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STUDY OF *IN VITRO* ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTICANCER POTENTIAL OF *BLUMEA OXYODONTA* DC.

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ABSTRACT

The present study aimed to establish scientific authenticity anticancer, antioxidant and anti-inflammatory activities of ethanol and aqueous extracts of the whole plant *Blumeaoxyodonta* DC. The antioxidant activity of the extracts were assessed by DPPH radical scavenging assay, ferric reducing power assay and hydrogen peroxide radical scavenging assay using ascorbic acid as the standard. Anti-inflammatory activity of the extracts was evaluated using HRBC membrane stabilization method where diclofenac sodium was used as the standard. The results indicated that the ethanolic and aqueous extracts have significant antioxidant, anti-inflammatory and cytotoxicity activity in a dose-dependent manner. The extract demonstrated potent scavenging of free radicals such as hydrogen peroxide and superoxide and possessed high reducing power against the standard ascorbic acid. Potent inhibition of HRBC membrane lysis and protein denaturation proved its anti-inflammatory activity when compared to standard diclofenac sodium. Brine shrimp lethality test provided strong results for the anticancer activity which was confirmed by the MTT assay performed on cell lines. The finding powerfully supports that the ethanolic extract of the plant *Blumeaoxyodonta*DC could be used as a source of anticancer drug attributed to its phenolic content that makes it prompting for further isolation of bioactive compounds.

Key words: *Blumeaoxyodonta*DC, antioxidant, anti-inflammatory, anticancer, MTT assay.

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INTRODUCTION

Cancer is defined as a cluster of diseases that are produced due to loss of cell cycle control or abnormal/uncontrolled cell growth (Krishnamurthy K, 2007). It may be caused due to several changes in the expression of genes which results in dysregulation of balance between cell proliferation and cell death. This ultimately leads to the evolution of a population of cells that has the potential to invade tissues and metastasize into distant sites and produce significant morbidity or death if left untreated (Mohan H, 1992).

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Due to insufficient early detection methods that are widespread and comprehensive, cancer has become a substantial health problem all over the world (Divisi D *et al*, 2006). This can be caused by factors that are both internal and external. Internal factors may involve inherited mutations, hormonal imbalances, immune problems or metabolic mutations whereas external factors can be tobacco, radiation, chemicals or infectious organisms (Chanda S and Nagani K, 2013). Cancer has become a prominent cause of death in especially less developed countries and the affliction is just expected to grow more (Torre LA*et al*, 2015). It was estimated that almost 18.1 million new cancer cases and 9.6 million cancer deaths will already be reported in the year 2018. The most common and leading cause of death that is diagnosed in both sexes is lung cancer which constitutes

almost 18.4% of total deaths followed by female breast cancer (11.6%) (Bray F*et al*, 2018).

One of the most important components that are involved in the multi-staged cancer process is the oxidative stress which is mainly produced by the environmental factors. Oxidative stress is produced as a result of overproduction of free radicals or due to the deficient antioxidant defense system that can lead to reversible/irreversible tissue injury (Dreher D*et al*, 1996). Usually free radicals are produced in normal/pathological cell metabolism, due to xenobiotics or due to the exposure of ionizing radiation. Overproduction occurs when they react with non-radicals through the chain reactions (Halliwell B and Gutteridge JMC, 1984). Persistent and cumulative oxidative stress that is produced due to these free radicals produce harmful modifications to the intracellular components such as DNA, proteins and lipids (Ziech D *et al*, 2010). At the same time reduced levels of antioxidant enzymes such as Superoxide dismutase, catalase, glutathione peroxidase as well as non-enzymatic antioxidants such as GSH, Vitamin C, thioredoxin also play a vital role in induction of human cancers (McEligot AJ *et al*, 2005). Another important endogenic cause that produces persistent oxidative stress is the inflammatory response (Cerutti PA and Trump BF, 1991). The process of inflammatory response generates oxidative stress that further enhances inflammation. Thus these two factors are common and can potentiate each other in the process of carcinogenesis (Kaushal N and Kudva AK, 2013). Normally inflammation is a protective response of the body to eliminate harmful agents or pathogens from the host, but when there is any kind of dysregulation; inflammation turns out to be injurious and induces necrosis and malignant transformations (Bermejo-Martin JF *et al*, 2014).

With substantial enhancement in the prognosis of cancer, it has been estimated that cancer is responsible for one out of four deaths (Siegel R *et al*, 2014). This situation creates an immediate need for the discovery of new approaches or chemotherapeutic agents that would fight against cancer (Davidson KT*et al*, 2016). Chemotherapy is defined as a crucial treatment approach for the management of advanced malignancies 16. Although the existing anticancer agents aim to eradicate the tumor cells, they also affect the distinct normal cells resulting in adverse effects in several organs (Lawal RA *et al*, 2012; Zhou H, 2007; Ahles TA and Saykin AJ, 2007; Han R *et al*, 2008; Nicolson GL, 2005; Constantinou C *et al*, 2008). Such devitalizing side effects limit the utility of the anticancer agents (Johnstone RW *et al*, 2002; Kovacic P, 2007). Therefore there is an increase in surge in the indication of other alternative or supplemental therapies for a higher success in treatment of cancer (Lamson DW and Brignall MS, 1999). One of the promising modality in the treatment of cancer in recent years has been the phytochemicals that can act either alone or as synergistic (Davidson KT*et al*, 2016). Medicinal plants are being used for the cure and prevention of cancer in many countries (Madhuri S and Pandey G, 2009). Due to the abundance of natural chemicals in plants they possess immense ability to produce newer drugs with chemoprotective capacity to fight cancer (Desai AG *et al*, 2008).

*Blumeaoxyodonta*D.C. belonging to the family Asteraceae is popular as spiny leaved blumea. It is a very common rabbi weed in India and found in the paddy fields in Konkan region. Essential oil present in the plant contains terpenes, sesquiterpenes, alkaloids and flavonoids and is used as styptic, anthelmintic, expectorant, febrifuge, antipyretic, diuretic and as liver tonic (Dinde AV *et al*, 2018). However there haven't been any studies on the anti-cancer potential of *Blumeaoxyodonta*D.C. Thus, the present study was aimed to investigate the antioxidant, anti-inflammatory and cytotoxic potential of *Blumeaoxyodonta*D.C. leaves extract on cancer cell lines.

MATERIALS AND METHODS

Collection and identification of plant material

The whole plant Blumeaoxyodonta (Asteracea) collected from campus of Pariyaram Medical College, Kannur district; Kerala, India was authenticated by Pramod. C., Assistant Professor ,Govt. Brennen College, Dharmadam, Thalassery, Kerala and Dr. M.K. Ratheesh Narayanan, Assistant Professor Govt. Payyannur College, Payyannur, Kerala. The voucher specimen (No: 0025) was deposited in the herbarium of Botany Department, Brennen College, Dharmadam, Thalassery, Kannur, India. After authentication the plants were collected, cleaned and dried in shade at room temperature. The dried whole plants were pulverized in a mechanical grinder to obtain coarse powder.

Extraction of the Powdered Plant Material

The dried and powdered plants were subjected to soxhlet extraction to produce ethanolic and aqueous extracts of whole plant of *Blumeaoxyodonta*DC (EEBO and AEBO respectively). To prepare ethanolic extract, the extraction was first run by petroleum ether for removing the fats and fatty constituent; then by 70% ethanol. A sticky mass was obtained after evaporation of solvent. And to prepare aqueous extract, the extraction was first run by petroleum ether for the removal of fats and fatty constituent; then by distilled water. A sticky mass were obtained after evaporation of solvent. Then the extracts were kept in refrigerator until further use.

Primary phytochemical screening

All the extracts obtained by extraction procedure were subjected to preliminary phytochemical tests in order to identify the nature of chemical constituents. Tests were carried out by the standard methods described in

Practical Pharmacognosy by Dr. C.K Kokate and K.R. Khandelwal.

In vitro Antioxidant Activity

DPPH radical scavenging activity

The solution of DPPH (0.3mM) was prepared in methanol and 1ml of this solution was added to 3ml of crude extracts dissolved in methanol at different concentrations (50, 100, 150, 200, 250µg/ml). The mixture was kept at room temperature for 30 minutes and the absorbance was measured at 517nm using a UV spectrophotometer. The percentage scavenging activity at different concentrations was determined using ascorbic acid as standard (Asirvatham R *et al*, 2013; Gowrish A *et al*, 2015). All the tests were performed in triplicates and percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{Ac - At}{Ac} \times 100$$

Where Ac is the absorbance of control and At is the absorbance of test

Ferric reducing power assay

One ml of varying concentrations of extracts (50 – 250 µg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH-6.6) and 2.5 ml of 1% potassium ferric cyanide. This mixture was kept at 50^{o}c in water bath for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (5 ml) was mixed with 5 ml of distilled water and 1 ml of freshly prepared 0.1% of ferric chloride solution and the absorbance was read at 700nm. Control was prepared in a similar manner excluding samples. Ascorbic acid was used as the standard (Ferreira ICFR *et al*, 2007). The percentage reducing power was calculated by using the formula:

$$\text{Inhibition (\%)} = \frac{Ac - At}{Ac} \times 100$$

Where Ac is the absorbance of control and At is the absorbance of test

Hydrogen peroxide radical scavenging assay

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4).Different concentrations of extracts (50 -250 µg/ml) from a stock concentration of 10 mg/ml was added to H_2O_2 solution (0.6 ml).A control without the test compound but an equivalent amount of distilled water was taken.Absorbance was read at 230 nm after 10 minutes (Keser S *et al*, 2012). The percentage of hydrogen peroxide scavenging of both extracts and standard compounds were calculated by using the formula:

$$H_2O_2 \text{ Scavenged (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where Ac is the absorbance of control and As is the absorbance of sample

In vitro Anti-Inflammatory Activity

The human red blood cell (HRBC) membrane stabilization method

The blood was collected from healthy human volunteer who are not taking any NSAID's for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8 % sodium citrate, 0.05 % citric acid and 0.42 % NaCl in water) and centrifuged at 3000 rpm. Packed cells were washed with isosaline (0.85 %, pH 7.2) and a 10 % v/v suspension was made with isosaline.

Various concentrations of extracts were prepared (50-250 µg/mL) using distilled water and to each concentration 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml hypo saline (0.36 %) and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 20 minutes. The hemoglobin content in the supernatant solution was estimated spectrophotometricaly at 560 nM.

To the control, added 1 ml of solvent, 2 ml phosphate buffer, 1 ml hyposaline and 0.5 ml HRBC suspension. Blank consists of 1 ml of solvent, 2 ml phosphate buffer and 1 ml hyposaline. Diclofenac sodium was used as the standard and control was prepared by omitting the extracts (Varghese CP *et al*, 2012; Nagaharika Y *et al*, 2013).

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

$$\text{\% protection} = \frac{\textit{ensity of drug treated sample}}{\textit{tical density of control}} \times 100$$

$$\text{\%inhibition} = \frac{\textit{ical density of drug treated sample}}{\textit{optical density of control}} \times 100$$

Brine shrimp lethality test

The assay was carried out according to the principle and protocol developed by Meyer *et al* with little modifications. Brine shrimp eggs (Ocean Star International INC, Snowville, USA) were purchased locally from (Matsyafed, Kannur, India) and placed on 500 ml beaker which is filled with filtered sea water. After 48 hours of incubation at room temperature under illumination and aeration. The cysts were hatched and the resulting nauplii were attracted towards the light source and collected with pipette. Samples for testing were prepared by dissolving 100 mg of the each concentrated extract with 100 ml water and prepared a 1 mg/ml stock solution. From this 100 µg/mL, 10 µg/mL solutions were prepared.

Ten brine shrimps were transferred to each test tube and tests for each concentration were done in triplicate. The total volume of solution in each vial was adjusted to 5 ml by adding sea water. Sea water was considered as negative control while standard cyclophosphamide was used as positive control. The test tubes are maintained at room temperature (25 – 29 °C) under illumination.

Survivors were counted after 24 hours (Meyer BN *et al*, 1982; Hasanat A, 2015).

Determination of in vitro antiproliferative effect of extracts on cultured HeLa cell lines by MTT assay

HeLa cervical cancer cell line was purchased from NCCS Pune was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO2 (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a CO2 incubator. The cells were trypsinized (100µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Himedia)) for 2 minutes and passaged to 24 well plate in complete aseptic conditions. Extracts were added to grown cells at a concentration of 6.25 µg, 12.5 µg, 25 µg, 50µg and 100µg from a stock of 1mg/ml and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation.

Cytotoxicity Assay by Direct Microscopic observation

Entire plate was observed at an interval of each of 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observations were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 30 µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°c in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100 µl of MTT Solubilisation Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 540 nm (Dinesh MD *et al*., 2016; Mazzucco MB *et al*.,2015).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ viability} = \frac{\text{of sample} \times 100}{\text{of control group}}$$

Statistical Analysis

Results of antioxidant and anticancer activities were expressed as Mean ± SEM. IC50 value were determined. One way ANOVA followed with Tukey-Kramer method was used for comparing control and test groups in anti inflammatory activity calculations.

RESULTS

Evaluation of Antioxidant Activity

In vitro antioxidant activity of extracts of whole plant of Blumea oxyodonta DC by DPPH radical scavenging method

In vitro antioxidant activity of the ethanol and aqueous extracts of whole plant of *Blumeaoxyodonta*DC was evaluated by DPPH radical scavenging method. Both ethanol and aqueous extracts exhibited antioxidant activity in a dose dependent manner. The results are shown in Table No 1 and depicted in Figure No 1. EEBO exhibited a slightly better antioxidant activity than AEBO. EEBO showed a maximum inhibition of 81.75%, while AEBO exhibited an inhibition of 75.49% and the standard ascorbic acid showed an inhibition of 91.86% at a concentration of 250 µg/ml.

entration g/ml

In vitro antioxidant activity of extracts of whole plant of Blumea oxyodonta DC by hydrogen peroxide radical scavenging method

Antioxidant potential of the whole plant extracts were evaluated using hydrogen peroxide radical scavenging method. Both the extracts showed antioxidant activity in a dose dependent manner. The activity was compared with standard ascorbic acid and is shown in Table No 2 and depicted in Figure No 2. EEBO showed a maximum inhibition of 86.07% while AEBO showed an inhibition of 79.98% and ascorbic acid showed an inhibition of 91.54% at a concentration of 250 µg/ml.

In vitro antioxidant activity of whole plant extract of Blumea oxyodonta DC by reducing power assay method

Antioxidant potential of the leaf extracts of *Blumea oxyodonta* was evaluated using reducing power assay method. Both ethanol and aqueous extracts exhibited anti-oxidant activity in a dose dependent manner. The results were compared to the standard ascorbic acid and shown in Table No 3 and depicted in Figure No 3. EEBO showed a maximum inhibition of 86.2553% while AEBO showed an inhibition of 70.1262%, and standard (ascorbic acid) at showed an inhibition of 92.8472% at 250µg/ml.

Evaluation of Anti-inflammatory activity

In vitro anti-inflammatory activity of whole plant extracts of Blumea oxyodonta DC by HRBC membrane stabilization method

In vitro anti-inflammatory activity of ethanol and aqueous extracts of whole plant extracts of *Blumea oxyodonta* were evaluated using HRBC membrane stabilization method. Both ethanol and aqueous extracts exhibited anti-inflammatory activity in a dose dependent

manner. The results are tabulated in Table No 4 and depicted in Figure No 4. Ethanol extract (EEBO) has exhibited a better anti-inflammatory activity than the aqueous extract (AEBO). Both ethanol and aqueous extracts exhibited extremely significant anti-inflammatory activity in a dose dependent manner. EEBO showed maximum inhibition of 89.9314%, while AEBO exhibited 84.8970% inhibition, and the standard diclofenac 91.0755% exhibited inhibition at 250µg/ml concentration.

Brine shrimp lethality assay

Cytotoxic activity of the whole plant extracts of *Blumea oxyodonta* DC was evaluated using brine shrimp assay. Both ethanol and aqueous extracts exhibited cytotoxic activity in a dose dependent manner. The results were compared to the standard cyclophosphamide and shown in Table No 5 and depicted in Figure No 5. The IC_{50} values of EEBO were found to be 20.3704 µg/ml while that of AEBO 32.2106 µg/ml and standard (cyclopposphamide) were found to be 5.7279µg/ml.

MTT Assay

Cytotoxic activity of the whole plant extracts of Blumea oxyodonta was evaluated using MTT assay. Both ethanol and aqueous extracts exhibited cytotoxic activity in a dose dependent manner. The results were compared to the standard cyclophosphamide and shown in Table No 6 and depicted in Figure 6. The IC 50 value of EEBO was found to be 37.9315 µg/ml while that of AEBO and standard (Doxorubicin) were found to be 65.1628 µg/ml and 68.987 µg/ml respectively.

Table 1. DPPH radical scavenging activity of *Blumea oxyodonta* DC

Sl. No.	Conc. (µg/ml)	Ethanolic extract		Aqueous extract		Ascorbic acid	
		Absorbance	% Scavenging activity	Absorbance	% Scavenging activity	Absorbance	% Scavenging activity
1	50	0.632±0.021	30.5495	0.716±0.009	21.3186	0.421±0.013	53.7362
2	100	0.498±0.017	45.2747	0.634±0.045	30.3297	0.302±0.009	66.8132
3	150	0.301±0.043	66.9231	0.493±0.037	45.8242	0.231±0.010	74.6154
4	200	0.237±0.026	73.9560	0.342±0.018	62.4176	0.108±0.025	88.79
5	250	0.172±0.080	81.75	0.223±0.084	75.4945	0.074±0.076	91.8681
6	Control	0.910±0.001		0.910±0.001		0.910±0.001	
	IC_{50}	103.585		160.407		38.123	

Absorbance values are in Mean ± SEM., n=3.

Table 2.Hydrogen peroxide radical scavenging activity of *Blumea oxyodonta* DC

Sl. No.	Conc. (µg/ml)	Ascorbic acid		Ethanolic extract		Aqueous extract	
		Absorbance	%Scavenging activity	Absorbance	%Scavenging activity	Absorbance	%Scavenging activity
1	50	0.312±0.034	51.1737	0.398±0.031	37.7152	0.456±0.013	28.6385
2	100	0.232±0.009	63.6933	0.302±0.004	52.7387	0.337±0.025	47.2613
3	150	0.187±0.027	70.7355	0.203±0.008	68.2316	0.268±0.038	58.0595
4	200	0.121±0.043	81.0642	0.142±0.018	77.7777	0.198±0.009	69.0141
5	250	0.054±0.012	91.5493	0.089±0.024	86.0719	0.128±0.076	79.9687
6	Control	0.639±0.007		0.639±0.007		0.639±0.007	
	IC_{50}	39.713		90.424		123.522	

Absorbance values are in Mean ± SEM., n=3

Table 3. Reducing power assay of *Blumea oxyodonta* DC

Sl. No.	Conc. (µg/ml)	Ascorbic acid		Ethanolic extract		Aqueous extract	
		Absorbance	%Scavenging activity	Absorbance	%Scavenging activity	Absorbance	%Scavenging activity
1	50	0.364±0.009	48.9481	0.494±0.005	30.7153	0.513±0.035	28.0505
2	100	0.421±0.010	59.8878	0.383±0.003	46.2833	0.485±0.027	31.9776
3	150	0.178±0.005	75.0351	0296.±0.010	58.4853	0.310±0.067	56.5217
4	200	0.084±0.013	88.2188	0.167±0.034	76.5778	0.254±0.045	64.3759
5	250	0.051±0.024	92.8472	0.098±0.006	86.2553	0.213±0.016	70.1262
6	Control	0.713±0.003		0.713±0.003		0.713±0.003	
	IC 50	51.027		115.823		149.097	

Values are in Mean ± SEM., n=3

Table 4. *In vitro* anti-inflammatory activity of *Blumea oxyodonta* by HRBC membrane stabilization method

Sl.No	Conc. (µg/ml)	Diclofenac		Ethanolic extract		Aqueous extract	
		Absorbance	% inhibition	Absorbance	% inhibition	Absorbance	% inhibition
1	50	0.394 ±0.008	54.9199***	0.421±0.005	51.8307***	0.586±0.012	32.9519***
2	100	0.293±0.013	66.4759***	0.354±0.030	59.4966***	0.456±0.004	47.8261***
3	150	0.221±0.010	74.7139***	0.246±0.009	71.8535***	0.327±0.090	62.5858***
4	200	0.154±0.006	82.3799***	0.186±0.012	78.7185***	0.256±0.087	70.7094***
5	250	0.078±0.004	91.0755***	0.088±0.034	89.9314***	0.132±0.041	84.8970***
6	Control	0.874±0.002		0.874±0.002		0.874±0.002	

Values are in Mean ± SEM., n=3, *** extremely significant at $p < 0.0001$ compared to control

Table 5. Brine shrimp lethality assay

Groups	Log Concentration	% mortality	LC_{50} (µg/ml)
Control	-	0	
Ethanolic extract	1	46.667± 3.333	20.3704
	2	6 0.0 ± 3.333	
	3	100.0 ± 3.333	
Cyclopposphamide	1	56.667 ± 3.333	5.7279
	2	76.667 ± 3.333	
	3	100.0 ±3.333	
Aqueous extract	1	36.667 ± 3.333	32.2106
	2	56.667 ± 3.333	
	3	100.0±3.333	

Values are in Mean ± SEM., n=3

Table 6. % Cytotoxicity determination by MTT Assay

S. No.	Conc. (µg/ml)	Doxorubicin		Ethanolic extract		Aqueous extract	
		Optical density	% Cytotoxicity	Optical density	% Cytotoxicity	Optical density	% Cytotoxicity
1	6.25	0.04184	25.0001	0.4225	24.26960	0.4379	21.51000
2	12.5	0.3619	35.1303	0.3776	32.32600	0.4013	28.06960
3	25	0.2676	52.0300	0.2846	48.98730	0.3376	39.48740
4	50	0.2496	55.2523	0.2732	51.02402	0.2969	46.78260
5	100	0.1600	71.314	0.2014	63.90034	0.2511	54.99193
6	Control			0.5579		0.5579	
	IC_{50}	28.7078		37.9315		65.1628	

Fig 1. DPPH radical scavenging activity of *Blumea oxyodonta* DC

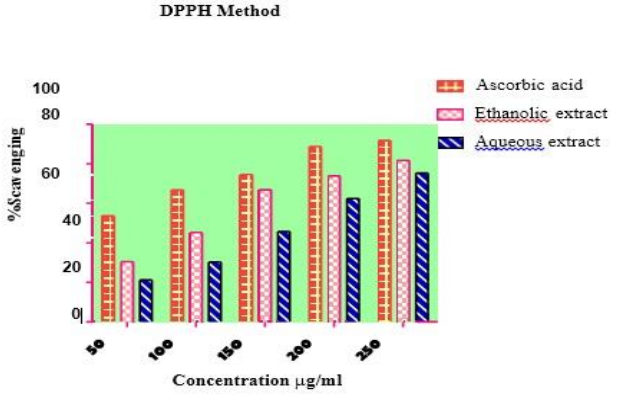


Fig 2. Hydrogen peroxide radical scavenging activity of *Blumea oxyodonta* DC

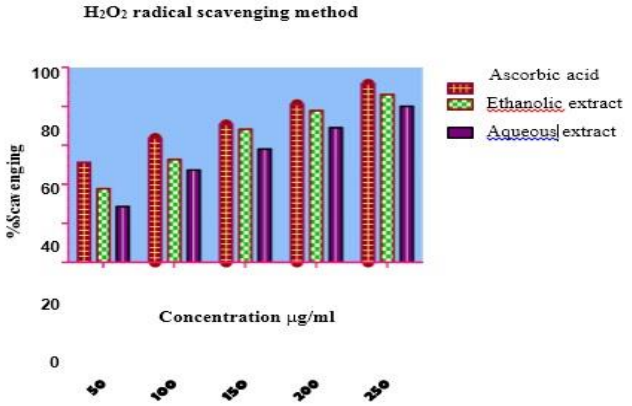


Fig 3. Reducing power assay of *Blumea oxyodonta* DC

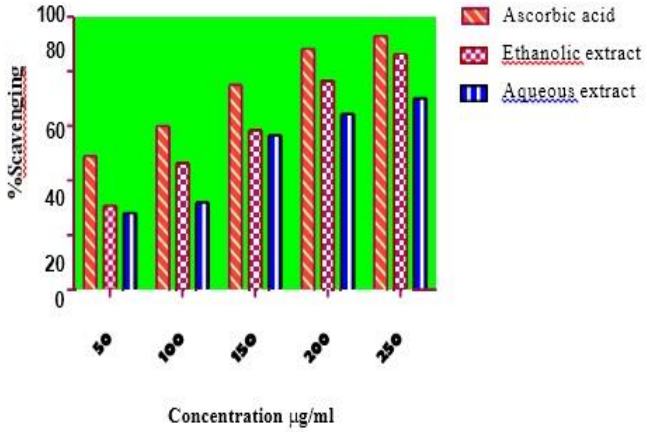


Figure No 4: In vitro anti-inflammatory activity of *Blumea oxyodonta* DC by HRBC membrane stabilization method

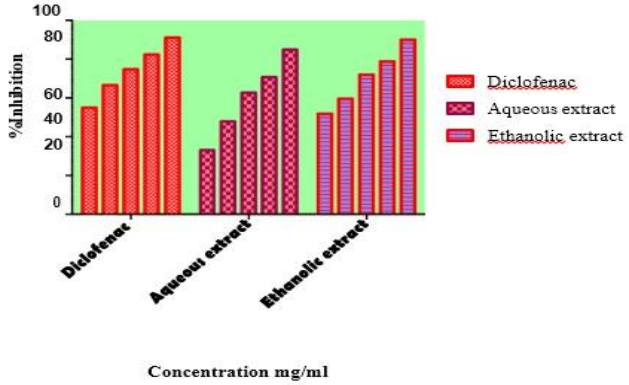


Fig 5. Brine shrimp lethality assay

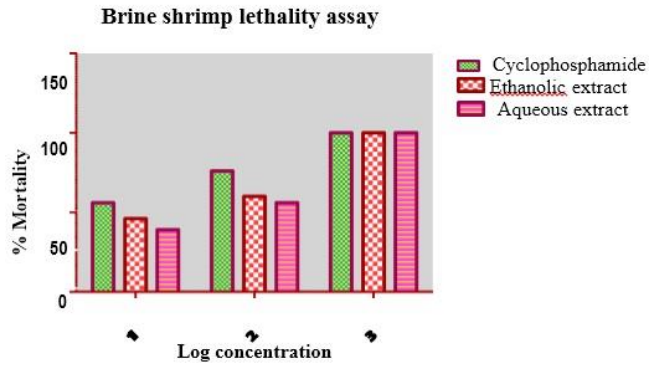


Fig 6. % Cytotoxicity determination by MTT Assay

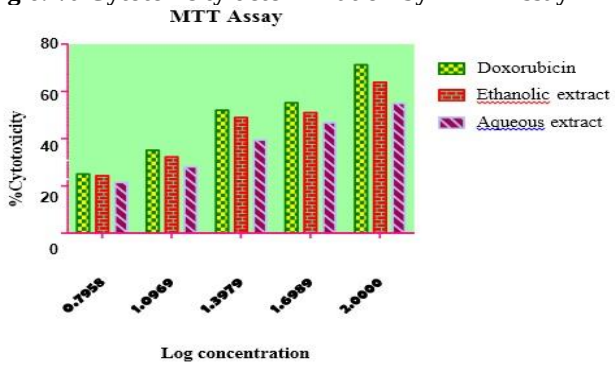


Fig 7. Action of various concentrations of doxorubicin on HeLa celllines

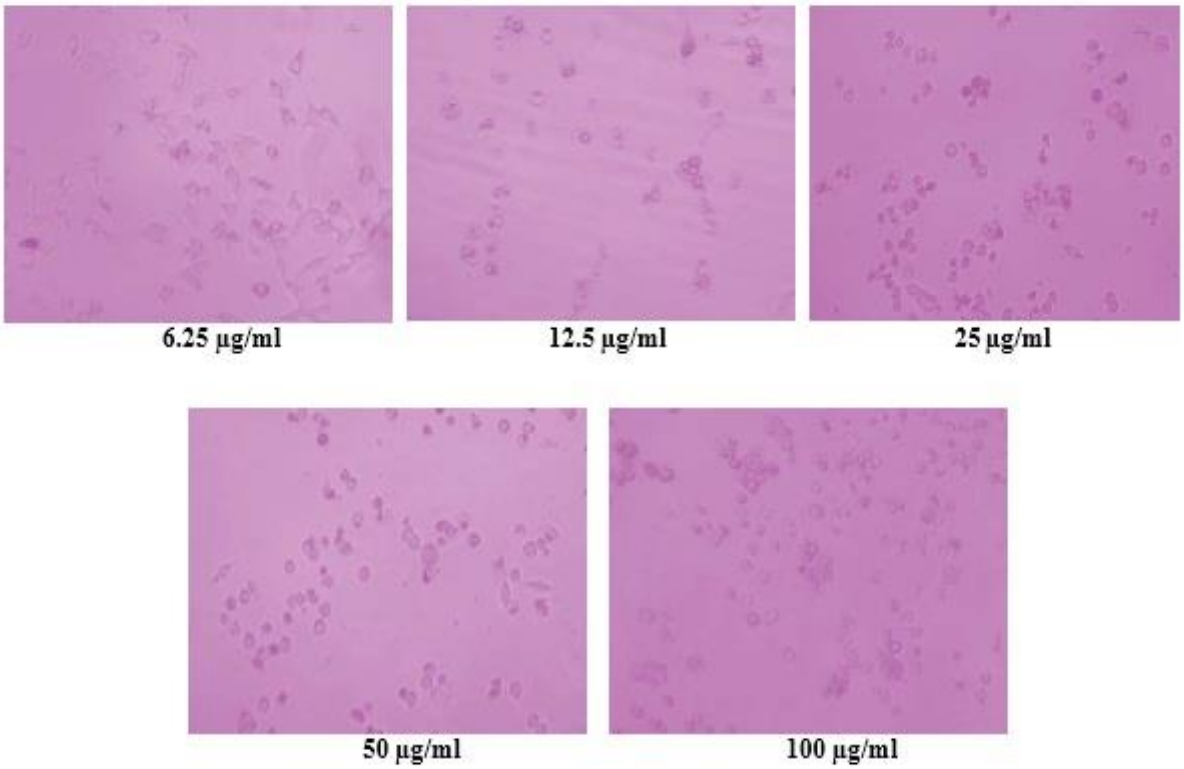


Fig 8. Action of various concentrations of ethanolic extract on HeLa cell lines

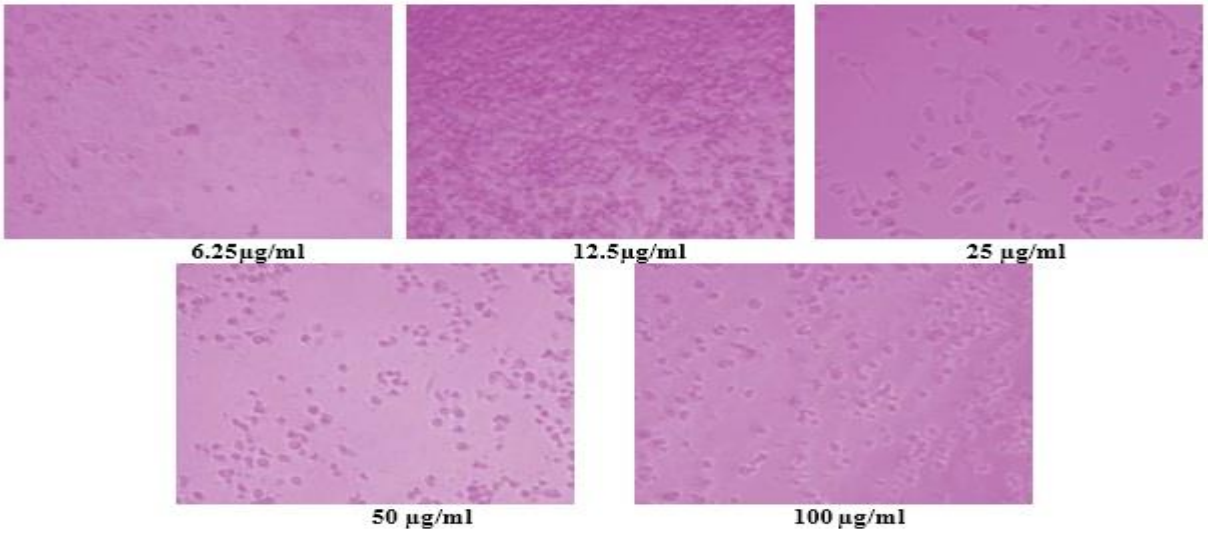
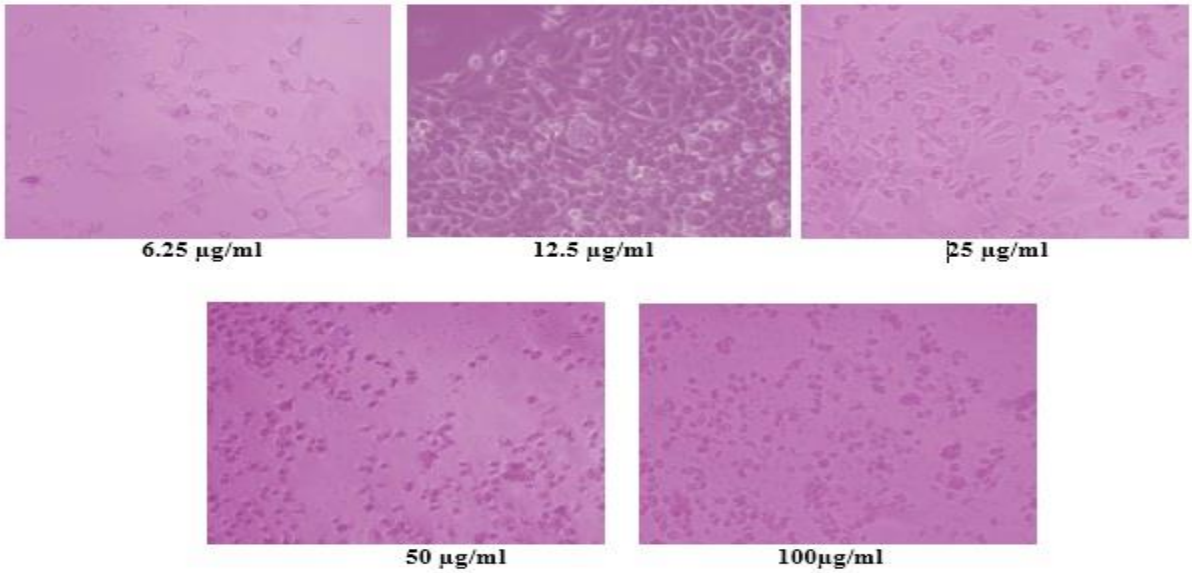


Fig 9. Action of various concentrations of aqueous extract on HeLa cell lines



DISCUSSION

Blumea oxyodonta D.C. belongs to the family Asteraceae and is vast in its species. It is popular known as spiny leaved blumea and is a very common rabbi weed found in India, in the paddy fields of Konkan region. Essential oil present in the plant contains terpenes, sesquiterpenes, alkaloids and flavonoids and is used as styptic, anthelmintic, expectorant, febrifuge, antipyretic, diuretic and as liver tonic (Dinde AV *et al*, 2018).

In the present study the preliminary phytochemical screening of *Blumea oxyodonta* DC was studied extensively to explore its chemical nature. The extracts revealed the presence of flavonoids, tannins, phenolic compounds and saponins. In vitro antioxidant, anti-inflammatory and cytotoxic activity of the extracts was performed to investigate its bioefficiency.

A rapid and sensitive method to assess the antioxidant activity is the DPPH radical scavenging method that uses 1, 1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. The DPPH radical abstracts an electron in the presence of an antioxidant that results in the reduction of absorbance value (Pourmorad F *et al*, 2006). The ethanolic extract (EEBO) was capable of neutralizing the DPPH free radicals via hydrogen donating activity by 30.55, 45.27, 66.92, 73.96 and 81.75% at concentrations of 50, 100, 150, 200, and 250 µg/ml respectively whereas aqueous extract (AEBO) showed 21.32, 30.33, 45.82, 62.42 and 75.5 % at concentrations of 50, 100, 150, 200, and 250 µg/ml.81 The IC50 was found to be 38.123 µg/ml for standard ascorbic acid and 103.585 µg/ml for ethanolic extract (EEBO) and 160.407 µg/ml for aqueous extract (AEBO)

respectively.

The hydrogen peroxide radical scavenging activity of the *Blumea oxyodonta* extracts are shown in Figure 2. Ascorbic acid was used as the positive control. The IC50 was found to be 90.424 and 123.522 for ethanolic extract (EEBO) and aqueous extract (AEBO) respectively and for standard ascorbic acid, it was found to be 39.713 µg/ml. The alcoholic and aqueous extracts scavenged H2O2 and this may be attributed to the presence of phenols and tannins which could donate electrons, thereby neutralizing it into water. A decrease in absorbance indicated the scavenging activity or consumption of hydrogen peroxide anion radicals present in the reaction mixture (Sasikumar V *et al*, 2014). It was observed that ethanol and aqueous extracts % scavenging activity ranged from 37.72% to 86.07% and 28.64 % to 79.97% respectively.

Figure 3 shows the reducing power of ethanolic and alcoholic extracts of *Blumea oxyodonta* DC as a function of their concentration. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe3+/ferricyanide complex to the ferrous form in the assay. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe2+concentration (Ferreira ICFR *et al*, 2007). It was observed that ethanol and aqueous extracts % scavenging activity ranged from to 30.72% to 86.26 and 28.05% to 70.12% respectively.

The study adopted HRBC membrane stabilization method for screening of anti- inflammatory activity. The results revealed the ethanolic extract (EEBO) of *Blumea oxyodonta DC* showed percentage inhibition of 78.7185% for 200 µg/ml followed by a maximum inhibition of 89.93% for 250 mg/ml whereas aqueous extract (AEBO) showed 70.70% for 200 mg/ml and a maximum inhibition of 84.9% for 250 µg/ml.

The brine shrimp lethality assay has been used routinely in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials (Shuaib MA *et al*, 2014). In the present study it was found that both the ethanolic and aqueous extracts of Blumeaoxyodonta showed prominent results in brine shrimp cytotoxic assay in a dose dependent manner (Table 5 and Figure 5). The lethal concentration (LC50) of the test samples after 24 hours was obtained by a plot of the percentage mortalities of shrimps against the logarithm of the sample concentration (toxicant concentration) and an approximate linear correlation was observed. LC50 values were compared with cyclophosphamide having LC50 value 5.7279 µg/ml. Ethanolic extract showed lowest lethality with LC50 value 20.3704 µg/ml while aqueous extract showed highest cytotoxic activity with LC50 value 32.2106 µg/ml.

The cell proliferation activity of ethanolic and aqueous extracts of *Blumeaoxyodonta*DC whole plant was carried out by MTT assay using HeLa cell line. Measurement of cell viability and proliferation forms was used as basis for this in vitro assay. We estimated the effect of extract on the growth of cell in vitro. The results obtained from assay (Table 6) showed IC50 value of 37.93µg/ml for ethanolic extract and 65.1693µg/ml. It confirmed cytotoxic activity of ethanolic and aqueous extracts of *Blumea oxyodonta* DC.

CONCLUSION

The results of the study revealed that the both the alcoholic and aqueous extracts of the *Blumea oxyodonta DC* possess significant antioxidant and anti-inflammatory activity. The results obtained from brine shrimp lethality bioassay is quite encouraging and thus it was followed by *in vitro* anticancer activity on HeLa cell line using MTT assay which revealed the potential cytotoxic activity of ethanolic extract of *Blumeaoxyodonta* DC probably because of its direct cytotoxic effects. Therefore, this plant could be used as a source of potent anticancer agent for anticancer drug development. The results are promising and hence future studies will be focused on characterization of active compounds.

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CONFLICT OF INTEREST

Nil.

REFERENCES

Ahles TA and Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. *Nature Reviews Cancer*, 7(3), 2007, 192–201.

Asirvatham R, Christina AJM, Murali A. In vitro antioxidant and anticancer activity studies on *Drosera indicia* (Droseraceae). *Advanced Pharmaceutical Bulletin*, 3(1), 2013, 115-120.

Bermejo-Martin JF, Martín-Loeches I, Bosinger S. Inflammation and infection in critical care medicine. *MediatInflamm*, 2014, 456256.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A.

Cerutti PA and Trump BF. Inflammation and oxidative stress in carcinogenesis. *Cancer Cells*, 3, 1991, l-7.

Chanda S and Nagani K. *In vitro* and *in vivo* methods for anticancer activity evaluation and some Indian medicinal plants possessing anticancer properties: an overview. *Journal of Pharmacognosy and Phytochemistry*, 2(2), 2013, 140-152.

Constantinou C, Papas A, Constantinou AI. Vitamin E and cancer: an insight into the anticancer activities of vitamin E isomers and analogs. *International Journal of Cancer*. 2008; 123(4), 2008, 739–52.

Davidson KT, Zhu Z, Fang Y. Phytochemicals in the Fight against Cancer. *PatholOncol Res,* 22(4), 2016, 655-660.

Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, Bedi YS, Taneja SC, Bhat HK. Medicinal Plants and Cancer Chemoprevention. *Curr Drug Metab*, 9(7), 2008, 581–591.

Dinde AV, Lokhande PB, Mujawar HA. Essential oil Extraction, Characterization and Antimicrobial Study of BlumeaOxyodonta D.C. From Konkan Region. *Int J Recent Sci Res*, 9(3), 2018, 24784-24789.

Dinesh MD, Athira PS, Ajma N, Abhisha NC, Carmel A. Anti-cancer Activity (Oral) of Betel Leaf Extract by *in-vitro*. *Int. J. Life. Sci. Scienti. Res*, 2(4), 2016, 412-414.

Divisi D, Di Tommaso S, Salvemini S, Garramone M, Crisci R. Diet and cancer. *Acta Biomed*, 77(2), 2006, 118-123.

Dreher D, Junod AF. Role of oxygen free radicals in cancer development. *Eur J Cancer*,32 A (1), 1996, 30-38.

Ferreira ICFR, Paula Bapista, Miguel Vilas-Boas, Lillian Barros. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chemistry*,100(4), 2007, 1511-15116.

Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 68(6), 2018, 394-424.

Gowrish A, Vagdevi HM, Rajashekar H. In vitro antioxidant and antitubercular activity of *Leucasmarrubioides*Desf. root extracts. *Journal of Applied Pharmaceutical Science*, 5(2), 2015, 137-142.

Halliwell B and Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J*. 1984; 219: 1-14.

Han R, Yang YM, Dietrich J, Luebke A, MayerProschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. *Journal of Biology*, 7(4), 2008, 12– 33.

Hasanat A, Jakaria,M, Ibrahim Md, Tanvir AC, Arifujjaman, Ali MS. Bioassay of brine shrimp lethality and thrombolytic activity of methanolic extract of *Macarangadenticulata*leaves. *Journal of Pharmacognosy and Phytochemistry*, 3(6), 2015, 43-46.

Howes RM. Dangers of antioxidants in cancer patients: a review. Philica, Article ID 153, 2009.

Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. *Cell*, 108(2), 2002, 153–164.

Kaushal N and Kudva AK. Oxidative stress and inflammation: the lesser of two evils in carcinogenesis. *Post Doc J*, 1(2), 2013, 89–101.

Keser S, Sait C, Semra T, Yilmaz O, Ismail T. Hydrogen peroxide radical Scavenging and total antioxidant activity of Hawthorn. *Chemistry Journal*, 2(1), 2012, 9-12.

Khandelwal KR. Practical Pharmacognosy. 16th Ed. Pune: NiraliPrakashan, 2006.

Kokate CK and Gokhale SB. Practical Pharmacognosy. 12th Ed. NiraliPrakashan, 2008.

Krishnamurthi K. Screening of natural products for anticancer and antidiabetic properties. *Health Administrator*, 1&2, 2007, 69-75.

Lamson DW and Brignall MS. Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. *Alternative Medicine Review*, 4(5), 1999, 304–329.

Lawal RA, Ozaslan MD, Odesanmi OS, Karagoz ID, Kilic IH, Ebuehi OAT. Cytotoxic and antiproliferative activity of *Securidacalongepedunculata*aqueous extract on Ehrlich ascites carcinoma cells in Swiss albino mice. *IJARNP*, 5(4), 2012, 19-27.

Madhuri S and Pandey G. Some anticancer medicinal plants of foreign origin. *Current Science*, 96 (6), 2009, 779-83.

Mazzucco MB, Talarico LB, Vatansever S, Carro AC, Fascio ML, D'Accorso NB, Garcia CC, Damonte EB. Antiviral activity of an N-allylacridone against dengue virus. *Journal of Biomedical Science.* 2015, 22-29.

McEligot AJ, Yang S, Meyskens FL Jr. Redox regulation by intrinsic species and extrinsic nutrients in normal and cancer cells. *Ann. Rev. Nutr*, 25, 2005, 261–295.

Mohan H. Text book of Pathology.6thed. New Delhi: Jaypee brother's medical publishers, 1992.

Nagaharika Y, Kalyani V, Rasheed S, Ramadosskarthikeyan. Anti-inflammatory activity of leaves of *Jatrophagossypifolia*L by hrbc membrane stabilization method. *Journal of Acute Disease*, 2013, 156-158.

Nicolson GL. Lipid replacement/antioxidant therapy as an adjunct supplement to reduce the adverse effects of cancer therapy and restore mitochondrial function. *Pathology and Oncology Research*, 11(3), 2005, 139–144.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol*, 5(11), 2006, 1142-1145.

Sasikumar V, Kalaisezhiyen. Evaluation of Free Radical Scavenging Activity of Various Leaf Extracts from

Kedrostisfoetidissim a Cogn. *Biochem Anal Biochem*, 3(2), 2014, 1-7.

Shuaib MA, Shumaia, Kader MA. Antimicrobial and preliminary cytotoxic effects of ethanol extract and its fractions of Anthocephaluscadamba (Roxb.). *International Journal OfPharmacyand Life Sciences*, 2014; 5(12), 2014, 4038-4044.

Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*, 64(1), 2014, 9–29.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*, 65(2), 2015, 87-108.

Varghese CP, Ambrose C, Jin SC, Lim YJ, Keisaban T. Antioxidant and Anti-inflammatory Activity of *Eurycomalongifolia*Jack, A Traditional Medicinal Plant in Malaysia. *Int J Pharm Sci Nanotech*, 5(4), 2013, 1875-1878.

Zhou H, Zou P, Chen ZC, You Y. A novel vicious cycle cascade in tumor chemotherapy. *Medical Hypotheses*, 69(6), 2007, 1230-1233.

Ziech D, Franco R, Georgkalikas AG, Georgakila S, Malamou-Mitsi V, Schoneveld O, *et al*. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. *ChemBiol Interact*, 188, 2010, 334–339.