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EVALUATION OF *IN-VITRO* ANTI-CANCER AND ANTIOXIDANT ACTIVITY OF *DURVILLAEA ANTARCTICA*

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ABSTRACT

The present study aimed to evaluate its antioxidant and anticancer potential using in vitro assay system using *Durvillaea antarctica* methanolic extract. Alkaloid, flavonoid, steroids, saponin and carbohydrates were present in the methanol extract. In quantitative system Steroids show higher content when compared to the other phytoconstituents. *In vitro,* antioxidant activity of the methanol extract was investigated by DPPH radical scavenging assays. Antifungal activity of *Durvillaea antarctica* was tested for the organisms *T.simii, A.niger, C.albicans* and *C.lunata* and the activity compared with the Fucanazole control. Breast cancer (MCF 7) cell line was used as the in vitro cancer model for MTT assay. It can be concluded that *Durvillaea antarctica* has potential antioxidant and anticancer activities.

KEYWORDS: Sea algae, phytochemical, antifungal, Antioxidant and cell line study.

INTRODUCTION

Cancer is a multistep disease incorporating physical, environmental, metabolic, chemical, and genetic factors, which play a direct and/or indirect role in the induction and deterioration of cancers. Cancer is a major public health burden in both developed and developing countries. Cancer is a complex multifactorial cell disease characterized by abnormal cellular proliferation. Cancer development and progression are dependent on the cellular accumulation of various genetic and epigenetic events (Giri *et al*., 2006; Mbaveng *et al*., 2011) and is an aberrant net accumulation of typical cells arising from excess proliferation, insufficient apoptosis. (Abdul *et al*., 2009). Cancer development is normally caused by oncogene, tumour suppressor gene and microRNA gene alterations (Burstein and Schwartz,

2008). It imposes a serious burden on the public health system and its treatment and cure are scientifically challenging.

The limited success of clinical therapies including radiation, chemotherapy, immunomodulation, and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management (Dai and Mumper, 2010). Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Balunas and Kinghorn, 2005). The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity. It was estimated that 14 cancer drugs of the top 35 drugs in year 2000 based on worldwide sales were natural products and natural product derivatives (Shoeb, 2006). Thus, it is urgent to find more and more safe new compounds that kill cancer cells.

Phytochemicals are biologically active, naturally occurring chemical compounds presents in plants, which provide health benefits for humans further than those attributed to macro-nutrients and micro-nutrients (Hasler and Blumberg, 1999). It is well known that many polyphenolic compounds, such as phenolic acids, flavonoids, anthocyanidins, and tannins, which possess remarkable antioxidant and anticancer activities, are rich in plant materials. Some studies have shown the positive correlation of the increased dietary intake of natural antioxidants with the reduced coronary heart disease and cancer mortality, as well as with longer life expectancy (Namiki, 1990; Nagendra *et al*., 2010). Many dietary polyphenolic constituents derived from the plants or plant extracts exhibited comparatively high antioxidant properties than the standard antioxidants, vitamins E or C by in vitro. Diet containing antioxidant rich fruits and vegetables significantly reduces the risk of many cancer diseases suggesting that antioxidants could be effective agents for the inhibition of cancer spread (Fresco *et al*., 2006). In recent years, researches about anticarcinogenic potential of quercetin have exhibited its promise as an anticancer agent. Likewise, *in vitro* and *in vivo* studies showed that quercetin was able to inhibit viability of leukemic cells, colon, and ovarian carcinoma cells, especially human breast cancer cells. In this study, we aimed to explore antioxidant potential and anticancer activity of methanol extract from the plant extract of *Durvillaea antarctica*.

MATERIALS AND METHODS

Collection and Extraction of Algal sample

The fresh sample of *Durvillaea antarctica* were collected randomly from the Rameshwaram sea shore, Tamil Nadu. Plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator. Crude sample extract was prepared by Soxhlet extraction method. About 20gm of powdered material was uniformly packed into a thimble and extracted with 250ml of ethyl acetate and methanol extract separately. Dried extract was kept in refrigerator at 4ºC till future use.

Phytochemical Screening

Preliminary phytochemical analysis was carried out for ethyl acetate and methanol extracts of *Durvillaea antarctica* as per standard methods described by Brain and Turner 1975 and Evans 1996.

Quantitative Phytochemical Analysis

Estimation of flavonoids

The determination 20g of plant sample was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hrs with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of normal butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven into a constant weight. The content was calculated in percentage (Nahapetian and Bassiri, 1975).

Estimation of Alkaloids

Alkaloid determination using Harborne (1973) method 5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Conc. ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Steroids

1ml of extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

***In Vitro* Antioxidant Activity**

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was carried out by the method of Molyneux (2004). To 1.0 ml of 100.0 µM DPPH solution in methanol, equal volume of the *Durvillaea antarctica* sample in methanol of different concentration was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation [(Absorbance of control - Absorbance of test)/ Absorbance of control)] × 100. IC50 value was calculated using Graph pad prism 5.0.

Antifungal Activity

The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (methanol, ethyl acetate and hexane). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37^{o}C for 72 hrs. The diameters of zone of inhibition observed were measured.

***In Vitro* Anticancer Activity (Cytotoxicity Activity)**

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^{5} cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of

test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

RESULT AND DISCUSSION

Yield Obtained

Yield from the *Durvillaea antarctica* were obtained from the solvents methanol and ethyl acetate. The yield obtained from methanol extract was higher than that of ethyl acetate extract by 20%. Since the yield was high in the methanol extract, this was used for further analysis.

Phytochemical Analysis

Qualitative phytochemical analysis of *Durvillaea Antarctica* was done for methanol extract and ethyl acetate extract. The phytochemical constituent in methanol was higher and it found to possess five constituents alkaloids, flavonoids, steroids, saponin and carbohydrates whereas the ethyl acetate extract showed only two phytochemicals tannin and carbohydrates. Our study is the first report for the phytochemical qualitative and quantitative analysis.

Table 2: Qualitative phytochemical analysis of *Durvillaea Antarctica.*

Phytochemicals	Observations	Extracts	
		Methanol	Ethyl acetate
Alkaloids			
Mayer's test	Cream color	+	-
Wagner's test	Reddish brown solution/ precipitate	+	-
Flavonoids			
Lead acetate test	Yellow orange	+	-
H_2SO_4 test	Reddish brown / Orange color precipitate	+	-
Steroids			
Liebermann-Burchard test	Violet to blue or Green color formation	+	-
Terpenoids			
Salkowski test	Reddish brown precipitate	-	-
Arthroquinone			
Borntrager's test	Pink color	-	-
Phenols			
Ferric chloride test	Deep blue to Black color formation	-	-
Lead acetate test	White precipitate	-	-
Saponin	Stable persistant	+	-
Tannin	Brownish green / Blue black	-	+
Carbohydrates	Yellow / brownish / blue / green color	+	+
Oils & Resins	Filter paper method	-	+

Phytochemical Quantitative Analysis

The quantitative analysis of phytoconstituents of *Durvillaea antarctica* from methanol extract was found to be 0.049 gm, 0.052 gm and 1.8879 gm respectively for flavonoids, alkaloids and steroids and the quantity of steroids obtained was highest.

Antioxidant Activity

For determining the DPPH radical scavenging activity, after the addition of required chemicals the samples were subjected to UV-spectrophotometer analysis. The absorbance was measured at 514 nm. The OD values for the concentrations of sample 50, 250, 500, 750 and 1000 µl were 24.48, 33.33, 39.45, 45.57 and 53.06 respectively. The inhibition concentration is 885.69 µl of the sample.

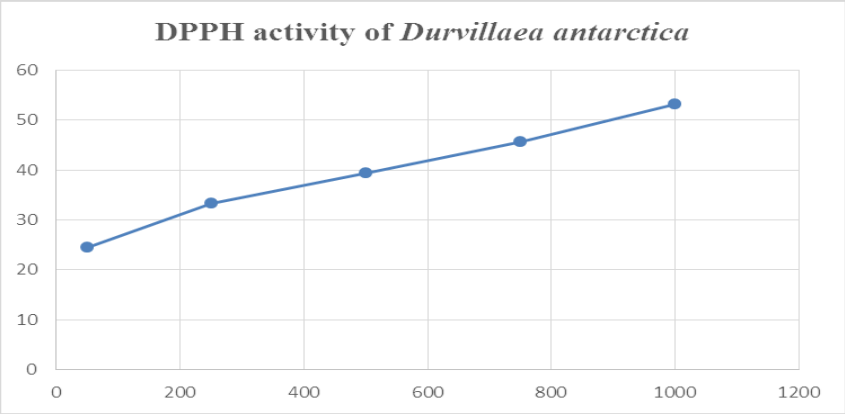

Figure 1: DPPH Activity of Methanol extracts of *Durvillaea Antarctica.*

Antifungal Activity

Antifungal activity of *Durvillaea antarctica* was tested for the organisms *T.simii*, *A.niger*, *C.albicans* and *C.lunata* and the activity compared with the Fucanazole control. The highest activity was found against the organisms *T.simii* and *C.lunata* and the methanol concentration 50. The highest activity for the other two organisms *A. niger* and *C.albicans* were also seen in 50 concentration. The activities were less in the methanol extract than the control.

Table 2: Antifungal activity of *Durvillaea Antarctica.*

S. No.	Organisms	Control	Methanol			
			20	30	40	50
1.	*T. simii*	20	9	12	15	18
2.	*A. niger*	23	10	12	15	17
3.	*C. albicans*	22	9	12	16	19
4.	*C. lunata*	25	10	11	13	15

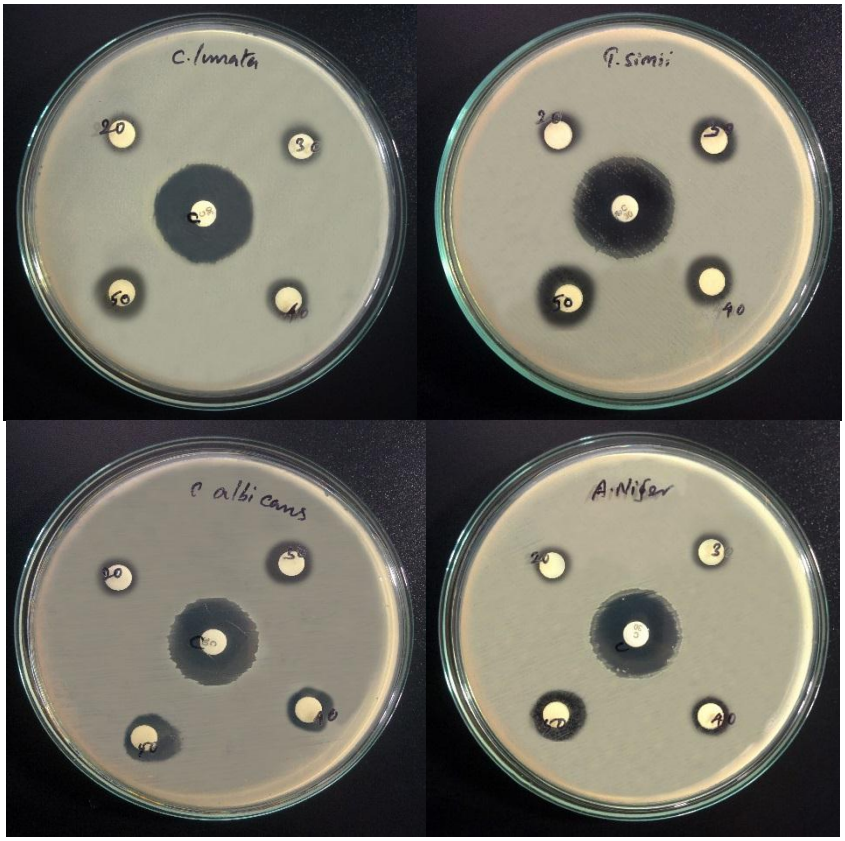

Plate photo: Antifungal activity of *Durvillaea antarctica*

In Vitro Anticancer Activity of *Durvillaea Antarctica*

The anticancer activity of methanol extract was measured using MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and cell viability (Lau *et al*., 2004). Methanol leaf extract was tested for cytotoxicity using MCF-7 cell line at concentrations 31.25 to 1000 and the cytotoxicity was highest in 1000 µg/ml and CTC_{50} value is 236.71 µg/ml.

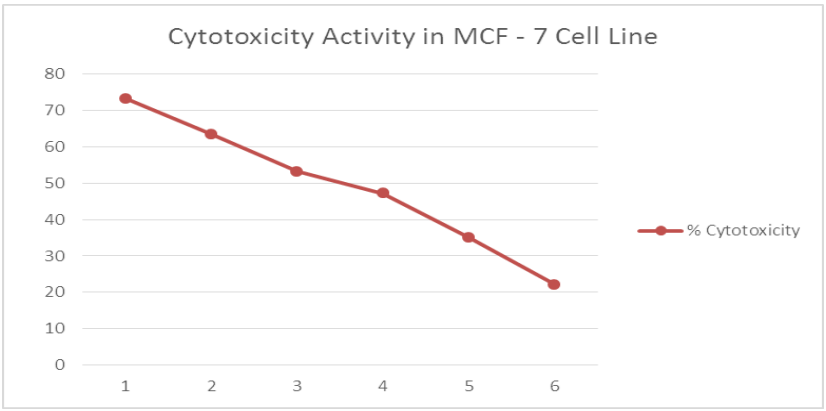

Figure 1: Cytotoxicity activity in MCF- 7 cell line.

CONCLUSION

Traditional Indian and Chinese medicinal herbs have been used in the treatment of different diseases in the country for centuries. There have been claims that some traditional healers can successfully treat cancer using herbal drugs. In this study, it is evident that the methanol extracts of *Durvillaea antarctica* possess effective antioxidant and anticancer activities. This is due to the presence of phytochemicals like alkaloids, flavonoids, steroids, saponin and carbohydrates. Cytotoxicity activity in MCF- 7 cell line showed a CTC_{50} value of 236.71µg/ml. Finally we conclude that the seaweeds are a source of bioactive compounds with potential applications, revealing activity to control pathogens.

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