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A BIOCHEMICAL STUDY ON THE PHYTOCHEMICAL CONSTITUENTS ANTIOXIDANT STATUS AND CYTOTOXIC EFFECT (BREAST CANCER CELL LINE - MCF-7) OF CHIA SEED EXTRACT

S. Sivasankari[1], M.D. Lakshmi Priya[2]*, S. Prem Kumar[3] and E.S. Anooj[4]

[1,2*]P.G. & Research Department of Biochemistry, Adhiparasakthi College of Arts and Science, GB Nagar, Kalavai, Vellore (Tamilnadu), India.
[3]Ramachandira Diabetic Hospital, Egmore, Chennai (Tamilnadu), India.
[4]Xcellogen Biotech India Pvt. Ltd., Nagercoil (Tamilnadu), India.

Abstract

Cancer is an umbrella term for altogether over 100 various types of the disease, which in the early twenty-first century became the acknowledged leading cause of the deaths worldwide; contextually, breast cancer plays a major role with around two million new cases and a half of million pathology-related deaths registered annually worldwide. Consequently, a lot of efforts have been made to explore the protective effects of a broad spectrum of plant-derived substances. The present study is one such effort exploring the beneficial role of *Salvia hispanica* seeds against the growth of breast cancer cells. Different extracts of *Chia seed* s(*Salvia hispanica*) were prepared and the following tests were carried out Phytochemical analysis of the aqueous, methanol, ethanol, chloroform, petroleum ether and n-hexane extracts were carried out. Determination of the Free Radical and Antioxidant scavenging activities of the Chia seed (*Salvia hispanica*) was performed. Analysis of MTT Assay on MCF-7 Breast Cancer cell line. The approval of chia seed as a Novel Food by the European Parliament has led to high degree of usage of chia seed in a wide range of foods. It is already well established that chia does not have anti-allergic, anti-nutritional and toxic effect on human health. Therefore, this preliminary work adds on and ensures the enormous possibilities of research in this area to bring out a effective drug against breast cancer for the benefit of human kind.

Key words: Cancer, Cytotoxicity, MTT assay

Introduction

The species *Salvia hispanica* produces numerous dry indehiscent fruits which are commonly called seeds (Abdul-Hamid & Luan, 2000; Alvarado-Sua´rez, 2008; American Institute of Nutrition, 1993; Arnason *et al.,* 1995; Ayerza and Coates, 2005). These small white and dark seds in pre-Columbian times, along with corn, beans and amaranth, were one of the basic foods in the diet of several Central American civilizations including Mayan and Aztec populations. The seeds had also been used like attribute to the capital of Aztec Empire and offered to Aztec god (Ayerza, 2010; Ayerza & Coates, 2001; Bhat *et al.,* 2005; Borderias *et al.,* 2005).

Chia seed belong to the family Laminaceae; Genera: Salvia, Species: hispanica, commonly known as chia, Spanish sage, Mexican chia and black chia (Hentry *et al.,* 1990). Plant is an annual herb bears flower in summer, with a height of about one meter with reverse Petiolate and serrated leaves (4-8 cm long; 3-5 cm wide) with hermaphrodite flowers. Plant can grow in a wide range of well drained clay and sandy soils with reasonable salt and acid tolerance. It can produce 500-600 kg seed/acre but under appropriate agronomic conditions the yield of 2500 kg/acre has also been reported (Anooj *et al.,* 2019; Joyce Boye *et al.,* 1997).

The culinary uses of chia seed were equally diverse and involved the use of whole seeds, seed flour, mucilage, and oil. Often processed in the same manner or together with maize, chia seeds Fig. 1 were roasted, ground to produce flour called chianpinolli, then integrated into tortillas, tamales, and beverages. Chianatoles, beverages made with chia flour, were extremely popular in the 16th century and were part of various cultural events and ceremonies (Brenna, 2002; Brenna *et al.,* 2009).

***Author for correspondence :** E-mail : lakshmipriya81@yahoo.com

Material and methods

Sample Collection: Chia seeds were purchased commercially in an organic shop, Velachery, Chennai.

Preparation of extracts: Extraction with different solvents in the order of increasing polarity like n-Hexane, Petroleum ether, Chloroform, acetone, Ethanol, methanol and distilled water were done using soxhlet apparatus. Briefly, for every 200 mL of the each solvent, 25 g of the crushed chia seed powder was used for soxhlet extraction. After extraction for 3 consecutive days, the crude liquids were placed in water bath at 55°C for excess solvent evaporation.

Phytochemical Screening:

Qualitative Analysis:

Phytochemical screening for various phytochemicals like Flavonoids, Tannins, Terpenoids, Alkaloids, Phlobatannins, Quinone, Steroids, Cardiac glycosides, Saponins, Carbohydrates and proteins were done using methanol & aqueous extract of Chia seed (*Salvia hispanica*).

Antioxidant and Free Radical Scavenging Activity:

DPPH Radical scavenging assay:

The DPPH free radical scavenging activity of methanol extract of Chia Seed (*Salvia. hispanica)* seed was carried out according to the method of Cheung, and Ooi *et al.,* 2003. Serial dilution from methanol stock solution (10mg/ml) was carried out to obtain concentrations of 10, 20, 30, 40, 50 and 60mg/ml. 1ml of 0.1mM DPPH solution in ethanol was added to the diluted extracts and allowed to stand for 30 minutes. The absorbance was determined at 517nm and corresponding percentage inhibition was calculated by the formula-

$$\frac{A-B}{A}\times 100$$

were A is the absorbance of control (1ml of 0.1mM DPPH solution in 1ml methanol) and B is the absorbance of the extract.

Cell Viability test using MTT assay:

The breast cancer cell line, MCF-7 was purchased from NCCS Pune. The cells were grown in a DMEM medium supplemented with 10% fetal bovine serum and antibiotics as mentioned earlier. Cell proliferation (MTT) assay was performed following the method described by Carmichael *et al.,* (1987) and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated controls. For the MTT assay, the cells were grown in 25 cm × 25 cm × 25 cm tissue culture flasks containing DMEM medium as culture medium supplemented with 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin (GIBCO) and grown at 37°C under a humidified atmosphere of 95% air and 5% CO_2. Cells were regularly passed and maintained before including for the experiment. When a cell density in a culture flask reached 70-80% confluence, they were trypsinized and seeded in 96-well plates in the density of 5000 cells per well in 100 µl and incubated for 24 hours at CO_2 incubator. Next day, test item was prepared as 100 mg/ml in DMSO. The working stock of 2X (2000, 600, 200, 60, 20, 6, 2 and 0.6 µg/ml) concentration to the cell in 100 µl volume and the final concentration range were: 1000, 300, 100, 30, 10, 3, 1 and 0.3 µg/ml. 100 µl of diluted stocks were added to the cell and the plate was further incubated for 48 hours in the CO_2 incubator at 37°C.MTT solution was composed of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) at 5 mg/ml in PBS . From this solution 50 µl was pipette out into each well to achieve 1mg/ml as final concentration. The plate was further incubated for 3 hours in incubator and the medium was carefully decanted. The formazan crystals were air dried in dark place and dissolved in 100 µLDMSO and the plates were mildly mixed at room temperature and the OD was measured using Synergy HT micro plate reader at 570 nm.From the optical densities the percentage growths were calculated using the following formula: Percentage Growth (%) = 100 × [(T-T0)/(C-T0)] where, T is optical density of test, C is the optical density of control, T0 is the optical density at time zero (at the time of compound addition will serve as blank to assess the cytotoxicity). From the percentage growths a dose response curve was

Phytochemical screening

Table 1: Phytochemical constituents of different extracts obtained from chia seeds (*Salvia hispanica*).

Phytochemicals	n-Hexane	Petroleum Ether	Chloroform	Acetone	Ethanol	Methanol	Aqueous
Tannins	+	+	+	+	-	++	-
Phlobatannins	-	-	-	+	+	++	+
Saponins	-	+	+	+	-	++	-
Flavonoids	-	-	-	-	-	++	+
Steroids	+	+	+	-	+	++	-
Alkaloids	-	+	+	-	-	++	+
Quinone	-	-	-	-	-	+	+
Carbohydrates	-	-	-	-	-	+	+
Terpenoids	-	-	+	-	-	++	+
Cardiac glycosides	-	-	-	-	-	+	-

generated and GI_{50} values were calculated.

Cell imaging:

After 48 hours before adding MTT solution, treated cells were observed under microscope for cell morphology analysis and images of each concentration was captured and recorded.

Statistical analysis:

Experimental values are expressed as Mean±SEM. Comparison of mean values between various groups was performed by one way-analysis of variance (one way-ANOVA).

Results and Discussion

Table 2: DPPH scavenging activity of different extracts obtained from chia seeds (*Salvia hispanica*).

Concentration mg/ml	Standard mg /ml	n-Hexane mg/ml	Petroleum ether mg /ml	Chloroform mg /ml	Ethanol mg /ml	Methanol mg /ml	Aqueous mg /ml
2	40	2	4	12	18	32	6
4	66	5	10	26	32	42	14
6	88	8	18	32	48	62	28
8	96	12	38	46	54	82	38
10	110	16	46	54	66	96	44

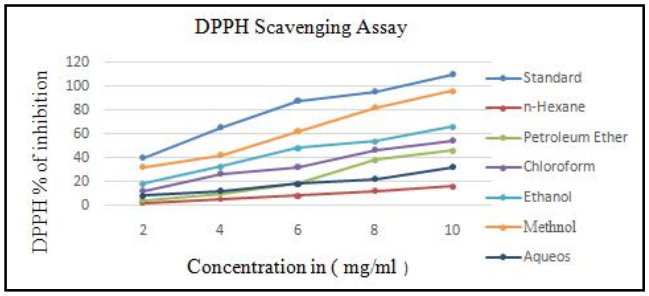

Fig. 1: DPPH scavenging activity of different extracts obtained from chia seeds (*Salvia hispanica*).

Cell growth inhibition property:

Table 3: 15 % Cell Viability of MCF-7.

Concentration (µg/ml)	% Viability	SD
1000	39.6	2.3
300	49.4	2.3
100	71.7	2.0
30	83.9	3.4
10	94.1	3.8
3	92.7	7.7
1	97.9	4.8
0.3	101.4	7.4
Control	100.0	8.2

Conclusion

This study has conducted the extraction of phytochemicals from Chia seed (*Salvia hispanica*) using various solvents given in the order of polarity as Water, methanol, ethanol, acetone, chloroform, petroleum ether and n- hexane. A good solvent is characterized by its optimal extraction and its capacity in conserving the stability of the chemical structure of desired compounds. Therefore the type of extraction solvent and its polarity may have a significant impact on the level of extracted polyphenols. It was elucidated that the methanol extracts showed higher phenolic content than other solvent extract. Methanol seed extract also exhibited a high content of flavonoid and condensed tannins. The total polyphenolic and flavonoid content in methanolic extract with regards to different solvents used for extraction proved to be high significantly. Antioxidant property of Chia seed (*Salvia hispanica*) is investigated in the present research and the methanolic extract exhibited high antioxidant ability when compared to other solvent extracts. Human breast cancer MCF-7 cells represent one of the most widely used experimental models for in vitro studies on breast carcinoma. The study conducted experiments to find out the anticancer activity of Chia seed (*Salvia hispanica*) by inhibiting the growth of cancer cells. Human breast epithelial MCF-7 cells when exposed to Chia seed (*Salvia hispanica*) at the concentrations of 0, 25, 50, 100 and 200 µg/ml for 24 h, profound cytotoxic effect was witnessed in s concentration dependent manner. This proves that Chia seed (*Salvia hispanica*) holds effective therapeutic property against breast cancer. The approval

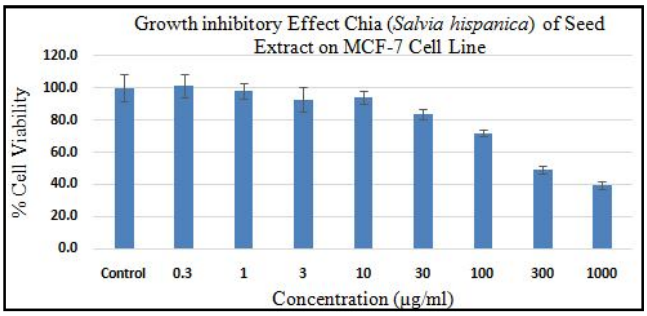

Fig. 2: Growth Inhibitory Effect of *Salvia hispanica* on MCF-7 Cell line.

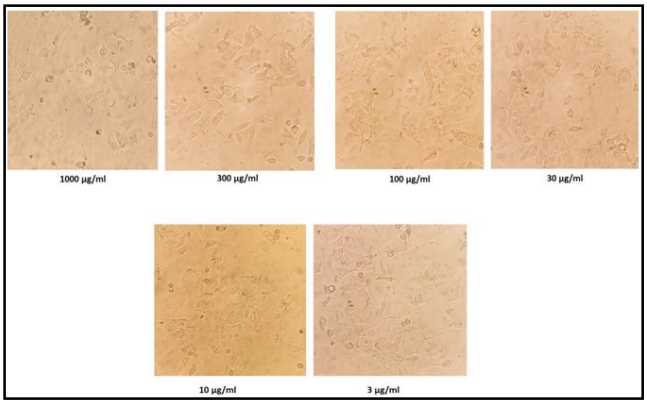

Fig. 3: Growth inhibition assay pictures.

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