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Research Article

Free radical scavenging and cytotoxic effect of *Stachytarpheta indica* vahl., against EAC cell lines

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Abstract

The present study was carried out to evaluate the anticancer property of ethanol extract of ***Stachytarpheta indica* vahl**., leaves against Ehrlich ascites carcinoma. The leaves powder was subjected to continuous extraction using ethanol. The ethanol extracts of the ***Stachytarpheta indica* vahl**., (EESI) was studied for its antioxidant potentials using various free radical models such a DPPH, Reducing power assay, super oxide scavenging activity and ABTS. *In vitro* cytotoxic assay such as trypan blue dye exclusion and MTT assays were carried out against EAC cell line. The plant extract significantly inhibits the DPPH, ABTS and superoxide radical and showed pronounced reducing power.

Keywords: Antioxidant, MTT, Trypan blue, EAC cell line and Anticancer.

INTRODUCTION

Cancer is a large group of diseases, all of which have one thing in common i.e. cells growing out of control or fundamentally a disease of tissue growth regulation failure. In order for a normal cell to transform into a cancer cell, the genes which regulate cell growth and differentiation must be altered [1]. Though many diseases (such as heart failure) may have a worst prognosis than most cases of cancer, cancer is the subject of widespread fear and taboos, there are 200 different types of cancer that afflict humans. [2]

Cancer is one of the leading causes of adult deaths worldwide. In India, the International Agency for Research on Cancer (IARC) estimated that indirectly the number of people died from cancer in 2008 was 8% of all projected global cancer deaths and about 6% of all deaths in India[3]. It is expected that average life expectancy of the Indian population will increase to 70 years by 2021-25 (Registrar General of India, 1996) [4]. There will be a substantial rise in the proportion of elderly people in the country. In-terms of absolute numbers, the increase will be from 14 million as recorded during the year 1971 to 113 million in the year 2016 [5]. India is facing a variety of nontransferable diseases or epidemics like cancer that need immediate attention.

Medicinal plants play a key role in human health care. About 80 percent of the world populations rely on the use of traditional medicines, which are predominantly based on plant materials. The traditional medicine refers to a broad range of ancient,

natural health care practices including folk/tribal practices as well as Ayurveda, Siddha and Unani. These medicinal practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles [6].Numerous useful drugs have been discovered from higher plants followed by ethno medical practices[7]. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs [8].

Materials and Methods

Plant source selected for the present study was ***Stachytarpheta indica* vahl**., Aerial parts of the selected plant was collected from in, around Trichy, identified with the help of Flora of Presidency of Madras[9], and authenticated by Taxonomist Rev. Fr. Dr.John Birtto, Director, RAPINAT Herbarium, St.Joseph's college, Trichy, Tamil nadu , India.

Cells

EAC (Ehrlich Ascites carcinoma) cell lines were obtained from Srimad Andavan Arts and Science College, Trichy and maintained at the dose of $1X10^6$cells/mouse by intraperitoneal inoculation at once in a week [10].

***In vitro* antioxidant activity**

Antioxidant activity was measured by using DPPH radical scavenging assay method [11], Reducing Power assay [12], Superoxide radical scavenging activity [13] and ABTS Radical scavenging activity [14]. Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

***In vitro* cytotoxicity assays**

The cytotoxic effect of the water extract of the test drug was screened against EAC cell lines using trypan blue dye exclusion method and MTT assay.
The ethanol extract of the plant *Stachytarpheta indica* vahl., belonging to the family Verbenaceae was selected and was screened for its anticancer activity against EAC cell lines employing various and *in vitro* studies.

Results and Discussion

Table 1 - DPPH radical scavenging potential of EESI

S.No	Concentration (µg/ml)	Scavenging of DPPH radical (%)
1	**10**	**12.45**
2	**20**	**27.81**
3	**30**	**48.61**
4	**40**	**68.97**
5	**50**	**78.52**
6	**Ascorbic acid (30µg/ml)**	**40**

IC_{50} value = 30.85 µg/ml

Table 2 -Reducing power of EESI

S.No	Concentration (µg/ml)	Reducing power (%)
1	100	17.03
2	200	26.10
3	300	56.30
4	400	64.39
5	500	74.62
6	Ascorbic acid (30 µg/ml)	80

IC_{50} value = 266.42 µg/ml

Table 3-ABTS⁺ Inhibition potential of EESI

S.No	Concentration (µg/ml)	Reducing power (%)
1	100	54.86
2	200	63.49
3	300	66.91
4	400	71.29
5	500	79.82
6	Ascorbic acid (50 µg/ml)	52.94

IC_{50} value = 91.4 µg/ml

Table 4-Superoxide radical inhibition potential of EESI

S.No	Concentration (µg/ml)	Inhibition of Superoxide radical Production (%)
1	100	29.83
2	200	30.52
3	300	43.18
4	400	57.55
5	500	63.99
6	Ascorbic acid (30 µg/ml)	75

IC_{50} value =347.52 µg/ml

Table 5 - Cytotoxic effect of EESI on EAC cell line (Trypan blue method)

Concentration of EESI (µg/ml)	Viable cells (%)	Death cells (%)
31.25	84.42	15.58
62.5	80.41	19.59
125	64.03	35.97
250	58.09	41.91
500	52.39	47.61

Dead cell = Stained with Trypan blue dye; Viable cell = Not stained with Trypan blue dye

Table 6 - Cytotoxic effect of EESI on EAC Cell line using MTT Assay:

S.NO	Concentration of Plant extract (µg/ml)	24 hours % of dead cells
1	Control	-
2	15.62	8
3	31.25	12.4
6	250	52.88
6	250	52.88
6	250	52.88

IC_{50} value = 236.38 µg/ml

Table – 4, Showed that it was evidenced that increased superoxide radical inhibition was linearly corresponds with concentration of the plant extract At 500 µg/ml. Superoxide radical scavenging activity of ethanol extract of *Stachytarpheta indica* vahl**.** , was found to be 63.99%. IC_{50} value of *Stachytarpheta indica* vahl., was found to be 347.52 µg/ml. These results were indicated that the ethanol extract of *Stachytarpheta indica* vahl**.** , was found to have good antioxidant activity.

The Trypan blue, MTT assay are the most commonly used for the detection of cytotoxicity or cell viability. Cytotoxic effect of EESI on EAC cell lines was tabulated (**Table 5**). The percentage of dead cells statically increased from concentration 31.25 to 500 µg/ml of EESI administration and the percentage of viable cell is decreased with increase in concentration of the plant extract.
The cytotoxic effect of ethanol extract of *Stachytarpheta indica* vahl**.** was analyzed and the results were tabulated in Table 6. At a concentration of 15.62µg/ml 8% of cytotoxicity was observed, whereas at high concentration 250 µg/ml 52.8 % of cytotoxicty was observed in 24 hours of incubation with test drug. The IC_{50} value was found to be 236.38 µg/ml.

Discussion

DPPH test is based on the ability of DPPH a stable free radical, to decolourize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515- 517nm and also for a visible deep purple colour. when DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance[15]. *Stachytarpheta indica* vahl extract showed 78.52% scavenging activity with IC50 value of 30.85µg/ml

In reducing power assay antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700nm, where in presence of reductants (antioxidants) in the plant extracts, causes the reduction ofFe3+/Ferricyanide complex to ferrous form[12] The EESI showed potent reducing power with IC50 of 266.42µg/ml

In the $ABTS^+$ assay technique, $ABTS^+$ cation is produced by incubating the ABTS with potassium persulphate. The test is based on the principle that the ability of the extract to scavenge the $ABTS^+$ cation by measuring the decrease in the OD at 700 nm. The result revealed that extract showed pronounced $ABTS^+$ cations scavenging activity and the IC_{50} value was found to be 91.4 µg/ml.

Superoxide anion (O^{-2}) is one of the most important representatives of free radicals. It acts as a precursor of more reactive oxidative species such as single oxygen and hydroxyl radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage, and plays a vital role in peroxidation of lipids. In the present study, the inhibitory effect of *Stachytarpheta indica* vahl extracts on superoxide radicals was in a concentration dependent manner. In the present study EESI showed high super oxide radical scavenging activity and it is comparable with standard ascorbic acid.

The ethanolic extract of the *Stachytarpheta indica* vahl was tested against EAC cell lines. Different concentrations of the plant extract were inoculated with selected cell line and the cytotoxicity was assessed using trypan blue dye exclusive method. The test based on the principle that the dead cell accepts dye and stain with blue colour. The plant drug would have disturbed the membrane integrity and have caused the cell death, which is one of the hall marks of apoptosis. The ethanolic extract showed 47.61% of cytotoxicity (500µg/ml) against EAC cell line. The loss of membrane integrity is considered as an indicator of apoptosis.

In-depth *In-vitro* cytotoxic study was carried out for ethanol extract of *Stachytarpheta indica* vahl against EAC cell lines employing MTT assay method. The yellow tetrazolium MTT (3-(4,5 dimethyl thiazolium -2)- 2, 5 di phenyl tetrazolium bromide) is reduced by metabolic activity of cells by the action of mitochondrial dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intra cellular purple formazan can be solubilized and quantified by spectrophotometric method. From the result it was observed that the plant drug under study has potent and maximum cytotoxicity 52.88% in 24 hrs and at a concentration of 250µg/ml and the IC_{50} value was

found to be 236.08µg/ml. The plant drug might have disturbed the mitochondrial assembly which resulted in the increased cytotoxicity of EAC cell line.

Summary and Conclusion

In-vitro antioxidant studies were carried out with four different models such as DPPH, Reducing power assay, superoxide inhibtion and $ABTS^{+}$ assay the results revealed that the ethanol extract of *Stachytarpheta indica* vahl., has a potent antioxidant activity.

The EESI was screened for its anticancer potentials against EAC cell lines employing In-Vitro methods; Trypan blue dye exclusion method. The results revealed that the ethanol extract showed a potent cytotoxic effect against EAC cell lines.

In the *In-Vitro* cytotoxic studies of EESI against EAC cell lines employing MTT assay method the plant showed maximum cytotoxic activity 52.88% at the concentration of 250µg/ml. The cytotoxic effect of the EESI may be due to the disruption of EAC cells.

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