Journal of Medicinal Plants Studies
Year: **2013**, Volume: **1**, Issue: **4**
First page: (**152**) Last page: (**159**)
ISSN: **2320-3862**
Online Available at **www.plantsjournal.com**

Journal of Medicinal Plants Studies

Drosera burmannii Vahl: Antioxidant Potential in Dalton's Ascites Lymphoma (DAL) Bearing Mice

Raju A [1*], AJM Christina [2]

1. Department of Pharmacology, Shri Rawatpura Sarkar Institute of Pharmacy, Datia, M.P, India. [E-mail: rajuasirvatham@yahoo.com]
2. Department of Pharmacology, School of Pharmacy, Taylor's university, Malaysia.

The aim of the present study was to evaluate the antioxidant role of *Drosera burmannii* Vahl. (Droseraceae) in Dalton's Ascites Lymphoma (DAL) bearing mice. The effect of ethanol and aqueous extracts of *D.burmannii* (EEDB and AEDB) were administered at a 250 and 500mg/kg once a day for 14 days, 24 h after the inoculation of tumor cell line. After the treatment period, blood was collected from the animals and subsequently they were sacrificed for isolation of liver, brain, kidney and lungs for the observation of antioxidant status level. The parameters analyzed were catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), peroxidase (P), total protein (TP) and carbonyl content. Treatment with EEDB and AEDB significantly reduced the levels of MDA and increased the levels of GSH, SOD, CAT, P and TP in cancer induced animal and are similar as that of normal mice. The results suggest that the ethanol and aqueous extract of *D. burmannii* Vahl. possess significant antioxidant effects in DAL bearing mice.

Keyword: *Drosera burmannii* Vahl, Antioxidant enzyme, Dalton's Ascites Lymphoma (DAL).

1. Introduction

Cancer is one of the ailment disease process mediated by a diversity of endogenous and environmental stimuli such as reactive oxygen species (ROS) and other free radicals. ROS are generated endogenously and exogenously as a function of biochemical reactions using oxygen. High levels of ROS are toxic and least quantity required for various physiological functions including activation and modulation of signal transduction pathways, modulation of activities of redox-sensitive transcription factors, and regulation of mitochondrial enzyme activities. Cell consists of various antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH) and catalase (CAT). They are capable for scavenging many harmful ROS and converted into harmless product. Oxidative stress (OS) occurs when there is an imbalance between the production of ROS and decrease in oxidation capacity. This stress may cause mutagenesis, cytotoxicity and changes in gene expression that initiate or promote carcinogenesis [1]. In recent years many researcher attention on plant derived natural products such as flavonoids, terpenoids, steroids etc have diverse pharmacological properties including antioxidant and antitumor activity [2, 3]. One of such a plant is *Drosera*.

Drosera species are popularly known as the Sundews. It is one of the largest geniuses of carnivorous plants with over 170 species belonging to the family Droseraceae [4]. Three species of Drosera found in India viz., *Drosera burmannii* Vahl, *Drosera indica* L., and *Drosera peltata* J.E.Sm. *Drosera burmannii* contain several medicinally active compounds including quinones (plumbagin) hydroplumbagin glucoside, flavonoids (kaempferol, myricetin, quercetin and hyperoside), rossoliside (7-methyl-hydrojuglone-4-glucoside [5]. These species are used as vital components in an Ayurvedic preparation called

'Swarnabhasma' (Golden ash).It has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness, bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders and incidence of early aging [6]. Quercetin, one of the flavonoid, is active against cancer [7].

Relevant scientifically proven data supporting this claim were lacking therefore this study was designed to find out the various antioxidant levels in blood and various organs such as brain, liver, kidney and lungs. Moreover the anticancer effect of the plant extract at different concentration.

2. Materials and Methods

2.1. Plant Material and Extraction

The whole plant of *D. burmannii* Vahl. was collected from the forests of Savanadurga, The plant material was identified and authenticated by Dr. S.N.Yoganarasimhan, Taxonomist and Research Coordinator at M. S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India. The plant specimen was deposited to Department of pharmacognosy, Shri Rawatpura Sarkar Institute of Pharmacy (Herbarium specimen no: SRIP/COGNOSY/2011-03). The shade dried and coarsely powdered was extracted successively with petroleum ether and ethanol in a Soxhlet extractor for 72 h. The ethanol extract was concentrated to dryness under reduced pressure and controlled temperature (40–50^0 C). The resultant marc was extracted by maceration with chloroform water (2.5 ml of chloroform in 1000 ml of water) for 24 h followed by filtration and concentration to dryness. The aqueous extract for animal studies was prepared using distilled water.

2.2. Tumor Cell lines

Dalton ascites lymphoma (DAL) cells were obtained under the courtesy of Amala Cancer Research Center, Thrissur, India, They were maintained by weekly intraperitoneal (i.p) inoculation of 2x10^6 cells/per mouse.

2.3. Animals

Male and female adult Swiss albino mice (20-25 g) were procured from KM College of Pharmacy, Madurai, Tamil Nadu, India. They were acclimatized to the experimental conditions for about 2 weeks before subjecting them to experimental procedures and were fed with standard pellet diet and water ad. *libitum*.

2.4. Treatment Protocol

Study protocol was approved by the Institution Animal Ethical Committee of K M College of Pharmacy, Madurai, Tamil Nadu (Protocol. No: A. Raju 0903PH2254/JNTUH 2009).

Swiss albino mice were divided in to seven group of ten each [5]. All the animals in groups 2- 6 were injected with DAL cells (2 x 10^6 cells per mouse/i.p.), and the remaining one group is a normal control group. Group 1 served as the normal control. Group 2 served as the DAL control. Group 1 and 2 received normal diet and water. Group 3 served as the positive control and was treated with injection 5- Fluorouracil (5FU) at 20mg/kg body weight, intraperitonealy. Group 4 served as the treatment control, which was treated with ethanol extract of *D. burmannii* Vahl (EEDB) at 250 mg/kg body weight orally. Group 5 served as the treatment control, which was treated with EEDB at a dose of 500 mg/kg body weight orally. Group 6 served as treatment control which was treated with aqueous extract of *D. burmannii* Vahl (AEDB) at 250 mg/kg of body weight, through orally. Group 7 served as treatment control which was treated with AEDB at 500 mg/kg of body weight orally. In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days.

2.5. Estimation of Blood Antioxidant Enzyme

After 14 days treatment, blood samples were collected by retro-orbital puncturing and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30ºC for 15 min and used for the estimation of serum antioxidant parameters such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) [8]. In addition to that protein carbonyl content (PCC) [9] also estimated from blood.

2.6. Estimation of various Tissue Antioxidant Enzymes

After collection of blood samples, the mice were killed by excess anesthesia and the brain, lungs, kidney and liver samples were isolated. All the tissue preparations were frozen on dry ice and then transferred to a -80°C freezer. The isolated organs were divided in to 2 parts for the preparation of homogenates.

One part was used for the preparation of 10 %w/v homogenate in potassium chloride (0.15M). It was centrifuged at 8000 rpm for 10 min and the supernatant thus obtained was used for estimation of total proteins (TP) [10, 11], peroxidase (P)[12], catalase (CAT) [13,14] and malondialdehyde (MDA)[15]. The second part was used for preparation of 10 % w/v homogenate in 0.25 % w/v sucrose in phosphate buffer (5 M, pH 7.4) and was centrifuged at 8000 rpm for 10 min. The supernatant thus obtained was used for estimation of superoxide dismutase (SOD) [16] and glutathione peroxidase (GSH) [17]. All the estimations were done according to the manufacturer manual of markedly available reagent kit. (Agappe, Kerala, India).

2.7. Statistical Analysis

The results are expressed as mean ± S.E.M. The evaluation of the data was done using one way ANOVA followed by Newman- Keul's multiple comparison tests.

3. Results

The levels of various blood antioxidants were summarized in Table 1. In the present study, the levels of MDA were significantly ($p< 0.01$) increased in DAL control animals when compared with normal control animals. After treatment with the doses of 250, 500 mg/kg of EEDB and AEDB was significantly increased the free radical defense enzyme level more or less similar to normal groups when compared with DAL control mice. The PCC content in hemolysate was increased in DAL control mice. Treatment with doses of 250, 500 mg/kg of EEDB and AEDB restored the PCC significantly ($p<0.01$) as that of normal group, whereas 250mg/kg of AEDB was not significant ($p>0.05$) to PCC.

Table 1: Effect of EEDB and AEDB on blood antioxidant status of DAL bearing mice

Parameters (BLOOD)	PCC nmol/mg protein	Catalase U/mg tissue	SOD U/mg tissue	Peroxidase nm/100mg tissue	Glutathion nm/100mg tissue	MDA nm/g protein
Normal	4.15±0.61	28.13±0.57	1.08±0.16	19.07±0.33	31.68±0.19	2.25±0.08
DAL Control	11.38±0.16	12.55±0.1	0.3±0.04	12.83±0.40	20.53±0.39	20.03±1.06
DAL+ 5FU 20mg/kg	2.5±0.32	26.5±0.78	0.95±0.1	18.1±0.32	29.55±0.41	4.1±0.44
DAL+ EEDB (250mg/kg)	2.78±0.09^{c}	23.28±0.47^{a}	0.9±0.06^{b}	15.08±0.44^{b}	28.53±0.22^{a}	7.57±0.22^{a}
DAL+ EEDB (500mg/kg)	3.42±0.27^{a}	25.54±0.52^{a}	1.2±0.18^{b}	17.23±0.44^{a}	30±0.49^{a}	2.98±0.51^{a}
DAL+ AEDB (250mg/kg)	1.25±0.15^{d}	14.34±0.5^{c}	0.4±0.11^{d}	14.43±0.18^{b}	25.05±0.46^{a}	9.15±0.31^{a}
DAL+ AEDB (500mg/kg)	2.35±0.13^{c}	17.56±0.49^{a}	0.98±0.09^{c}	16.1±0.43^{a}	27.1±0.43^{a}	6.13±0.39^{a}

The data were expressed as mean± S.E.M. n =10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test
a- p<0.001, compared to the DAL control group
b- p<0.01, compared to the DAL control group
c- p<0.05, compared to the DAL control group
d- p>.0.05, compared to the DAL control group

Table 2: Effect of EEDB and AEDB on liver antioxidant enzymes level

Parameters (LIVER)	Total protein mg/dl	Catalase U/mg tissue	SOD U/mg tissue	Peroxidase nm/100mg tissue	Glutathion nm/100mg tissue	MDA nm/g protein
Normal	11.13±0.24	7.78±0.12	2.65±0.14	22.5±0.56	52.65±0.65	3.02±0.12
DAL Control	3.53±0.24	0.93±0.08	1.05±0.08	16.35±0.62	42.88±0.55	24.77±0.99
DAL+ 5FU	9.17±0.4	5.58±0.38	2.18±0.17	19.1±1.52	50.02±1.25	4.98±1.1
DAL+ EEDB (250mg/kg)	6.12±0.36[b]	3.82±0.52[b]	1.58±0.24[c]	19.32±0.35[c]	48.88±1.58[b]	8.3±0.86[a]
DAL+ EEDB (500mg/kg)	10.68±0.9[a]	6.78±1.22[a]	2.58±0.14[a]	22.08±0.71[a]	53.02±1.02[a]	3±0.07[a]
DAL+ AEDB (250mg/kg)	5.54±0.27[b]	1.74±0.16[d]	1.06±0.09[d]	15.48±0.79[d]	46.1±0.85[c]	17.9±2.18[a]
DAL+ AEDB (500mg/kg)	8.42±0.57[a]	3.28±0.20[b]	1.7±0.16[a]	19.9±0.54[c]	48.86±1.47[b]	10.2±0.76[a]

The data were expressed as mean± S.E.M. n =10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test
a- p<0.001, compared to the DAL control group
b- p<0.01, compared to the DAL control group
c- p<0.05, compared to the DAL control group
d- p>.0.05, compared to the DAL control group

Table 3: Effect of EEDB and AEDB on brain antioxidant enzyme level

Parameters (BRAIN)	Total protein mg/dl	Catalase U/mg tissue	SOD U/mg tissue	Peroxidase nm/100mg tissue	Glutathion nm/100mg tissue	MDA nm/g protein
Normal	23.81±0.49	12.88±0.32	5.98±0.22	33±0.84	73.05±0.8.	7.37±0.32
DAL Control	9.13±0.37	3.18±0.22	1.75±0.23	19.33±0.4	42.12±0.45	20.6±0.41
DAL+ 5FU	21.64±0.45	13.2±1.52	5±0.16	31.51±0.37	71.92±0.19	6.58±0.35
DAL+ EEDB	18.88±0.21[a]	8.62±0.19[a]	2.82±2.32[c]	26.68±0.67[a]	62.54±0.82[a]	9.9±0.23[a]

(250mg/kg)						
DAL+ EEDB (500mg/kg)	22.62±0.49[a]	11.52±0.09[a]	3.94±0.3[a]	31.5±0.1[a]	70.82±0.24[a]	7.28±0.42[a]
DAL+ AEDB (250mg/kg)	10.52±0.3[c]	3.9±0.27[d]	2.7±0.07[b]	20.8±0.15[c]	47.46±0.85[a]	16.8±0.41[a]
DAL+ AEDB (500mg/kg)	18.86±0.55[a]	7.94±0.22[a]	3.58±0.12a	26.06±0.3[a]	53.65±0.73[a]	13.04±0.32[a]

The data were expressed as mean± S.E.M. n =10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test
a- p<0.001, compared to the DAL control group
b- p<0.01, compared to the DAL control group
c- p<0.05, compared to the DAL control group
d- p>.0.05, compared to the DAL control group

Table 4: Effect of EEDB and AEDB on kidney antioxidant enzyme level

Parameters (KIDNEY)	**Total protein mg/dl**	**Catalase U/mg tissue**	**SOD U/mg tissue**	**Peroxidase nm/100mg tissue**	**Glutathion nm/100mg tissue**	**MDA nm/g protein**
Normal	21.12±0.26	10.05±0.16	22.69±0.64	61.11±0.35	81.47±0.48	11.53±0.35
DAL Control	15.42±0.34	3.18±0.33	14.49±0.37	42.58±0.65	30.6±0.12	17.3±0.24
DAL+ 5FU	19.9±0.22	8.4±0.29	19.48±0.45	59.45±0.43	77.41±0.71	9.45±0.43
DAL+ EEDB (250mg/kg)	20.4±0.33[a]	7.9±0.7[a]	17.62±0.1[a]	56.45±0.71[a]	61.44±2.27[a]	5.76±0.18[a]
DAL+ EEDB (500mg/kg)	21.67±0.26[a]	8.38±0.37[a]	20.86±0.26[a]	59.09±0.48[a]	74.44±0.55[a]	9.25±0.77[a]
DAL+ AEDB (250mg/kg)	16.94±0.98[a]	6.7±0.22[a]	16.39±0.64[a]	46.88±0.47[a]	41.51±0.74[a]	3.94±0.36[a]
DAL+ AEDB (500mg/kg)	19.78±0.28[a]	8.28±0.15[a]	17.18±0.31[a]	51.7±0.52[a]	49.6±2.33[a]	6.7±0.34[a]

The data were expressed as mean± S.E.M. n =10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test
a- p<0.001, compared to the DAL control group

Table 5 Effect of EEDB and AEDB on lung antioxidant enzyme level

Parameters (LUNG)	Total protein mg/dl	Catalase U/mg tissue	SOD U/mg tissue	Peroxidase nm/100mg tissue	Glutathion nm/100mg tissue	MDA nm/g protein
Normal	26.97±0.62	19.62±0.37	12.78±0.42	41.1±0.33	61.7±0.52	6.46±0.4
DAL Control	13.17±0.79	5.13±0.22	2.87±0.18	29.42±0.45	23.1±0.85	23.42±1.1

DAL+ 5FU	23.15±0.67	18.52±0.38	11.3±0.36	37.97±0.73	59.48±0.39	6.32±0.3
DAL+ EEDB (250mg/kg)	22.55±0.58[a]	16.92±0.19[a]	9.75±0.27[a]	39.63±0.31[a]	58.43±0.46[a]	7.4±0.79[a]
DAL+ EEDB (500mg/kg)	23.92±0.13[a]	20.1±0.32[a]	12.95±0.19[a]	40.35±0.2[a]	60.98±0.22[a]	6.12±0.46[a]
DAL+ AEDB (250mg/kg)	18.89±0.24[d]	7.22±0.44[a]	5.97±0.35[a]	32.53±0.39[a]	29.06±0.35[a]	19.64±0.68[a]
DAL+ AEDB (500mg/kg)	14.82±1.28[b]	10.18±0.37[a]	8.95±0.32[a]	36.97±0.42[a]	35.21±0.43[a]	15.44±0.29[a]

The data were expressed as mean± S.E.M. n =10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test
a- $p<0.001$, compared to the DAL control group
b- $p<0.01$, compared to the DAL control group
d- $p>.0.05$, compared to the DAL control group

The inoculation of DAL cells to tumor control animals caused significant increases in the levels of MDA level in the liver, brain, lung and kidney when compared to normal and extract treatment group animals. Significant ($p<0.001$) decreases in the levels of TP, CAT, GSH, P and SOD was also observed in the liver, brain, lung and kidney samples. The treatment with EEDB at doses 250 and 500 mg/kg body weight reversed these changes to near normal values (Tables 1 to 5). Most of the results were found to be significant. Almost similar results were observed with 5-FU treatment. The extract treatment at 500 mg/kg body weight of EEDB was found to be more potent than 250mg/kg dose. Both doses of AEDB restored these changes towards the normal values, where 500mg/kg showed equivalent significant as that of 250mf of EEDB. The lower dose of AEDB did not show significant effect ($p>0.05$) in restoring the SOD in blood and liver, CAT levels in liver TP in lungs and liver.

4. Discussion

Antioxidants are capable of neutralizing free radicals or their actions in different stages. They act at the different levels as prevention, interception and repair. Preventive antioxidants attempt to stop the formation of ROS. These include SOD that catalyses the dismutation of superoxide to H_2O_2 and CAT that breaks it down to water. Interception of free radicals is mainly by radical scavenging, while at the secondary level scavenging of peroxyl radicals are effected. The effectors include various antioxidants like vitamins C and E, glutathione, other thiol compounds, carotenoids, flavonoids, etc. At the repair and reconstitution level, mainly repair enzymes are involved [18].

The present study was carried out to evaluate the effect of EEDB and AEDB on the antioxidant status in blood and different organs in DAL bearing mice. This extracts possessed certain degrees of antioxidant potential by scavenging free radicals and were reported [19].

The significantly elevated levels of MDA and decrease in the levels of SOD in serum, liver, brain, lung and kidney of tumor inoculated animals. The elevated level of MDA in cancer indicated that unsaturated chain of membrane fatty acids can readily react with free radicals and undergo peroxidation. This process can become autocatalytic after initiation and yields lipid peroxides, lipid alcohols and aldehydic byproducts, such as, 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA)[20, 21].

SOD plays an important role in the antioxidant enzyme defense system. SODs convert superoxide radicals into hydrogen peroxide [18]. SOD defends cells against potentially damaging superoxide radicals. There are three known

human isoforms of SOD, which defends cells against potentially damaging superoxide radicals: they are SOD1, SOD2 and SOD3 [22]. EEDB and AEDB enhance the activity of SOD mice by acceleration of SOD mediated catalyzing reaction. Therefore our study result reveled that increased in level of SOD and protective effect on various organ from cancer induced ROS.

Glutathione ia a natural tripeptide found within almost all cells. It play a vital roles within a cell including antioxidation, maintenance of the redox state, modulation of the immune response and detoxification of xenobiotics. With respect to cancer, glutathione metabolism is able to play both protective and pathogenic roles. Elevated levels of glutathione in cancer treatment can able to protect the other cell from violent ROS [23]. Moreover GSH synthesis is upregulated during oxidative stress and inflammation. In practice, oxidants such as ozone, hyperoxia, H_2O_2, etc. cause short-term falls in intracellular GSH which associate with higher oxidized glutathione (GSSG) levels; this is followed by increases in GSH levels .Therefore, oxidants and oxidant-generating systems (if their levels do not compromise cell viability) can upregulate GSH synthesis-linked genes, thus providing paradoxically a protective mechanism against oxidative stress [24]. In our research also showed that extract treatment group showed an elevation of GSH in blood, liver, kidney, lungs and brain.

Even though cancer cells generate reactive oxygen species, it has been demonstrated that antioxidant enzyme levels are low in most animal and human cancers. Morphologic studies of animal and human cancer have confirmed that although the majority of tumor cell types from several organ systems have low antioxidant enzymes such as GSH and CAT [25]. CAT subsequently reacts with hydrogen peroxide which was produced by SOD, and decomposes it into water and molecular oxygen. These results indicated that lipid peroxidation may contribute to the development of DNA damage in the development of cancer[26]. CAT also decreased in the DAL control group and were increased significantly in extract treatment group.

During oxidative stress triggers detoxification pathway, result of involvement of multiple enzymes, with SOD catalyzing the first step and then CAT and various peroxidases removing hydrogen peroxide from cell in order to prevent the formation of free radicals. These enzymes protect DNA from oxidative stress and prevent the individual's risk of cancer susceptibility[27]. There is a reduction of peroxidase enzyme level in DAL control group which was normalized by 14 days extract treatment.

Carbonyl group level in proteins has been introduced as a good marker of oxidative stress damage of protein and increased in tumors [28]. Protein carbonyl contents (PCC) are byproducts of oxidation of proteins. PCCs are generally more stable; hence PCCs have a major advantage over lipid peroxidation products as markers of oxidative stress. Moreover, PCCs are formed early and circulate in blood for longer periods, compared with other parameters of oxidative stress, such as glutathione disulfide and MDA[29]. Accumulation of carbonyl groups on protein results in series of chemical modifications and formation of protein oxidation products [5]. In the present study we have observed a highly significant increase in protein carbonyl content in DAL bearing mice which were brought back to normal by the administration 250, 500 mg/kg doses of EEDB and AEDB.

Hypoproteinemia is a condition at which there is a decrease in albumin content. Albumin, which is considered as a sacrificial antioxidant, via its thiol groups, fight against free radicals. Moreover hypoproteinemia in cancer may be an expression of cachexia, representing homeostatic derangement in which the utilization and destruction of albumin by the tumor cannot be compensated by the organism, especially by the liver [30]. Total protein (TP) level was significantly reduced in DAL control mice and was brought back to normal by the administration 250, 500 mg/kg doses of EEDB and AEDB.

It was reported that plant-derived extracts containing antioxidant principles such as plumbagin, showed cytotoxicity towards tumor cells. Antitumor activity of these antioxidants is either through induction of apoptosis or by inducing cell cycle arrest at SG2/M phase. The implication of free radicals in tumors is well documented [5].

5. Conclusion

The present study concluded that the ethanol and aqueous extract of *D. burmannii* Vahl exhibited anticancer potential against DAL in Swiss albino mice, where the higher dose of the ethanol extract showed significantly good activity when compared with the lower dose, similarly higher dose of the aqueous extract showed better activity than the lower dose but the ethanol extract was comparatively better than the aqueous because most of the parameters are less significant or more or less non-significant with the lower dose of aqueous extract. Both the extracts protect heamatological system, lipid profile, liver enzyme and steroidal hormone in cancer bearing mice. The possible mechanism of anticancer effect may be due to its antioxidant effect. It may be possible that the natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restore the optimal balance by neutralizing the reactive species.

6. Acknowledgments

The authors gratefully acknowledge KM College of Pharmacy for providing support and facilities for this research work. The authors also thank Dr. SN Yoganarasimhan for his guidance in the selection and collection of plant material.

5. References

1. Fiaschi AI, Cozzolino A, RuggieroG, Giorgi G. Glutathione, ascorbic acid and antioxidant enzymes in the tumor tissue and blood of patients with oral squamous cell carcinoma. European Review for Medical and Pharmacological Sciences 2005; 9: 361-367.
2. De Feudis FV, Papadopoulos V, Drieu K. *Ginkgo biloba* extracts and cancer: a research area in its infancy. Fundam Clin Pharmacol 2003; 17: 405-17.
3. Takeoka GR, Dao LT. Antioxidant constituent of almond [Prunusdulcis (Mill.) D.A. Webb.] hulls. J Agric Food Chem 2003; 51: 496-501.
4. Jayaram K, Prasad MNV. *Drosera indica* L. and *D. burmanii* Vahl., medicinally important insectivorous plants in Andhra Pradesh – regional threats and conservation, Current Science 2006; 91:943-946.
5. Raju A, Christina AJM, Anita Murali. Antioxidant potential of *Drosera burmannii* Vahl. (Droseraceae) in Ehrlich Ascites Carcinoma (EAC) cell line inoculated Swiss albino mice. International Journal of Chemical And Pharmaceutical Research 2013; 2(1): 015-027.
6. Raju Asirvatham, Arockiasamy Josphin Maria Christina. Anticancer activity of *Drosera indica* L., on Dalton's lymphoma ascites (DLA) bearing mice. J Intercult Ethnopharmacol 2013; 2(1):9-14.
7. Wang Q, Su J. Zeng L. The isolation and identification of flavonoids from *Drosera burmanii*. Zhong Yao Cai 1998; 21: 401–403.
8. Senem D, Zeynep O, Handan AKC, Arif BA, Hikmet HA. The interaction between antioxidant status and cervical cancer: a case control study. Tumori 2011; 97:290-295.
9. Levine RL, Williams JA, Stadtman ER, Shacter E: Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 1994; 233: 357-63.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folinciocal phenol reagent. J. Bio. Chem 1951; 93(1): 265-276.
11. Sapan CV, Lundblad RL, Price NC. Colorimetric protein assay techniques. Biotech Appl Biochem 1999; 29: 99-108.
12. Alexander NM. A spectrophotometric assay for Iodide Oxidation by thyroid peroxidase. Anal.Biochem 1962; 4: 341-345.
13. Catherine W, Hosni MH. Introduction and inactivation of catalase and superoxide dismutase of Escherichia coli by ozone. Arch Biochem Biophy 1987; 259(2):464-471.
14. Aebi H. Catalase. In: Bergmeyer, editor. Mehtods in enzymatic analysis, Vol.2, New York, Academic Press, 4, 674-84, (1974).
15. Ohkawa H, Onishi,Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Bio Chem 1979; 95: 351-358.
16. Kakkar P, Dos B, Viswanathan PN,Maehly AC, Chance B. In: Methods of Biochemical Analysis Vol. I, Glick D,editors. New York, Interscience, 1954, 357.

17. Beutler E, Kelly BM. The effect of sodium nitrate on red cell glutathione. Experientia 1963; 18: 96-97.
18. Devasagayam TPA, Tilak JC, Boloor KK, Ketaki Sane S, Saroj Ghaskadbi S, Lele RD. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. Journal of the Association of Physicians of India 2004; 52: 794-804.
19. Raju A, Christina AJM. *In vitro* antioxidant and anticancer activity of *Drosera burmannii* Vahl. (Droseraceae).International Journal of Science Innovations and Discoveries2012; 2 (2): 316-324.
20. Kurata M, Suzuki M, Agar NS. Antioxidant systems and erythrocyte lifespan in mammals. Comp Biochem Physiol 1993;106(3):477-87.
21. Hagihara M, Nishigaki I, Maseki M, Yagi K. Age-dependent changes in lipid peroxide levels in the lipoprotein fractions of human serum. J Gerontol 1984;39(3):269-72.
22. Fridovich I. Superoxide dismutases. Adv. Enzymol. relat. areas mol. biol. 1986; 58: 61-97.
23. Balendiran GK, Dabur R, Fraser D. The role of glutathione in cancer. Cell Biochem Funct. 2004; 22(6):343-52.
24. Angel L. Ortega, Salvador Mena, Jose M Estrela. Glutathione in Cancer Cell Death. Cancers 2011; 3: 1285-1310.
25. Oberley TD, Oberley LW. Antioxidant enzyme levels in cancer. Histol Histopathol. 1997; 12(2) : 525 -35.
26. Halit Demir, Zeynel Abidin Akkus, Aysegul Cebi, Tahir Cakir, Mustafa Izmirli. Catalase, Carbonic anhydrase and other biochemical parameters in esophageal cancers in Turkey. Asian Pacific Journal of Cancer Prevention 2010; 11: 1029-1032.
27. Elaine Hardman W, Jesus Munoz, Ivan L Cameron. Role of lipid peroxidation and antioxidant enzymes in omega 3 fatty acids induced suppression of breast cancer xenograft growth in mice. Cancer Cell International 2002; 2:10 1-9.
28. Popadiuk S, Korzon M, Renke J, Woźniak M. Carbonyl groups content on the basis of protein peroxidation analysis with total antioxidant status in blood of children with cancers. Wiad Lek 1998;51(4):107-112.
29. Rajendiran S, Zachariah B, Hamide A. Increased protein carbonylation and decreased antioxidant status in anemic H. pylori infected patients: Effect of treatment. Saudi J Gastroenterol 2012; 18:252-6
30. Anita Mukthapura, Avinash Sidhuraju Shimogga, Vinodchandran Sudha K, Beena V Shetty, Gayathri M Rao. Oxidative products of proteins and antioxidant potential of thiols in gastric carcinoma patients. Journal of Medical Biochemistry 2010;29: 102–106.