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Phytochemical Analysis, Antioxidant Activity and Acute Toxicity Study of *Cicerarietinum* and *Brassica oleracea var. italica*

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Abstract
Plants have various bioactive components which are responsible for their different biological activities and provides the basis for using plants for treatment of several diseases. The present study involves two different plants *Cicerarietinum* and *Brassica oleracea var. italica*, locally available in Dehradun, Uttarakhand. Sprouts of *Cicerarietinum* and leaves of *Brassica oleracea var. italica* were washed, air dried and then powdered. The aqueous and methanolic extracts of these plants were used for the phytochemical anaysis. Besides phytochemical assays, antioxidant activity of these plants were also done. Acute toxicity study was done on Wistar rats to determine the toxicity level of these extracts.

The methanolic extract of *Cicerarietinum* sprouts (CAM) and aqueous extract of *Cicerarietinum* sprouts (CAA); methanolic extract of *Brassica oleracea var. italica* leaves (BOM) aqueous extract of *Brassica oleracea var. italica* leaves (BOM) possess various active constituents. CAA exhibit tannins, saponins and phenols, whereas CAM possess tannins, saponins, alkaloids, flavonoids and phenols. In contrast, BOA and BOM both containalkaloids, tannins, flavonoids and phenols. While saponins are present only in BOA. The TPC results showed that BOM (247.79±0.710mg GAE g^{-1}) has the highest phenolic content in it. In TFC analysis, BOA showed the highest level of flavonoids (104.20±0.10mg QE g^{-1}). In DPPH scavenging assay, BOM (87.22±0.011) showed maximum % inhibition. After acute toxicity study plant extracts were found to be safe even at highest concentration of 2000 mg kg^{-1}.

Keywords-Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Diphenylpicrylhydrazyl (DPPH).

1. Introduction

Medicinal plants are comprised of enormous and varied active compounds known as secondary metabolites, which are used since a long time to treat human diseases. Many important phytocompounds are phenolics, flavonoids, tannins, alkaloids, terpenoids and saponins. Secondary metabolites are important as they possess therapeutic properties for a wide range of diseases and disorders along with low toxicity as compared to the synthetic drugs (Inayatullah et al., 2012). Several phytocompounds isolated from plants are used as a lead compounds to slow down the growth of bacterial and fungal pathogens and to destruct Reactive Oxygen Species (ROS) with probably small toxicity to the host cell and new mechanism of action (Ahmad and Aqil, 2007). Present research deals with phytochemical

analysis, estimation of total phenolic content, total flavonoid content, DPPH free radical scavenging activity and acute toxicity study of CAA, CAM, BOA and BOM. Free radicals are extremely toxic and are concerned with the occurrence of many persistent diseases which finally leads damage to nucleic acids, proteins and lipids. Even though a natural arrangement of antioxidant present in our body, but to dispose of tremendous free radicals, some extrinsic antioxidants are suggested (Yanishlieva et al., 2006). Two types of antioxidants are known, natural and synthetic, but due to their harmful effects such as toxicity and carcinogenicity, synthetic antioxidants namely butylhydroxyanisole and butylhydroxytoluene are being substituted with natural antioxidants (Botterweck et al., 2000).

Cicerarietinum (chick pea or Bengal gram) be a part of the family Fabaceae. It is reported that *Cicerarietinum* contains high amount of protein content and one of the oldest cultivated vegetables, some residue have been found in the Middle East, 7500-years ago. The seeds are brown with an important characteristic “beak” present in the embryonic axis. Seeds are generally consumed entire, and sometimes they are peeled and used. The seeds are of various therapeutic uses which includes the cure of different ailments burning sensation, hyperdipsia, leprosy, inflammation, skin diseases and bronchitis (Sandeep et al., 2012).

Brassica oleracea L. var. italica (Broccoli) be a part of the family Brassicaceae. It serves worldwide an important part of the human feed. Brassicaceae vegetables are considered as imperative food crops in India, Japan, China and European countries. The plant has bundle of fully distinguished flower buds on main head which are less compactly set with longer peduncles. Broccoli attain a height of 400 mm during vegetative phase. It is an annually growing herb and 1-2 m high at the end of flowering. Broccoli has anticancer, antioxidant and antimicrobial activities (Sibi et al., 2013).

2. Materials and Methods

2.1 Plant Collection

The seeds of *Cicerarietinum* and leaves of *Brassicaoleracea var. italica*plants (30° 19' N, 78° 04' E, Dehradun) were collected and authenticated by Dr. S. K. Srivastava, Scientist ‘D’ Botanical Survey of India, Dehradun, Uttarakhand.

2.2 Preparation of Plant Extracts

The dried sprouts of *Cicerarietinum* seeds and leaves of *Brassica oleracea var. italic* were grinded to make fine powder. 10 g of crushed sample was soaked in 100 ml of distilled water and methanol, separately for 72 hrs at room temperature. The extracts were then filtered, dried and then subjected to phytochemical analysis.

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Table 1. Phytochemical analysis of methanolic extract of *Cicerarietinum* seed sprouts (CAM), aqueous extract of *Cicerarietinum* seed sprouts and (CAA), methanolic extract of *Brassica oleracea var. italica* leaves (BOM) and aqueous extract of *Brassica oleracea var. italica* leaves (BOA). (+) indicates presence of phytoconstituent, (-) indicates absence of phytoconstituent

Phytochemicals	CAM	CAA	BOM	BOA
Alkaloids	+	-	+	+
Phenols	+	+	+	+
Tannins	+	+	+	+
Steroids	-	-	-	-
Saponins	+	+	-	+
Flavonoids	+	-	+	+

2.3 Preliminary Phytochemical Screening

Presence or absence of phytoconstituents was done by the qualitative tests. Plant extracts were screened for flavonoids, alkaloids, steroids, phenols, saponins and tannins by using standard methods.

2.3.1 Test for Alkaloids

One ml sample extract was taken in a test tube and then 1ml Wagner's reagent was added. A brown colour precipitation confirms that alkaloids were present in the sample (Wadood et al., 2013).

2.3.2 Test for Phenols

In a test tube 1 ml of sample extract was taken and some drops of ferric chloride were added to it. Emergence of dark green colour indicates that phenols were present in the sample (Ramya et al., 2012).

2.3.3 Test for Tannins

In one ml of sample extract, 1 ml of 0.1% ferric chloride containing 0.1 N HCl was added. A blue-black colouration confirms that tannins were present in the sample (Iyengar, 1995).

2.3.4 Test for Steroids

In one ml crude sample extract, 2 ml of acetic anhydride and 2 ml of H_2SO_4 was added. The change in colour from violet to blue indicates that sterols were present in the sample (Siddiqui and Ali, 1997).

2.3.5 Test for Saponins

One ml of the sample extract was taken in a test tube and mixed with 5 ml of distilled water, then shaken strongly until the froth formation. A froth formation indicates the presence of saponins (Kalita et al., 2013).

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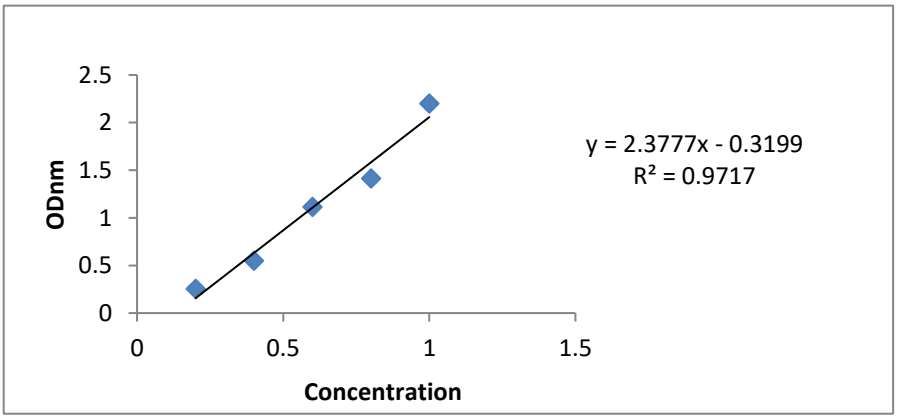
2.3.6 Test for Flavonoids

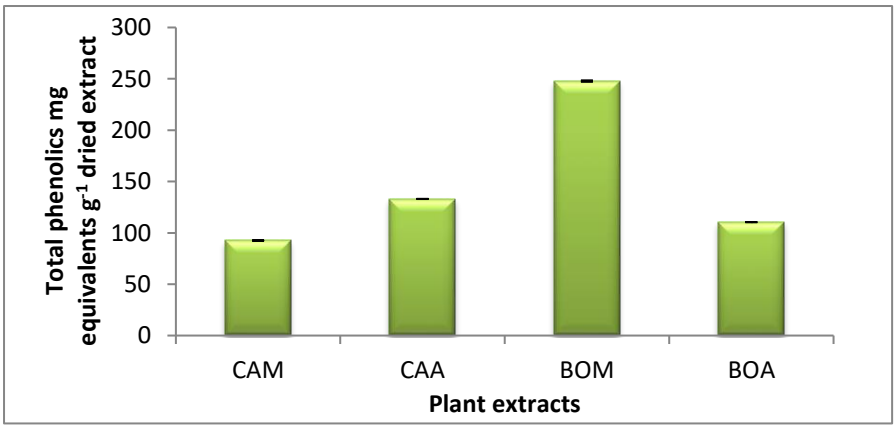
In 1 ml of sample extract, 1 ml of dilute ammonia solution was added, subsequently with the addition of concentrated H_2SO_4. A yellow colour indicates that flavonoids were present in the sample extract (Edeoga et al., 2005).

2.4 Total Phenolic Content

The Total Phenolic Content (TPC) of *cicerarietinum* sprouts and *Brassica oleracea var. italica* leaves extracts, Folin-Ciocalteau method was determined as described by Skerget et al. (2005). In a test tube containing sample, standard or positive control (150 µl), 500µl of 1:10 Folin-Ciocalteau phenol reagent was added to it. Then mixture present in the test tube was allowed to incubate for 5 min and 350 µl of 10 % sodium carbonate (Na_2CO_3) was added to it. The reaction mixture was further incubated for 2 h in the dark, at Room Temperature (RT). Absorbance was taken at 765 nm using a spectrophotometer. A standard solution of gallic acid was prepared in methanol with concentration 0.2, 0.4, 0.6, 0.8 and 1 mg ml^{-1}. The sample exract with concentration of 1 mg ml^{-1} was also prepared in methanol. The TPC was expressed as milligrams of gallic acid equivalents per g (mg GAE g^{-1}) of extract.



(A)



(B)

Figure 1. (A and B) Total phenolic content. Total phenolic content of methanolic and aqueous extracts of *Cicerarietinum* and *Brassicaoleracea var. italica*. Each value is expressed as the mean ± standard error

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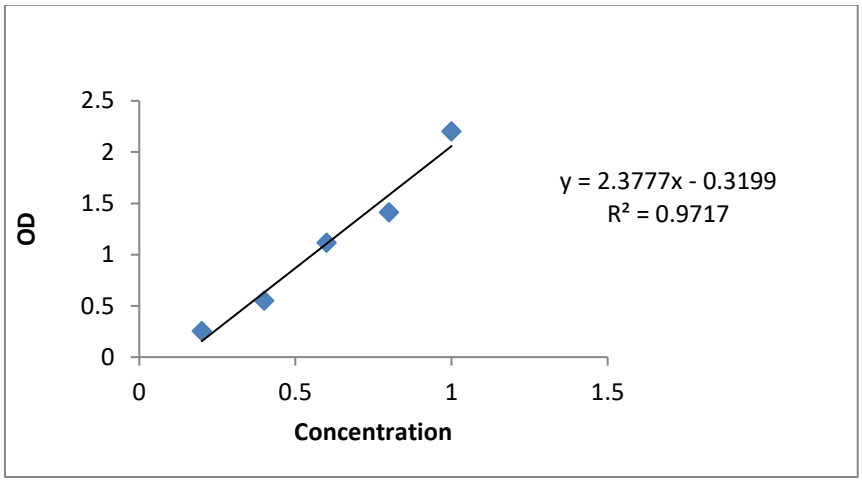
Table 2. Total phenolic content

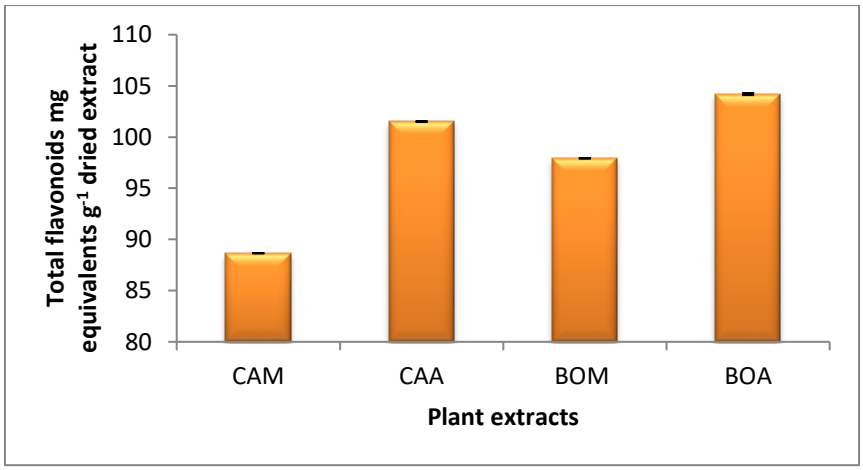
S. No.	Plant extracts	Total phenolic content (mg GAE g^{-1})
1.	CAM	92.55±0.007
2.	CAA	133.05±0.087
3.	BOM	247.79±0.710
4.	BOA	110.43±0.028

2.5 Total Flavonoid Content

To determine total flavonoid content aluminium chloride colorimetric assay was used (Kalita et al., 2013). In a test tube, 0.3 ml of sample extracts, 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$ (0.5 M) and 0.15 ml of $AlCl_3.6H_2O$(0.3M) were mixed. After 5 min, 1 ml of NaOH was added to reaction mixture. The absorbance was measured at 506 nm using UV-Vis spectrophotometer. The standard curve of quercetin was made to quantify total flavonoid content and it was expressed as QE $g^{-1}$of extract.



(A)



(B)

Figure 2. (A and B) Total flavonoid content. Total flavonoid content of methanolic and aqueous extracts of *Cicerarietinum* and *Brassicaoleracea var. italica*. Each value is expressed as the mean ± standard error

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Table 3. Total flavonoid content

S. No.	Plant extracts	Total flavonoid content (mg QE g^{-1})
1.	CAM	88.65±0.0007
2.	CAA	101.52±0.008
3.	BOM	97.92±0.016
4.	BOA	104.20±0.10

2.6 DPPH Assay

The antioxidant activity of the extracts was estimated by 1, 1- Diphenyl 2-Picrylhyorazyl (DPPH), which is a stable free radical, as per the method stated by Brand-Williams et al. (1995) with few modifications. One ml of sample extract solution of varying concentrations (50, 100, 150, 200 and 250 µg ml^{-1}) was mixed with 1ml of DPPH (0.1mM) solution in methanol. The L-Ascorbic acid (1-100 µg ml^{-1}) was used as reference standard. Control was used as 1ml methanol and 1ml DPPH solution. After 30 minutes incubation in dark, the decreased absorbance was measured at 517 nm, using UV-Vis spectrophotometer. The inhibition % was calculated as.

$$\text{Inhibition \%} = A(c)-A(s)/A(c)\times 100$$

Where A(c) is the absorbance of the control and A(s) is the absorbance of the sample.

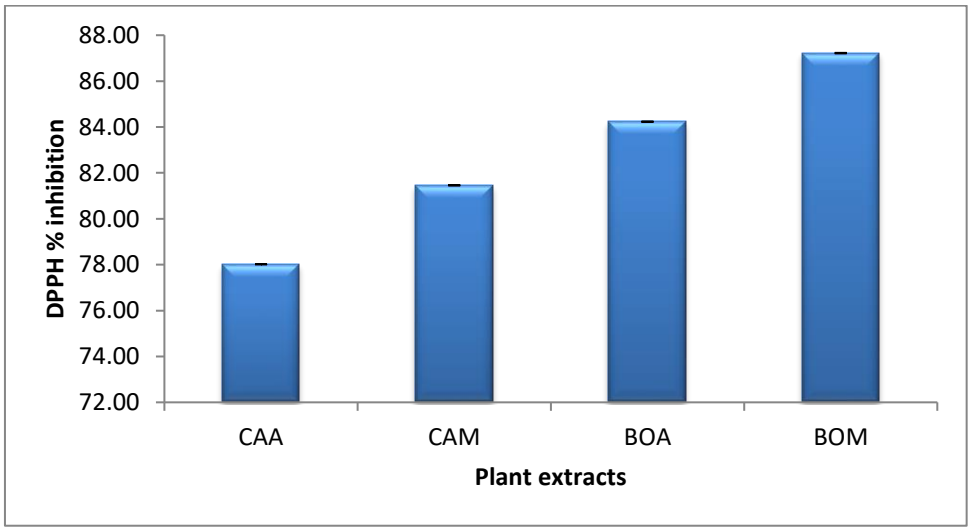

Figure 3. DPPH % Inhibition. Free radical scavenging activity of methanolic and aqueous extracts of *Cicerarietinum* and *Brassicaoleracea var. italica*. Each value is expressed as the mean ± standard error

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Table 4. DPPH % Inhibition

S. No.	Plant extracts	DPPH % Inhibition
1.	CAM	81.46±0.003
2.	CAA	78.02±0.004
3.	BOM	87.22±0.011
4.	BOA	84.23±0.003

2.7 Acute Toxicity Study

Acute toxicity indicates the effects of a single dose of a chemical on the whole body, usually manifested over a period of 14 days. Male Wistar rats of Seven weeks old, weighing within 200-250 g, obtained from the Pinnacle Biomedical Research Institute, Bhopal, M. P. India, were used for this study. The experimental protocol was approved by the Institutional Animal Ethics Committee (Reg. No. 1824/PO /ERe /5115/ CPCSEA and Protocol approval number is PBRI/IAEC/PN-017). A stepwise procedure of acute toxic class method was used with 3 animals of single sex per step. They were kept in standard environmental conditions of temperature at 24 ± 2 °C, humidity 45 ± 5% under a 12 h dark-light cycle, and were permitted free entrance to water and food. Animals were kept in the animal house for one week prior to dosing to allow them to be acclimated in the environment. The animal experiments were performed as per the guidelines of CPCSEA (*Committee for the Purpose of Control and Supervision of Experiments on Animals).*The methanolic and aqueous extracts of sprouted seeds of *Cicerarietinum* and leaves of *Brassica oleracea var. italica*, administered at various doses (5, 50, 300 and 2000 mg kg^{-1}) orally to Wistarrats. After the drug administration animals were observed continually, during the first three hours for behavioural changes. Other symptoms such as convulsions, hyperactivity, hypothermia, sedation and mortality were noted up to 14 days.

Table 5. *In vivo* acute toxicity study of *Cicerarietinum* sprouts

Extracts treatment	Dose	Signs of toxicity (hyperactivity, convulsions, sedation)	Death
CAM and CAA	5 mg kg^{-1}	No signs	0/3
	50 mg kg^{-1}	No signs	0/3
	300 mg kg^{-1}	No signs	0/3
	2000 mg kg^{-1}	No signs	0/3

2.8 Statistical Analysis

The results were expressed as mean ± S.E.M. The differences were compared using one way Analysis of Variance (ANOVA) and subsequently followed by Bonferroni's test.

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Table 6. In vivo acute toxicity study of *Brassica oleracea var. italica* leaves

Extracts treatment	Dose	Signs of toxicity (hyperactivity, convulsions, sedation)	Death
BOM and BOA	5 mg kg^{-1}	No signs	0/3
	50 mg kg^{-1}	No signs	0/3
	300 mg kg^{-1}	No signs	0/3
	2000 mg kg^{-1}	No signs	0/3

3. Results and Discussion

The present study reveals the presence of various active phyto constituents in CAM, CAA, BOM and BOA (Table 1), also showed significant level of total phenolic (Figure 1 and Table 2) and total flavonoid content (Figure 2 and Table 3). Antioxidant activity showed good range in all the extracts (Figure 3 and Table 4). The Phytochemical constituents contribute to various biological activities like antioxidant, antidiabetic, antimalarial activities. In the screening analysis *Cicerarietinum* or Bengal gram, phenols were found to be present, which showed that antioxidant activity is present and also hepato protective property of *C.arietinum.* Tannins were also present in *C.arietinum* and helps to fasten the healing of wounds and reduce inflammation in mucous membranes. *C. arietinum* also contain flavonoids which accounts for powerful anticancer and hepato protective activity. Flavonoids are putative antioxidants, helps in mitigating liver diseases, diabetes as well. Terpenoids plays important rolein treatment of various diseases like necrosis and cancer. It is known to have antimicrobial, anti-inflammatory, antiviral properties. Steroids helps in reducing cholesterol levels and regulating immune system. Proteins are the building blocks of life and presence of proteins helps in repairing and maintaining the body (Sultana and Ata, 2008; Rabi and Bishayee, 2009). Sprouted seed extract of *Cicerarietinum* shown high level of total phenolic, flavonoids and antioxidant activity as compared to non-sprouted seed extracts (Singh and Kaur, 2015). *Brassica oleracea var. italic* (Broccoli) contains health-promoting properties which attributes to its antioxidant and anti-carcinogenic compounds. It is primarily composed of sulforaphane, glucosinolates, polyphenols and selenium. Sulforaphane prevents neurodegenerative diseases like Parkinson's disease and Alzheimer's disease. Some active components such as Isothiocyanates (ITCs) are known for their anticancer activity (Ravikumar, 2015**)**. As reported earlier the higher dose (2g kg^{-1}) of *C. arietinum* taken for acute toxicity study was non-toxic and no signs of mortality was observed (Mekky et al., 2016). No toxicity was observed in Wistar rats administered with CAA, CAM, BOA and BOM at a dose of 2000 mg kg^{-1} (Table 5 and 6).

4. Conclusion

The current study showed that CAA, CAM, BOA and BOM possess various active components in it. The BOM possess good antioxidant activity and highest phenolic content among all the extracts, while BOA exhibit highest flavonoid content in it. Acute toxicity study showed no toxicity at 2000 mg kg^{-1} of all the extracts. Therefore, these extracts with various activities are safe for therapeutic use.

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References

Ahmad, I., & Aqil, F. (2007). In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiological Research, 162(3), 264-275.

Botterweck, A. A. M., Verhagen, H., Goldbohm, R. A., Kleinjans, J., & Van den Brandt, P. A. (2000). Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. Food and Chemical Toxicology, 38(7), 599-605.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30.

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4(7), 685-688.

Inayatullah, S., Prenzler, P. D., Obied, H. K., Rehman, A. U., & Mirza, B. (2012). Bioprospect-ing traditional Pakistani medicinal plants for potent antioxidants. Food Chemistry, 132(1), 222-229.

Iyengar, M. A. (1995). Study of crude drugs, Manipal Power Press, Manipal, India. 8^{th} edition, 2.

Kalita, P., Tapan, B. K., Pal, T. K., & Kalita, R. (2013). Estimation of total flavonoids content (TFC) and anti oxidant activities of methanolic whole plant extract of *Biophytumsensitivum* Linn. Journal of Drug delivery and Therapeutics, 3(4), 33-37.

Mekky, R. H., Fayed, M. R., El-Gindi, M. R., Abdel-Monem, A. R., del Mar Contreras, M., Segura-Carretero, A., & Abdel-Sattar, E. (2016). Hepatoprotective effect and chemical assessment of a selected egyptian chickpea cultivar. Frontiers in Pharmacology, 7(344), 1-9.

Rabi, T., & Bishayee, A. (2009).Terpenoids and breast cancer chemoprevention. Breast Cancer Research and Treatment, 115(2), 223-239.

Ramya, S., Krishnasamy, G., Jayakumararaj, R., Periathambi, N., & Devaraj, A. (2012). Bioprospecting Solanumnigrum Linn. (*Solanaceae*) as a potential source of Anti-Microbial agents against selected Bacterial strains. Asian Journal of Biomedical and Pharmaceutical Sciences, 2(12), 65-68.

Ravikumar, C. (2015). Therapeutic potential of Brassica oleracea (Broccoli) - A review. Ravikumar, Int J Drug Dev & Res, 7(2), 009-010.

Sandeep, D., Sandya. L., Chandra Kalyan Reddy, Y., Nagarjuna, S., Padmanabha Reddy, Y., & Shafeen, S. (2012). Anti-inflammatory activity of *cicerarietinum* seed extracts. Asian Journal of Pharmaceutical and Clinical Research, 5, 64-68.

Sibi, G., Shukla, A., Dhananjaya, K., Ravikumar, K. R., & Mallesha, H. (2013).In vitro antibacterial activities of Broccoli (*Brassica oleracea L. varitalica*) against food borne bacteria. Journal of Applied Pharmaceutical Science, 3(05), 100-103.

Siddiqui, A. A., & Ali, M. (1997).Practical pharmaceutical chemistry.1st edn, CBS Publishers and Distributors, New Delhi, 126-131.

Singh, S., & Kaur, J. (2015). Effect of sprouting on in vitro antioxidant potential of some varieties of chickpea seeds (CICER ARIETINUM LINN). Asian Journal Pharmaceutical and Clinical Research, 8(6), 265-268.

Skerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonic, M., & Knez, Z. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry, 89(2), 191-198.

Sultana, N., & Ata, A. (2008). Oleanolic acid and related derivatives as medicinally important compounds. Journal of Enzyme Inhibition and Medicinal Chemistry, 23(6), 739-756.

Wadood, A., Ghufran, M., Jamal, S. B., Naeem, M., Khan, A., Ghaffar, R., & Asnad (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochemistry and Analytical Biochemistry, 2(4), 1-4.

Yanishlieva, N. V., Marinova, E., & Pokorný, J. (2006). Natural antioxidants from herbs and spices. European Journal of Lipid Science and Technology, 108(9), 776-793.