IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Www.Iosrphr.Org Volume 3, Issue 8 (September 2013), Pp 32-37

Antioxidant And Cytotoxic Activities Of Crude Methanolic Extract Of *Medicago Polymorpha*

Muhammad Imran Khan[1]*, Suhaib Asad[2], Goher Zaman[1], Hamid Rehman[1], Syed Rehman[1], Asif Iqbal[1], AminUllah[1], Ihsan Ullah[1], Shahnoor[1], Sher Ali[3]

[1] *Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology Bannu, Khyber Pakhtunkhwa, Pakistan.*
[2] *Department of zoolagy,, Faculty of Biological Sciences, University of Science and Technology kohat, Khyber Pakhtunkhwa, Pakistan*
[3] *Department of Biological Sciences, Quaid-e-azam university Islamabad, Pakistan*

ABSTRACT: *Free radicals are the source of many diseases such as diabetes mellitus, cancer, arthritis, anemea, Parkinson disease, alzimer and ageing etc. Antioxidants are very important for treatment of this disease by scavenging free radicals. The present study was conducted to determine the Cytotoxic and antioxidant activities of metanolic extract of Medicago polymorpha against free radicals such as DPPH and ABTS etc in vitro. These activities of Medicago polymorpha are count for its anticancer properties. The results were analyzed by the comparison of the extract with the standards. Ascorbic acid was used as a standard for antioxidants assays and see salt solution without extract was used a standard for cytotoxic assays. The antioxidant property may be related to the polyphenols and flavonoids present in the extract. These results clearly indicate that Medicago polymorpha is effective against free radical mediated disease and thus it has potential for treating cancer.*

KEY WORDS*: DPPH, lipid per oxidation, cytotoxic, Medicago polymorpha, superoxide.*

I. INTRODUCTION;

1.1 Free radicals and anti oxidants;

Free radicals are highly active substances due to the presence of unpaired electrons. These free radicals can damage DNA, proteins and other macro molecules causing various severe diseases including diabetes, cancer, neurological diseases such as Parkinson, Alzimer diseases cardiovascular disease such as coronary artery disease and stroke etc. The cases of free radicals generation may be environmental, genetically or dietary etc. reactive oxygen species and reactive nitrogen species are the main types of free radicals. These are also the major cause of cancer because of mutation of DNA.Antioxidants are substances which protect the body from the hazards effect of free radicals. Antioxidants stabilizes are scavenge free radicals by balancing their electrons. Enzymatic Antioxidants includes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase etc vitamins A, C, and E, glutathione, and lipoic acid, mixed carotenoids, several bioflavonoids, antioxidant minerals (copper, zinc, manganese, and selenium), etc. and different vitamins such as vitamins A, C etc are come from dietary sources.

1.2 Role of anti oxidants in the prevention of Cancer

The term cancer is used in the medical sciences for malignant neoplasm, the mass of cell which is irregular and undergoes uncontrollable cell growth. Cancer cells grow in uncontrollable manner, and result in malignancy hence affecting the nearby parts of the body. The cancerous cell metastasized to other parts of the body through lymphatic or blood stream. The affected tumors cell loses the ability of differentiation and apoptosis. More than 200 types of cancer have been identified that harm the human body. Free radicals produced oxidative stress which causes mutation, in tamer suppressor genes leading to cancer. Active oxygen may cause cancer through two possible mechanisms: gene mutations and the effects on signal transduction and transcription factors. Oxidative stress causes damages to DNA, phospholipids, proteins and carbohydrates on the cell membrane. Oxidation and injury to DNA induce genetic mutation. The presence of free radicals may enhance the mutation of some genes. The oxidative stress can be avoided if there present a sufficient amount of antioxidants

1.3 Medicinal plants are chief source of antioxidants

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illnesses (Zhang X *et al* 2004). Traditional remedies have a long-standing history in many locations in Yemen and continue to provide useful and applicable tools for treating ailments (Fleurentin J *et al* 1982 Schopen A *et al* 1983 Al-Dubai *et al* 1996). Nevertheless, little scientific research was done to investigate the plants of Yemen used in herbal medicine.

Plants are good source of Natural products which are used in the formation of drugs for the treatment of almost all types of human diseases including immunological disorders microbial infection, cancer and (D. J. Newman *et al*). According to an estimate 25% of modern drugs in the world originate from plants [S. M. K. 2001] about 3000 species of plants have been screened for anticancer properties [J. G. Graham *et al* 2000]. Traditional medicines from plants are used by about 80% of the population in developing countries [FAO 2004]. Pakistan is a rich source of herbal medicines among the South Asian countries. There is a huge number of plants species found in Pakistan However, many of these plants have not yet screened for chemical, pharmacological and toxicological studies to investigate the presence of bioactive compound(s) [M. Yusuf, *et al* 1994]. It is cleared from Traditional records that plants in Pakistan represent an exciting resource for possible lead structures in drug design. Rababah *et al* ; 2004 , studied that different plants parts such as fruits, leaves, oil seed, roots and vegetables act as natural antioxidants. Thus the poly phenolics or the other metabolites of different plants scavenge the oxygen and nitrogen free radicals and control the oxidative stress (Sun *et al*; 2002). So for this purpose recently scientists focus on the isolation and development of natural anti oxidants which have no or less side effects.

The term **"**Cytotoxicity" is made by the combination of two wards "cyto" which means the living cell and "toxicity" means toxic/dangerous. Thus by cytotoxicity we mean those bioactive compounds which are toxic/fatal to living cells. Some toxic chemicals, toxic venom of different animals and immune cell are the main cause of cytotoxicity. In pharmaceutical industry the cytotoxicity assays are mostly performed for screening/isolation of those natural bioactive compounds which are used for the treatment of cancer by MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide, clonogenic and SRB (sulforhodamine B) assay. It is estimated that more or less 200 types of different cells are affected by the cancer. Both in the developed and developing countries of the world cancer, which is one of the most life threatening diseases and serious public health problem has given much massive damage to the society. Cancer is a group of diseases which is responsible for the disruption of the healthy surrounding tissues by the dis-regulate propagation of abnormal cells either by direct attack or to the distant sites in the body by metastasis. Metastasis can be defined as the stage in the body by which the cancer cells are transported through the blood stream or lymphatic system (Gennari et *al.,* 2007, and Hanahan *et al.,* 2000). In the treatment of cancer, there are many difficulties but the drug resistance, toxicity, and low specificity are the most serious major one. Because of the severe toxicity and adverse side effects, the synthetic drugs are failed in controlling of cancer or tumor. Conventional treatments are not also very fruitful against cancer therapy. At last, to improve and satisfy our present and future health needs, herbal medicine has a crucial rule. (Harun-ur-Rashid *et al.,* 2002). The most important sources of antitumor and anticancer throughout the world are the different plant molecules, their semi-synthetic and synthetic derivatives. More than 50% of anti cancer drugs are based on natural sources (Newman and cragg, 2007). For this purpose, plant kingdom has remained a very rich source search for natural products which are used for cancer treatment, and shows an area of great interest for providing many anticancer and antitumor agents with novel structures and unique mechanisms of action, (Change *et al.*, 1999).

Vinblastine and vincristine, the effective anticancer agents are isolated from the *Catharanthus roseus* (L) G. Don. Thus it provides strong evidence that plants are the real rich source of novel anticancer agents throughout the world (Cragg *et al.,* 1996). It is noted that up to now there is no such drug or therapeutic strategies which destroy only the cancer cells by inhibiting their growth and not to harm the normal cells of the host. For this purpose therefore attention has been focused on the development of such modern immunotherapy (active and passive immunotherapy) which only to damage the cancer cells and have no adverse effect on the normal cells of the host. Such compounds are classified as biological response modifiers (BRMS). BRMS have a great potential to control homeostasis, improve quality of life and to increase the immune response system of the body. They also increase the antitumor immunity power and remove all those pathologies associated with the diseases in the body (Lull *et al.,* 2005). *Medicago polymorpha* is a plant species of the genus *Medicago*. It is found throughout the world. The bacterium *Sinorhizobium medicae* forms a symbiotic relationship with this plant, play important role in the nitrogen fixation. *Medicago polymorpha* has several Common names including toothed medick, California burclover, toothed bur clover, and burr medic

II. MATERIALS AND METHODS

2.1 Extraction procedure and preparation of crude extract.

Plant of *Medicago polymorpha* was collected from the main Township campus of University of Science and Technology, Bannu, Khyber Pakhtunkhwa, Pakistan in the month of July 2010. The plant was identified by Taxonomist Prof: HASHAM KHAN: Department of Botany, Government Post Graduate College, Bannu.

The plant materials were washed by the distilled water and were shade dried at room temperature for two weeks, chopped and grinded mechanically of mesh size 1 mm. 3 kg powder of *Medicago polymorpha* was extracted in 3 liter of 70% methanol by random shaking. After a week, the extract was filtered by using Whatman filter paper No.1. After filtration, the filtrate was further concentrated by using rotary vacuum evaporator at 38 C^0 in order to get the methanolic crude extract of the plant. The methanolic crude extract was stored at 4 oC in the refrigerator for further phytochemical studies and in vitro investigation.

2.2 Cytotoxic assay (Brine shrimp assay)

Samples preparation for this assay, the stock solution of 15 mg/15 ml of methanolic crude extract was prepared in the respective solvent methanol. From this stock solution further dilution was made to 10 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml each one of 1ml by using sea salt solution, instead of methanol due to its toxicity (M1V1=M2V2). Saline preparation 2.8 g of commercial sea salt (Sigma) was dissolved completely in 100 ml distilled water The commercial sea salt solution (media) was put in the two compartment rectangular tray contained with large number of small pores for passage of larvae. Eggs were scattered in dark compartment of tray (covered by aluminum foil) and were placed under table lamp for incubation for 24 hours. After 24 hours, the larvae were hatched, migrated to the lightened side by pores and was collected by pasture pipette. This assay was done according to the methods of Solis et al (1992) and Potduang et al, (2007) with modification to determine the inhibitory activity. 50µl of different concentrations of crude methanolic extracts (1000, 500, 250, 200, 100 and 50 µg/ml) was taken and methanol was used as a control. All these concentration were added into graduated vial bottles. 5ml of artificial sea water was taken in each bottle containing 10 hatched brine shrimps, all concentration were repeated in triplicate. After incubation at room temperature for 24 hours the living brine shrimps were counted with the help of a hand magnifying lens. Using potassium dichromate as positive control same procedure was followed
Antioxidant assays

2.3 DPPH radical scavenging activity

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay was made according to the method of Gyamfi et al. (1999) with some modification.Assay procedure 100 µl of plant extract, from each concentration i.e. (50, 100, 150, 200, 250 µg/ml) was taken in a separate test tube and 900 µl of DPPH solution was mixed with it for each concentration and was taken in duplicate. Similar process was repeated for ascorbic acid concentration used as a reference. 1ml (1000 µl) was taken directly from the DPPH solution in a separate test tube. Now all these test tubes labeled separately as (extract, ascorbic acid, and DPPH) were shaken well and incubated in the dark at 25 OC for 30 minutes. Then the absorbance was taken at 517 nm by spectrophotometer. The potential to scavenging the DPPH radical was calculated using the following equation: DPPH radical scavenging effect (%) = (A1-A2/A1) x100 Where A1 is the absorbance of the control (DPPH solution without test sample) and A2 is the absorbance in the presence of the test sample.

2.4 ABTS radical scavenging activity

The ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) ABTS have high ability to act as an electron donor for the reduction of oxo species. ABTS assay was made according to the method of Gyamfi et al. (1999) with some modification. Assay procedure Equal volumes of 7mM ABTS and 35mM K2S2O8 (potassium per sulfate) were mixed and incubate for 24 hours. 100 µl of plant extract, from each concentration i.e. (50, 100, 150, 200, 250 µg/ml each of the following concentration was taken in duplicate) was taken in a separate test tube and 900 µl of ABTS solution was mixed with it for each concentration. Similar process was repeated for ascorbic acid concentration used as a reference. 1ml (1000 µl) was taken directly from the ABTS solution in a separate test tube. Now all these test tubes labeled separately as (extract, ascorbic acid, and DPPH) were shaken well and incubated in the dark at 25 OC for 30 minutes. Then the Kinetic absorbance was taken at 760 nm by spectrophotometer after 1 and 6minutes for each concentration and mean was taken for each reading. The potential to scavenging the ABTS radical was calculated using the following equation: ABTS radical scavenging effect (%) = (A1-A2/A1) x100 Where A1 is the absorbance of the control (ABTS solution without test sample) and A2 is the absorbance in the presence of the test sample.

III. RESULT AND DISCUSSIONS

3.1 DPPH free radical scavenging assay

DPPH (1, 1-diphenyl 1-2-picryl-hydrazyl) has strong potential to oxidize a range of compounds. Therefore, it is widely used for estimation of *in vitro* antioxidant scavenging activities of medicinal plants. The Fig.4.1 shows the % scavenging activity of *Medicago polymorpha* methanolic extract (MDME) for free radicals of DPPH.

In this study we used various concentration of *Medicago polymorpha* methanolic extract the graph show that by increasing concentration of *Medicago polymorpha* methanolic extract the scavenging activities also increasing (50 µg/ml < 100 µg/ml < 150 µg/ml < 200 µg/ml < 250 µg/ml < 500 µg/ml). Ascorbic acid was used as a reference compound and Similar result was presented by various concentration of Ascorbic acid (50 µg/ml < 100 µg/ml < 150 µg/ml < 200 µg/ml < 250 µg/ml and 500µg/ml).

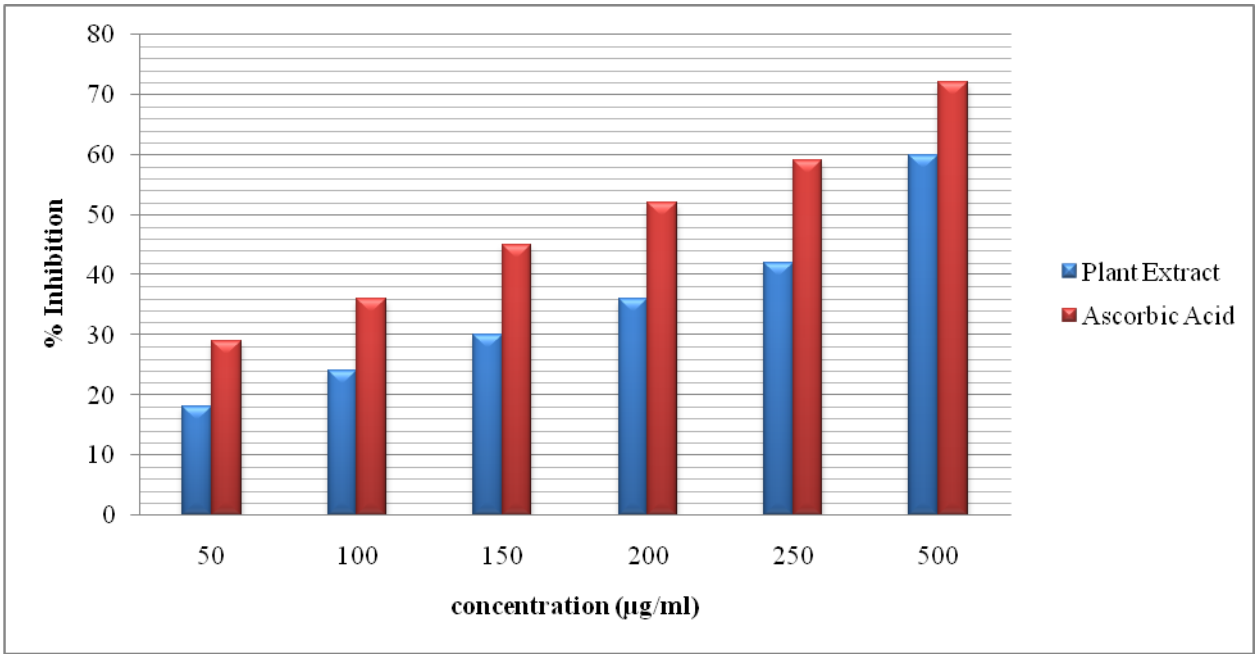

Fig 1 DPPH (free radicals) scavenging activities of various concentrations of *Medicago polymorpha* extract and ascorbic acid

3.2 ABTS free radical scavenging assay

In this study we used various concentration of *Medicago polymorpha* extract. Scavenging activities of *Medicago polymorpha* metanolic extract show a sequential increase with increasing concentration of the plan extract (50 µg/ml < 100 µg/ml < 150 µg/ml < 200 µg/ml < 250 µg/ml < 500 µg/ml). Ascorbic acid which was used as a standard show Similar results I,e scavenging activities increased with increasing of Ascorbic acid concentrations (50 µg/ml < 100 µg/ml < 150 µg/ml < 200 µg/ml < 250 µg/ml and 500µg/ml).

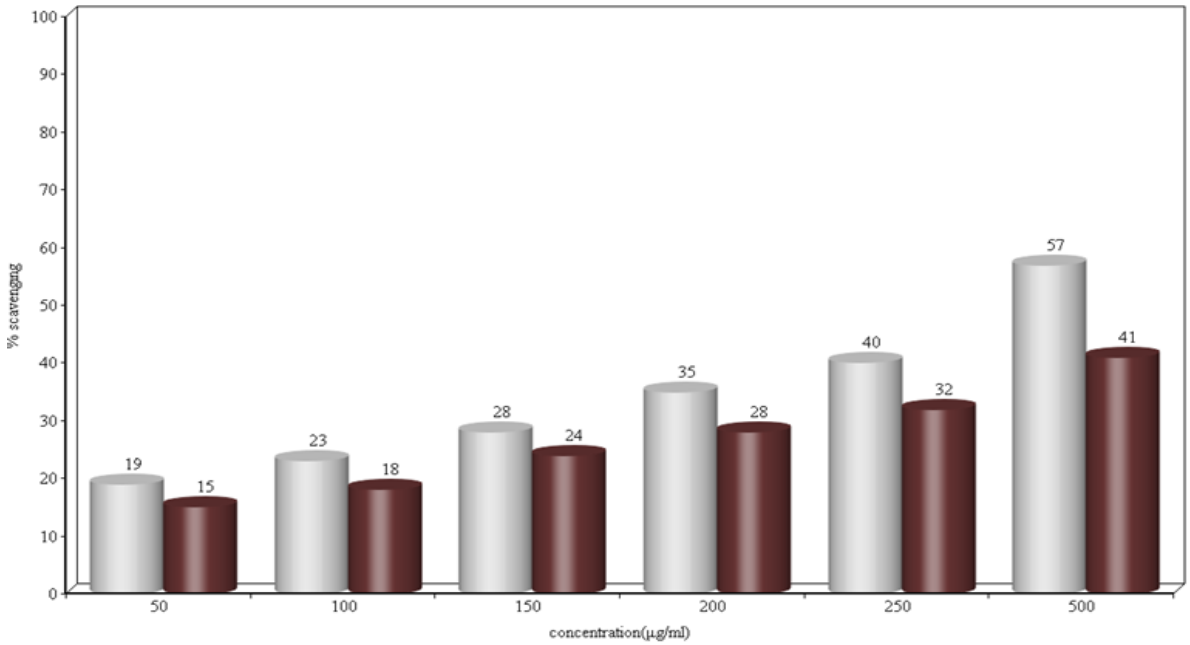

Fig 2 ABTS (free radicals) scavenging activities of various concentrations of plant extract and ascorbic acid

3.3 Cytotoxic brine shrimp assay

Cytotoxicity of plant extract conform its antitumor property. Cytotoxic effect of *Periploca* was measured against brine shrimps growth Fig 2From figure it is clear that at low concentration *Medicago polymorpha* methanolic extract (MPME) survival rate of the brine shrimp is high and at high concentration *Medicago polymorpha* extract (MPME) death rate of brine shrimp is high, it clearly indicates that possess cytotoxic properties and can be utilized for the treatment of tumors and cancer.

conc.	No of total brine shrimp	No of alive brine shrimp
10	8	8
50	8	6
100	8	5
150	8	3
200	8	2

Table 1, Cytotoxic effect of different concentrations of methanolic extract of *Medicago polymorpha* extract and ascorbic acid

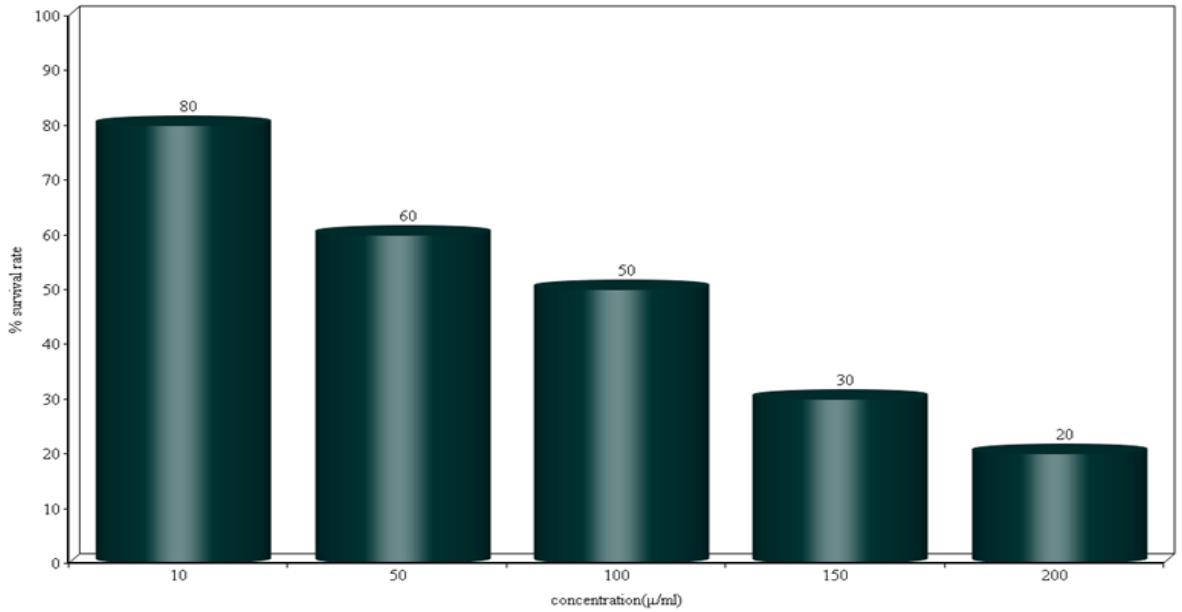

Fig3 Cytotoxic effect of different concentrations of methanolic extract of *Medicago polymorpha* and ascorbic acid

REFERENCES

[1] A.Ghani. Medicinal Plants of Bangladesh with Chemical Constituents and Uses, Asiatic Society of Bangladesh, Dhaka, Bangladesh 2003.

[2] Al-Dubai AS, Al-khulaidi AA. Medicinal and Aromatic Plants of Yemen (In Arabic), Sana'a, Yemen: Obadi Center for studies and Publishing 1996.

[3] Cragg GM, Simon JE, Jato JG, Sander KM. Drug discovery and development at the National Cancer Institute: Potential for New Pharmaceutical crops. In: Progress in new crops, Ed., Janick, J. ASHS Press, 1996; 554-560.

[4] Cragg GM, Newman DJ. Plants as source of anticancer agents. J Ethanopharmacol 2005; 100:72-79.

[5] D. J. Newman and G. M. Cragg, "Natural products as sources of new drugs over the last 25 years," Journal of Natural Products, vol. 70, no. 3 2007; 461–477.

[6] FAO, Trade in Medicinal Plants, Economic and Social Department, Food and Agriculture Organization of the United Nations, Rome, Italy, 2004.

[7] J. G. Graham, M. L. Quinn, D. S. Fabricant, and N. R. Farnsworth, "Plants used against cancer—an extension of the work of Jonathan Hartwell," Journal of Ethno pharmacology, vol. 73, no. 3, pp. 2000; 347–377. View at Publisher · View at Google Scholar

[8] M. Yusuf, J. U. Chowdhury, M. A. Wahab, and J. Begum, Medicinal Plants of Bangladesh, Bangladesh Council of Scientific & Industrial Research (BCSIR), Dhaka, Bangladesh, 1994.

[9] Gennari C, Castoldi D, Sharon O. Natural products with taxol-like anticancer activity. Synthetic approach to eleutherobin and dictyostatin. Pure and Appl Chem 2007; 79 (2): 173-180.

[10] Harun-ur- Rashid M, Gafur MA, Sadik GM, Rahman MAA. Biological activities of a new acryl amide derivative from Ipomoea turpithum. Pakistan J Biol Sci 2002; 5(9): 968-969.

[11] L.-C. Chiang, H.-Y. Cheng, C.-C. Chen and C.-C. Lin, "In vitro anti-leukemic and antiviral activities of traditionally used medicinal plants in Taiwan," American Journal of Chinese Medicine, vol. 32, no. 5, pp. 2004; 695–704.

[12] Lull C, Wichers HJ, Savelkoul HFJ. Anti-inflammatory and immunomodulating

[13] Properties of fungal metabolites. Med Inflammation 2005; 2: 63-80.

[14] S. M. K. Rates, "Plants as source of drugs," Toxicon, vol. 39, no. 5, pp. 2001; 603–613.

[15] Sun J, Chu Y, Wu X, Liu R (). Antioxidant and anti proliferative activities of common fruits. J Agric Food Chem 2002; 50 (25): 7449-7454.

[16] World Health Organization. WHO Traditional Medicine Strategy 2002–2005. Geneva: World Health Organization, 2002.

[17] Zhang X. Traditional medicine: its importance and protection. In: Twarog S, Kapoor P (eds). Protecting and Promoting Traditional Knowledge: Systems, National Experiences and International Dimensions. Part 1. The Role of Traditional Knowledge in Healthcare and Agriculture. New York: United Nations; 2004, 3–6.

[18] Fleurentin J, Pelt J-M. Repertory of drugs and medicinal plants of Yemen. J Ethnopharmacol 1982; 6:85–108.

[19] Schopen A. Traditionelle Heilmittel in Jemen. Berlin: Franz Steiner

[20] Verlag GmbH, 1983.