Amaranthus spinosus leaf extracts and its anti-inflamatory effects on cancer

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ABSTRACT

The fruits and vegetables generate phytochemicals by its nature such as flavonoids and antioxidants which can reduce the oxidative stress and lower risk of chronic sustainment like cancer. The present study was to investigate and evaluate the antitumor capacity and chemoprotective effects of Amaranthus Spinosus leaves. The methanol extracts of Amaranthus Spinosus leaves tested with different doses for different cell lines such as breast, colorectal, liver and normal cell lines. This was observed that the methanol leaf extract at different doses causes a decrease significant level of tumor development and viable cell count. Percentage of leaf Methanol extracts HEP G2, MCF-7 and HT-29 cells shows IC 50 values of less than 30ug/ml. Amaranthus Spinousus leaf extracts shown significant membrane stability property which shows better anti-inflammatory responses. FACS used to analysis the inhibition of cell cycle progressionsby using PI Staining for HEp G2 Cells. The results suggest that the methanol extracts of Amaranthus spinosus leaves reveals significant antitumor effects in cancers of breast, colorectal, liver and normal cell lines. Further studies are required to elucidate the precise molecular mechanisms and targets for cell growth inhibition.

Key words: *Amaranthus spinosus,* Anti-inflammatory, Cancer

INTRODUCTION

During the last decades, chemists and biologists havebeen intensively investigating tropical and subtropicalplants species with potential medicinal properties inorder to assess the feasibility of developing natural, sustainable, and affordable drugs and cosmetics (AkéAssi and Guinko, 1991). Medicinal plant exhibits antibacterial activitysince they contain innumerable biologically activechemical constituents. The use of plant preparation asfoodstuff, insecticides, CNS active, cardio active, antitumorand antimicrobial agents are some examples ofimmense chemical diversity in plants, which are as old asmankind (Al-Sereiti, 1999).Plants produce a diverse range of bioactive molecules, making them a rich source of different types ofmedicines. A rich heritage of knowledge to preventive and curative medicines was available in ancientscholastic works included in the Atharva Veda, Charaka, Sushruta etc. Over 50% of all modern clinicaldrugs are of natural product origin (Stuffness, 1982) and natural products play an important rolein drug development programs in the pharmaceutical industry (Baker, 1995).

Herbal drugs havegained importance in recent years because of their efficacy and cost effectiveness. About 80% of individuals from developed countries use traditional medicine, derived from medicinal plants. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of immense therapeutic value. Even now, approximately 80% of the third world population is almost entirely dependent on traditional medicines for maintaining general health and combating many diseases Plants and plant products both as extracts and derived compoundsare known to be effective and versatile chemo preventive agents various types of cancers. The plant *A.spinosus* Linn (Amaranthaceae) is an annual herb found in throughout India and also many tropical countries. Traditionally the plant is used to treat various diseases. Although the leaves part are used as a laxative and an applied as an emollient poultice to abscesses, boils and burns and reported as antimalarial,antioxidant and anti-hepatotoxic actions. The presence of spinoside, new coumaroyl flavone glycoside lectins, betacyanins and phenolic compounds has been reported.

Amaranthus spinosus Linn. (Amaranthaceae) is commonly known as "KateWali Chaulai (Kanatabhajii)" in Hindi, and is used as avegetable and cultivated throughout India, Sri Lanka, andmany other tropical countries. In Ayurveda (Indian traditionalsystem of medicine) the plant is used as a digestive,laxative, diuretic, stomachic, and antipyretic, to improveappetite, biliousness, blood diseases, burning sensation,leprosy, bronchitis, rat bite, piles, and leucorrhea, whilethe boiled leaves and root are given to children as a laxative,emollient, and poultice

for abscesses, boils, and burns (Kirtikar & Basu, 2001). The leaves are usedto treat rheumatic pain, stomach-ache, eczema, gastroenteritis,gallbladder inflammation, boils, abscesses, snakebite, colic menorrhagia, and arthritis. *Amaranthus spinosus* is also used as an anti-inflammatory, antimalarial, antibacterial,antimicrobial, antidiuretic, and antiviral agent and in hepatic disorders (Olajide, 2004; Stintzing, 2004).

A. spinosus were identified as amaranthine, Isoamaranthine, Hydroxycinnamates, Quercetin, and Kaempferol Glycosides (Hilou, 2006; Rastogi & Mehrotra, 1999; Srinivasan, 2005; Stintzing, 2004). The plant has a high concentration of antioxidant components, high nutritive value due to the presence of fiber and proteins, and a high concentration of essential amino acids, especially lysine (Cao, 1996; Odhav, 2007).

Scientific studies indicate that the promising phytochemicals can be developed from the medicinal plants for many health problems. Moreover, the herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts or fractions thereof or mixtures of fractions/extracts from different plants, which have been carefully, standardized for their safety and efficacy. *Amaranthus spinosus* Linn. (Amaranthaceae) commonly known as Spiny amaranth or Pig weed, is an annual or perennial herb, native to tropical America and found throughout India as a weed in cultivated as well as fallow lands. Though whole plant is used as laxative, the root are regarded as highly specific for colic by Hindu physicians (Sivarajan & Balachandran 1994) and in Madagascar they are considered as laxative (Kirtikar & Basu 1987).Traditionally boiled leaves and roots of *Amaranthus spinosus* are given to children as laxative. However the drug is also used traditionally as diuretic, antidiabetic, antipyretic, anti-snake venum, antileprotic, and anti-gonorrheal (Kirtikar & Basu 1987). In Malaysia, *Amaranthus spinosus* is used as an expectorant and to relieve breathing in acute bronchitis. Some tribes in India apply *A. spinosus* to induce abortion. The *A. spinosus* is reported for its anti-inflammatory properties, effect on hematology, immunomodulatory activity, antiandrogenic activity, anthelmintic properties and effect on biochemical changes in epididymis.

MATERIALS AND METHODS

Plant material: *Amaranthus Spinosus leaves* were obtained from Anna medicinal farm, Chennai. The Leaves were washed with sterile water, dried in shade, finely powdered & stored in air tight bottles.

Preparation of plant extract: 25 g of air-dried powder of *Amaranthus Spinosus* leaves was immersed in 100 mL of Methanol in a conical flask. It was incubated at room temperature for 48 hour at 150 rpm in an orbital shaker. The suspension was filtered and concentrated to dryness at 40^{o}C in hot air oven. The extract was dissolved in 0.25% Dimethyl Sulphoxide (DMSO, Merck) to a concentration of 100mg/mL.

Cell lines: Cells lines representing the most common human cancers (WHO, 2006) were obtained from the King Institute of Preventive Medicine, Chennai. These included the breast adenocarcinoma (MCF-7), the human Liver Adenocarcinoma (HEP G2) , Human Laryngeal cancer cell lines (HEp2) , African Green Monkey Kidney Cell lines (VERO) and the colon adenocarcinoma (HT-29).

Phytochemical analysis of plant extracts: Phytochemical screening of the extracts was carried out according to the methods described by (Jacob et al., 2011) for the detection of active components like saponins, tannins, alkaloids, phlobatanins, etc.

a) Alkaloids- 1 ml of 1% HCl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered about 2 drops of Mayer's reagent to1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.

b) Tannins- 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of tannins.

d) Saponins- Frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 min. No frothing was observed.

e) Flavonoids- 1 ml of 10% NaOH was added to 3 ml of the extract

Cell viability assay: The antitumor assay was performed on Human laryngeal epithiloma cells (HEp2) obtained from King Institute of Preventive Medicine, Chennai, India with non-toxic dose of the plant extract and its dilutions. The cells were grown in a 24-wellplate in Eagle's Minimum Essential Medium (Hi Media) supplemented with 10% fetal bovine serum (Gibco Laboratories) and antibiotics (streptomycin, penicillin-G, kanamycin, Amphotericin B). About 1 mL cell suspension (10^5 cells/mL) was seeded in each well and incubated at 37º C for 48 hour in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to

various dilutions of the plant extract. The cell viability was measured using MTT assay (Mosmann, 1983) with MTT (5 mg/mL) and DMSO. This tetrazolium salt is metabolically reduced by viable cells to yield a blue formosan product measured at 540nm spectrophotometerically (Mosmann, 1983).Controls were maintained throughout the experiment (untreated wells as cell control and diluents treated wells as diluents control). The assay was performed in triplicate for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract on cells. A graph was plotted against the % cell viability (HEp2 cells) Vs dilution of the plant extract.

DNA Fragmentation: Inter-nucleosome cleavage of DNA was analyzed as given below. *Detection of DNA* Fragmentation—Brifely 1 x $10^6$was seeded in 6 well plates and allowed to settle and attach. Cells were then treated with the IC_{50} concentrations of Plant extracts for 24 h. For analysis of genomic DNA, cells were harvested and collected together with nonattached cells in the supernatant. Cells were resuspended in 0.5 ml of lysis buffer (50 mM Tris-HCl, 100 mM EDTA, 0.5% sodium dodecyl sulfate (pH 8.0) containing 0.1 mg/ml RNase A. After incubation at 37 °C for 30 min, extracts were treated with 1 mg/ml proteinase K at 37 °C. DNA was extracted with phenol/chloroform and Isoamyl alcohol with and precipitated with ethanol and stored in Tris EDTA Buffer and gel loading buffer was added and DNA was separated on a 1 % agarose gel. DNA in the gel was stained with Ethidium bromide, visualized under UV light, and photographed.

Cell cycle analysis by flow cytometry: HepG2 cells (5×10^5) were seeded into a 6 well plates and pre-cultured for 24 h. The cells were treated with IC_{50} Concentration of the extracts. The cells were trypsinized, washed twice with PBS, and suspended in 500µL PBS containing for 15 min on ice. The cell suspension was mixed with 5mL of cold 70% ethanol and stored at 4ºC until analysis. On the day of analysis, cells were washed twice with and re-suspended in 1mLPBS. After incubation with RNase A (250µg/mL) for 30 min and staining with propidium iodide (PI, 10 µg/mL) for 10 min, cell cycle analysis was determined using the FACS system (BD Biosciences, San Jose, CA, USA). Histograms were generated and cell cycle analysis was carried Cell Quest software.

DPPH radical scavenging activity: The DPPH assay was performed according to the method of Brand-Williams et al. (28), with a few modifications. To 2 mL of extract solution made in methanol (at concentrations of 10, 20, 40, 60, 80, and 1000 µg/mL, respectively) 1 mL of DPPHsolution (0.2mM/mL methanol) was added and mixed vigorously.The mixture was incubated in the dark at 20 °C for 40 min. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer with methanol as a blank. Butylated hydroxyl toludine (BHT) was used as a positive control. IC50 values for the percentage of DPPH radical scavenging were estimated

In-vitro Anti-inflammatory activity:

Membrane stabilizing activity: This test was followed by the method described by (Chinnasamy Velmurugan, 2010) with some modifications. Whole human blood was obtained from a healthy human volunteer and transferred to heparinized centrifuge tube. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) for 10 minutes at 3000 rpm. The test sample consisted of stock erythrocyte (RBC) suspension (0.5 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the BV extracts (0.2- 1.0 mg/ml) and indomethacin (0.1 mg/ml). The control sample consisted of 0.5 ml of RBC suspension mixed with hypotonic buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000rpm and the absorbance of the supernatant was measured at 540 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

% Inhibition of haemolysis = 100 x (A1-A2/A1)

Where: A1=Absorption of hypotonic buffered saline solution alone,

A2 = Absorption of test sample in hypotonic solution

Effect on protein denaturation: Test solution (1ml) containing different concentrations (50 - 250 µg/ml) of plant extracts and indomethacin (100 µg/ml) was mixed with 1ml of egg albumin solution (1mM) and incubated at 27 ±1 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling the turbidity was measured spectrophotometrically at 660nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was carried out in triplicate and the average was taken

Statistical Analysis: Results were expressed as mean values with ± standard error of the mean (SEM). All

data werePerformed in triplicates and analyzed using Student t' test one-way ANOVA, where differences were considered significant at $p \leq 0.05$.

RESULTS

Phytochemical screening: The Methanolic extracts showed the presence of alkaloids, flavonoids, proteins, steroids and anthroquinine and absence of tannins and saponins showed in Table 1.

DPPH Analysis:

The in- vitro antioxidant mechanism: Extracts were subjected for the evaluation of antioxidant activity by using in vitro model systems. DPPH radical scavenging activity was observed in the extract.The results of the DPPH radical scavenging activity of Methanolic extracts of Amaranthus spinosus shows that it possesses very high percentage antioxidant activity, 98.22% at a concentration of 1000µg/ml. Cancer is one of the most widespread diseases in humans and there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them. A number of active compounds have been shown to possess anticancer activity; these include flavonoids, diterpenoids, triterpenoids, and alkaloids.Several mechanisms have been proposed to explain the cancer-preventive effects of plants. These include inhibition of mutagenesis by inhibiting the metabolism, inhibition of DNA adduct formation, free-radical scavenging, and effects on cell proliferation and tumor growth (Shukla, 2007)

Determination of Cytotoxicity by MTT assay is based on the formation of formazan blue colour due to reduction of MTT by mitochondrial Succinate dehydrogenase that was directly proportional to the number of viable cells and the absorbance was read out by 540 nm. The cytotoxicity study was carried out for the plant extract of *A.spinosus*. These extract was screened for its cytotoxicity against four different cancercell lines (HT 29, HEp G2, HEP 2 & MCF 7) at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. The extracts showed IC 50 ranges from less than 10µg/ml.

Membrane stabilizing activity: In the study of membrane stabilization extract activity at concentration range of 1000µg/ml-10µg/ml protect significantly the erythrocyte membrane against lysis induced by hypotonic solution. Also indomethacin (100 µg/ml) offered a significant protection of the RBC's against the damaging effect of hypotonic solution. At a concentration of 1 mg/ml, the methanolic extracts of amaranthus spinosus showed 95.52% respectively, whereas indomethacin at 0.1 mg/ml showed 60.85 % inhibition of RBC haemolysis.

Inhibition of Protein Denaturation: The inhibitory effect of different concentrations of methanolic extracts on protein denaturation is shown in Table 3. A.spinosus extracts (6.25-250 µg/ml) showed significant inhibition of denaturation of egg albumin in concentration dependent manner. Methanolic extract of plant at concentration of 250 µg/ml and indomethacin at concentration of 100µg/ml showed significant inhibition (62.52, and 85.23%) of protein denaturation when compared with control.

Cell cycle arrest on G2 /m phase: G2/M Arrest in *Amaranthus spinosus* Extracts-treated HEP G 2 The profile of the DNA content of the plants extract-treated HEP G2 cells (62.5 µg/ml, 31.25 µg/ml and 15.625 µg/ml for 24 h) was obtained using flow cytometric analysis to measure the fluorescence of PI-DNA binding. The stage at which extract-induced growth inhibition occurred in cell cycle progression was determined, along with cellular distributions in the different phases after treatment. Figure show that the extract exposure resulted in progressive and sustained accumulation of cells in the G2/M phase in a dose-dependent manner. Furthermore, the percentage of S and G2/M phase cells increased, while those in the G1 phase decreased after treatment with the extracts. Our findings suggest that promotes cell growth inhibition by inducing G2/M phase arrest in cancer cells.

Table.1.Phytochemical Screening

Contents	1mg/ml
Tanins	-
Saponins	-
Flavonoids	+
Alkaloids	+
Proteins	+
Steroids	+
Anthraquinones	+

Table.2. *In-vitro* Antioxidant for methanolic extracts of *A. spinosus*

Concentration	% DPPH Activity
1000	98.22±0.22
750	91.25 ±0.56
500	83.63 ±0.66
250	75.12 ±0.45
125	70.11 ±0.32
100	65.32 ±0.45
75	60.15 ±0.12
50	55.23 ±0.66
25	40.12 ±0.32
10	33.12 ±0.18
Control	100

Table.3. Inhibitory growth effect of *Amaranthus spinosus* On Different Cancer cell line

Concentration (mg/ml)	*Amaranthus spinosus*				
	HEp 2	**HEP G2**	**HU7**	**HT 29**	**VERO**
5	11.60 ± 0.73	14.61 ± 0.44	15.39± 0.52	18.56±0.95	30.56±1.47
2.5	18.74 ± 0.73	20.49 ±0.62	24.65 ± 0.79	24.25 ±0.23	45.76±0.82
1.25	28.56 ± 1.45	31.92 ±1.24	32.41 ± 0.45	29.83 ±0.58	52.79±0.49
0.625	41.96 ± 0.72	34.15 ±0.53	38.35 ± 0.79	34.25 ±0.96	68.97±0.87
0.3125	48.21 ± 1.46	43.99 ± 0.87	53.71 ± 0.79	39.66 ±1.23	76.24±0.73
0.156	53.56 ±1.45	51.64 ± 0.47	58.95 ± 0.29	48.53 ±0.95	81.86±1.45
0.078	61.60 ± 0.73	54.47 ± 0.88	61.64 ± 0.79	59.98 ±0.25	84.78±0.98
0.039	76.78 ± 1.45	75.58 ± 0.47	76.25 ± 0.45	64.52±0.32	92.39±1.23
0.0195	88.39 ± 0.72	82.62 ± 0.46	84.93 ± 0.79	74.56±0.58	95.57±1.17
0.09	97.31± 0.73	96.24 ± 0.47	98.17 ± 0.45	88.93±0.65	98.84±0.76
Cell control	100	100	100	100	100

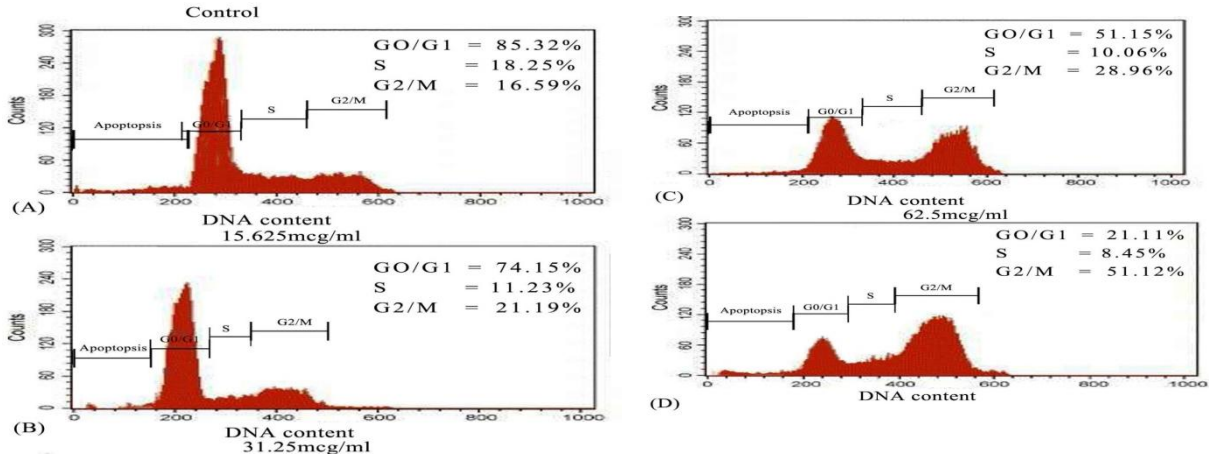

Figure.2.Inhibition of cell cycle progression in liver cancer cell lines analysed by flow cytometry. (A)Cell control (B) to (d) different concentration of extract after 24h

Table.4. Effect of methanolic extracts of *A. spinosus* on erythrocytes hemolysis

Concentration	Activity (% Protection)	Standard From 100µg/ ml
1000	**95.52 ± 0.08**	**60.85**
750	**90.15 ±0.02**	**51.23**
500	**84.45 ±0.15**	**48.25**
250	**80.23 ±0.18**	**44.52**
125	**75.56 ±0.26**	**38.85**
100	**58.59 ±0.68**	**35.56**
75	**48.85 ±0.45**	**30.26**
50	**41.13 ±0.95**	**25.56**
25	**32.26 ±0.35**	**19.95**
10	**25.53 ±0.19**	**15.65**
Control	**-**	**--**

DNA fragmentation Analysis: Gel electrophoresis results revealed fragmentation in cells treated with 50 µg/ml concentration of methanol extract of Indigofera tinctoria, while DNA fragmentswere absent in the control cellsThe growth of the Cancer cells was markedly suppressed by the treatment at more than 15µg/ml , as compared with the control without treatment In the treatment with 31.25µg/ml of plants extracts for 24 h, we observed apoptotic changes, whichwere assessed by morphological parameters (nuclearcondensation and fragmentation) and DNA ladder formation in HEp G2 cells. These findings were further confirmed by the analysis of FACS using PI staining, as shown inFig.2. Taken together, it was indicated that the marked cytotoxicity by methanolic extracts was attributed to apoptotic cell death.

Table.5. Effect of methanolic extracts of *A. spinosus* on Protein denaturation

Concentration	Activity (% inhibition of protein Denaturation)	Standard (100mcg/ml)
250	62.52	85.23
200	59.25	78.95
150	54.46	75.56
100	48.52	68.95
75	44.56	59.24
50	38.56	55.62
25	32.21	48.25
12.5	28.52	41.12
6.25	18.42	33.29
Control	-	-

DISCUSSION

Further research is needed to unravel the specific bioactive compounds responsible for the anticancer properties of the extracts of *A. spinosus* In conclusion; the study has not only established the anticancer property of the extracts of *A. spinosus* but also its antioxidant activity. Thus, the plant could be employed in ethno-medicine for the management of cancerous diseases.
In this study, there may be compounds in *Amaranthus spinospus* extracts that can induce a cytotoxic action against various cancer cell lines and initiate cell death. The American National Cancer Institute guidelines set the limit of activity for crude extracts at a 50% inhibition (IC50 value) of proliferation of less than 30 μg/ml. The plants exhibited various responses depending on the cell line used. Thepercentage of plant extracts with IC50 values less than 30 μg/ml was against HEP G2, MCF-7 and HT-29 cells anticancer activity against all three cell lines, with an IC50 value of less than 30 μg/ml.

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair. The vitality of cells depends on the integrity of their membrane, exposure of RBC's (red blood corpuscles) to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of hemoglobin. The hemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. It is therefore expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances Compounds with membrane-stabilizing properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators

The exracts has shown significant membrane stabilizing property, which suggests that its anti-inflammatory activity observed in this study, may be related to the inhibition of the release of phospholipases that trigger the formation of inflammatory mediators like Prostoglandinand Leukotriene etc through COX (cyclooxygenase) and LOX (lipooxygenase) pathway.

Denaturation of proteins is a well documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Ability of BV extract to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity.The anti-inflammatory activity ofplant extracts found may be due to the presence of therapeutically active flavonoids.

CONCLUSION

Our study provides evidence that methanolic extracts of *Amaranthus spinosus*have the ability to inhibit cell proliferation and induce apoptosis in cancers of breast, colorectal, liver and normal cell lines. So the plant extracts also have the properties of anti-inflammatory and antioxidant at different dose levels. In conclusion, that *in-vitro* data demonstrate that consumption of the parts of these plants or ingestion of extract may impart anticancer effects. Further studies are required to elucidate the precise molecular mechanisms and targets for cell growth inhibition which will allow the rationale design for more effective molecules for the eventual use as cancer chemo-preventive or therapeutic agents.

REFERRENCES

Aké Assi L, Guinko S, Plants used in traditional medicine in West Africa, Basel, Switzerland, Roche, 1991, 90.

Al-Sereiti MR, Abu-Amer KM, Sen P, Pharmacology of Rosemary and its threpeutic potentials, Indian J. Exp. Biol, 37, 1999, 124-130.

Aruna K and Sivarama Krishnan VM, Plant products as protective agents againstcancer, Ind. J. Exp. Bio, 1990, 28, 1008-1011.

Amin I, Norazaidah Y, Hainida K. I. E, Antioxidant activity and phenolic content of raw and blanched Amaranthus species, Food Chemistry, 94 (1), 2006, 47-52.

Azhar-ul-Haq, Malik, Abdul Khan, Anwar-ul-Haq Sher Bahadar, Shah, Muhammad Raza, Muhammad Pir, Spinoside, new coumaroyl flavone glycoside from Amaranthus spinosus, Archives of pharmacal research, 27 (12), 2004, 1216-1219.

Baker JT, Borris RP, and Carte B, Natural product drug discovery and development: New perspective on international collaboration, Journal of Natural Product, 58, 1995, 1325-1357.

Cao G, Sofic E, Prior RL, Antioxidant capacity of tea and common vegetables, J Agric Food Chem, 44, 1996, 3426–3431.

Ellof, J.N. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacol, 60, 1998, 1-6.

S.Justin Packia Jacob and S.Shenbagaraman Evaluation of antioxidant and antimicrobialactivities of the selected green leafy vegetables, 3(1), 2011, 148-152.

Kirtikar KR, Basu BD, Indian Medicinal Plants, Vol. I, 2nd ed. Dehradun, India, Oriental Enterprises, 2001, 2832–2836.

NostroA, Germano MP, D'Angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity, Letters in Applied Microbiology, 30, 2000, 379- 384.

Olajide O, Ogunleye B, Erinle T, Anti-inflammatory properties of Amaranthus spinosusleaf extract. Pharm Biol, 42, 2004, 521–525.

Odhav B, Beekrum S, Akula US, Baijnath H, Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. J Food Compost Anal 20, 2007, 430–435.

Prasanna R, Harish CC, Pichai R, Sakthisekaran D and Gunasekaran P. Anticancer Effect of Cassia auriculata leaf extract in vitro through cycle arrest and induction ofapoptosis in human breast and larynx cancer cell lines. Cell Biology International, 33, 2009, 127-134.

Rastogi RP, Mehrotra BN, Compendium of Indian MedicinalPlants, Vol. 2. Lucknow, India, CDRI and NISCAIR, 1999, 38.

Srinivasan K. Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts. International Journal of Food Science and Nutrition, 56, 2005, 399 - 414.

Srinivasan K. Plant foods in the management of diabetes mellitus: Spices as beneficial antidiabetic food adjuncts. International Journal of Food Science and Nutrition, 56, 2005, 399 - 414.

Shukla Y, Kalra N. Cancer chemoprevention with garlic and its constituents, Cancer Lett, 2007; 247:167-81.

Srinivasan K, Kaul CL, Ramarao P, Partial protective effect of rutin on multiple low dose streptozotocin induced diabetes in mice. Indian J Pharmacology, 37, 2005, 327–328.

Stintzing FC, Kammerer D, Schieber A, Hilou A, Nacoulma O, Carle R, Betacyanins and phenolic compounds from Amaranthus spinosus and Boerhaavia erecta. Z Naturforsch, 59, 2004, 1–8.

Singh, J., Kamboj, K.K., Kamboj, S. S., Sandhu, R. S., Shangary, S. S, Affinity purification and characterization of lectins from two Amaranthus species, Plant Science, 94 (1), 1993, 47-53.

Stuffness M. and J. Douros, Current status of the NCI plant and animal product program, Journal of Natural product, 45, 1982, 1-14.

WHO (World Health Organization), 2006. Cancer. www.who.int/cancer/ 2006 29th January.

Yang, L. L., Yen, K. Y., Kiso, Y., Hikino, H., Antihepatotoxic action of formosan plant drugs, Journal of Ethnopharmacology, 19 (1), 1987, 103-110.