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Research Article

Dietary Evaluation, Antioxidant and Cytotoxic Activity of Crude Extract from Chia Seeds (*Salvia hispanica* L.) against Human Prostate Cancer Cell Line (PC-3)

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ABSTRACT
Natural products have continually played an important role in drug discovery because it serves as active principles in drugs as well as templates for synthesis of new drugs. Present study is attempted to evaluate the dietary evaluation, antioxidant property and cytotoxicity (human prostate cancer cells) of crude extract of Chia seeds. Results, phytochemical screening and dietary evaluation revealed the presence of tanins, saponins, flavonoids, alkaloids, proteins, cardio glycosides, phenols and important minerals were present in the crude extract of Chia seeds. The total estimation of secondary metabolites of chia seed were found to be in moderate level. The cytotoxicity assay exposed that, crude extract of chia seeds inhibited the growth of prostate cancer cell lines (PC-3) in a dose dependent manner. In conclusion, the crude extract of Chia seeds can be used as a therapeutic option in prostate cancer cells. However, further studies are warranted to substantiate the current findings.

Keywords: Chia seeds; Dietary evaluation; Antioxidant activity; Cytotoxicity**.**

INTRODUCTION

Cancer greatly contributes to human mortality and is considered as a major threat to humankind and morbidity globally[1]. The incidence of cancer is a growing health problem around the world particularly the prostate cancer is one of the leading causes of cancer related deaths in men worldwide[2]. Prostate cancer (PCa) is recognized as one of the principal health care problems facing the male population worldwide. The world wide prostate cancer burden is expected to grow to 1.7 million new cases and 499 000 new deaths by 2030[3]. According to the 2015 statistics of the American Cancer Society estimates the current prostate cancer incidence in about 220, 800 new cases for prostate cancer and 27, 540 deaths from prostate cancer. Cancer is a major health threat in India. In India, prostate cancer ranks fifth in its incidence and fourth in mortality rate[4]. Plants have many phytochemicals with various bioactivities including antioxidant, anti inflammatory and anticancer activities[5]. Extracts of medicinal herbs such as sterols, phenolic compounds, flavonoids and tannins have positive effects against cancer, compared with chemotherapy or hormonal treatment. Natural antioxidants protect the human body against free radicals, inhibit many chronic diseases[6]. *Salvia hispanica* L., also known as chia, is an herbaceous plant, and it belongs to the family Lamiaceae. Chia is native to the region that stretches from North Mexico to Guatemala and now it is also cultivated in Southern parts of India (Mysore District, Karnataka). Its seeds were widely used by Aztec tribes for food, medicine and paints[7]. Chia seed oil contains the highest natural percentage of omega-3 fatty acids, which are essential in the human diet. Omega-3 fatty acids help to make up the phospholipids that are fundamental components of cell membranes[8]. Unsaturated Omega-3 fatty acids are nutritionally important for good health and are beneficial for individuals suffering from heart disease, diabetes and immune response disorders. Chia seeds are good source of dietary fiber, protein and antioxidants[9]. The consumption of dietary fiber improves fecal bolus formation and proper evacuation of stool, which helps prevent obesity and colon cancer. Chia seeds are losses source of antioxidants such as polyphenols which protects cardiovascular diseases and cancers[10]. Therefore the present study is aimed to evaluate the the dietary evaluation, antioxidant property and cytotoxic activity of crude extract from Chia seeds in human prostate cancer cells.

MATERIALS AND METHODS

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Table 1: Phytochemical screening of Chia seeds.

S. No	Contents	Sample
1.	Tanins	+
2.	Saponins	+
3.	Flavonoids	+
4.	Alkaloids	+
5.	Proteins	+
7.	Quinones	-
8.	Terpenoids	-
9.	Cardio glycosides	-/+
10.	Phenols	+

Table 2: Quantification of secondary metabolites compounds.

Particulars	Ethanolic extract of Chia seeds
Total phenolic content (mg/g of gallic acid equivalent)	76.32 ± 0.22
Total flavonoids (mg/g of quercetin equlvalent)	38.25 ± 1.18
Tannins (mg/g)	190.62 ± 1.29

Sample Collection and authentication

The seeds of *Salvia hispanica* L. were collected from Mysore District, Karnataka, India. The plant of *Salvia hispanica* L. was authenticated by Dr. G.V.S. Murthy, Director, Botanical Survey of India, Coimbatore, Tamil Nadu, India. A Voucher specimen was deposited in the laboratory for future reference (BSI/SRC/5/23/2015/Tech./2411).

Chemicals

DMEM, fetal bovine serum (FBS), trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and antibiotics were purchased from Himedia & Sigma Aldrich.

Plant seed extract preparation

The powdered seeds of *Salvia hispanica* L (100 g) were extracted with 500 ml of 95% ethanol.

Preliminary Phytochemical Screening

The phytochemical screening was carried out for ethanolic extract of *Salvia hispanica* L. as per procedure[11].

Quantitative analysis of secondary metabolites

Determination of total alkaloid

The alkaloid content of sample was determined as described by[12]. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. The mixture was filtered through Whatman no 1 filter paper and the filtrate concentrated to ¼ of its original volume on a water bath maintained at 90°C. Alkaloid was precipitated from each sample, using a concentrated ammonium hydroxide solution (NH_4OH) and then allowed to sediment. The whole solution was allowed to settle and the precipitated was collected and washed with concentrated NH_4OH and then dried in a hot air oven.

The residue is alkaloid and is calculated thus: (%) Alkaloid = W2-W1 / W x 100,

Where, W1 = Initial weight before drying, W2 = Final weight after drying, W = weight of sample

Estimation of Total Flavonoid

Total flavonoid content was determined using the method of[13]. 0.5 ml of 2% $AlCl_3$ in ethanol solution was added to 0.5 ml of sample solution. After one hour incubation at room temperature, yellow colour was developed. This was measured at 420 nm with UV-Visible spectrophotometer. A standard graph was prepared using the quercetin and the total flavonoid content was expressed as quercetin equivalent (mg/g).

Estimation of total phenol content

Total phenol content was assayed according to the spectrophotometric method[14]. Test samples of different concentration (1-10 mg) in 1 ml ethanol were prepared and 0.25 ml of Folin ciocalteau was added. After 2 min, 0.75 ml of 20% sodium carbonate was added and the volume made upto 5 ml with distilled water. The mixture was vortexed, left for 2 h and the absorbance was measured at 760 nm. The mixture without test solution was used as a blank. A standard curve of gallic acid was plotted for the calculation of polyphenolic content. The concentration of polyphenols was expressed in terms of mg gallic acid equivalent per gram dried leaves.

Nutritional analysis

Total ash was taken for the analysis of mineral contents. Two ml of conc. HNO_3 was added to the ash and heated for 2 minutes. One drop of hydrogen peroxide was added into the solution. The solution was then transferred into a volumetric flask and total volume was made 50 ml by adding deionized distilled water. This was then used to analyze the contents of iron, calcium, magnesium, dietary fiber, carbohydrate, protein, fat, ash, moisture and potassium by flame and graphite method with atomic absorption spectrophotometer.

In vitro antioxidant activity

DPPH antioxidant assay

Aliquot 3.7 ml of absolute methanol in all test tubes along with blank. Then, add 100µl of absolute methanol was added to the blank. 100 µl of Ascorbic acid (1mg/ml) was added to tube marked as standard and 100 µl of respective samples were added to all other tubes marked as tests. Then, finally 200 µl of DPPH (1,1-diphenyl-2-picrylhydrazyl 1mg/ml in methanol) reagent was added to all the test tubes including blank and incubated at room temperature in a dark environment for 30 min. The absorbance of all samples were then read at 517nm[15].

Cytotoxicity analysis

MTT-cell proliferation assay

The anticancer activity of samples on PC-3 cells (Prostate cancer cells) was determined by the MTT assay[16]. Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 48hrs at 5 % CO_2 incubator. After removal of the sample solution and 20µl/well (5mg/ml) of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) in phosphate- buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. Measurements were performed and the concentration required for a 50% inhibition of viability

Table 3: Nutritional analysis of Chia seeds.

Analysis	Method	Result	Unit
Iron	AOAC-985.29	288.16	mg/kg
Calcium	AOAC-985.29	5422.17	mg/kg
Magnesium	AOAC-985.29	3445.53	mg/kg
Dietary fiber	AOAC-985.29	31.66	g/100g
Carbohydrate	On Calculation Basis	34.92	g/100g
Protein	AOAC-984.13	18.06	g/100g
Fat	AOAC-920.39	32.72	g/100g
Ash	AOAC-942.05	7.05	g/100g
Moisture	AOAC-925.40	7.25	g/100g
Potassium	AOAC-985.29	5639.13	mg/kg

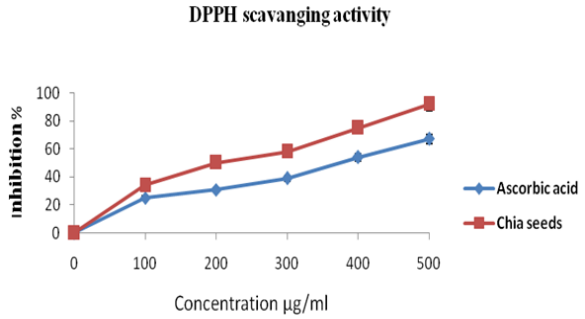


Figure 1: DPPH radical scavenging activity.

(IC_{50}) was determined graphically. The effect of the samples on the proliferation of PC-3 cells was expressed as the % cell viability.

Statistical analysis

The results were expressed as mean ± SD of three independent experiments ($P<0.01$). IC_{50} values were calculated from MTT assay and subjected to statistical analysis.

RESULTS

Initially, the phytochemical screening (Table 1) revealed the presence of tanins, saponins, flavonoids, alkaloids, proteins, cardio glycosides and phenols in ethanolic extract of chia seeds. The total phenolic flavonoids and tannins content of chia seed (Table 2) were found to be 76.32 ± 0.22, 38.25 ± 1.18 and 190.62 ± 1.29 mg/g respectively. These components have been shown to exert anticarcinogenic actions. Table 3 shows the presence of important minerals. 1kg of Chia seeds contained iron (288 mg/kg), calcium (5422 mg/kg), magnesium (3445 mg/kg), dietary fiber (31 g/100g), carbohydrate (34 g/100g), protein (18 g/100g), fat (32 g/100g), ash (7 g/100g), moisture (7 g/100g) and potassium (5639 mg). The antioxidant activity (DPPH approach) suggest that, ethanolic extract of Chia seeds IC_{50} value was 162±1.25 µg/ml which was compared with standard Ascorbic acid (326 ±1.02 µg/ml). The Chia seeds percentage of inhibition was higher than the standard drug at 500µg/ml (92% and 67% respectively, Figure 1). Cytotoxicity of ethanolic extract of *Salvia hispanica* L. have significant cell growth inhibitory activity (Figure 2) at low concentrations (IC_{50} value 1.48±0.72 µg/ml) in human prostate cancer cell line (Figure 3).

DISCUSSION

To the best of existing information, the present study is the first report the anticancer activity of chia seeds in prostate cancer. Medicinal plants are very ancient and true natural medicines which are useful for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites[17]. They provide the modern medicine with numerous plant derived therapeutic agents. Many plants contain a variety of phyto-pharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine[18]. *Salvia hispanica* seeds has been shown to inhibit melanocyte proliferation[19]. Currently, the synthetic therapeutic agents are being regarded unsafe due to its toxicity. It is generally assumed that frequent use of plant- derived phytochemicals may contribute to shift the stability in the direction of a sufficient antioxidant status. As a result, attention in natural antioxidants, in particular plant origin, has gained prominence in recent years[20]. In hypertensive subjects,

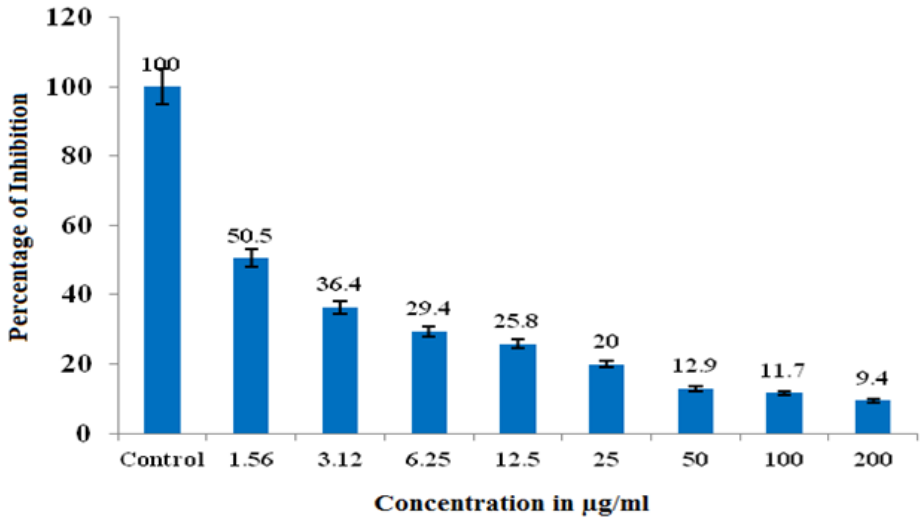
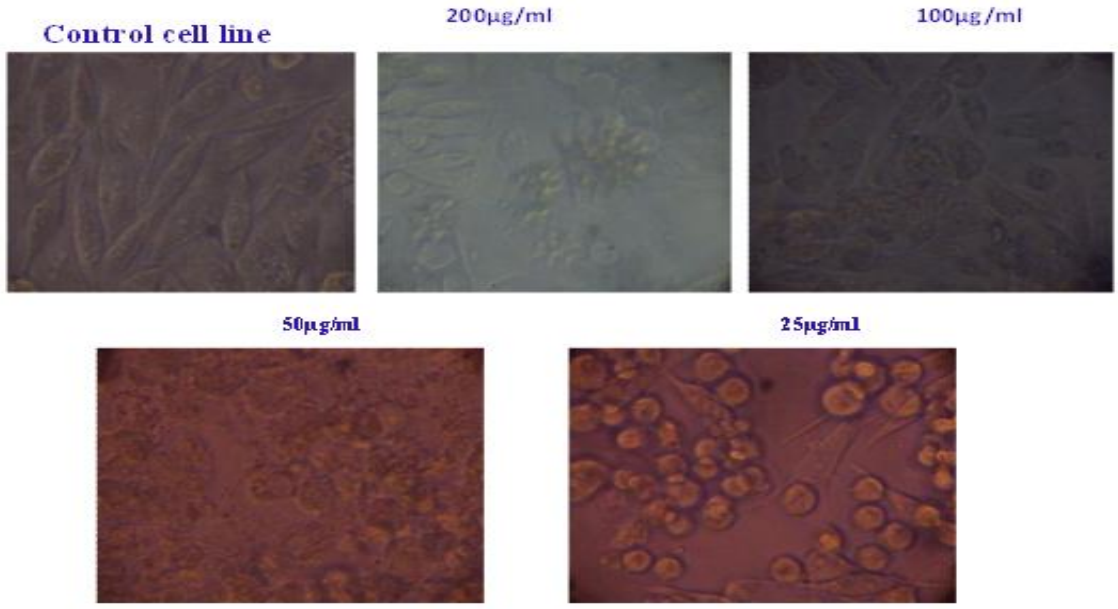


Figure 2: Percentage of inhibition of chia seed extract against PC-3 cell line.

MORPHOLOGICAL FEATURES OF PC-3 CELL LINES TREATED BY CHIA SEEDS EXTRACT WITH VARIOUS CONCENTRATIONS.



Chia flour supplementation reduced blood pressure. The current study provides the estimated amounts of mineral elements such as Iron, Calcium and Phosphorus present in the Chia seeds extract. This may provide knowledge on the biological activities of Chia seeds. Further the proximate analysis and mineral elements estimation may aid in the detection of the bioactive dietary elements that are responsible for the therapeutic properties of Chia seeds. Ash contains inorganic material of the plant because ashing destroys all the organic material present in the sample. Ash is also indicative of high digestibility of the plant[21]. A strong correlation may be suggested between moisture contents and fiber, which could be of interest to human health as the fibrous are easily digested and disintegrated. Fibers in the diet are necessary for digestion and for effective elimination of wastes[22]. It can lower the serum cholesterol, the risk of coronary heart disease, hypertension, constipation, diabetes, and cancer[23]. Discovery of plant natural products with chemotherapeutic property had spurred numerous studies on plant extracts and eventually compounds with the potential for drug development[24]. Flavonoids are known to exert anticancer activities in variety of cells Hence, the anticancer activity of Chia seeds can be attributed to the flavonoids and tannins present in the extract[25].

CONCLUSION

In the present study preliminary phytochemical screening assessed rich amount of secondary metabolites were present in the crude extract of Chia seeds. The dietary evaluation revealed the presence of important minerals in Chia seeds. Cytotoxicity of crude extract of Chia seeds has lower viability to the against human prostate cancer cell line (PC-3). Therefore based on the results this study can be concluded that, the crude extract of Chia seeds may provide a vital alternative to chemotherapeutic options. However, the present study warrants further investigation to identify the mechanism behind this antiproliferative effect.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this paper.

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