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Effect of germination on *in vitro* Antidiabetic activity of *Cicer arietinum* L

1* Manjunatha K S
Assistant Professor
Depatrtment of Biotechnology
Sir M V Government Science College, Bhadravathi, India
E-mail: manjuks1@gmail.com

2. Dr C K Ramesh
Professor
Department of Biotechnology,
Sahyadri Science College, Shivamogga, India
E-mail: ckramck@gmail.com

Abstract

Chana (*Cicer arietinum)* is a common food plant worldwide. This crop is well documented as a medicinal plant in traditional medicine systems. The antidiabetic activity of Chana seed extracts were examined *in vitro* and compared with the activity in germinated seed extracts. The results indicated the enhancement of activity upon germination. The dry seeds (C1) showed minimum activity in *in-vitro* α-glucosidase inhibition compared to the germinated sprouts of different intervals (C2, C3 and C4). *In vitro* α—amylase inhibition activity also proved to enhanced upon germination. The germination therefore is said to boost up the antidiabetic activity in Chana seeds.

Introduction

Pulses are apprehended as the ultimatum protein source in the vegetarian Indian diet with fair amount of minerals, fatty acids, vitamins and dietary fiber or non-starch polysaccharides and hence recently, pulses have also been established as functional foods. They have been supplementing the cereal based diets and hold a crucial position in human nutrition (Mbithi-Mwikya *et al*. 2000, Rao, 2002).

Cicer arietinum L., commonly known as chickpea or Chana, is a major pulse crop of the globe. It confers positive health impacts and, combined with other legumes and grains, employed to treat some of the prevalent human diseases (Jukanti *et al*. 2012).

Chana is a prominent member of Leguminosae or Fabaceae family, and is also familiar by names such as Bengal gram and Egyptian pea. It is hrld as one of the initially farmed legume crops since 7500-year old remains of the plants are discovered in the Middle East. It is the only cultivated species among the 43 species of the *Cicer* genus. India being one of the major Chana producers has Chana as an important ingredient in Indian and Middle Eastern cuisine. India

recorded a yield of around 90,75,000 tonnes of Chana in 2017 as per the data of FAO. India hails to be the world's largest producer of Chana (Heuzé *et al*. 2015; Wallace *et al*. 2016).

Taxonomic Classification of Cicer arietinum

Kingdom: Plantae

Subkingdom: Viridiplantae

Super division: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Super order: Rosanae

Order: Fabales

Family: Fabaceae

Genus: Cicer

Species: *Cicer arietinum*

(Source: Integrated Taxonomic Information System (ITIS) report April, 2019)

Chana is a premium resource of nutrients with a high amount of protein, folic acid, dietary fiber, and minerals including iron, phosphorus, magnesium, zinc, thiamin, and Vitamin B6. Both the cooked and germinated Chana have a commendable amount of essential amino acids and total aromatic amino acids on par to the reference standards of the FAO and WHO. Chana seeds are generally devoid of sulfur containing amino acids, which is usually complemented by cereal grains added to the food (Jukanti *et al*. 2012).

In Ayurveda, Chana seeds are extensively used as tonic, stimulant, aphrodisiac, anthelminthic, appetizer and to treat blood dyscrasias, ear infections, liver and spleen disorders. In Chinese herbal medicine, Chana has been extensively used for treating hypertension and diabetes for over the past 2500 years (Harini *et al*. 2015). Chana is commended as one of the food candidates with potentials in the management of diabetes. The phytochemicals have demonstrated the capacities in lowering the clinical complications related to several diseases (Gupta *et al*. 2016; Aisa *et al*. 2019).

Germination is held as the most effective and inexpensive processes to increase the dietary values of legumes. Germination enhances protein digestibility, breaks down proteins to simple peptides and lowers the amount of amino acids, sulphur and total aromatic amino acids, though the concentration is greater than the proposed standards. Germination is known to enhance the

enzyme synthesis required to eliminate or reduce the antinutrients. (El-Adawy, 2002; Tarzi, 2012).

Antidiabetic activity

Diabetes mellitus, a chronic metabolic impairment marked with elevated blood glucose concentrations, is due to defective glucose metabolism that appears when the pancreas fails to produce required level of insulin and when the body flounders to utilize it. As per the reports of International Diabetes Federation till 2010 India had highest number of diabetics than any other country, which is now surpassed by China. Diabetes is said to be affecting more than 72 million Indians, which almost equates to 8.8% of the adults. This high incidence could be blamed to the genetic vulnerability, leading a high-calorie and poor-activity lifestyle by India's emerging middleclass society and combination of all these. Diabetes is also linked with an increased level of serum lipids which leads to the coronary heart diseases. It has been reported that hyperglycemic condition conjoined with the variations in carbohydrate, lipid and protein metabolisms along with oxidative stress are more likely to disturb hepatic adrenal functions too (Nabi *et al.* 2013; Godavari and Amutha, 2017).

Besides using insulin for treating the insulin dependent diabetes mellitus (IDDM), other way to manage is to use amylin analogues that slow down the gastric emptying and delay postprandial glycemic level rise. Sulphonylureas and Metformin are also commonly used but these drugs also lead to few side-effects like induction of hypoglycemia if high doses are administered, liver disorders, lactic acidosis as well as diarrhea. Therefore inferring the side effects of these drugs, there is definitely a need for a remedial substitute that is safe with least possible negative impacts and that can be administered for longer durations (Bhan *et al.* 2015).

Certain indigenous plants are helpful in managing diabetes. One of the winning edge qualities in such plants is that these are easy to get and have no or very negligible side effects. Plants are always regarded as excellent sources of pharmacologically active compounds and many of the present medicines are being extracted from plants (Arumugam *et al.* 2013).

Many clinical and experimental proofs clearly imply the inclusion of oxidative stress in the induction of diabetes as well as its complications. Thus, it becomes crucial to scale down the oxidative stress in diabetics to minimize the severity. Since plants synthesize a good amount of antioxidant molecules to manage oxidative stress induced by photons as well as oxygen, they are regarded a promising resource of novel elements bearing antioxidant and antidiabetic properties (Bhutkar and Bhise, 2011).

Antidiabetic property of plants can be ascertained *in vitro* employing a variety of biological test systems. Such systems have a pivotal role as an initial screening tool before the in vivo studies. In vitro assay provides a basic platform for assessing these plant extracts and help us understand various mechanisms that would alleviate hyperglycemia in diabetes (Dsouza and Lakshmidevi, 2015).

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Despite human consumption of Chana has a long history because of its superior dietary attributes and being a member of the "founder crop package" with exceptional pharmacological values, it has barely gained due research attention as functional foods on par with other founder crops such as wheat and barley. Although a few researches have been documented on the physicochemical and nutritional attributes, still the information is inadequate (Jukanti *et al*. 2012).

For the present investigation, Chana was selected due to its immense value in providing optimal nutrition and as medicine. The present study is determined to provide a validation for the germination associated medicinal properties.

Materials and Methods

The dried seeds of Chana – *Cicer arietinum* var., JG-11 were procured from the Institute of Horticultural Sciences, Bengaluru. The samples were cleaned. Half a kilogram of dried chana seeds were blended and the powder was stored separately. One and half kilogram of dried seeds of chana was divided into 3 batches of 500 g each, and immersed in distilled water for 24 hrs and later the seeds were spread on wet cotton cloth. One batch of 500 g seeds was allowed for 24 hrs germination, the second batch of seeds was allowed for 48 hrs germination and the last batch for 72 hrs germination. The germinated seeds were dried separately at 45^{o}C using hot air oven, grounded and stored for further use.

The process of sample extraction was performed as per the protocol explained by Jamuna *et al*. (2015). Exactly 200 g of sample was extracted in 70% ethanol for 7 days in dark at room temperature with intermittent shaking. Then the extracts were subjected to filtration with muslin cloth and condensed employing a rotary evaporator (Buchi, Flaweli, Switzerland). The sample-yield is noted, stored in desiccators for 3-4 days, and later stored in a deep freezer in separate containers. The extracts were labeled as C1 (ethanolic extract of dried Chana seeds), C2 (ethanolic extract of 24 hrs germinated Chana seeds), C3 (ethanolic extract of 48hrs germinated Chana seeds) and C4 (ethanolic extract of 72 hrs germinated Chana seeds).

In-vitro Antidiabetic activity

1) In vitro α-glucosidase inhibition assay

The α-glucosidase inhibitory capacity of Chana extracts of seeds and sprouts were measured by the standard method as described by Matsui *et al*. (1996) with few modifications. Briefly, 50 µL of sample and 50 µL of 0.1M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.1µ/mL) was thawed at 37°C for10 min. Later, 50 µL of 2.5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) solution in the same buffer was added to each well and incubated for 20 min. Then, the reaction was stopped by adding 100 µL of 0.2M Na2CO3 and absorbance (A) was recorded at 405 nm by microplate reader (BioTek, USA) and compared to control, which had 50 µL of buffer solution in place of the extract.

2) In vitro α-amylase inhibition assay

Chana extracts of seeds and sprouts were subjected to the α-amylase inhibitory activity assay as per the standard method of Xiao *et al* (2006) with modifications. The 50 µL of sample (0.02M phosphate buffer, pH 6.9) was premixed with 100 µL of α-amylase solution (1.0 µ/mL in the pH 6.9 buffer), and incubated at 25°C for10 minutes. After, 200 µL of a 0.25% starch solution was added to start the reaction for 5 minutes and terminated by addition of 1.0 mL of the DNS reagent. The test tubes were then kept on a boiling water bath for 5 minutes and cooled to room temperature, and absorbance was measured at 540 nm using an ultraviolet-visible spectrophotometer (Shimadzu, Japan). The control had 200 µL of buffer solution in place of α amylase solution. The enzyme inhibitory activity was expressed as percentage inhibition and was calculated as follows:

Enzyme inhibitory activity (%) = $(A_{control} - [A_{test} - A_{background}]) / {}_{Acontrol} \times 100$ where $A_{control}$, A_{test}, and $A_{background}$ are the absorbance of 100% enzyme activity (only the solvent with the enzyme), test sample with the enzyme and test sample without the enzyme, respectively.

The concentration of inhibitors required for inhibiting 50% of the enzyme activity under the assay conditions was presented as the IC50 value.

Results and Discussions

1. ***In vitro* α-glucosidase inhibition**

α-glucosidase inhibition activity assay in Chana revealed that the activity increases upon germination. C4 reported the maximum inhibitory capacity in comparison with the extracts of dried seeds and other sprouts. Minimum IC50 value was recorded for C4 (150.7±1.5) and maximum for C1 (232.5±2.5).

Table– *In vitro* α- glucosidase inhibition assay of Chana extracts

Conc	**C1**	C2	**C3**	**C4**
30	5.25±1.6	6±2.3	7.9±1.1	9.8±2.8
60	7.45±2.2	7.35±2.5	10.5±2.4	12.5±1.9
90	12.4±2.1	11.3±1.8	11.75±2.3	25.3±2
120	17.3±1.7	18.5±1.4	19.5±1.8	35.24±2.4
150	24.5±1.6	26.5±2.2	35.7±2.6	49.8±1.5
180	30.45±1.4	34.25±1.2	44.5±0.9	56.45±2.7
210	43.25±2.6	47.75±1.3	48.9±1.2	65.6±2.3

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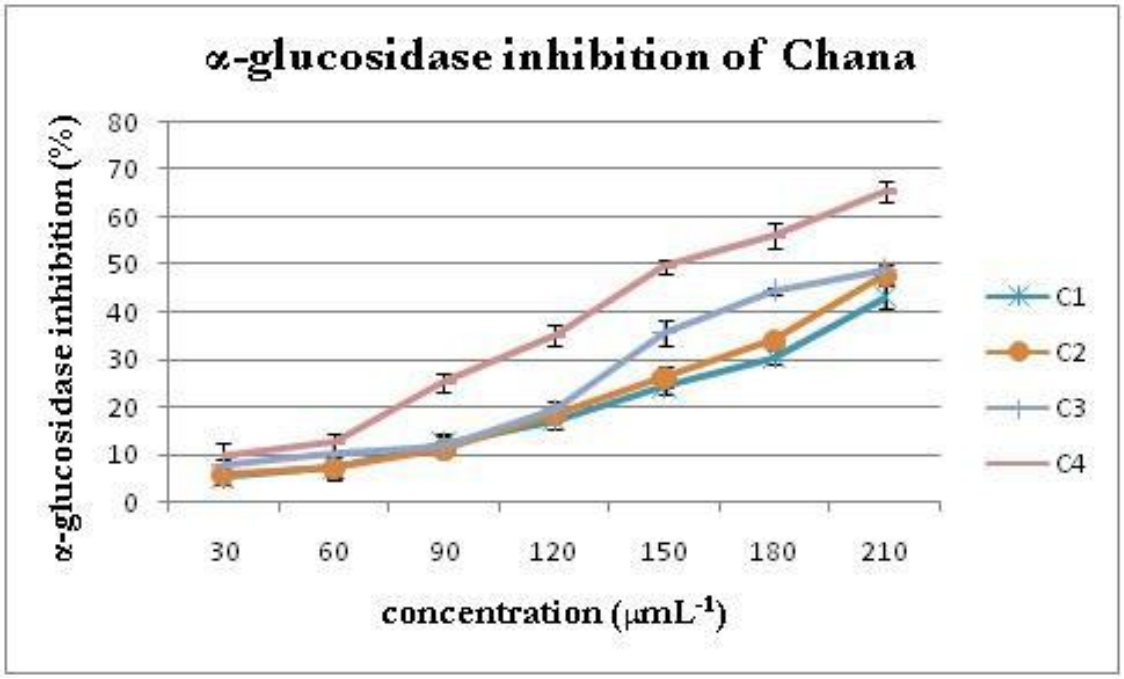

2. *In vitro* α—amylase inhibition activity

Chana recorded a maximum inhibition against α—amylase in germinated stage. C4 showed to be having a better inhibitory activity than C1, C2 and C3. The results are shown in Table 22 and Figure 21. Minimum IC50 value was recorded for C4 (115.28±2.2) and maximum for C2 (237.5±2.7).

Conc	**C1**	**C2**	**C3**	**C4**
30	9.52±1.7	15.13±1.6	22.87±1.6	24.86±1.6
60	13.5±1.8	18.2±2.9	25.7±1.5	30.1±1.3
90	20.04±1.9	24.75±1.8	30.5±1.2	38.8±1
120	32.6±1.6	40.02±1	42.12±1.8	52.1±1.2
150	46.9±1.3	57.2±1	59.5±1.4	70.3±1.4
180	52.45±1.2	64.6±1	69.7±1.2	80.5±1.2
210	59.93±1.3	73.6±1.2	77.9±1.7	87.75±1.5

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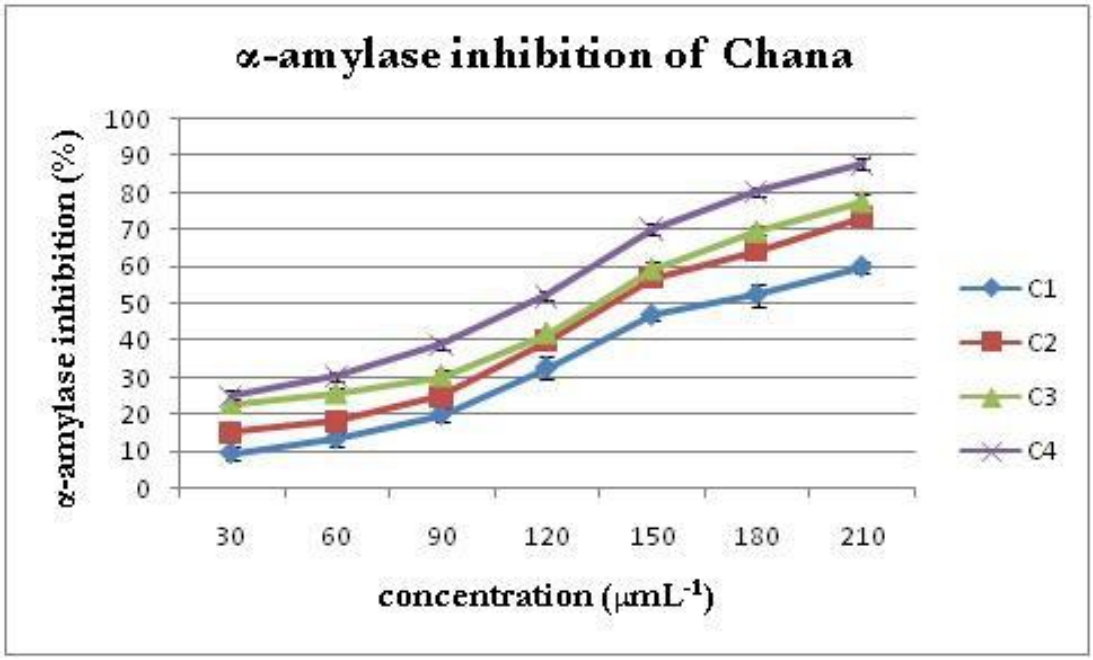

IC50 values of Antihyperglycemic activities - The IC50 values of α-glucosidase inhibition and α-amylase inhibition activities of ethanolic extracts of seeds and sprouts of Chana are shown in Table

IC50 value of In vitro antihyperglycemic activities

	IC50 value of α-glucosidase inhibition				IC50 value of α-amylase inhibition			
Chana	C1	C2	C3	C4	C1	C2	C3	C4
	232.5±2.5	217.5±2.1	213.6±2.1	150.7±1.5	166.8±1.85	137.5±2.7	133.6±2.9	115.48±2.2

The result of antidiabetic activity of Chana extracts is in consistent with the results of several earlier investigations. Prathapan *et al*. (2010), Gan *et al*. (2017) and Domínguez-Arispuro *et al*. (2018) reported that germination of Chana seeds results in an increase in the level of phytochemicals that have major role in the antidiabetic activity. Yang *et al*. (2007), Xue *et al*. (2012), Mollard *et al*. (2014) and Zakaria *et al*. (2016) suggested that Chana seeds have a low glycemic index and significantly improve insulin resistance preventing post-prandial hyperglycemia and hyperinsulinemia. Because of the low glycemic index (GI) and the high content of undigestible fibres, dry legumes are claimed to help glycemic control in diabetic individuals (Duranti, 2006). Vasishtha and Srivastava (2012) reported that a component called resistant-starch of Chana that induces slow glycemic condition which helps in treating in insulin-resistant diabetics. Chana have certain enzyme inhibitors, phytic acid and phenols that are

accountable for the antioxidant, antidiabetic, antiobese and other such pharmacological activities (Milán-Carrillo *et al*. 2007).

Conclusion

The current study obviously generated important information regarding the antidiabetic potentialities of a pulse crop. The seeds of these plants and their sprouts were subjected to phytochemical analysis and plausible antidiabetic potentialities by utilizing standardized experimental protocols. From the research it is proven that both dry seeds and their sprouts possess prominent activities for the parameters evaluated. The study also emphasizes on the fact that many of human ailments such as diabetes and lipidemia in particular could be managed and/or curated by consumption of appropriate diets.

References

1. Aisa, H.A., Gao, Y., Yili, A., Ma, Q. and Cheng, Z., 2019. Beneficial Role of Chickpea (Cicer arietinum L.) Functional Factors in the Intervention of Metabolic Syndrome and Diabetes Mellitus. In Bioactive Food as Dietary Interventions for Diabetes (pp. 615-627). Academic Press.

2. Arumugam, G., Manjula, P. and Paari, N., 2013. A review: Anti diabetic medicinal plants used for diabetes mellitus. Journal of Acute Disease, 2(3), pp.196-200.

3. Bhan, C., Kannaujia, P.K., Shrivastava, A.K., Bairwa, S.K., Duhan, S., Prasad, H. and Prasad, D., 2015. Antidiabetic potential of bioactive extracts from fruits and vegetables: A review. Journal of progressive agriculture, 6(2), pp.1-4.

4. Bhutkar, M.A. and Bhise, S.B., 2011. Comparative Studies on Antioxidant Activity of Some Antidiabetic Plants. Research Journal of Pharmacy and Technology, 4(9), pp.1409-1412.

5. Domínguez-Arispuro, D.M., Cuevas-Rodríguez, E.O., Milán-Carrillo, J., León-López, L., Gutiérrez-Dorado, R. and Reyes-Moreno, C., 2018. Optimal germination condition impacts on the antioxidant activity and phenolic acids profile in pigmented desi chickpea (Cicer arietinum L.) seeds. Journal of food science and technology, 55(2), pp.638-647.

6. Dsouza, D.O.R.I.N. and Lakshmidevi, N., 2015. Models to study In-vitro antidiabetic activity of plants: A review. International Journal of Pharma and Bio Sciences, pp.732-741.

7. Duranti, M., 2006. Grain legume proteins and nutraceutical properties. Fitoterapia, 77(2), pp.67-82.

8. El-Adawy, T.A., 2002. Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. Plant Foods for Human Nutrition, 57(1), pp.83-97.

9. Gan, R.Y., Lui, W.Y., Wu, K., Chan, C.L., Dai, S.H., Sui, Z.Q. and Corke, H., 2017. Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. Trends in Food Science & Technology, 59, pp.1-14.

10. Godavari, A. and Amutha, K., 2017. In vitro Antidiabetic Activity of Garcinia mangostana by Enzymatic Inhibition Assay. Research Journal of Pharmacy and Technology, 10(2), pp.508-512.

11. Gupta, R.K., Gupta, K., Sharma, A., Das, M., Ansari, I.A. and Dwivedi, P.D., 2016. Health risks and benefits of chickpea (Cicer arietinum) consumption. Journal of agricultural and food chemistry, 65(1), pp.6-22.

12. Harini, S., Adilaxmamma, K., Mohan, E.M., Srilatha, C. and Raj, M.A., 2015. Antihyperlipidemic activity of chickpea sprouts supplementation in ovariectomy-induced dyslipidemia in rats. Journal of Ayurveda and integrative medicine, 6(2), p.104.

13. Heuzé, V., Tran, G., Boudon, A., Bastianelli, D. and Lebas, F., 2015. Chickpea (# Cicer arietinum#).

14. Jamuna, K.S., Suma, M.S., Ramesh, C., Mahmood, R. and Prabhakar, B., Evaluation on Lipid Peroxidation and Antiproliferative Activities of Raphanus Sativus L. IJRDPL, 5, pp.2405-2409.

15. Jukanti, A.K., Gaur, P.M., Gowda, C.L.L. and Chibbar, R.N., 2012. Nutritional quality and health benefits of chickpea (Cicer arietinum L.): a review. British Journal of Nutrition, 108(S1), pp.S11-S26.

16. Matsui, T., Yoshimoto, C., Osajima, K., Oki, T. and Osajima, Y., 1996. In vitro survey of α-glucosidase inhibitory food components. Bioscience, biotechnology, and biochemistry, 60(12), pp.2019-2022.

17. Mbithi-Mwikya, S., Van Camp, J., Yiru, Y. and Huyghebaert, A., 2000. Nutrient and antinutrient changes in finger millet (Eleusine coracan) during sprouting. LWT-Food Science and Technology, 33(1), pp.9-14.

18. Milán-Carrillo, J., Valdez-Alarcon, C., Gutierrez-Dorado, R., Cardenas-Valenzuela, O.G., Mora-Escobedo, R., Garzon-Tiznado, J.A. and Reyes-Moreno, C., 2007. Nutritional properties of quality protein maize and chickpea extruded based weaning food. Plant foods for human nutrition, 62(1), p.31.

19. Mollard, R.C., Wong, C.L., Luhovyy, B.L., Cho, F. and Anderson, G.H., 2014. Second-meal effects of pulses on blood glucose and subjective appetite following a standardized meal 2 h later. Applied Physiology, Nutrition, and Metabolism, 39(7), pp.849-851.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

20. Nabi, S.A., Kasetti, R.B., Sirasanagandla, S., Tilak, T.K., Kumar, M.V.J. and Rao, C.A., 2013. Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. BMC complementary and alternative medicine, 13(1), p.37.

21. Prathapan, A., Fahad, K., Thomas, B.K., Philip, R.M. and Raghu, K.G., 2011. Effect of sprouting on antioxidant and inhibitory potential of two varieties of Bengal gram (Cicer arietinum L.) against key enzymes linked to type-2 diabetes. International journal of food sciences and nutrition, 62(3), pp.234-238.

22. Rao, B.N., 2002. Pulses and legumes as functional foods. NFI BULLETIN, 23(1), pp.1-4.

23. Tarzi, B.G., Gharachorloo, M., Baharinia, M. and Mortazavi, S.A., 2012. The effect of germination on phenolic content and antioxidant activity of chickpea. Iranian journal of pharmaceutical research: IJPR, 11(4), p.1137.

24. Vasishtha, H. and Srivastava, R.P., 2012. Changes in lipids and fatty acids during soaking and germination of chickpea (Cicer arietinum). Indian Journal of Agriculture and Biochemistry, 25(1), pp.14-19.

25. Wallace, T., Murray, R. and Zelman, K., 2016. The nutritional value and health benefits of chickpeas and hummus. Nutrients, 8(12), p.766.

26. Xiao, Z., Storms, R. and Tsang, A., 2006. A quantitative starch? Iodine method for measuring alpha-amylase and glucoamylase activities. Analytical biochemistry, 351(1), pp.146-148.

27. Xue, Z., Gao, J., Zhang, Z., Yu, W., Wang, H. and Kou, X., 2012. Antihyperlipidemic and antitumor effects of chickpea albumin hydrolysate. Plant foods for human nutrition, 67(4), pp.393-400.

28. Zakaria, Z., Aziz, R., Lachimanan, Y.L., Sreenivasan, S. and Rathinam, X., 2008. Antioxidant activity of Coleus blumei, Orthosiphon stamineus, Ocimum basilicum and Mentha arvensis from Lamiaceae family. Int J Nat Eng Sci, 2(1), pp.93-5.