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Antimicrobial, Antidiabetic and Antioxidant Property of Chia Seeds (*Salvia hispanica* L)

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Abstract: Chia (*Salvia hispanica*) is an annual herbaceous plant that belong to the Lamiaceace family. It is a medicinal and dietary plant species used since ancient times. There are various essential micronutrients including minerals and vitamins in the diet which play vital roles in maintaining and reinforcing antioxidant performance affecting the complex network of genes and encoding proteins for carcinogenesis. Present study is an attempt to evaluate the antioxidant property and cytotoxicity activity of chia seeds against human breast cancer cells. The cytotoxicity assay revealed that chia seeds inhibited the growth of breast cancer cell lines in a dose dependent manner. Thus chia seed can be used as a therapeutic option in breast cancer cells. However, further studies are warranted to substantiate the current findings.

Keywords: Antioxidant activity, Chia seeds, Cytotoxicity, Dietary evaluation

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Introduction

Salvia hispanica (Chia) mainly originated from Mexico and Guatemala. It has been considered as a part of human food for about 5500 years. The seeds were traditionally used by Aztecs and Mayas people for the medicine's formulation, food and canvases (Cahill, 2003; Ayerza and Coates, 2005). Chia was the second main crop after beans cultivated in Columbian societies during pre-historic times. Chia (*Salvia hispanica* L.) is a well-known plant with 900 different species of Salvia Family. The plant is an annual herb which exhibit flowers in summer, with a height of 1 m with petiolate reversely and serrated leaves (4-8 cm long; 3-5 cm wide). Plant can be cultivated in well drained clay and sandy soils with limited salt and acid tolerance (Armstrong, 2004).

Chia seeds have been used as a food, folk medicines, primary cosmetics and a part of religious rituals in pre-Columbian societies traditionally (Craig, 2004). This is due to its enormous nutritional and therapeutic components in chia seed (Fernanderz *et al.*, 2006). Chia seed

should be recommended as the part of diet for good health and longevity. The word chia is derived from Spanish word means oilseed, with a huge amount of omega 3 fatty acids, superior quality protein, higher extent of dietary fibre, vitamins, minerals and wide range of polyphenolic antioxidants (Capitani *et al.*, 2012).

Chia seeds possess significant amount of omega-3alpha (α)–linolenic acid (64%) and omega-6 linolenic acids (19%) (Ali *et al.*, 2012). The chia seed is said to be known as power house of the omega fatty acids. Eicosapentaenoic acid, and docopsahexaenoic acid have cardiovascular protective effects (Manzella and Paolisso,, 2005). α–linolenic acid and eicosapentaenoic acids play an important action in the formation of prostaglandins, leukotrienes, and thromboxanes which are encountered in cardio protective functions.

The presence of phytochemicals of caffeic acid, chorogenic in chia seed is potentially a massive source of antioxidants. Ayerza and Coates (2001) isolated polyphenolics-- Chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol from chia seed. Crosby (2005) reported that the phenolics of chia seed analysis by HPLC contained chlorogenic acid, caffeic acid, quercetin, phenolic glycoside k and glycoside Q in amounts. The antioxidant property of chia seed was greater than *Moringa olleifera* and seasame cake extract (Azeem *et al.*, 2015).

Various unhealthy life style habits stimulate the risk of chronic inflammation, including smoking, lack of exercise or a poor diet. On the other way, certain healthy foods may reduce the blood levels of inflammatory markers. Chia seeds have some nutritional benefits, but it has not been shown to treat or prevent cancer. Data from human studies showed that chia seed may help to regulate blood sugar but do not affect weight loss.

Among the most powerful anticancer foods are flax and chia seeds which are a rich source of lignans. Lignans have anti-estrogenic effects that inhibit cell growth in breast tumors (Crosby, 2005). Flax seeds are clearly super foods, even with a mediocare diet they offer powerful protection against breast cancer. This study deals with antioxidant value of chia seeds and cytotoxic activity against breast cancer cell lines.

Materials and Methods

Salvia hispanica L was identified by a Botanist. The seeds of chia were shade dried. The dried seeds were then pulverized to make a coarse powder using a sterilized mixer grinder and stored in air tight bottle.

Preparation of Extract:

100 g of the chia seeds powder was taken and macerated in 600 ml of distilled water in a sterile glass container for aqueous extract preparation and similarly same procedure was followed for ethanolic extract. Cold maceration was maintained for two days by shaking at regular intervals. Both extracts were subjected to filtration using Whatmann filter paper to obtain a clear filtrate. This was kept on a water bath set at 60◦C to obtain a crude extract of chia seeds. The extract was used as test sample for further analysis.

Analysis of antioxidants by DPPH Assay:

The radical scavenging activity of chia seed was determined by using DPPH assay. The decrease of the absorption at 517 nm of the DPPH solution after the addition of the antioxidant was measured. The setup was kept at dark at room temperature and the absorption was monitored after 30 min. The ability of the test sample to scavenge DPPH radical was calculated by the following equation.

% of DPPH radical scavenging activity (%RSA) = Abs. Control - Abs sample

Abs. control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + test sample.

Antibacterial activity by Disc diffusion assay:

Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. Growing of bacterial culture of bacterial pathogen

allowed on it for 24 h. Then the ethanolic extract of chia seed sample was loaded in various concentrations on the well. Tetracycline was used as positive control. The plates were then incubated at 37◦C for 24 h. After incubation the inhibition diameter was measured (mm).

Antifungal activity by Well diffusion assay:

Potato dextrose agar was prepared and poured in the sterile Petri dish and allowed to solidify. 48 h growing fungi culture of *Aspergillus niger* was swabbed on it. Then, the text sample in different concentrations was loaded in the wells made using Cork borer. Fluconalzole was used as positive control. The plates were then incubated the inhibition diameter was measured using zone scale.

Antidiabetic assay α – amylase inhibition test:

α-amylase activity can be measured *in vitro* by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue color with starch. The reduced intensity of blue color indicates the enzyme-induced hydrolysis of starch into monosaccharides. If the substance/extract possesses α-amylase inhibitory activity, the intensity of blue color will be more. In other words, the intensity of blue color in test sample is directly proportional to α-amylase inhibitory activity.

Analysis of lipase activity in chia seeds:

50 g of chia seed was added to 100 ml ethanol. The mixture of ethanol and acetone (1:1) act as stopper solution. 50 mm sodium hydroxide solution was used as titration solution. Enzyme dilution buffer: 0.1M Potassium dihydrogen phosphate–sodium hydroxide buffer, pH 8 containing 0.1% bovine serum albumin and 0.1% sodium nitrate. 10 mg of lipase was added to 100 ml with enzyme dilution buffer. 5 ml of substrate suspension and 2 ml of distilled water were mixed to start the pre-incubation at 37◦C. 0.5 ml of enzyme (lipase) solution was added. In case of test blank added 0.5 ml of enzyme dilution buffer in place of enzyme solution. The reaction mixtures were stopped after 20 min using 16 ml of stopper solution, three drops of phenolphthalein indicator and titrated the whole mixture against sodium hydroxide.

Cell growth inhibition studies by MTT assay:

MCF 7 cells were obtained from NCCS (National Centre For Cell Science, Pune) and cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/ml), gentamycin (100 µg/ml) andamphotericin B (1 mg/ml). MTT and Acridine orange were obtained from Sigma Chemicals, MO, USA. The Cell viability was measured with the conventional MTT reduction assay. Briefly, MCF 7cells were seeded at a density of 5×10^3 cells/well in 96 well plates for 24 h, in 200 µl of RPMI with 10% FBS. Then culture supernatant was separated and RPMI containing various concentrations of test samplewas added and incubated for 48 h. After treatment, cells were incubated with MTT (10 µl, 5 mg/ml) at 37 ◦C for 4 h and then with DMSO at room temperature for 1 h. The absorbance of the plates were measured at 595 nm using UV spectrophotometer. Data represented the mean values for six independent experiments (Evelyn *et al.*, 2012).

Cell viability (%) = (Average test OD/Control OD) x 100

Results and Discussion

Chia seeds showed high nutritional aspects of proteins, tannins, saponins , flavonoids, alkaloids, quinines, terpenoids, phenols and cardioglycolsides. Tepe *et al.* (2006) reported that phenolics of chia seed can block the lipid peroxidation phenomenon. The antioxidant activity (DPPH) of ehanolic extract of chia seeds was 56.49±1.25 µg/ml as compared to standard ascorbic acid (326±1.02 µg/ml). Reyes-Caudillo *et al.* (2008) also reported that chia seeds possess wide range of antioxidant compound and can be regarded as a great source of antioxidants.

The fat reduction was found to be higher in 500 µg of chia seeds than 100µg. It showed rapid reduction of fat into fatty acids. It showed 62% of

Table 1: Antibacterial activity by disc diffusion assay

Organism	Concentration of sample (µg/ml)	Zone of inhibition in mm		
		Aqueous extract	Ethanolic extract	Control (0.2% chlorhexidine)
Escherichia coli	Control	-	-	19
	5-10	R*	R	
	50-100	R	R	
	200-500	R	2 mm	
Staphylococus aureus	Control	-	-	17
	5-10	R	R	
	50-100	R	R	
	200-500	R	4 mm	

*R= Resistant

fat reduction activity.

Antimicrobial activity of chia seeds:

Chia seeds exhibit antioxidant, antimicrobial, anticoagulant and antineoplastic property.The antibacterial property of aqueous and ethanolic extract of chia seeds extract) was assessed against *Escherichia coli* and *Staphylococus aureus*. The chia seeds percentage of inhibition was higher at 50-100 µg/ml in ethanolic extract and mild activity in aqueous extract. The antifungal activity of ethanolic extract of *S. hispanica* (Chia seeds) against *Aspergillus niger* and *A. actinomycetemcomitans* were observed.

The results of this study showed that the ethanolic extract of seeds of *S. hispanica* (Chia) had a minimum inhibitory concentration (MIC) of 50 to 100 g/ml against *Staphylococus aureus* and that the aqueous extract of Chia seeds had no antibacterial activity (Table 1). The minimum inhibitory concentration of ethanolic extract of *A. actinomycetemcomitans* exhibited antifungal activity (21 mm). The antimicrobial activity of chia seeds probably is due to its presence of antioxidants like omega 3 fatty acids, linoleic acid and linolenic acid. The chia seeds showed rapid fat reduction activity. Divyapriya *et al*. (2016) investigated antibacterial effect of chia seeds extract against all the three main pathogens of periodontal disease by using disc diffusion method. Similarly, in micro dilution assay test, both the aqueous and ethanolic extract of *S. hispanica* exhibited antibacterial effect.

Antidiabetic activity of chia seeds:

The α-amylase inhibition of *Salvia hispanica* was analyzed in terms of zone of clearance and quantitatively. The maximum activity of anti-diabetic activity was observed in ethanolic extract and found to be 67% (Fig. 1).

Cytotoxicity assay on cancer cell lines:

Medicinal plant having seeds are true medicines which are useful for the treatment of different disease. They can be used directly due to the presence of secondary metabolites. Natural antioxidants, in particular of plant origin, has

gained importance in cancer treatment in recent years. Breast cancer cell line (MTT assay): Cytotoxicity of ethanolic extract of *Salvia hispanica* L exhibit substantial cell growth inhibitory action at low concentration, IC_{50} value = 15.1 ± 1 ng IC value = 71.0 ± 100 g concentration (Figs. 2, 3).

Fig. 1: The α-amylase inhibition of *Salvia hispanica.*

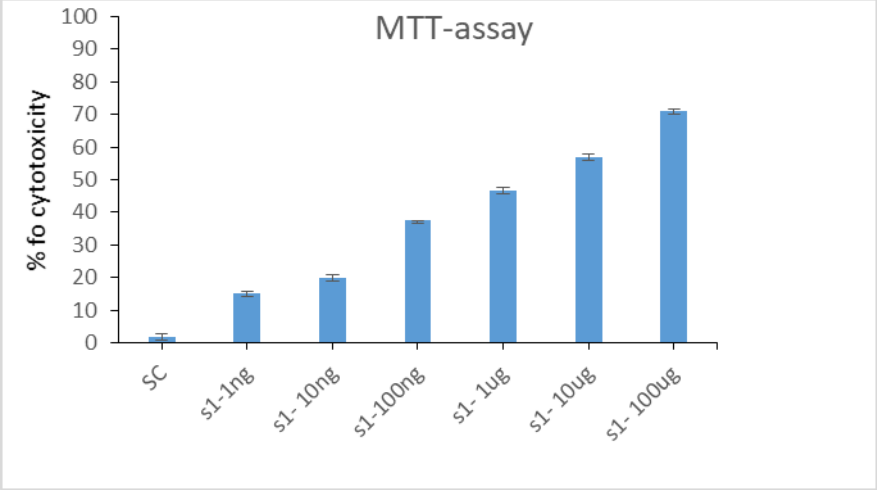

Fig. 2: Cytotoxicity assay on breast cancer cell lines.

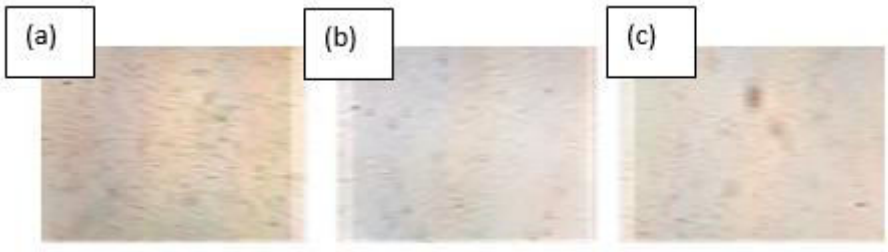

Fig. 3 (a): Control MCF-7; (b): MTT (100 µg) of sample, and (c): MTT (100ng) of sample.

Conclusion

Food security is a major problem faced by whole world. To secure nutritional benefits, improved use of under utilized grains like chia seeds can be best option due to extensive nutritional constituents. Now a days people showing interest at healthy aspects of rare foods because of their excellent physical and functional properties. Chia can help in the prevention of current disorders because it contains maximum concentration of fatty acids, essential amino acids, good quality proteins and adequate amount of dietary fiber.

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