 0.56, 0.2
			S2=2		0.03, 0.5
EE	Toluene: Ethyl acetate: formic acid: chloroform	3:3:0.8: 0.2	S1=3	A.S.	0.6, 0.9, 0.8
			S2=3		0.66, 0.8, 0.92
HE	Toluene: Acetone:$CHCl_3$	4: 2.5: 3.5	S1=3	A.S.	0.68, 0.73, 0.81
			S2=3		0.66, 0.69, 0.8
ME	Toluene: Ethyl acetate: formic acid: chloroform	3:3:0.8: 0.2	S1=3	A.S.	0.21, 0.74, 0.9
			S2=3		0.23, 0.72, 0.87

A. Toluene:Acetone:$CHCl_3$ (4:2.5:3.5)	B. Chloroform : Methanol (9.7:3)	C. Chloroform : Methanol (9.7:3)
		
TLC of ethanol extract of Cicer arietinum (S1, S2)	TLC of chloroform extract of Cicer arietinum (S1, S2)	TLC of ethyl acetate extract of Cicer arietinum (S1, S2)

D. Toluene: Ethyl acetate: formic acid: chloroform (3:3:0.8:0.2)	E. Toluene: Acetone:$CHCl_3$ (4:2.5:3.5)	F. Toluene: Ethyl acetate: formic acid: chloroform (3:3:0.8:0.2)



TLC of chloroform extract of cicer Arietinum Linn. (S1, S2)	TLC of hexane extract of Cicer arietinum Linn. (S1, S2)	TLC of methanol extract of cicer arietinum Linn. (S1, S2)

Figure I: (A-F): Chromatogram for different solvents

A- EE (Toluene: Acetone: $CHCl_3$:: 4: 2.5: 3.5)
B- CE (Chloroform: Methanol:: 9.7: 3)
C- EAE (Chloroform: Methanol:: 9.7: 3)
D-CE (Toluene: Ethyl acetate: formic acid: chloroform:: 3:3:0.8:0.2)
E-HE (Toluene: Acetone: $CHCl_{3::}$ 4: 2.5: 3.5)
F-EE (Toluene: Ethyl acetate: formic acid: chloroform:: 3:3:0.8: 0.2)
EE- Ethanol extract; CE- Chloroform extract; EAE- Ethyl acetate extract., S1-seed sample from retail store 1; S2- seed sample from retail store 2.

V. CONCLUSION

Cicer arietinum Linn.seeds S1and S2 were purchased, authenticated and powered for successive extraction with solvents in increasing order of their polarity viz. hexane, chloroform, ethyl acetate, ethanol and water. Phytochemical analysis indicated the presence of various phytoconstituents viz. phytosterols, flavonoids, phenolic compounds, tannins, carbohydrates, proteins, amino acids, fixed oils and fats etc. Thin layer chromatography study on extracts revealed the presence of a number of compounds. The chickpea studies on these two samples have shown variation qualitatively and quantitatively regarding different parameters.. Cicer Arietinum could contribute extensively to the management and avoidance of degenerative diseases associated with free radical damage. The data obtained will be useful for development of nutraceutical food products from this plant.

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