Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

ANTIPROLIFERATIVE AND ANTIOXIDANT ACTIVITY OF *GYNANDROPSIS PENTAPHYLLA* LINN ON MCF-7 CELL LINE

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Received: 27 May 2014 Revised and Accepted: 09 Jul 2014

ABSTRACT

Objective: To investigate the *in vitro* Antiproliferative and Antioxidant Activity of *Gynandropsis pentaphylla* Linn. Leaves on MCF-7 Cell line.

Methods: Phytochemicals were analysed by using standard methods. *In Vitro* antioxidant studies were carried out for the methanol extracts of the *Gynandropsis pentaphylla* using various free radical models such a DPPH, Reducing power assay and Hydrogen peroxide. *In vitro* cytotoxic assay such as trypan blue dye exclusion and MTT assays were carried out in methanolic extract against MCF-7 cell line.

Results: The results of the present study demonstrated that the qualitative phytochemical analysis of the methanol, aqueous and chloroform extract of *Gynandropsis pentaphylla* showed the presence of carbohydrates, flavonoids, tannins, glycosides, phenols, saponins, protein and terpenoids. The antioxidant activity of methanolic extract of leaves *G.pentaphylla*was confirmed by free radical scavenging activity and reducing (FRAP) and it was found to be significant. Inhibitory concentration (IC_{50}) for the methanolic extract of *Gynandropsis pentaphylla* on MCF-7 cell was found to be 96.67 determined by MTT assay.

Conclusion: It revealed that the crude methanolic extract of *G. pentaphylla* has antiproliferative activity against MCF-7 cell lines. From our study it is concluded that, the phytochemicals present in the *Gynandropsis pentaphylla* possess antioxidant and anticancer activity.

Keywords: *Gynandropsis pentaphylla* Linn, Antiproliferative, Antioxidant, Anticancer.

INTRODUCTION

Cancer is one of the major killing diseases, worldwide and more than 6 million die of the disease and over 22 million people in the world are cancer patients. It is predicted that cancer incidences increasing every year in both developed and developing countries. The disease affects men and women. Thus, public and private sector institutions are focusing their research towards the development of anticancer agents. Cancer chemotherapy now plays a significant role in treating malignancies, it acts as either curative (by itself or as an adjuvant to surgery and/or radiation) or palliative care, depending upon the specific tumor situation [1].

Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is second only lung cancer as a cause of cancer mortality, and it is the leading cause of death for American Women between the ages of 40 and 55. The lifetime risk of a woman developing invasive breast cancer is 12.6% - one out of 8 females in the United States will develop breast cancer at some point in her life [2].Genetics plays a limited but important role as a risk factor for breast cancer. Only 5% to 6% of breast cancers are considered hereditary. BRCA-1 and BRCA-2 account for an estimated 80% of hereditary breast cancer, but again, this only represents 5% to 6% of all breast cancers. BRCA-1 and / or BRCA-2 positive women have a 50% to 85% lifetime risk of developing breast cancer and 15% to 65% risk of developing ovarian cancer beginning at age 25 [3].

Women treated for breast cancer have about a 1% greater chance per year of developing a new second cancer in either the treatment of breast or the other breast. Therefore, previous breast cancer is an accepted risk factor for development of breast cancer [4]. The most commonly used treatments are surgical, radiation and hormone treatment but they have severe side effects. Medicinal plants can be promising sources of novel chemotherapeutic agents especially for cancer. It has been estimated that, out of total 250,000 plant species existing on earth approximately one thousand species are known to have anticancer potential. Thousands of plant species have been screened through bioassays for search of novel plant based anticancer drugs. Bioactivity guided isolation is an important strategy for discovery of potent anticancer agents [5]. The aim of the present work is to investigate the phytochemical constituents, antiproliferating and antioxidant activity of *Gynandropsis pentaphylla Linn* in MCF – 7 cell lines (Breast cancer). The plant has been traditionally used as an anthelmintic and rubefacient. Leaves are applied externally over the wounds to prevent the sepsis.

The plant also used in the treatment of malaria, piles, rheumatism and in tumour. The decoction of the root is used to treat fevers. The juice of the root is used to relieve scorpion stings. The leaves, applied as a poultice, are used as a vesicant and rubefacient in the treatment of rheumatism. The juice of the leaves is a remedy for pain in the ear. The whole plant is used in the treatment of rheumatism [6].

MATERIAL AND METHODS

Collection of plant materials

The fresh plant materials belong to the *Gynandropsis pentaphylla* were collected from Ariyalur District. The plant was authenticated by the Department of Botany, Government Arts Colleage for Men, Kumbakonam. The plant leaves were shade dried for seven days. The dried sample was powdered with electrical blender.

Preparation of plant extracts

Aqueous and Methanolic extract

50g of dry leaf powder of *Gynandropsis pentaphylla* was taken for cold maceration with aqueous (250ml) and methanolic (250ml) extract preparation. They were kept in sterile condition for 72hours with occasional shakings. The content is filtered and evaporated using water bath for 30 minutes. The obtained plant extract was used to determine antioxidant, anticancer activity and the presence of phytochemicals [7].

Chloroform extract

50g of dry leaf powder of *Gynandropsis pentaphylla* was taken for soxhlet apparatus with chloroform (250ml). The content is filtered

and evaporated using water bath for 30 minutes. The obtained plant extract was used for screening phytochemical [8].

Qualitative analysis of phytochemical constituents

Phytochemical analysis was carried out qualitatively to identify the presence of various secondary metabolites [8].

***In vitro* Antioxidant activity**

Antioxidant activity measured by DPPH radical scavenging assay method [9], Reducing Power assay [10] and Hydrogen peroxide radical scavenging activity. Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

***In vitro* cytotoxicity assay**

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). All cells were maintained at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week [11].

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted by tryphan blue exclusion assay using a hemocytometer. The cell suspension was diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microliters per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dispersed in neat dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software.

RESULT AND DISCUSSION

Nature has been a source of medicinal agents for thousands for years and an impressive number of modern drugs have been isolated from natural sources; many of this isolation are based on the uses of the agents in traditional medicine. This plant based, traditional medicine system continues to play essential role in health care [12]. Traditional uses of plants for medicinal purposes provide a basis for the use of specific medicinal condition [13]. Hamilton, [14] reported that, the plant continue to be a vital part of Western medicine, and are still considered as an important source of novel compounds in the field of drug discovery.

Medicinal herbs are steadily increasing in complementary use for chronic and alternative therapies. The health risks associated with herbal supplements have been identified as a top research priority [15]. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active compounds as antimicrobial agents. Medicinal plants are the richest bio-resources of drugs of traditional medicinal system, modern medicines, food supplements, folk medicines, intermediate and chemical entitled for synthetic drugs [16, 17].

Table 1 represents the qualitative analysis of *Gynandropsis pentaphylla* leaves extracts (methanol, aqueous and chloroform). Preliminary phytochemical screening of methanol, aqueous and chloroform extracts of leaves revealed that the presence of flavonoids, tannins, glycosides phenols, saponins, carbohydrate, protein and terpenoids.

Flavonoids, tannins saponins, terpenoids, carbohydrate and protein are present in all the three extracts. Glycoside is present in aqueous and chloroform extracts. Phenols are present in methanol and aqueous extracts. Cardiac glycosides and amino acids are present in methanol and chloroform extracts. Alkaloid, phlobatannins and Anthroquinone are completely absent in all three extracts.

Table 1: Phytocompounds of *Gynandropsis pentaphylla* leaves extract.

S. No.	Components	Methanol extract	Aqueous extract	Chloroform extract
1.	Alkaloids	-	-	-
2.	Flavonoids	+	+	+
3.	Tannins	+	+	+
4.	Glycosides	-	+	+
5.	Phenols	+	+	-
6.	Saponins	+	+	+
7.	Terpenoids	+	+	+
8.	Carbohydrate	+	+	+
9.	Protein	+	+	+
10.	Phlobatannins	-	-	-
11.	Anthroquinone	-	-	-
12.	Cardiac glycoside	+	-	-

(+) Present (-) Absent

Flavonoids are found in almost all plant families. Flavonoids are present in different plant parts including the leaves, stem, roots, flowers and seeds and are among the most popular anti-cancer candidates worldwide. Flavonoid derivatives have a wide range of biological actions such as antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities. Some of these benefits are attributed to the potent antioxidant effects of flavonoids, which include metal chelation and free-radical scavenging activities [18]. Tannins, phenolic phytochemicals, which are natural constituents of green tea, are considered to have cancer- preventive properties [19]. Condensed tannins, isolated from black beans, did not affect the growth of normal cells, but induced cell death in cancer cells in a dose-dependent manner [20]. Studies in animal models and with cultured human malignant cell lines have demonstrated both the antitumor and cancer preventive activities of methanolic extract of *Gynandropsis pentaphylla* and its main ingredients. It was suggested that these effects of methanolic extract might be due to their content of flavonoids, tannins, alkaloids and saponins. [21].

Table 2, 3 & 4 represent DPPH radical scavenging, reducing activity and hydrogen peroxide radical scavenging activity of*Gynandropsis pentaphylla* leaves extract respectively.

1, 1-diphenyl-2-picrylhydrazyl (DPPH), is a kind of stable organic radical. The capacity of biological reagents to scavenge DPPH radicals can be expressed as its magnitude of antioxidant ability. The DPPH oxidative assay is used worldwide in the quantification of radical scavenging capacity. The antioxidant activities of plant

extracts and the standard were assessed on the basis of the free radical scavenging effect of the stable DPPH free radical activity[22]. The results are expressed as the ic50 value (the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%). The results of the DPPH free radical scavenging assay suggest that leaves of all *Cleome* species have potent antioxidant property of scavenging free radicals. These species could be used as a potent source for the cancer chemo protective therapy. The most abundant ROS represented in living inflammatory cells, is superoxide, as well as hydrogen peroxide, singlet oxygen and highly toxic hydroxyl radicals. The special feature of these agents is their high oxidative reactivity to living cells. Natural system, like reduced glutathione, vitamins, and free fatty acids are considered an essential pool of antioxidants. Oxidative stress refers to a situation where in the production of oxidants exceeds the capacity to neutralize them, leading to damage to cell membranes, lipids, nucleic acids, proteins, and constituents of the extracellular matrix, such as proteoglycans and collagens. Different therapeutic approaches can be used to decrease the oxidative stress, and include scavenging of free radicals, inhibition of free radical producing enzymes, enhancing the antioxidant system or targeting the signaling routes and expression of molecules involved in the inflammatory cascade. Amongst the intracellular ROS generated, superoxide plays a pivotal role in inflammation [23].

Table 2: DPPH radical scavenging activity of methanolic extract of *Gynandropsis pentaphylla* leaves.

S. No.	Concentration (µg/ml)	DPPH radicals scavenging activity (%)	
		% of inhibition (Standard ascorbic acid)	% of inhibition (Leaves)
1.	200	50±3.50	28±1.96
2.	400	58±4.06	40±2.80
3.	600	66±4.62	44±3.08
4.	800	70±4.90	56±3.92
5.	1000	75±5.25	60±4.20

Values were expressed as mean ±SD

Table 3: Reducing Power of methanolic extract of *Gynandropsis pentaphylla* leaves

S. No.	Concentration (µg/ml)	Reducing power activity (%)	
		% of inhibition (Standard ascorbic acid)	% of inhibition Leaves
1.	200	50 ±3.50	20±1.4
2.	400	66±4.62	33±2.31
3.	600	75±5.25	42±2.94
4.	800	80±5.60	50±3.50
5.	1000	83±5.81	66±4.62

Values were expressed as mean ±SD

Table 4: Hydrogen peroxide scavenging activity of methanolic extract of *Gynandropsis pentaphylla* leaves.

S. No.	Concentration (µg/ml)	Hydrogen Peroxide Scavenging activity (%)	
		% of inhibition (Standard ascorbic acid)	% of inhibition (Leaves)
1.	200	57±3.99	9.0±6.3
2.	400	71±4.97	30±2.1
3.	600	75±5.25	48±3.36
4.	800	82±5.74	63±4.41
5.	1000	85±5.95	78±5.46

Values were expressed as mean ±SD

Free radicals are atoms that cause damage to our cells. They harm our immune system leading to much degenerative disease. Free radicals are formed by our cells being exposed to a variety of substances such as radiation, chemicals, pollution, smoke, drugs, alcohol, pesticides and sun and through various metabolic processes such as when our bodies utilize stored fat for energy. A poor diet also aids in the formation of free radicals. Antioxidants consist of a group of vitamins, minerals and enzymes that have health enhancing effects for our bodies. Antioxidants work to neutralize free radicals before they do harm to our bodies. Antioxidants are a type of complex compounds found in our diet that act as a protective shield for our body against certain disastrous enemies(disease) such as arterial and cardiac disease, arthritis, cataracts and also premature ageing along with several chronic disease. [24]. The methanol extract of *Cleome gynandra*possesses very good free radical scavenging and also antioxidant property. Its anticancer activity is already reported in Ehrlich ascites carcinoma cell bearing mice. Recently, it has been published that the antioxidant activity of *Cleome gynandra* is responsible for its anticancer activity. Therefore, the major flavonoid fraction was isolated and characterization was performed by TLC and HPLC to determine the active principles present in Cat's whiskers for the scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in some inflammatory cells [25]. The antioxidant potentials of folk plants are largely attributed to certain classes of low molecular weight secondary plant metabolites, they may also be influenced by certain essential and trace antioxidant micronutrients. In fact, studies have shown that additive and synergistic combination of scores of phytochemicals and trace elements, which are either directly or indirectly involved in various redox processes are responsible for the observed health benefits of fruits and vegetables. Around 30% of enzymes and several other biomocules contain a trace metal at the active site and thus play a vital role in human metabolism. Micronutrient deficiencies affect nearly half the world's population, impairing child development, reducing work productivity, and increasing mortality and morbidity rates. All essential elements are primarily supplied through diet and therefore determination of the elemental composition of the food stuff and evaluation of their daily dietary intake becomes a necessity. Indian council of Medical Research (ICMR) has recommended selective studies of individual foodstuff as an important step in the estimation of dietary intake of trace elements. Consequently the essential trace metal composition. Hydrogen peroxide liberated by GO caused direct oxidative attack on cells membrane leading to increased rigidity to lipid bilayer, osmotic fragility, aggregation of membrane protein and decreased activity of membrane bound protein of the erythrocytes. CWF decreased oxidative damage to the RBC cells in a concentration dependent manner which correlated with the previously described *in vitro* free radical scavenging activity [25, 26]. The ferric reducing antioxidant plasma activity of *cleomegynandra* leaves is presented in Table 3. It is clear from the results that the FRAP activity of leaves of *cleome gynandra*is found to have significant antioxidant activity. The most common *in vitro* assays include MTT. These assays have been successfully used in the assay-guided chemotherapy for certain cancers, including breast, ovarian, melanoma and colorectal cancers. Kim *et al.*,[27] reported that, MTT assay has been most widely used in different cancers, and is sensitive, accurate, and efficient in the *in vitro*evalution of anticancer or immunological agent prior to preclinical and clinical testing. Anticancer activity was examined by MTT assay using breast cancer (MCF-7). The extent of cytotoxicity can be measured by MTT dye reduction assay. The percentage viability was analyzed by MTT assay after treatment of methanolic extract of *Gynandropsis pentaphylla* at 18.75µg/ml,37.5µg/ml, 75µg/ml, 150µg/ml, 300µg/ml concentration.

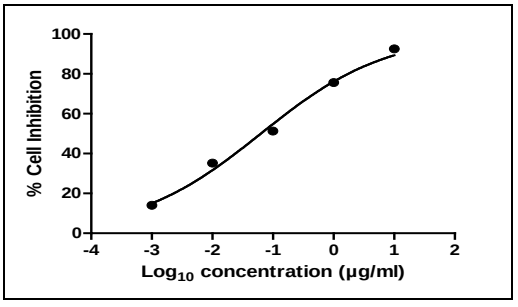

Fig. 1: Effect of anticancer activity of *G. Pentaphylla* on MCF-7 Cell line

Table 5: Anticancer activities of methanolic extracts of *G.pentaphylla* against MCF-7 cell lines by MTT assay.

S. No.	Concentration of test (µg/ml)	% of Cell inhibition	Concentration of standard(µg/ml)	% of Cell inhibition
1.	18.75	3.36±0.23	0.001	14.02±0.58
2.	37.5	15.48±0.62	0.01	35.24±1.85
3.	75	31.05±2.34	0.1	51.27±3.89
4.	150	75.31±8.24	1	75.59±8.28
5.	300	96.99±9.45	10	92.53±96.25

Values were expressed as mean ±SD

Strong cytotoxicity was reported by the *SporithrixSpp* (kk 29 FLI) isolated from *Costus specious* against Colorectal carcinoma (HCT-116) and human breast adenocarcinoma (MCF- 7) cell lines confirmed by MTT assay [28]. The volatile fractions from *Astragaluscorniculatusbicb* were analyzed for its cytotoxic effects in SKN- 3 cells [29].Lipophilic and hydrophilic fraction of *Menthaspica*showed a considerable cytotoxic effect on human prostate cancer cell line by MTT assay [30].

Table 5 and fig 1 represented the methanolic extraction of *G.pentaphylla* which exhibited the antiproliferative potential which showed IC_{50} values of 96.65µg/ml. The antiproliferative activity of plant extract was measured using MTT (3-(4,5dimethylthiazol- 2-yl)-2,5-diphenyltetrazolum 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 [31]. Similar results were also reported by [32], who studied effects of different concentrations of *Carallumatuberculata* crude extract against MCF-7 cell line. Maximum growth inhibition shown by the crude extract was 96.99454 at a concentration 300µg/ml. The results of the present study demonstrated that the qualitative phytochemical analysis of the methanol, aqueous and chloroform extract of *Gynandropsis pentaphylla* showed the presence of carbohydrates, flavonoids, tannins, glycosides, phenols, saponins, protein and terpenoids.

The antioxidant activity of methanolic extract of leaves *G. pentaphylla*was confirmed by free radical scavenging activity and reducing (FRAP) and it was found to be significant. Inhibitory concentration (IC_{50}) for the methanolic extract of *Gynandropsis pentaphylla on* MCF-7 cell was found to be 96.67 determined by MTT assay. It revealed that the crude methanolic extract of *G. pentaphylla* has anticellprliferative activity against MCF-7 cell lines. From our study it is concluded that, the phytochemicals present in the *Gynandropsis pentaphylla* possess antioxidant and anticancer activity.

ACKNOWLEDGEMENT

The authors are grateful to Dr. S. Velavan, Director, Harman Institute of Science Education and Research (www. Harman research centre.com), Thanjavur, Tamil Nadu for their support.

REFERENCES

1. Carter SG, Livingston ER. eds.). "Priniciples of Cancer Treatment". New York, McGraw-Hill2001. 95-110.
2. Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. CA Cancer J Clin 2001;51(1):15-36.
3. Haber D. Prophylactic oophorectomy to reduce the risk of ovarian and breast cancer in carriers of BRCA mutations. The New England journal of medicine 2002;346(21):1660-2.
4. Fisher B, Dignam J, Wolmark N, Wickerham DL, Fisher ER, Mamounas E, et al. Tamoxifen in treatment of intraductal breast cancer:National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial. Lancet 1999;353(9169):1993-2000.
5. Abu-Dahab R, Afifi F. Antiproliferative activity of selected medicinal plants of Jordan against a breast adenocarcinoma cell line (MCF7), Sci. Pharm. Ann Trop Paediatr 2007;75:121-36.
6. Thenmozhi S, Subasini U, Kameshwaran S, Dhanalakshmi M, VictorRajamanickam G. Morpho-anatomical and preliminary phytochemical studies of leaves of Gynandropsis pentaphylla Linn. Ann Trop Paediatr 2013:0976-7126.
7. Ajaiyeoba, EO.Phytochemical and antimicrobial studies of *gynandropsisgynandra*and *Buchholziacoriaceae*extracts, *Afr. J. Biomed. Res. 20003;161–65*.
8. Kokate K, Lakshmiah R, Ramasastri M. Chemical component of plants. Journal of Nutrition and Dietetics 200 1995;6.
9. Gyamfi MA, Aniya Y. Antioxidant properties of Thonningianin A, isolated from the African medicinal herb, Thonningia sanguinea. Biochem Pharmacol 2002;63(9):1725-37.
10. Oyaizu M, J. Studies on product of browning reaction prepared from glucose amine. Jap 1986;44:307-15.
11. Mosmann T. Rapid colorimetric assay for cellular growth and survival:application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65(1-2):55-63.
12. Owalabi OJ, Omogbai EKI, African J. Analgesic and Anti inflammatory activities of Ethanolic stem bark extract of KigeliaAfricana,Biotechnol. Ann Trop Paediatr 2009;6:582-5.
13. Nariman TZ, Emama SA, Mousari SH. Analyzing cytotoxic and apoptogenic properties of scutellarialitwinowiiroot extract on cancer cell lines, Evid. Based Complement Alternat. Med 2009;1:1-9.
14. Hamilton AC. Medicinal plants, conservation and livelihoods. Biodivers Conserv 2004;13:1477-517.
15. Krasteva I, Platikanov S, Momekov G, Konstantinov S, Nikolov S. Phytochemical analysis and in vitro cytotoxic activity of volatiles from Astragalus corniculatus. Natural product research 2008;22(11):969-74.
16. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agents. JMed Plant Res 2010;4(2):104-11.
17. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999;86(6):985-90.
18. Amin A, Buratovich M. The anti-cancer charm of flavonoids:a cup-of-tea will do! Recent Pat Anticancer Drug Discov 2007;2(2):109-17.
19. Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 2001;91(3):453-62.
20. Awika JM, Rooney LW. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 2004;65(9):1199-221.
21. Abdullaev FI, Espinosa-Aguirre JJ. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detect Prev 2004;28(6):426-32.
22. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from Licania licaniaeflora. J Ethnopharmacol 2002;79(3):379-81.
23. Kundu S, Bala A, Ghosh P, Mukhopadhyay D, Mitra A, Sarkar A, et al. Attenuation of oxidative stress by allylpyrocatechol in synovial cellular infiltrate of patients with Rheumatoid Arthritis. Free Radic Res 2011;45(5):518-26.
24. Mayo JL. Black cohosh and chaste berry:herbs valued by women for centuries. Clinical Nutrition Insight 1998;6:1-4.
25. Bala A, Haldar PK, Kar B. Antioxdiant activity of the fractions of cleome gynandra promotes antitumor activity in Ehrlich ascites carcinoma [J] chem. Ann Trop Paediatr 2011;11:5055-60.
26. Velavan S, Nagulendran K, Mahesh R. *In vitro* antioxidant activity of *Asparagus racemosus* root. Pharmacog Magaz 2007;26-33.
27. Kim R, Emi M, Tanabe K, Uchida Y, Toge T. Chemosensitivity testing for gastrointestinal cancer:survival benefit potential and limitations. Anticancer Drugs 2003;14(9):715-23.

28. Hazalin NAMN, Ramasamy K, Lim SM, Wahab IA, Cole ALJ, Abdul Majeed AB. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. BMC Complement Altern Med 2009;9:46.
29. Krasteva I, Platikanov S, Nikolov S. Phytochemical analysis and invitro cytotoxic activity of volatiles from *Astragaluscorniculatus*, *Nat. Pro. Res*., 2008;22, 969-74.
30. Arumugam P, Ramamurthy R, Mentaspicata L. Antioxidant and cytotoxic activity of lipophilic and hydrophilic fraction of Int. J Food properties 2010;1:23-31.
31. Lau CBS, Ho CY, Kim CF, Leung KN, Fung KP, Tse TF, et al. Cytotoxic activities of Coriolus versicolor (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. Life Sci 2004;75(7):797-808.
32. Ahmed S, Owen CP, WaheedA, Nisa S, Bibi Y, Chaudhary MF. Biochemical evalution of extracts from Carallumatuberculata against hormone-dependent breast cancer cells. J. Pharm. Pharmacol. 183 supplement Issue. 2009.