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# Phytochemical Profile and *in vitro* Anti-oxidant activity of Seeds of *Cicer arietinum* L. (Gram or Chik Pea)

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***Abstract:*** *Cicer arietinum L. is a much branched erect or spreading annual, 25-50 cm tall. The leaves are imparipinnate with 9 to 15 pairs of ovate, elliptic or obovate leaflets with serrate margin. All parts of the plant are clothed with clavate grandular hairs, the secretions of which are rich in oxalic and malic acids that impart a sour taste to the leaves and fruits. The flowers, varying in color from white to pink, are usually borne singly. The pods are small (up to 3x2 cm) but inflated and contain one or two seeds which are angular with a prominent beak and small hilum at the anterior end. The seed coat may be wrinkled, smooth or rough, ranging in color from white, yellow, red, brown to nearly black. The cotyledons are thick and yellowish.*

*In the present investigation phytochemical profile of Chik pea seeds was assayed qualitatively and quantitatively in six solvent extracts viz. acetone, petroleum ether, ethanol, methanol, benzene and distilled water. The results revealed the presence of various phytochemicals such as tannins, phlobatannins, saponins, terpinoids, diterpinoids, emodins, flavonoids, cardiac glycosides, anthraquinones, carotenoids, reducing sugars, alkaloids, anthocyanin, coumarins, steroids, phytosterols, phenol, fatty acids, proteins and amino acids. terpenoid, diterpinoid, emodin, cardiac glycoside, anthraquinone, carotenoid, reducing sugar, alkaloids, anthocyanin, coumarin, fatty acid and protein were detected in all the six solvent extracts. Tannins, Flavonoids, Saponins, Phenols and amino acids were not detected in ethanol and methanol extracts. Phlobatannin, steroid and phytosterol were also not detected in ethanol, methanol and distilled water extracts. Emodin was detected in all extracts except petroleum ether and benzene. The seeds of Cicer arietinum contained a significant amount of alkaloid, flavonoids, phenolic, saponins and tannin content.*

*The antioxidant activity of the methanol extracts of seeds of Cicer arietinum in vitro has been assayed. The results revealed that methanol and hexane extracts of leaves and seeds of C. arietinum caused bleaching of the purple colour of DPPH radical that caused the development of pale spots over a purple background. This indicated that the extracts contained some active phytochemicals exhibiting antioxidant activities. The DPPH scavenging activity of the methanol and n-hexane extracts (0.5 -2.5 mg/ml) exhibited concentration-dependent free radical scavenging activity. The extracts (0.5-2.5 mg/ml) and the standard antioxidant n-propyl gallate (3.0 mg/ml) caused a concentration- dependent reduction of Fe3+ to Fe2+. The extracts (0.5 – 2.5 mg/ml) and n-propyl gallate (3.0 mg/ml) caused a concentration- dependent inhibition of linoleic acid auto-oxidation.*

*This versatile plant is the source of many important bioactive compounds that exhibit anti-oxidant activity. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future prospective study.*

***Key Words:*** *Cicer arietinum, Phytochemicals, Acetone,Ppetroleum ether, Ethanol, Methanol, Benzene, Distilled water, Antioxidant, DPPH, Linoleic acid, Seeds*

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## I. Introduction

Gram or Chick-pea (*Cicer arietinum* L.) is believed to have originated in southwest Asia. The plants are growing wild in the Mediterranean region having in face escape from cultivation. It is said to be one of the oldest pulse crops known and cultivated in Asia and Europe and was known to the ancient Egyptians, Hebrews and Greeks (Rossato *et al.*, 1999) [1].

Chickpea has been introduced all over the world, including tropical America, Africa and Australia, Chickpea is the most important legume grown in India, ranking fourth among all food sources, being grown over 7 600 000 ha of land, mostly in Uttar Pradesh, Madhya Pradesh, Rajasthan, Haryana, Maharashtra, Punjab and Bihar.

**Botanical Profile:**

The plant is a much branched erect or spreading annual, 25-50 cm tall. The leaves are imparipinnate with 9 to 15 pairs of ovate, elliptic or obovate leaflets with serrate margin. All parts of the plant are clothed with clavate grandular hairs, the secretions of which are rich in oxalic and malic acids that impart a sour taste to the leaves and fruits. The flowers, varying in color from white to pink, are usually borne singly. The pods are small (up to 3x2 cm) but inflated and contain one or two seeds which are angular with a prominent beak and small hilum at the anterior end. The seed coat may be wrinkled, smooth or rough, ranging in color from white, yellow, red, brown to nearly black. The cotyledons are thick and yellowish.

In India, chickpea, like wheat and linseed, is grown as a *rabi* crop (winter crop) in the middle of October after the monsoon rains are over. It is well adapted to arid and semi arid regions with low to moderate rainfall and a cool and dry climate. Heavy rain after sowing or at flowering and fruiting stages of growth. It is essentially a crop of clay soils, thriving best in deep alluvial clay. Chickpea may be grown as a pure crop or inter-mixed with others such as wheat, barley or mustard.

Analysis of the whole dried seed gives approximately: moisture, 9.8%; proteins, 17.1%; fats, 5.3%; carbohydrates, 61.2%; fibre, 3.9% and ash, 2.7%.

Chickpea is extensively used as a nutritious pulse crop in India. The seeds may be eaten raw, roasted, parched or boiled in split form (dhal). Tender leaves are used as a leafy vegetable. Flour (besan) is one of the major ingredients, with ghee (clarified butter) and sugar, of many forms of Indian confectionery. The dry stems and leaves, after threshing, are used as a cattle feed. Chickpeas are occasionally included in the concentration of animals. Germinated seed are often recommended to prevent scurvy. An acrid juice (94% malic acid + 6% oxalic acid), collected by spreading a cloth over the crop overnight, is used medicinally for intestinal disorders.

**Systematic Position**

**Kingdom**: Plantae; **Division**: Magnoliophyta; **Class**: Magnoliopsida; **Order**: Fabales; **Family**: Fabaceae; **Subfamily**: Faboideae; **Genus**: *Cicer;* **Species**: *Cicer arietinum* L

**Common name:** kala chana, Bengal gram, Gram, Chick Pea

**Traditional uses of Gram:**

Leaves, seeds and seedpod (Duke, 1981; Doppalapudi et al., 2012; Liu et al., 1986) [2, 3 and 4] of Gram are traditionally used to treat many diseases.The seeds are used as aphrodisiac, for bronchitis, catarrh, cholera, constipation, diarrhea, dyspepsia, flatulence, snakebite, sunstroke, and warts. Acids particularly malic acid and oxalic acid of gram are used to lower the blood cholesterol levels. In India these acids are harvested by spreading thin muslin over the crop during the night. In the morning the soaked cloth is wrung out, and the acids are collected and used as hypolipidemic. Seeds are also considered antibilious (Al Snafi, 2015) [5]. *Cicer arietinum* is a good source of protein and traditionally used in pacifying the burning sensation in stomach, hepatomegali, stomatitis, inflammations, skin diseases and bronchitis (Doppalapudi et al., 2012) [3]. Chickpeas have also been widely used in traditional Uighur medicine to treat and prevent hypertension, hyperlipidemia, diabetes, itchy skin, flatulence, low libido, tumor formation and osteoporosis (Liu et al., 1986) [3].

**Phytochemicals of gram:**

The preliminary phytochemical screening of *Cicer arietinum* seeds revealed the presence of carbohydrates, proteins, amino acids, fixed oils, phytosterols, alkaloids, Phenolic compounds and tannins, flavonoids, glycosides, saponins, amino acids, iron, phosphate, sulphate, and chloride (73-76). Chick peas are an excellent source of carbohydrates and proteins, which constitute about 80% of the total dry seed weight. Dried chickpeas contain about 20% protein. The bulk of the seed is made up of carbohydrates (61%) and 5% fat. Crude fiber is mostly located within the seed coat. The seeds were relatively rich in lecithin, potassium, phosphorus, calcium, folate and vitamin C, and also have small quantities of vitamins A and B.

The seed proteins contain lysine, methionine, arginine, glycine, histidine, isoleucine, leucine, phenylalanine, tyrosine, threonine, valine, alanine, aspartic acid, glutamic acid, hydroxyproline, proline, and serine (Duke, 1981; Huisman and Vander Poel, 1994; Williams et al., 1984) [2, 6, 7]. Fatty acid compositions of Chick pea include oleic, linoleic, myristic and steatic acids (Duke, 1981) [2]. The volatile compounds identified in the Roasted Chickpea (*Cicer arietinum* L) include 61 aroma-active compounds viz. aldehydes (25%), hydrocarbons (25%), terpenoids (20%), esters (8%), ketones (8%), alcohols (8%) and heterocyclic (8%) (Lasekan et al., 2011) [8]. Phyto-oestrogen content of *Cicer arietinum* include daidzein, genistein and secoisolariciresinol (Mazur and Aldercreutz, 1998)[9]. The chick pea seeds contain at least eight phytoestrogens viz. biochanin A, formononetin, genistein, biochanin A-7-O-β-D-glucoside, calycosin, trifolirhizin, ononin, and sissotrin (Zhao et al., 2009) [10].

**Pharmacological importance:** *Cicer arietinum* exhibits following pharmacological abilities.

**1. Effects on Reproductive systems: Aphrodisiac effect:**

The seeds of *Cicer airetinum* show enhancement of sexual behavioral parameters like mount frequency (MF), intromission frequency (IF), ejaculation frequency (EF), ejaculation latency (EL), mount latency (ML) and intromission latencies (IL). The seed extract significantly increases the serum cholesterol and testosterone levels (Saija et al., 2014) [11].

**2. Estrogenic effects:** Aqueous, alcoholic and chloroform extract of *Cicer arietinum* is found to increase the reproductive organ weight and possess estrogenic activity in female albino rats. Isoflavones, the important chemical components of the seeds and sprouts of chickpea, have drawn attention due to their potential therapeutic use. Isoflavones extracted from chickpea sprouts (ICS) stimulates estrogen responsive element (ERE)-promoter activity in cells, and concurrent treatment with the nonselective estrogen receptor antagonist ICI 182,780 abolished the estrogenic activity induced by ICS (Hai Rong et al., 2013) [12].

**Antioxidant effects:** The free radicals scavenging, antioxidant properties and intestinal α-glucosidase inhibitory activity of methanol extract of two varieties of *Cicer arietinum* have been evaluated. The extract of Cicer arietinum shows strong free radical scavenging properties and reduces postprandial glycemic load (AK sahana et al., 2013) [13].

**ACE- inhibition:** Treatment of legumin of *Cicer arietinum* with alcalase yields a hydrolysate that inhibits the angiotensin I converting enzyme. Hydrolysates of chickpea legumin obtained by treatment with alcalase are good source of peptides with angiotensin-1converting enzyme inhibitory activity (Pedroche et al., 2003) [14].

**Antidiabetic effect:** The seeds of Cicer arietinum have been found to reduce postprandial plasma glucose and are useful in the treatment of diabetes (The wealth of India, 2003; Pullaih and Naidu, 2003) [15, 16].

**Anti-inflammatory effects:** The anti-inflammatory potency of methanolic and ethanolic extracts of *Cicer arietinum* seeds at different doses (250 mg/kg and 500 mg/kg body weight) have been investigated against carrageenan and histamine induced paw edema in rats. All doses of the extracts show a significant ($p<0.001$) anti-inflammatory activity when compared to control groups and with standard drug (Indomethacin 10 mg/kg, orally). Both the methanolic and ethanolic extracts show the dose dependant anti-inflammatory activity (Doppalapudi et al., 2012) [17].

The chik pea also exhibits Hypocholesterolaemi (Yust et al., 2012) [18], Antidiarrhoeal (Dalal et al., 2011) [19], Anticonvulsant (Motahareh et al., 2009; Sardari et al., 2015) [20, 21], Hepatoprotective (Ramachandra et al., 2014) [22], Antimicrobial (Kan et al., 2010; Dalal et al., 2010; Thanekar et al., 2013) [23, 24, 25]. Anticancer (Valligatla et al., 2014) [26], Diuretic and anti-nephrolithiasis (Divya and Banda, 2014) [27] activities.

The present investigation is aimed to study the phytochemical profile and *in vitro* anti-oxidant activity of seeds of *Cicer arietinum* (Chik pea).

## II. Materials and Methods

In the present investigation the dried seeds of deshi *Cicer arietinum* (Chik pea) were powdered using a mixture grinder and stored in air-tight container for future use. Six different solvents (five non polar viz. Acetone, Petroleum ether, Ethanol, Benzene and Methanol and one polar solvent, the Distilled water) were used for preparation of solvent extracts. The dried seed sample was soaked separately with acetone, petroleum ether, ethanol, benzene, methanol and distilled water under reflux condition for the solvent extract preparation. About 1 gm of the dried sample of seeds was added respectively into the test tubes containing 5 ml of solvents, and was extracted at room temperature. The important phytochemicals of seeds of *Cicer arietinum* have been qualitatively and quantitatively analyzed for alkaloids, flavonoids, tannins, saponins and total phenols.

**Phytochemical Analysis:** Phytochemicals in seeds of *Cicer arietinum* were analyzed qualitatively and quantitatively in all the six solvent extracts

**Qualitative Phytochemical Analysis**

The extracts in all the six solvents of seeds of *Cicer arietinum* were tested for the presence of biological compounds by using following standard methods.

**Test for Carbohydrates**

**Fehling's test:** Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

**Benedict's test:** Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

**Iodine test:** Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

**Test for Phenols and Tannins**
Crude extracts were mixed with 2ml of 2% solution of $FeCl_3$. A blue–green or black coloration indicated the presence of phenols and tannins.

**Test for Flavonoid**
**Alkaline reagent test:** Crude extracts were mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids.

**Test for Saponins (Frothing test):** Crude extracts were mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponin.

**Test for Glycosides**
**Liebermann's test:** Crude extracts were mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated $H_2SO_4$ was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

**Salkowski's test:** Crude extracts were mixed with 2ml of chloroform. Then 2ml of concentrated $H_2SO_4$ was added carefully and shaken gently. A reddish brown color indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

**Keller-kilani test (Cardiac Glycosides):** Crude extracts were mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated $H_2SO_4$. A brown ring at the inter phase indicated the presence of cardiac glycoside.

**Test for Alkaloids:** The crude extract of all the six solvents was boiled in 10 ml methanol and filtered separately. 1% HCl was added followed by 6 drops of Dragendroff reagent, and the brownish-red precipitate was taken as evidence for the presence of alkaloids.
**Phlobatannins**: The deposition of a red precipitate denoted the presence of phlobatannins when crude extract of all the six solvent of plant material was dissolved in 10 ml of aqueous extract and few drops of 1% HCl were added in the boiling tube.

**Anthraquinones**: All the six solvent extracts of leaves were boiled in 10% HCl for 5 mins separately and the filtrate was allowed to cool. An equal volume of $CHCl_3$ with few drops of 10% $NH_3$ was added to the 2ml filtrate. The formation of rose-pink colour implies the presence of anthraquinones.

**Quantitative estimation of phytochemicals**
**Determination of Alkaloids:** Alkaloids content was measured by method suggested by Harborne (Harborne, 1973) [28]. A suspension was prepared by dispersing 5 gm of the dried leaves in 10% acetic acid solution in ethanol and kept at $28^0$C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous $NH_4OH$ were added. Finally, the precipitate was washed with 1% ammonia solution and dried at $80^0$C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

**Determination of Flavonoids:** The flavonoids content was also determined by Harborne (Harborne, 1973) [28] method. 5 gm of seed powder were boiled in 2M HCl for 30 min under reflux condition and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and recorded as mg/g.

**Determination of Tannins:** The finely powdered seeds of Gram were kept in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at $80^0$C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% $Na_2CO_3$ for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

**Determination of Saponins:** 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered.

2 ml of 5% FeCl3 solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

**Determination of total phenols:** Five gms of the powdered seeds were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

For measuring alkaloids a suspension was prepared by dispersing 5 gm of the dried powdered seeds in 10% acetic acid solution in ethanol and kept at $28^0$C for 4hrs which was further filtered through Whatman No. 42. Thereafter alkaloid was precipitated by concentrating the filtrate to one-quarter of its original volume and drops of conc. aqueous $NH_4OH$ were added. Finally, the precipitate was washed with 1% ammonia solution and dried at $80^0$C in the oven. The content of alkaloid was calculated and expressed as mg/g of sample.

For determining flavonoids 5 gm of dried seed powder were boiled in 2M HCl for 30 min under reflux and filtered after cooling. An equal volume of ethyl acetate was then added drop wise to the filtrate. The weight of precipitated flavonoid was determined and reported as mg/g.

For measuring tannin the finely powdered seeds of *Cicer arietinum* were kept in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at $80^0$C in a water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and rinsed with 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% $Na_2CO_3$ for the development of a bluish-green colour and was allowed to stand for 20 mins. The absorbance was measured at 760 nm and the amount of tannin was calculated by comparing it with a standard curve prepared in the range of 0-10 ppm.

For determining saponin content 100 ml Isobutyl alcohol was added to 1 gm of the finely powdered seed sample and stirred for 5 hrs. 20 ml of 40% saturated solution of Magnesium carbonate was added to the mixture and filtered. 2 ml of 5% $FeCl_3$ solution and 50ml volume of distilled water was added to 1ml of colourless solution and kept for 30 mins for colour (blood red) development The absorbance of the samples as along with the standard were read at 380 nm and calculated in mg/g. Standard saponin solution was prepared in the reference range of 0-10 ppm.

For determining total phenolic content five gms of the powdered leaves were boiled with 50 ml of ether for 15 mins and distributed in the ratio 1:2 (extract: distilled water). 2ml of ammonium hydroxide followed with 5ml of pentanol was added to it and incubated at the room temperature for 30mins. The absorbance was read at 505 nm wavelength.

**Quantitative analysis of phytochemical constituents in six different solvent extracts**

Six solvent extract of seeds of *Cicer arietinum* viz. acetone, petroleum ether, ethanol, methanol, benzene and distilled water were prepared by soaking 10gm of the powdered sample in 200 ml of each of the solvent separately for 12 hrs. The extracts were then filtered using filter paper. The extracts were then concentrated to ¼ of the original extracts i.e. 50 ml.

The amount of total phenolics in extracts was determined by the Folin–Ciocalteu method. Gallic acid was used as a standard by using different concentrations of (20-200µg) from which the total phenol content in the extract was expressed in terms of gallic acid equivalent (mg GAE /gm) extract. Different aliquots of 0.1 to 1.0 ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin- Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 mins at room temperature. Phenols react with the phosphomolybdic acid in Folin- Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue). The absorbance of the resulting solutions was measured at 760 nm against reagent blank. A standard calibration curve was prepared by plotting absorbance against concentration and it was found to be linear over this concentration range. The concentration of total phenol in the test sample was determined from the calibration graph. The assay was carried out in triplicate and the mean values with ± SD are presented.

The aluminium chloride colorimetric method was used for flavonoids determination. Each solvent extract (0.5 ml of 1:10 gm $ml^{-1}$) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 418 nm. The percentage of total flavonoids were calculated from the calibration curve of Quercetin (200-1000µg) plotted by using the same procedure and total flavonoids was expressed as Quircetin equivalents (QE) in mg per gm sample.

**Anti-oxidant assay**

The anti-oxidant activity was assayed in methanol extract of seeds of Gram (*Cicer arietinum*) qualitatively and quantitatively employing following methods:

***In vitro* qualitative DPPH test:** The qualitative test for antioxidant activity was performed using the rapid DPPH radical scavenging assay (Cuendet *et al.,* 1997) [29]. 10 µl of the leaf and seed extracts were applied on silica gel plates 60 F254 (Merck, 0.25 mm thick) and allowed to dry completely. The plate was then sprayed with a solution of 2% DPPH in methanol. A pale yellow to white spot over a purple background indicated a radical scavenging activity of the particular extract.

**Quantitative antioxidant assays of extracts**

**Reducing power assay:** Reducing activity of the methane extract was assayed as follows. Different concentrations (0.5 – 2.5 mg/ml) of both the extracts as well as the standard drug *n*-propyl gallate (3.0 µg/ml) were prepared in aqueous methanol (50% v/v) and 1 ml was taken into test tubes in triplicates. To the test tubes, 2.5 ml of sodium phosphate buffer (pH 7.0) and 2.5 ml of 1% potassium ferric cyanide solution was added. The contents were mixed well and incubated at $50^0$C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added and the mixture centrifuged at 3000 revolutions per minute for 10 minutes. After centrifugation 2.5 ml of supernatant was added to 2.5 ml of distilled water. To this about 1 ml of 0.1% ferric chloride was added. The absorbance was then recorded at 700 nm. A graph of absorbance was then plotted against the concentration of the extracts. Increase in absorbance was indicative of higher reducing power of the extract.

**Scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay:** The free radical scavenging activity of methanol extract was determined as follows. 1 ml each of the methanol extract in five different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml in methanol) was added to 3 ml methanolic solution of DPPH solution (20 mg/l) in a test tube. The reaction mixture was kept at 25°C for 30 mins. The absorbance of the residual DPPH was determined at 517 nm in a spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). One milliliter (1 ml) methanol (50%) was added to 3 ml DPPH solution, incubated at 25 °C for 30 minutes and used as control. *n*-propyll gallate (3.75-30 µg/l) was used as a standard free radical scavenger. The absorbance decreases with increasing free radical scavenging ability. Results were expressed as percentages of blank (100%). The concentration required to cause a 50% decrease in the absorbance was calculated (EC50). Each test was carried out using three replicates. The % DPPH scavenging effect (% of control) of the antioxidant was calculated as follows:

$$\% \text{ DPPH scavenging effects} = (Ac - At)/Ac \times 100$$

Where

Ac = Absorbance of the control; At = Absorbance of the test drug/ extracts

**Total antioxidant capacity assay:** The assay is based on the reduction of molybdenum, Mo +6 to Mo +5, by the extracts and subsequent formation of a green phosphate-molybdate (Mo +5) complex at acidic pH. Test tubes containing l ml of the methanol extract in five different concentrations (0.5-2.5 mg/ml) and 3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) were incubated at $95^0$C for 90 minutes. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm. Four concentrations of ascorbic acid (0.025, 0.05, 0.1 and 0.2 mg/ml) was used to construct a calibration curve. A blank solution was prepared by adding every other solution but without extract or standard drug. The antioxidant capacity was expressed as mg of ascorbic acid equivalent (AAE) per g of extract. This procedure was used for both methanol and n-hexane extracts.

**Determination of total phenolic content:** The presence of phenol in the methanol extract of seeds of *Cicer arietinum* was determined qualitatively using ferric chloride test. An intense positive colouration indicating the presence of phenols led to further quantification of total soluble phenols in the extract. The total phenol in the extract was determined by spectrophotometric assay using the Folin-Ciocalteu‟s reagent as described by Singleton *et al.,* (1999) [30] using tannic acid as standard.

1ml of the extracts (0.25-2 mg/ml) in distilled water was added to 1 ml Folin-Ciocalteu‟s reagent in a test tube. The content of the test tube was mixed and allowed to stand for five minutes at $25^0$ C in an incubator. 1 ml of sodium bicarbonate solution (2%) was added to the mixture. The reaction mixture was allowed to stand for 2 hours with shaking at $25^0$C in an incubator. The mixture was then centrifuged at 3000 rpm for 10 minutes and absorbance of the supernatant determined at 760 nm. Three replicates were prepared for each concentration of tannic acid and extracts. 1 ml distilled water was added to 1 ml Folin-Ciocalteu‟s reagent processed in the same way as the test drugs and used as blank. Tannic acid was used as reference. Four concentrations of tannic acid

(0.025, 0.05, 0.1, 0.2 mg/ml) were used to construct a calibration curve and the total phenols expressed as mg of tannic acid equivalents (TAE)/g of extract.

This method depends on the reduction of Folin-Ciocalteau reagent by phenols to a mixture of blue oxides which have a maximal absorption in the region of 760 nm. The reaction equation is as follows:

Folin: $Mo^{+6}$ (yellow) + è (from antioxidant) → $Mo^{+5}$ (blue)

Where the oxidizing reagent is a molybdophosphotungstic heteropolyacid comprised of $3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$, in which the hypothesized active centre is $Mo^{+6}$.

**Linoleic acid auto-oxidation assay:** The methanol extract in five different concentrations (0.5-2.5 mg/ml) in absolute alcohol were compared with *n*-propyl gallate (3.0 µgm/l) in absolute alcohol as a reference antioxidant. 2 ml of the extract, 2 ml of 2.5% linoleic acid in absolute ethanol, 4 ml of 0.05 M phosphate buffer (pH =7) and 1.9 ml of distilled water were put into test tubes with a screw cap and placed in an oven at $40^0$C in the dark for 7 days. After the seven day period, 2 ml each of the extracts and standard drug was added to 20 % aqueous trichloroactetic acid solution and 1 ml of 0.6 % aqueous thiobarbituric acid solution. This mixture was placed in boiling water bath for 10 minutes and after cooling, was centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured 535 nm. Each test was carried out in three replicates. Percentage inhibition of lipid peroxidation by the test drugs was assessed by comparing the absorbance of the drug test with that of the control (linoleic acid mixture without any drug). Data was presented as percentage inhibition of lipid peroxidation against concentration. The % inhibition of linoleic acid auto-oxidation was calculated as follows:

$$\% \text{ inhibition} = 1 - \left( \frac{D - D_O}{C_O - C} \right)$$

Where

Co= (Full reaction mixture) is the degree of lipid peroxidation in the absence of antioxidant

C= is the underlying lipid peroxidation before the initiation of lipid peroxidation

D= is any absorbance produced by the extract/ linoleic acid mixture

Do= is the absorbance produced by the extract alone

**Statistical analysis:** All the experiments were conducted in replicates of three and data was recorded as mean value ± SE. The statistical analysis was performed by one way analysis of variance (ANOVA) and means were compared by least significance difference test ($P< 0.05$) using the SPSS statistical software package (SPSS, ver. 10.0; Chicago. IL, USA).

## III. Results

The present study was carried out on six solvent extracts of dried seeds of *Cicer arietinum* to investigate the presence of important phytochemicals. All the six extracts revealed the presence of various phytochemicals such as tannins, phlobatannins, saponins, terpinoids, diterpinoids, emodins, flavonoids, cardiac glycosides, anthraquinones, carotenoids, reducing sugars, alkaloids, anthocyanin, coumarins, steroids, phytosterols, phenol, fatty acids, proteins and amino acids (Table-1) Of these 20 phytochemicals terpenoid, diterpinoid, emodin, cardiac glycoside, anthraquinone, carotenoid, reducing sugar, alkaloids, anthocyanin, coumarin, fatty acid and protein were detected in all the six solvent extracts. Tannins, Flavonoids, Saponins, Phenols and amino acids were not detected in ethanol and methanol extracts. Phlobatannin, steroid and phytosterol were also not detected in ethanol, methanol and distilled water extracts. Emodin was detected in all extracts except petroleum ether and benzene (Table-1).

From the results (Table- 2; Fig- 1) it is evident that the dried seeds of *Cicer arietinum* contained a significant amount of alkaloid, flavonoids, phenolic, saponins and tannin content. The amount of flavonoids was maximum (53.45mg/gm) followed by phenols (29.75mg/gm), alkaloids (22.25mg/gm), saponins (18.75mg/gm) and tannins (16.45mg/gm) (Table- 2; Fig- 1).

The comparative analysis of phytochemicals viz. total alkaloids, flavonoids, phenols, saponins and tannins in six different solvent extracts from dried seeds of Chik pea has been presented in Table- 3 and Fig- 2. From the results it is evident that the concentration of total alkaloids was maximum in distilled water extract (42.85mg/gm), followed by methanol extract (28.65mg/gm), acetone extract (28.45 mg/gm), ethanol extract (26.75mg/gm), petroleum ether extract (18.65mg/gm), benzene extract (17.75mg/gm). The concentration of total

flavonoids was minimum in ethanol and methanol extracts (11.65mg/gm and 11.75mg/gm respectively). The distilled water extract contained maximum amount of total flavonoids (42.75mg/gm), followed by benzene extract (39.35mg/gm), petroleum ether extract (35.45mg/gm) and acetone extract (33.55mg/gm). The amount of total phenol was minimum in ethanol and methanol extracts (11.35 and 11.45mg/gm respectively). The distilled water extract contained 28.35 mg/gm of total phenol, followed by petroleum ether extract (26.75 mg/gm), benzene extract (25.75 mg/gm) and acetone extract (22.65mg/gm). Saponin concentration was minimum in ethanol and methanol extract (12.65mg/gm and 12.35mg/gm respectively) and maximum in distilled water extract (37.45mg/gm), followed by benzene extract (26.25 mg/gm) petroleum ether extract and acetone extracts (22.45 mg/gm and 22.55mg/gm respectively). The total tannin concentration was minimum in ethanol and methanol extract (10.25mg/gm) and maximum in distilled water extract (24.45mg/gm) followed by petroleum ether and acetone extract (22.45 mg/gm and 22.65 mg/gm respectively) and benzene extract (18.45 mg/gm) (Table- 3; Fig- 2).

**Table- 1: Phytochemicals of seeds of *Cicer arietinum* analysed qualitatively in six different solvent extracts**

| Solvent extracts of leaves | Ta | Phl | Sap | Terp | Dter | Emd | Fla | Car | Anth | Crt | Res | Alk | Anc | Cou | Str | Pst | Phe | Fa | Prt | Aa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acetone | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Petroleum ether | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Ethanol | - | + | - | + | + | + | - | + | + | + | + | + | + | + | - | - | + | + | + | + |
| Methanol | - | + | - | + | + | + | - | + | + | + | + | + | + | + | - | - | + | + | + | - |
| Benzene | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Distilled water | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | + | + | + |

**Ta= Tannin; Phl= Phlobatannin; Sap= Saponin; Ter= Terpinoid; Dtr = Diterpinoid ; Emd= Emodin ; Fla= Flavonoid; Car= Cardiac glycoside; Anth= Anthraquinones; Crt= Carotenoids; Res= Reducing sugar; Alk= Alkaloid; Anc= Anthocyanin; Cou= Coumarin; Str= Steroids; Pstr= Phytosterol; Phe= Phenol; FA= Fatty acids; Prt= Protein; Aa= Aminoacids**

**Table- 2: Quantitative estimation of Phytochemicals in dried seeds of *Cicer arietinum***

| Phytochemicals | Amount in mg/gm |
|---|---|
| Alkaloids | 22.25±0.21 |
| Flavonoids | 53.45±0.32 |
| Phenols | 29.75±0.51 |
| Saponins | 18.75±0.36 |
| Tannins | 16.45±0.25 |

**Mean ± SD of five measurements**

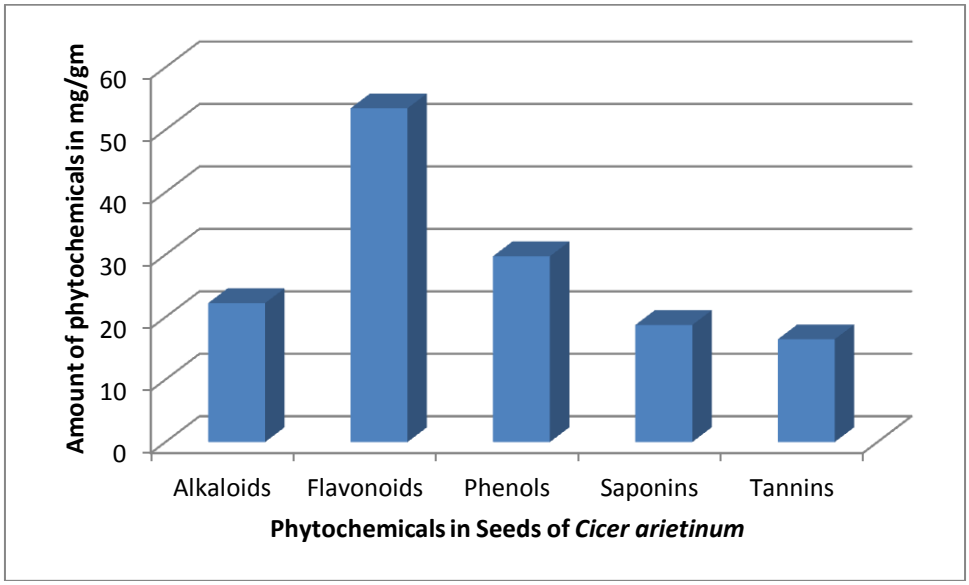


**Figure-1: Phytochemicals in seeds of *Cicer arietinum***

**Table- 3: Comparative analysis of total Alkaloids, Flavonoids, Phenol, Saponins and Tannins in six different solvent extracts of dried seeds of *Cicer arietinum* (amount in mg/gm)**

| Solvent Extracts | Total Alkaloids | Total Flavonoids | Total Phenol | Total Saponins | Total Tannin |
|---|---|---|---|---|---|
| Acetone | 28.45±0.32 | 33.55±0.45 | 22.65±0.45 | 22.55±0.32 | 22.65±0.19 |
| Petroleum ether | 18.65±0.21 | 35.45±0.17 | 26.75±0.62 | 22.45±0.21 | 22.45±0.14 |
| Ethanol | 26.75±0.33 | 11.65±0.17 | 11.35±0.25 | 12.65±0.21 | 10.25±0.25 |
| Methanol | 28.65±0.18 | 11.75±0.18 | 11.45±0.26 | 12.35±0.34 | 10.25±0.17 |
| Benzene | 17.75±0.17 | 39.35±0.45 | 25.75±0.11 | 26.25±0.43 | 18.45±0.17 |
| Distilled water | 42.85±0.15 | 42.75±0.25 | 28.35±0.12 | 37.45±0.41 | 24.45±0.18 |

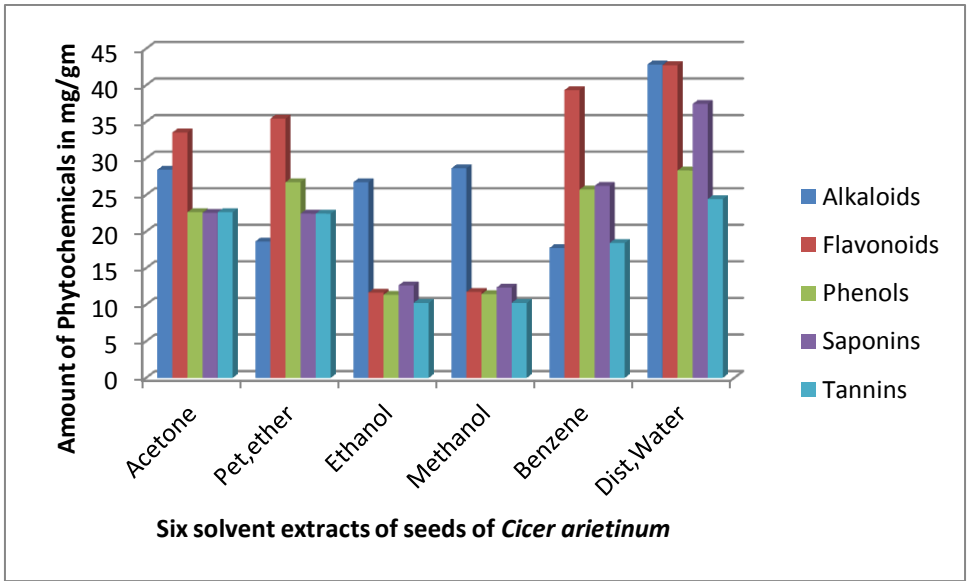


**Figure-2: Comparative analysis of phytochemicals in six different solvent extracts of seeds of *Cicer arietinum***

**Tatal phenol in mg/gm is measured as Gallic Acid Equivalent (GAE/g extract); Total flavonoids in mg/gm is measured as Quercetin Equivalent (QE)/g extract.**
**Mean ± SD of five measurements**

The results related to anti-oxidant activity of dried seeds of *Cicer arietinum* were as follows:
**Qualitative DPPH (2, 2-diphenyl-1-picrylhydrazyl) test for Antioxidant Activity:** The methanol extracts of seeds of *Cicer arietinum* caused bleaching of the purple colour of DPPH radical that caused the development of pale spots over a purple background. This indicated that the extracts contained some active phytochemicals exhibiting antioxidant activities.
**Quantitative antioxidant assay of methanol and n-hexane extracts:** The methods used to determine quantitative antioxidant activity of the methanol extract of seeds of *Cicer arietinum* included total phenolic content, total anti-oxidant capacity, reducing power, DPPH radical scavenging activity and linoleic acid autoxidation assays.

**1.** The total phenolic content of the methanol and n-hexane extracts was determined using the Folin- Ciocalteu's reagent and tannic acid was used as standard. The total phenolic content of the extracts was expressed as mg of tannic acid equivalents (TAE) per g of extract. The four different concentrations of the methanol extract of seeds were used for quantitative assay. The total phenolic content in methanol extract of seeds of *Cicer arietinum* has been presented in Table-4; Fig-3

**Table-4: Total Phenolic content in methanol extract of seeds of *Cicer arietinum***

| Concentration of methanol extract (mg/ml) | Seed |
|---|---|
| | Methanol extract |
| | Mean mgTAE/g ±SE |
| 1.0 | 47.25±0.51 |
| 1.5 | 51.25±0.42 |
| 2.0 | 58.45±0.35 |
| 2.5 | 68.27±0.35 |

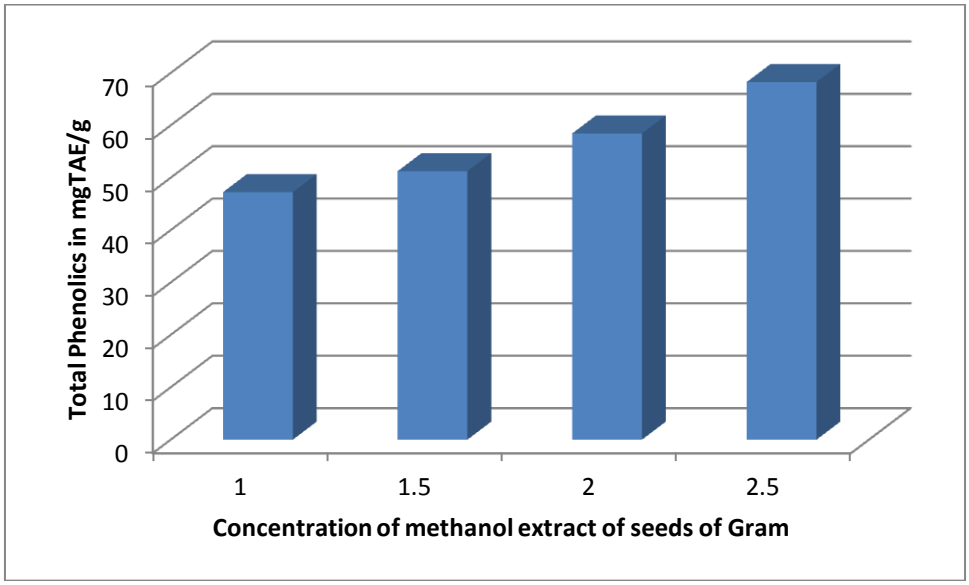


**Figure-3: Phenolic content in methanol extracts of seeds of *Cicer arietinum***

The results revealed that the seeds of *Cicer arietinum* contained appropriate phenolics. The methanol extracts contained high concentration of phenolics. The total phenolic content in 1.0 mg/ml of methanol extract was 47.25±0.51 mgTAE/g. Their concentration increased on increasing the concentration of methanol extracts. At 2.5 mg/ml of methanol the concentration of total phenolics was 68.27±0.35 TAE/g. (Table-4; Fig-3). The results clearly indicated that the seeds of *Cicer arietinum* contained more phenolic compounds.

**2. Free Radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity:** The results of the free radical scavenging potential of methanol extracts of seeds of *Cicer arietinum* using DPPH free radical scavenging method are depicted in Table-5 and Fig-4. The DPPH scavenging activity of the methanol extracts (0.5 -2.5 mg/ml) exhibited concentration-dependent free radical scavenging activity (Table- 5; Fig-4). Low free radical DPPH scavenging activity was observed at 0.5 mg/ml of methanol extracts of seeds ($IC_{50}$ 81.4 ± 0.025 µg/ml). The highest free radical scavenging activity was observed at concentration 2.5 mg/ml of methanol extract ($IC_{50}$ 925.7 ± 0.064 µg/ml).

**Table-5: DPPH scavenging activity of methanol and extracts of seeds of *Cicer arietinum***

| Dose of extract in mg/ml | Methanol seed extract |
|---|---|
| | IC50 (µg/ml) ±SEM |
| 0.5 | 81.4 ±0.025 |
| 1.0 | 235.5 ±0.065 |
| 1.5 | 566.5 ±0.064 |
| 2.0 | 727.5 ±0.083 |
| 2.5 | 925.7 ±0.064 |

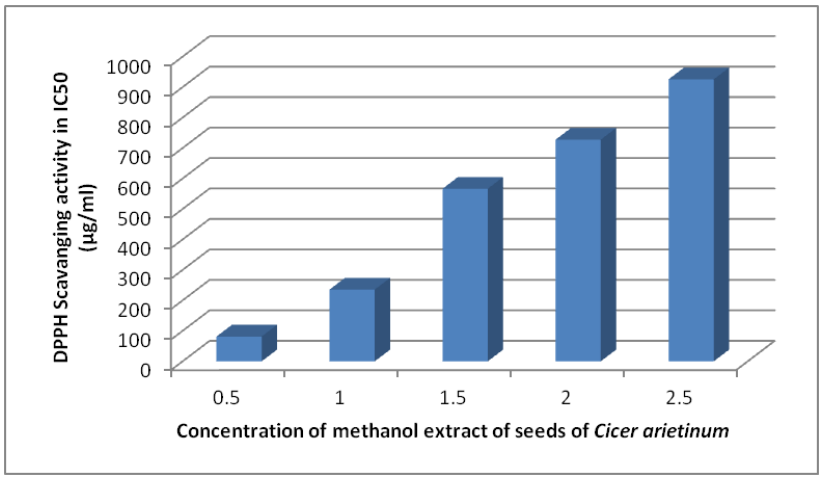


**Figure-4: DPPH scavenging activity of methanol and extracts of seeds of *Cicer arietinum***

**3. Reducing power:** The extracts (0.5-2.5 mg/ml) and the standard antioxidant *n*-propyl gallate (3.0 mg/ml) caused a concentration- dependent reduction of Fe3+ to Fe2+. From the IC50 values (Table-6; Fig-5), the methanol extracts of seeds of *Cicer arietinum* showed the highest reducing power activity in concentration dependent manner. At concentrations of 0.5 mg/ml methanol extracts of seeds caused reducing power activity of $IC_{50}$190.15 ± 0.013. Maximum reducing power activity was noticed for methanol extract of seeds of *Cicer arietinum* at concentration of 2.5 mg/ml. At this concentration of extract the reducing power activity was $IC_{50}$ 968.45 ± 0.035 µg/ml (Table-6; Fig-5).

**Table-6: Reducing power activity of methanol extracts of seeds of *Cicer arietinum***

| **Dose of extract in mg/ml** | ***Methanol seedextract*** |
|---|---|
| | **$IC_{50}$ (µg/ml) ±SEM** |
| **0.5** | **190.15 ±0.013** |
| **1.0** | **236.17 ±0.012** |
| **1.5** | **573.25 ±0.015** |
| **2.0** | **757.15 ±0.017** |
| **2.5** | **968.45 ±0.035** |
| **3.0 n-PG** | **70.25 ±0.003** |

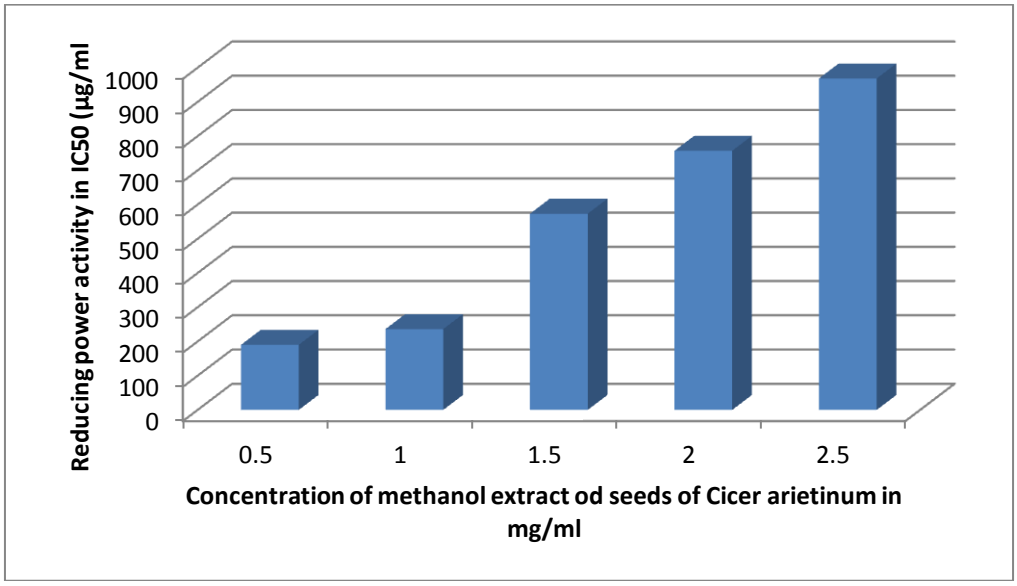


**Figure-5: Reducing power activity of methanol extracts of seeds of *Cicer arietinum***

**4. Lipid peroxidation:** The ability of the methanol extracts and test drug to inhibit linoleic acid auto-oxidation was investigated. The extracts (0.5 – 2.5 mg/ml) and *n*-propyl gallate (3.0 mg/ml) caused a concentration-dependent inhibition of linoleic acid autoxidation (Table-7; Fig-6). The per cent inhibition of lipid peroxidation by methanol extracts of *Cicer arietinum* increased with increasing the concentration. At concentration of 0.5 mg/ml, seed extracts caused minimum inhibition of lipid peroxidation (35.5 ± 0.14%). Percent inhibition of lipid

peroxidation increased on increasing the concentration of seed extracts. At concentration of 2.5 mg/ml the extracts caused maximum inhibition of lipid peroxidation. At this concentration methanol seed extract caused 98.5 ± 0.21% inhibition of lipid peroxidation (Table-7; Fig-6).

**Table-7: % inhibition of lipid peroxidation by methanol extracts of seeds of *Cicer arietinum***

| Dose of extract in mg/ml | *Methanol seed extract* |
|---|---|
| | % inhibition of lipid peroxidation ±SEM |
| 0.5 | 35.5±0.14 |
| 1.0 | 62.5 ±0.16 |
| 1.5 | 88.4±0.15 |
| 2.0 | 95.5 ±0.21 |
| 2.5 | 98.5 ±0.20 |
| 3.0 n-PG | 65.5 ±0.12 |

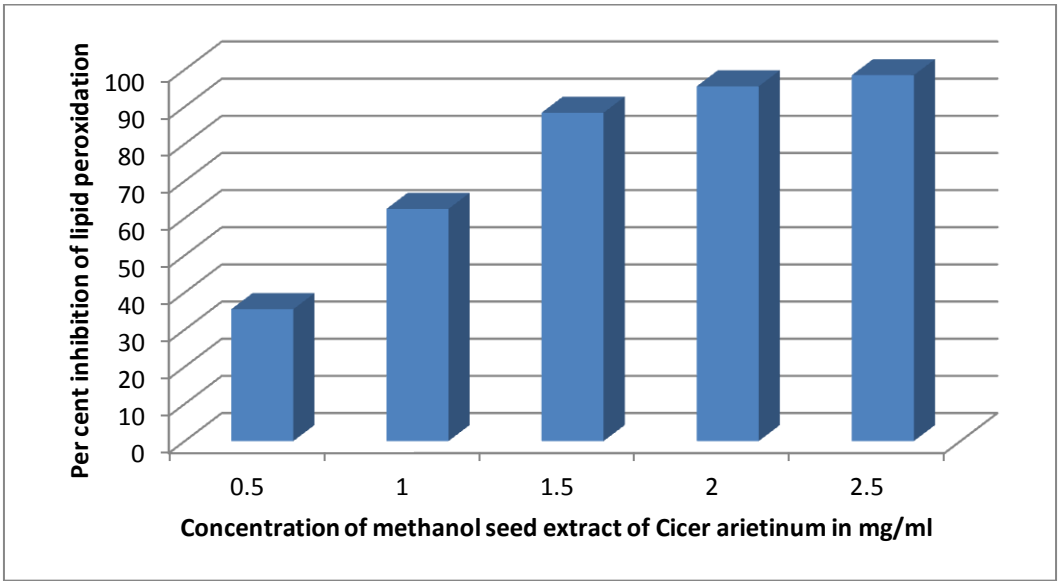


**Figure-6: % inhibition of lipid peroxidation by methanol extracts of seeds of *Cicer arietinum***

## IV. Discussion

The dried seed extracts of *Cicer arietinum* showed the presence of terpenoids, steroids and phytosterols, tannins, alkaloids, glycosides, saponins, reducing sugars, phenols and flavonoids. The extraction of various phytochemicals was seen to be more effectively done in methanol, a polar solvent. Hence, it can be reported that the polar solvent extract (methanol) was the best one for extracting the active principle. Flavonoids are water-soluble polyphenolic compounds which are extremely common and widespread in the plant kingdom as their glycosides. The flavonoids are known to act through scavenging or chelating process.

In the present investigation, antioxidant activity of the methanol extracts of seeds of *Cicer arietinum* was assayed in their methanol extracts by total antioxidant capacity, total phenolic content, DPPH scavenging activity, reducing power and lipid peroxidation activity. In all these assays, the antioxidant activity increased with increasing concentration of the methanol seed extracts of *Cicer arietinum* (Table-4-7; Fig-3-6).The methanol extracts showed higher reducing power and percent inhibition of linoleic acid lipid peroxidation considerably than the standard antioxidant *n*-propyl gallate. Antioxidant activity of plant extracts is not limited to phenolic compounds. The antioxidant activity may also be due to the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins. Thus the present study has shown that the seeds of *Cicer arietinum* possess significant antioxidant properties and may contribute to the retardation of the inflammatory process. This is because inflammatory tissue injuries are mediated by reactive oxygen metabolites from phagocytic leukocytes (e.g neutrophils, monocytes, macrophages and eosinophils) that invade the tissues and cause injury to essential cellular components (Parfenov, E. A. and Zaikov, G. E. (2000) [31]. Compounds that have scavenging activities toward these radicals have been found to be beneficial in inflammatory diseases (Auddy., et al 2003; Koo et al., 2006) [32, 33]. The ability of the seed extracts to inhibit the peroxidation of linoleic acid supports the use of *Cicer arietinum* in the preservation of palm oil in indigenous societies (Umerie *et al*., 2004) [34]. The antioxidant activity of the extract may also support its traditional use for wound healing. This is because in acute and chronic wounds, oxidants cause cell damage and thus inhibits wound healing (Thang *et al*., 2001) [35]. The administration of antioxidants or free radical scavengers is reportedly helpful,

notably to limit the delayed sequale of thermal trauma and to enhance the healing process (Thang *et al*., 2001) [35].

Various scientific studies show that aberrance in redox balance with elevated level of oxygen-free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) plays an important role in the origin and progression of most human diseases including cancer (Halliwell, 2012; Valko *et al*., 2007; Halliwell , 1991; Cross, *et al*., 1987; Bhattacharyya et al., 2014 (36, 37, 38, 39, 40]. Reactive oxygen species (ROS) act as secondary messenger in intracellular signalling cascades and elevated level of ROS associated with carcinogenesis by promoting initiation, progression, and metastasis of cancer cells. It also induced DNA damage leading to genetic lesions that initiate tumorigenicity and subsequent tumor progression (Volko, 2004; Wang and Yi, 2008; Storz, 2005; Khanna *et al*., 2014 [41, 42, 43]. However, many studies also suggested that free radicals are essential mediators of apoptotic pathway for triggering cell death and therefore function as anticancer agents.Thus, free radicals production approach is used in nonsurgical therapeutic methods for cancer therapy, including chemotherapy, radiotherapy, and photodynamic therapy (Wang and Yi, 2008; Salganik, 2001; Seifried *et al*., 2003) [42, 44, 45]. Free radicals produced in cancer therapy are associated with serious side effects. Furthermore, elevated level of ROS in cancer cell leads to intercellular transfer of hydrogen peroxide ($H_2O_2$) to neighbouring cells, and stimulates them to acquire uncontrolled ROS production (Storz, 2005) [43]. Free radical scavenger activity plays a protective role in normal healthy cells. They prevent the ROS from spreading and ultimately protect the adjacent cells from oxidative DNA damage and check the cancer progression. Many clinical trials have also suggested that intake of exogenous antioxidants can protect the healthy cells from oxidative stress as well as ameliorate toxic side effects of cancer therapy without affecting therapeutic efficacy (Salganik, 2001) [44]. Extracts of medicinal plants have been used for the treatment of various diseases, including cancer all over the globe, as they are easily prepared, standardized, and stored. Herbal extracts are also cost effective which increase their accessibility to the patients of all economic status (Eder and Mehnert, 1998; Vickers, 2002 [45, 46]. Global health policies promote the therapeutic use of herbal extract.World Health Organization (WHO) also encourages the use of medicinal plants in the treatment of disease (Debas *et al*., (2006); Winslow and Kroll, 1998; Pal and Shukla, 2003) [47, 48, 49].

Medicinal plants used as therapeutic agents are considered nontoxic for human consumption, while many studies reported the various side effects of medicinal plant (Chan, 2003; Ergil *et al*., 2002) [50, 51]. Medicinal plants uses for health benefit are not taken under the appropriate instruction and consultant of physician. Although people are using medicinal plants from ancient time, safety evaluation of these medicinal plants is required (Hwang *et al*., 2013) [52].

## V. Conclusions

The present study revealed the presence of various phytochemical components such as carbohydrates, flavonoids, saponins, phenols, tannins, glycosides and steroids in the dried seeds of *Cicer arietinum*. This herb has many medicinal uses and is a nontoxic traditional medicinal plant. Numerous medicinal therapies treat their patients with herbal medicines for its extraordinary influence, though relatively little knowledge about their mode of action is available. This versatile plant is the source of many bioactive compounds. The present work can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant material in future prospective study. The bioactive phytochemicals of *Cicer arietinum* responsible for various pharmacological activities require further investigation at scientific level.

Plants are sources of new natural products used in pharmaceutical, cosmetic and food production. An *in vitro* antioxidant assay provides scientific evidence to prove the traditional claims to the *Cicer arietinum* (Chik pea). On the basis of the present results it can be concluded that the methanol extracts of seeds of this plant possess significant antioxidant activity. Presence of an adequate amount of phenolics and flavonoids accounts for this fact. So the present investigation suggests that Chik pea plant is a potential source of natural antioxidant. The active phytochemicals responsible for antioxidant activity and their mechanism of action *in vivo* as well as *in vitro* require further investigation at scientific level.

**Conflict of interest:** Authors declare no conflict of interest directly or indirectly.

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