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IN VITRO CYTOTOXICITY STUDIES AND ANTIOXIDANT ACTIVITIES OF TWO WILD YAMS

Dioscorea pentaphylla* and *Dioscorea oppositifolia

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Abstract

Biomedical application of Dioscorea pentaphylla and Dioscorea oppositifolia by determining the cytotoxicity and antioxidant properties of tuber extracts. Plant extracts expressed cytotoxicity against human lung carcinoma cell line (A-549) which was ascertained by MTT Assay. From this the D. pentaphylla tuber was found to be more effective on (A-549) cell line. The present work also designed the antioxidant activities of D. pentaphylla and D. oppositifolia methanol extract were assessed by using DPPH and $ABTS^+$activity. In DPPH activities among the five different concentrations (100µg/ml to 500µg/ml) of extracts tested. In $ABTS^+$ activity D. pentaphylla and D. oppositifolia were assayed by using five different concentrations (50µg/ml to 250µg/ml). The absorbance was increased in both assays with increasing concentrations of the extracts.

Key words - Cytotoxicity, Antioxidant, D.pentaphylla, D.oppositifolia, MTT Assay, (A-549).

I. INTRODUCTION

Yam is important as it become a staple food in many African countries because of its eating quality. People consumed yams as a cooked vegetable; whether fried or roasted. In West Africa, boiled yam is often pounded into a thick paste and is eaten with soup. From recent studies, most people throughout the West African yam belt eat whole roasted yam as a snack when they are away from home and this became a popular snack among them [1]. Yams can also produce some secondary metabolites like alkaloids, flavonoids, phenols. It may have the potential of antioxidant and anti cancerous activity.

Antioxidants prevent oxidation and oxidative damage cells by scavenging oxygen radicals or hydroxy radicals, which have been implicated to attack polyunsaturated fatty acids in cell membranes giving rise to lipid peroxidation [2, 3,4]. At high levels, this type of oxidation has been linked not only to aging but also to mutagenesis, carcinogenesis, and atherosclerosis [5, 6,] Free radicals such as superoxide anion, hydroxyl and peroxyl radicals, which are produced in biological systems and foods, are responsible for oxidation of cell lipids and DNA damage, and they may cause serious diseases like cancer, coronary, [7]. Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, which are rich in antioxidant activity [8]. The therapeutic potentials of *Dioscorea* has been documented in many parts of the world include; the treatment of sore throat, gastric cancer and carcinoma of rectum, and goiter in China [9]. The tribal people also used *Dioscorea* as medicine for ailments related to digestive tract and respiration [10].

The leaves, flowers, tender shoots and tubers of *Dioscorea* are used for cooling and demulcent. They are used in the form of decoction for leprosy and cancerous lesions. The leaves are antiseptic; the paste is applied on ulcers and abscesses. The root is chewed to cure toothache aphthae. The whole plant is used in application for tumours and the ash extract of flowering twigs along with tender leaves cure cancer and leprosy [11].The present study was aimed to investigate the *in vitro*

antioxidant and cytotoxic activity of methanol extract of tubers of *D. pentaphylla* and *D. oppositifolia.*

II. MATERIALS AND METHODS

A. Source of plants materials

The tubers of *Dioscorea pentaphylla* (L.) and *Dioscorea oppositifolia* (L.) belongs to the family Dioscoreaceae were collected from Matheswaran hills Mysuru. The collected plant material was identified and authenticated by Dr. M. Murugasen- Scientist-B, BSI Eastern Regional Centre, Shillong.

B. Cytotoxicity assay

Cell Line Used: A-549 (Human, Lung Carcinoma)
Assay: MTT Assay
Concentration used: 250– 62.5 µg/m

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based.

The principle involved is the cleavage of tetrazolium salt MTT (3-(4, 5 dimethyl thiazole-2 yl) - 2, 5-diphenyl tetrazolium bromide) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The numbers of cells were found to be proportional to the extent of formazan production by the cells used [12].

The percentage growth inhibition was calculated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves for each cell line.

% Cell Viability = (Mean OD of individual test group/ Mean OD of control group) x 100

C. Anti-oxidant DPPH activity

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduce DPPH [13]. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517nm. Various concentration of methanol extract of the sample (4.0ml) was mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture were shaken vigorously and left to stand for 30min, and the absorbance was measured at517nm.Ascorbic acid was used as a control. The percentage of inhibition in DPPH radical scavenging activity was calculated as follows;

$$\% \text{ Inhibition} = A0-A1/A0 \times 100$$

D. ABTS$^+$ radical scavenging activity

ABTS$^+$ decolourisation assay involves the generation of the ABTS$^+$ chromophore by the oxidation of ABTS$^+$ with potassium persulphate . It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the extract on ABTS$^+$ radical cation was measured at 734nm[14] (Giao *et* al.,2007). ABTS$^+$ solution : Equal volume of the 7mM of ABTS$^+$ was mixed with 2.45mM potassium persulphate and the mixture was allowed to stand in the dark room temperature for 12-16 hours before use. ABTS$^+$ solution was diluted to an absorbance of 0.7±0.5 with methanol 734nm. The reaction initiated by the addition of 1.0ml diluted ABTS$^+$ to 10µl of different concentration (50-250µg/ml) of tuber extracts and also to 10 µl of methanol as control. Ascorbic acid was used as positive control. The absorbance was read at 734nm after 6minutes and the percentage inhibitions were calculated. The inhibition was calculated according to the equation.

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$$I = Ao - A1/Ao \times 100$$

Where, Ao is absorbance of control reaction,
A1 is absorbance of test compound.

III. RESULTS AND DISCUSSION

The results of cytotoxicity assay reveal that the methanolic extracts of *Dioscorea pentaphylla* and *Dioscorea oppositifolia.* The cytotoxicity action on A-549(Human lung) carcinoma dying cells exhibited ultra structural and biochemical features that characterize loss of viability. According to MTT test results, at the end of 48h, 250µg/ml of *D. pentaphylla* and *D.oppositifolia* methanolic tubers caused 67.71% and 62.00% inhibition in growth of A-549 cells, respectively. As can be seen from the percentage of the inhibition rates, at the end of the result *D. pentaphylla* tuber found to be more effective on A-549 cells than *D.oppositifolia.* The results are tabulated in table 1&2

The antioxidant activities in methanol extracts of *D. pentaphylla* and *D.oppositifolia* were assessed by using DPPH and $ABTS^+$ activity. The DPPH and $ABTS^+$ activity of different concentration of methanol extracts (100µg/ml to 500µg/ml) along with standard Vitamin C & Ascorbic acid is presented in the table 3&4. Among the five different concentrations (100µg/ml to 500µg/ml), the higher percentage of inhibition (44.35±0.12) and (53.55 ± 0.76) was observed *D. pentaphylla* against the standard (Vitamin C & Ascorbic acid) in DPPH and $ABTS^+$ activity respectively .

Table1. In Vitro Cytotoxicity activity of Methanolic extracts of Dioscorea pentaphylla

S.No	Concentration µg/ml	% viability	Average
1	250	36.29167	0.0871
2	125	37.95833	0.0911
3	62.5	51.29167	0.1231
4	31.25	93.54167	0.2245

IC50value of *Dioscorea pentaphylla*: 105.549

Table2. In Vitro Cytotoxicity activity of Methanolic extracts of Dioscorea oppositifolia

S.No	Concentration µg/ml	% viability	Average
1	250	38	0.0912
2	125	38.79167	0.0931
3	62.5	60.45833	0.145
4	31.25	105.875	0.2541

IC50value of *Dioscorea oppositifolia*: 123.0152

(25*R*)-Spirost-5-en-3β-ol 3-*O*-α-L-rhamnopyranosyl- (1→2) - *O* - [β - D - g l u c o p y r a n o s y l - (1→ 4)] - β - D -glucopyranoside, the first isolated spirostanol glycoside from yam had higher cytostatic activity than diosgenin for Hep G2, HEK293 and MCF7 cells [15]. Similarly the earlier reports of *Dioscorea* sps show the potential anticancer activity of the extract from tubers against human breast adenocarcinoma cell line (MCF-7) [16]. The study showed DALM exhibited moderate cytotoxicity towards MCF-7 cells whereas, DALA showed no or slight cytotoxicity. These results confirm the possible role of the 70% methanolic extract as a promising free radical scavenger and as a potent anticancer source. Viji *et al*. (2016) accounted the biomedical application of *Dioscorea alata* by determining the antimicrobial and cytotoxic properties of tuber extracts. The tests gave positive results [17].

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Table1. DPPH activity of different concentration of ethanolic extract of *Dioscorea oppositifolia and Dioscorea pentaphylla*

S. No	Aqueous Extracts	% inhibition				
		100 (µg/ml)	200 (µg/ml)	300 (µg/ml)	400 (µg/ml)	500 (µg/ml)
1	*Dioscorea oppositifolia*	9.31 ± 0.32	16.52 ± 0.36	29.32 ± 0.22	34.21 ± 0.32	43.33± 0.13
2	*Dioscorea pentaphylla*	10.62 ± 0.08	18.58 ± 0.22	32.32 ± 0.32	36.21 ± 0.51	44.35± 0.12
3	Standard (Vitamin C)	21.20 ± 0.30	32.52 ± 0.21	41.22 ± 0.23	53.12 ± 0.24	62.13 ± 0.35

The experiment was conducted in triplicates (n=3)

IC_{50} value of *Dioscorea oppositifolia* : 459.02µg/ml
IC_{50} value of *Dioscorea pentaphylla* : 439.17µg/ml
IC_{50} value of Vitamin C (standard) : 409.82µg/ml

Table 2. $ABTS^+$ activity of different concentration of aqueous extract of *Dioscorea oppositifolia and Dioscorea pentaphylla*

S. No	Aqueous Extracts	% inhibition				
		50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)
1	*Dioscorea oppositifolia*	11.41 ± 0.30	17.30 ± 0.21	26.80± 0.58	38.34 ± 0.15	51.52 ± 0.94
2	*Dioscorea pentaphylla*	13.43 ± 0.15	21.32 ± 0.56	33.57 ± 0.68	45.34 ± 0.66	53.55 ± 0.76
3	Ascorbic acid	24.45 ± 0.47	32.32 ± 0.39	46.76± 0.72	58.17 ± 0.40	76.16 ± 0.77

The experiment was conducted in triplicates (n=3).

IC_{50} value of *Dioscorea oppositifolia* : 241.13µg/ml
IC_{50} value of *Dioscorea pentaphylla* : 239.56µg/ml
IC_{50} value of Ascorbic acid (standard) : 184.21µg/ml

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical, to become a stable diamagnetic molecule [18]. Ethanolic extract of *D.villosa* shows the good antioxidant activities [19]. *D. alata has* effective free radical scavenging potential against 2, 2-diphenyl-1-picrylhydrazyl, nitric oxide and lipid per oxidation [20].Our work on the DPPH radical scavenging activity of *D. pentaphylla* and *D.oppositifolia* agrees with the findings of the DPPH radical scavenging of *D. tomentosa*. [21]

IV. CONCLUSION

The present data supports that methanolic tuber extract of *Dioscorea pentaphylla* is promising to be used in cancer therapy and antioxidant treatments. Further investigation is to be carried out to determine the mode of action and the molecules responsible for the anti proliferation activity observed.

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