EVALUATION OF ANTIOXIDANT AND ANTICANCER POTENTIAL OF *SESBANIA ACULEATA* – A MULTIPURPOSE LEGUME CROP

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Abstract
With the increasing side effects and decreasing effectiveness of synthetic drugs with time, trend has been moving towards the use of natural and safe drugs with less or no side effects and equal effective like synthetic drugs for curing diseases. So, in this view the present research has been done to investigate the antioxidant and first time the anticancer property of Sesbania aculeata. Plant belongs to legume family and is mainly grown for green manure. Extracts of different parts of the plant were prepared by using Soxhlet apparatus and methanol was used as choice of solvent. The antioxidant activity was checked by four different methods including DPPH free-radical scavenging, hydrogen peroxide scavenging activity, hydroxyl radical scavenging activity and iron reducing power FRAP. The anticancer activity was checked by using SRB assay against the human lung cancer cell line Hop-62. Extracts of all the tested parts of Sesbania aculeata showed excellent in vitro antioxidant activity with maximum activity of about 71-76% in seed coat extract when checked with different methods. Similarly, the seeds coat extracts exhibit anticancer potential against the tested cancer cell line while other parts showed negligible activity. The results have shown that the methanolic extracts could be the source of potential antioxidant and anticancer agents.

Keywords: Antioxidant; Dhaincha; Plant extract; Reducing power; Scavenging activity

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1. INTRODUCTION

With the increase in health concerns and side effects of synthetic drugs, the trend has been started moving towards the use of natural and safer medicines. The plants are the blessings in disguise as the source of many valuable compounds. Of the various compounds, natural oxidants are the subject of scientific research. Natural oxidants due to their property plays a vital role in reducing the oxidative damage associated with various diseases like ageing, cardiovascular diseases, cancer etc. (Ivanova et al, 2005; Katalini et al, 2006). Of the diseases, cancer is the deadliest disease of the modern world. According to WHO, about 8.2 million people died because of cancer in 2012 and it is estimated to increase to 11.4 million by 2030. The chemotherapy treatments for cancer are very painful and with side effects and even sometimes non-effective. So, need to look for alternative that are less painful and with no or little side effects as well as also acts as preventive measures. In this concern, plants have served as a source of new drugs such as vinblastine and vincristine derived from *Catharanthus roseus* (Gueritte and Fahi, 2005), taxol from *Taxus brevifolia* (Cragg, 1998), camptothecin from *Camptotheca acuminate* used as precursor for drugs like topotecan and irinotecan (Rahier et al, 2005). This has led to the screening of more and more plants for therapeutically important phytochemicals exhibiting anticancer potential.

In this regard, *Sesbania aculeata* – a multipurpose legume crop plant commonly known as 'Dhaincha' has been selected, which is a member of the Fabaceae family and is native to India, Pakistan and other Asian particularly South Asian countries (Duke, 1981). It is a fast-growing, erect, low annual sub shrub crop mainly cultivated for soil enrichment, various medicinal purposes, fibre, fuel wood, fodder, paper and dye industry and other agro-forestry use etc. Its root is alexeteric, antihelminthic and diuretic. It is also useful in snake-bite and disease of eye. Its seeds are useful in ringworm and skin diseases.

Though anticancer properties have been reported from another species of the genus *Sesbania* but till now there is no report for *in vitro* testing of anticancer activity of *Sesbania aculeata*. Therefore, present study has been carried out for the first time to check the antioxidant activity using diverse methods and anticancer potential of different parts of the *Sesbania aculeata*.

2. MATERIALS AND METHODS

Plant materials and Preparation of extracts

Different plant parts (stem, roots, seeds and seeds coat) of *S. aculeata* were collected. The parts were cleaned, washed, shade dried and powdered for the further study. The extraction was performed using Soxhlet apparatus and methanol as choice of solvent for extraction. The extracts were prepared under the previously optimized conditions used for the preparation of extracts for *Sesbania* plant parts (Mehta et al, 2018). The extracts were obtained in the form of powder (concentrated form) and stored at 4°C until further use.

Antioxidant activity by the reduction of the DPPH

DPPH (2,2-diphényl-1-picrylhydrazyl) method modified and used by Elansary et al, 2012, was used to determine the free radical scavenging activity of the samples. An aliquot of 2 ml of stock solution of 0.1 mM DPPH reagent dissolved in methanol was added to each test tube containing 2 ml of the sample solution of different concentration in methanol (20-100 µg/ml) and pure gallic acid which was taken as control. The reaction mixture was mixed for 10 sec and left to stand in the dark for 30 min at room temperature. The absorbance was measured at 517 nm, using a UV spectrophotometer. Pure methanol was used to calibrate the spectrophotometer. The decrease in the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. Total antioxidant activity (TAA %) was expressed as the percentage inhibition of the DPPH radical.

H_2O_2 scavenging activity

The H_2O_2 scavenging activity of all the extracts was determined according to the method of Da Silva et al. 2011. One ml of extract of various concentrations (20-100 µg/ml) was mixed in 2.5 ml of 0.1 M phosphate buffer (pH 7.4) and 400 µl of H_2O_2 (5 mM). The mixture was then mixed and incubated for 20 minutes. Absorbance of samples and ascorbic acid (control) was measured at 610 nm while mixture without sample is used as blank.

Hydroxyl radical scavenging activity (HRSA)

The hydroxyl radical scavenging activity of all the extracts was determined according to the method of Rathee et al. 2007. Extracts (100 µl) of various concentrations (20-100 µg/ml) and 100 µl of pure ascorbic acid (2 mM) which was used as control, individually mixed in buffer (containing 0.1M phosphate buffer (pH 7.4), 10 mM deoxyribose and 170 mM EDTA). Then added 100 µl of 10 mM H_2O_2 to the reaction mixture and incubated at 85ºC for 20 minutes. After that, the reaction was terminated by using 1ml of 10% trichloroacetic acid (TCA). Absorbance of each sample was read at 532 nm by taking ascorbic acid as control and reaction mixture without sample as blank. The hydroxyl radical scavenging activity of all the extracts was calculated.

Reducing power

The reducing power of all the extracts was determined according to the method of Loo et al. 2007. Two ml of extract of various concentrations (20-100 µg/ml) was mixed in 0.2M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide solution (1%). The mixture was then mixed vigorously and incubated at 50ºC for 20 minutes. After that, the reaction was terminated by using 1ml of 10% trichloroacetic acid (TCA). The reaction mixture was then centrifuged at 3000 rpm for 15 minutes. The supernatant was separated and 0.1% ferric chloride was dissolved in it. The reducing power of all the extracts was calculated by reading the absorbance of all the samples at 700 nm against ascorbic acid as

control and reaction mixture without sample as blank.

Anticancer potential tested by SRB assay (Skehan et al, 1990)

The anticancer activities of extracts were studied at Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai against the Human lung cancer cell line Hop-62 maintained in ideal laboratory conditions. For experiment, cells were inoculated into 96 well microtiter plates 90µL/well at appropriate plating densities. After 24 hrs, cells from one plate of cell line were fixed in-situ with TCA (trichloro acetic acid), to represent a measurement of the cell population at the time of drug addition (Tz). Experimental extracts were solubilised in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test article at a concentration of 100, 200, 400 and 800µg/ml. Aliquots of 10 µl of these different dilutions were added to the appropriate microtiter wells already containing 90µl of cell suspension, resulting in the required final drug concentrations of 10, 20, 40 and 80 µg/ml. For the experiment Adriamycin (Doxorubicin), a known anticancer drug was used as a positive control. After that, the results were interpreted by end point measurement. In this method, assay was terminated and cells were fixed by the addition of cold TCA and staining was done with Sulforhodomine B solution. The absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated and expressed as the ratio of average absorbance of test well to that of control wells * 100. Percentage growth inhibition was calculated as: [(Ti-Tz)/(C-Tz)] x 100, where Ti is test growth; Tz is growth at time zero; C is control growth. Linear regression method was used for estimation of experiment data of cell viability against drug concentration of tested compounds.

3. RESULTS AND DISCUSSION

Antioxidant activity

The natural antioxidants particularly phenols and flavonoids have been exploited since the last few decades due to their action as potential curative agent against several diseases like diabetes, ageing and cancer. During carcinogenesis, oxidative stress plays important role as it changes the redox status of the cell and drive the unregulated cancer cell proliferation and survival. Cancer cells exhibit increased oxidation levels due to signalling and metabolic aberrations which can be treated by scavenging the reactive species by using antioxidants to impair the oxidative stress signalling and cancer cell growth (Chahar et al, 2011). The radical scavenging potential of phenols is contributed by the hydroxyl groups (Asha et al, 2012) and that of tannins due to the presence of C-ring (Gulcin et al, 2003) and multiple hydroxyl groups (Aleksic and Knezevic, 2014). The most commonly used method to screen the phenolic compounds for free radical scavenging ability is by using DPPH radical which is artificial organic stable free radical (Kedare and Singh, 2011). DPPH free radical act by accepting the hydrogen atom from the antioxidant compound and becomes diamagnetic thus changes the colour from deep purple to faded purple (Verma et al, 2009). The extracts showed significant antioxidant potential when checked with DPPH free radical scavenging activity. Seeds coat showed a high antioxidant potential followed by stem, seeds and roots. . It has also been found that seeds coat extract contain phenols, tannins and flavonoids along with some other active metabolites (Mehta et al, 2018). Natural antioxidant flavones glycoside has also been previously reported from the stems of *S. aculeata* (Satnami and Yadava, 2012). The free radical scavenging activity was less than the pure gallic acid which was taken as control. This may be due to other components present in the extracts. The TAA% value of the seeds coat, stem, seeds and roots were found to be 76%, 74%, 70% and 68% respectively and that of gallic acid (Control) was 81% at the concentration 100 µg/ml (Figure 1A).

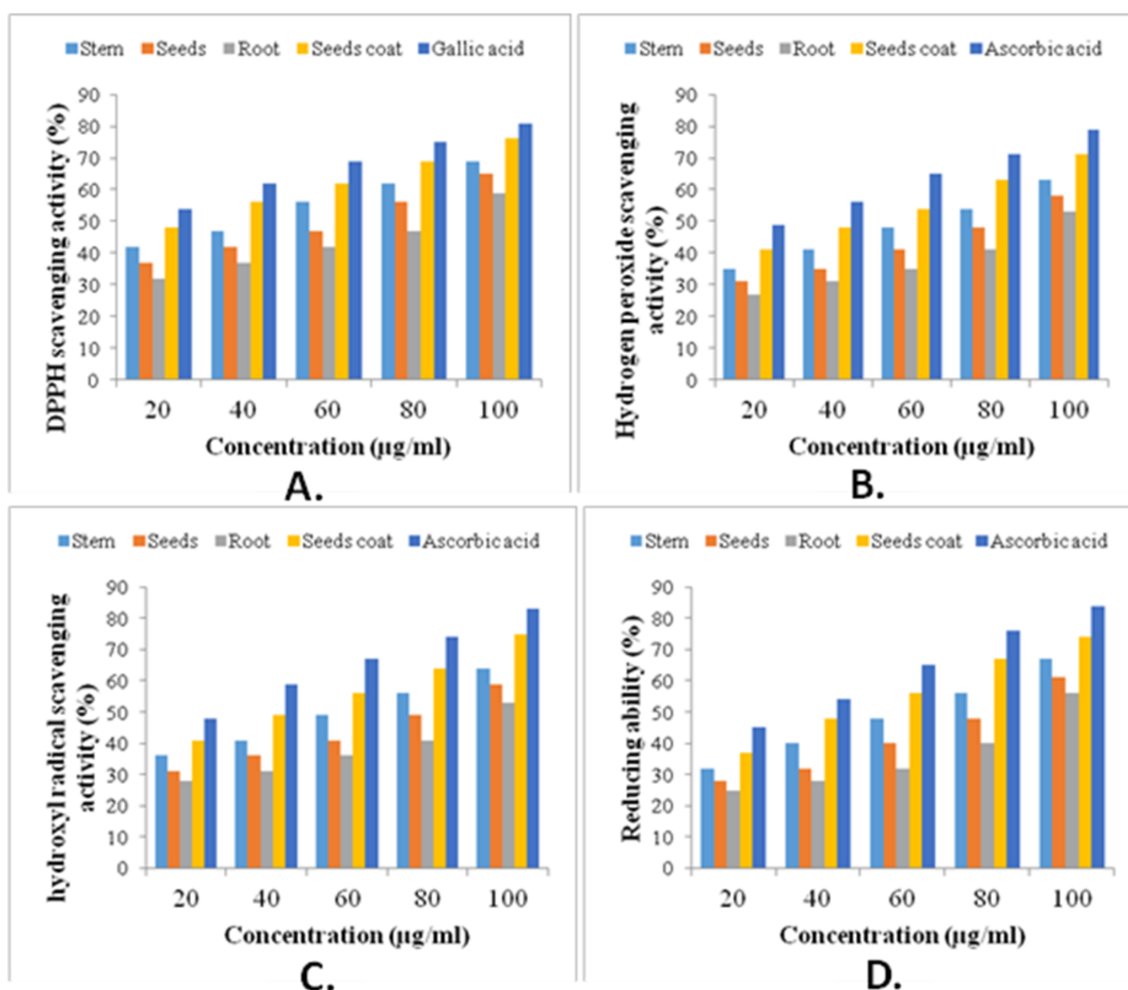

Figure 1: Antioxidant activity of different extracts of *Sesbania aculeata* at different concentration. A. DPPH activity, B. hydrogen peroxide scavenging activity, C. hydroxyl radical scavenging activity and D reducing power ability.

Thus, the extracts used in our study showed good antioxidant activity capable of quenching the DPPH free radicals either by providing hydrogen atoms or electrons. The activity of the extracts is better than the activity found in the other species of the same genus, *Sesbania grandiflora* where the activity was lower even at the higher concentrations (Gowri and Vasantha, 2010; Ouattara et al, 2011). Another method of antioxidant activity screening includes the scavenging activity against hydrogen peroxide and hydroxyl radical. Among the reactive oxidative species, hydrogen peroxide plays important role in oxidative stress. Hydrogen peroxide can lead to cell death by oxidation of important macromolecules like lipids, proteins and DNA (Sheline and Wei, 2006) where as hydroxyl radical is highly reactive and can cause damage to nearby essential biomolecules (Sakanaka et al, 2005) or may lead to tissue damage and cell death (Khan et al, 2012). All extracts showed adequate hydrogen peroxide scavenging activity with seed coat extract with maximum activity nearly equal to the control (ascorbic acid) followed by stem, seeds and root. The H_2O_2 scavenging activity of seed coat, stem, seed and root are 71, 63, 58 and 53% respectively and that of control is 79% at concentration of 100µg/ml (Figure 1B). Another reactive oxidative species that cause severe damage to biomolecules is hydroxyl radical. Extracts also showed good scavenging activity against the hydroxyl radicals with seed coat extracts showing highest activity followed by stem, seed and root at 75, 64, 59 and 53% respectively at concentration of 100µg/ml (Figure 1C). The hydroxyl scavenging activity

of control (ascorbic acid) was 83% which was close to the activity of seed coat extract. Extracts also showed reducing power which increased with increase in the concentration of the extracts. Though the reducing power of seed coat extracts was highest among the extracts tested but was lesser than the control (ascorbic acid) used (Figure 1D). Reducing activity demonstrated by the extracts showed the potential antioxidant activity and in particular the seeds coat extracts possess very good reducing activity. The results showed that the extracts could act by protecting the cells against oxidative stress via reducing the oxidative intermediates produced during lipid peroxidation processes. Reduction of potassium ferricyanide is an excellent mechanism to evaluate the antioxidant ability of the extracts (Jayaprakasha et al, 2001). The reducing ability of the extracts increased with increase in the concentration of the extracts but was slightly less than the control used.

Anticancer activity

The evaluation of cytotoxicity is the first step in search for new anticancer metabolites. Previously there is no report of work done related to anticancer activity in *S. aculeata*. In the present study, the *in vitro* cytotoxicity has been checked by SRB assay. The anticancer activity of the extracts was checked against the human lung cancer cell line Hop-62. Though the seeds coat extract has been found to exhibits anticancer potential against the tested cell line by inhibiting the growth of the cells with average viability 70% at the concentration of 80 µg/ml, whereas other extracts showed no or negligible activity (Figure 2 & 3). But, the activity of the plant extracts was very less than the control drug (Adriamycin) used which may be contributed by the fact that the extract is a mixture of compounds whereas the drug is a pure compound. Cell homeostasis is disrupted by biochemical and genetic alterations that could be estimated by different methods viz enzyme release, cell viability, survival and death assays (Sumantran, 2011). The results of SRB assay correlates with the changes in the cell proteins which varies with the cell number or the protein turnover of the cells tested. Upon exposing the cells to toxicants, alteration in the morphology is also the most observed effect (Ekwall et al, 1990). The results have shown that the cytotoxicity of the seed coat extract increases with increase in the concentration of the extract. Whereas other extracts tested showed no or negligible cytotoxicity in the concentration range tested. There have been reports for the extracts showing anticancer activity in other species of the *Sesbania* genus. Extracts of the *Sesbania grandiflora* have shown anticancer activity against the neuroblastima cell line (Ponnanikajamideen et al, 2015) and colon cancer cell line (Laladhas et al, 2010) when tested *in vitro* and both *in vivo* and *in vitro* respectively.

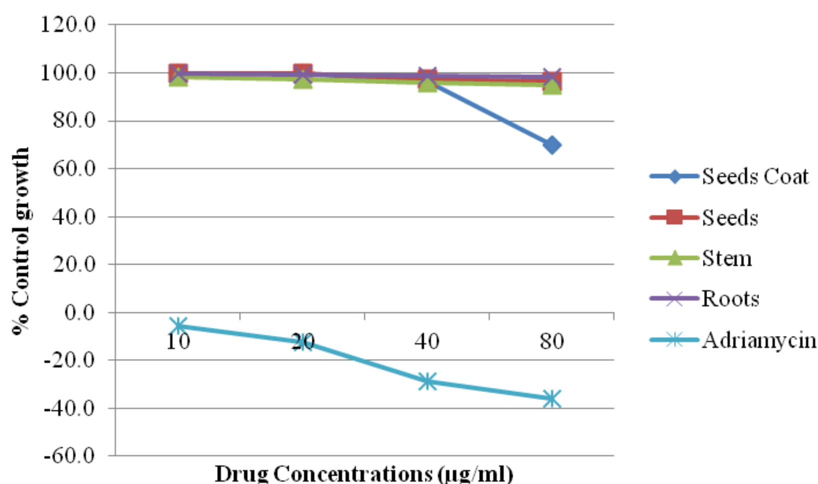

Figure 2: Effect of different extracts of *S. aculeata* on growth of Human Lung Cancer Cell Line Hop-62.

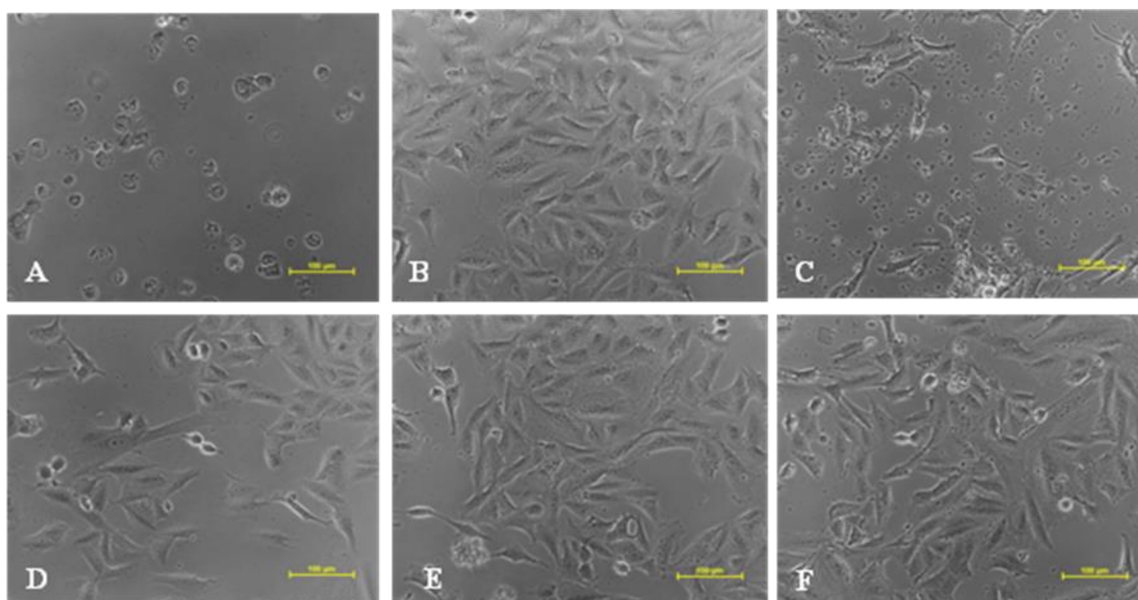

Figure 3: Effect of different extracts on the growth of Human lung cancer cell line Hop-62. A. Positive control (Adriamycin); B. Control; C. Seed coat; D. Stem; E. Root; F. Seed.

4. CONCLUSION

So, it can be concluded that the extracts of *Sesbania aculeata* can be used as a potential source of natural antioxidants that can be used to treat various oxidative stress related disorders. Seed coat extracts which has shown good anticancer activity against the human lung cancer cell line should be further explored for the active compound that is responsible for such activity as well as with other type of cancer cell lines, so that it could be used as control measures against deadly dangerous diseases like cancer.

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