Investigation of the Biological Activities of *Asparagus setaceus* Kunth.

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Abstract

The records collected from the fossils of 60,000 years back reveals the fact of utilization of plants as medicines. Therefore present research work was planned to investigate the antioxidant, cytotoxic, antibacterial, anti-inflammatory potential and melanin inhibitory effect of methanolic (MeOH) and dichloromethane (CH_2Cl_2) extracts of leaves and rhizomes of *Asparagus setaceus* Kunth (is a perennial herb or sub shrub and belongs to family Asparagaceae). Traditionally it is used for skin diseases, as tonic, demulcent, diuretic, in problems of urination, andrology, pulmonary infections, cough and also has antiepileptic potential. Antioxidant and melanin inhibitory potential was evaluated by DPPH and tyrosinase inhibitory assay in which *ascorbic acid* and *kojic acid* ware used as standards respectively.

Cytotoxic potential was carried out by brine shrimp lethality assay, *etoposide* was used as standard, antiinflammatory activity was performed by using oxidative burst assay in which *ibuprofen* was used as standard drug and microplate alamar blue assay was used to determine antibacterial activity of *asparagus setaceus* (*AS*) respectively. Results showed that the antioxidant, melanin inhibitory, antibacterial, anti inflammatory potential of MeOH and CH_2Cl_2 extracts of leaves/L and rhizomes/R of *Asparagus setaceus* Kunth have very low significant medicinal value while methanolic extract of leaves/L and rhizomes/R as compared to dichloromethane extracts showed a significant cytotoxic activity. It was concluded from the present research work leaves and rhizomes have significant dose dependant cytotoxic potential.

Keywords

Asparagus setaceus, Antibacterial, Antioxidant, Anti-inflammatory and Cytotoxic.

1. INTRODUCTION

Invention of this planet and human existence on it, by using hit and trial methodology man has acquired knowledge about animals and

plants having food and medicinal values (Tyler et.al., 1976). Therefore, The plants are considered oldest and most valuable source of medicines as reflect by the records collected from the fossils of 60,000 years back revealing the fact of utilization of plants as medicines (Fabricant and Farnsworth, 2001). Ayurveda, Siddha and Unani systems of medicine provide good base for scientific exploration of medicinally important molecules from nature. The ethnobotany provides a rich resource for natural drug research and development. In recent years, the use of traditional medicine information on plant research has again received considerable interest.

The western use of such information has also come under increasing scrutiny and the national and indigenous rights on these resources have been acknowledged by most academic and industrial researchers (Garg et al., 2007). According to the World Health Organization (WHO), about three quarters of the world's population currently use herbs and other forms of traditional medicines to treat various diseases. In USA, use of phytomedicines has increased dramatically in the last two decades (Rao et al., 2004). It has been also reported that more than 50% of all modern drugs in clinical use are of natural origion, many of which have been recognized to have the ability to induce apoptosis in various cancer cells of human origin (Rosangkima et al., 2004).

Asparagus setaceus Kunth is a perennial herb or sub shrub and belongs to family Asparagaceae.Traditionally it is used for skin diseases, as tonic, demulcent, diuretic, in problems of urination, andrology, pulmonary infections, cough and also has antiepileptic potential (de Boer et al., 2005). The plant contain active chemical constituents like yamogenen glycosides I, II, two furostanol glycosides III, IV, three spirostanol glycosides, anthocynin, malvin and asparagine (Negi et al.,2010). Despite the above mentioned uses of the plant the antibacterial, antioxidant, cytotoxic, anti-inflammatory and tyrosinase inhibitory activity has not been addressed. Therefore the present study was designed to find out the biological potential of *asparagus setaceus* kunth.

2. Material and Methods:

2.1. Collection and Authentication of Plant:

Plant was collected from Faiz-e-Aaam nursery Multan, Pakistan, authenticated to *Asparagus Setaceus* Kunth (Fam. Asparagaceae), by the botanist of Bahauddin Zakariya University, Dr Zaffar Ullah Zaffar with Voucher No. ''Stewart F.W. Pak. 49(10)'' submitted into herbarium of Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan. Leaves and rhizomes were separated, shade dried for 40 days at room temperature and grin to coarse powder.

2.2. Extracts Preparation:

Extraction of accurately weighed powder 150 g of leaves and rhizomes was carried out by macerated in dichloromethane (solvent) using air tight containers for a period of 7 days. For maximum extraction the macerated powder was shaken occasionally during maceration. After filtration process, the residue obtained was macerated in methanol for a period of 7 days.The macerated residue was occasionally shaken during maceration followed by filtration and converted semi solid mass by using Rotavapor (R-200).The semisolid extracts obtained by successive extraction was weighed and stored in amber glass

containers. The following activities were conducted.

a) Antioxidant activity:

In order to perform the assay a volume of 100 µl (mixture of 10 µl test and 90 µl DPPH solution) was used in test. A free radical compound 2, 2 diphenyl 1-picrylhydrazyl is abbreviated as DPPH, occurs as purple color solvent and turns into yellow upon presence of antioxidant in the sample. In solvent (previously made) the extract was dissolved. DPPH was dissolved in concentration of 100 µM of methanol. The assay solution in 96 well plates was incubated for 30 minutes at 37ÚC using ascorbic acid as standard. Absorbance was measured at 517nm. Software (Amherst USA) was used for computing IC_{50} values of serial dilutions. Decrease in absorbance is considered as scavenging activity of free radical (Ratshilivha; 2014). Percentage of inhibition was determined using the following formula:
Percentage inhibition= (a-b) / a × 100
Where,
a = Absorbance of control, b= Absorbance of test solution.

2.3. Tyrosinase inhibitory assay:

Tyrosinase (60 units), extract solution (10 µl) and buffer (150 µl) adjusted pH of 6.8 were poured in 96 well. Incubated for 30 minutes at 25ÚC and initial reading was recorded at 480 nm of 96 plate reader. For reference purposes *kojic acid* was used. Incubation process was repeated again by adding substrate (1µM) in each well. The reading was recorded at 480 nm and percentage inhibition was calculated as follows:
Percentage inhibition (%) = 100- (b/a) × 100
Where,
a = absorbance of control, b= absorbance of test
IC_{50} value of prepared using serial dilutions was determined.

2.4. Antibacterial activity:

Microplate Alamar Blue Assay

Mueller Hinton Media was used for growing *E.coli, B.subtillis.S.aureus, P. aeruginosa, and S.typhi*. Turbidity Index of 0.5 McFarland was adjusted of the prepared inoculums. Stock solutions of methanol and dichloromethane extracts from both leaves and rhizomes were prepared as 60 mg weight of both extracts and dimethyl sulphoxide (1:1) with a concentration of 3000 µg/ml of the compound. A 96 well micro-plate method was used and prepared media was poured into wells with no compound (control) and adjusted volume up to 200 µl. Ofloxacin was used as positive control. About 50, 00000 cells were introduced into the wells including the control. Parafilm covered plates were incubated for 18-20 hours. *Alamar Blue* dye was added in each well and shaken for 2 -3 hours in an incubating shaker at 80 rpm. Observation of the colour change from blue to pink was observed. Absorbance at 570 nm and 600 nm using ELISA reader was measured (Pettit *et al*; 2005) (Sarkar *et al*; 2007).

2.5. Anti-inflammatory activity:

Oxidative burst assay:

A technique chemiluminiscense was used to perform the assay. Incubation of equal volume of the extract and diluted whole blood Hanks Balanced Salt Solution containing magnesium chloride and calcium (HBSS++) at different concentrations (1 µg/ml, 10 µg/ml and 100 µg/ml) was performed. Extract was absent in control wells and Ibuprofen (standard) with IC_{50} of 11.2 ± 1.9 was used to perform the assay procedure by using 96 well micro-plates at 37º C for 15 minutes in luminometer¼s thermostat chamber. In all the

wells 25 µl of opsonized zymosan serum, species with intracellular reactive oxygen detecting probe and luminol was added except blank. Specific light units of luminometer were used to record different levels of reactive oxygen species (Helfand *et al*; 1982).

2.6. Brine shrimp lethality assay:

This bioassay is a general, rapid and cost effective technique for screening of physiologically active natural products. Shrimp eggs (50 mg) were sprinkled in hatching tray with perforations containing artificial sea water it and Incubated at 37 ºC. Sample extract (20 mg) in 2 ml of the respective solvent in vials were evaporated. Larvae 10/vial were placed using pasteur pipette after two days. One vial with an adjusted volume up to 5 ml with sea water was incubated at 27 ºC for a period of one day. Drug and solvent was added to other remaining vials for positive and negative control respectively. Finney computer program with 95% confidence interval was used to calculate LD_{50} value. Eggs hatched into larvae within two to four days (Alves *et al*; 2000) (Kivack *et al*; 2001) (Carballo et *al*; 2002) (Meyer *et al*; 1982) (Finey; 1971).

3. RESULTS AND DISCUSSION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998).

Asparagus setaceus kunth previously has been used in the folk medicine for the cure of different disease states such as diarrhoea, abdominal pain, antiepileptic potential etc (de Boer et al., 2005).This frequent use proved its medicinal chemical constituents; but there was a lack of research work on these lines. Leaves and rhizome were studied.

Oxidative stress (OS) is the imbalance between cellular production of reactive oxygen species (ROS) and the ability of cells to scavenge them. OS has been implicated as a potential contributor to the pathogenesis of several diseases, such as cancer, diabetes and heart disease (Gilgun et al.,2002) The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Wu et al., 2011). In the current study antifungal activity of the CH_2Cl_2 and MeOH extracts of leaves and rhizomes of the plant by using Ascorbic acid as standard was evaluated (Table 1) CH_2Cl_2 and MeOH extracts of both leaves and rhizomes of the plant showed minor antioxidant activity.
Melanin is essential for protecting human skin against radiation, but the accumulation of abnormal melanin induces pigmentation disorders, such as melasma, freckles, ephelides, and senile lentigines (Slominski et al.,2004).Tyrosinase inhibitors such as arbutin, kojic acid and hydroquinones have been used as whitening or anti hyperpigment agents because of their ability to suppress dermal-melanin production (Maeda

et al.,1991). In the present work Tyrosinase inhibitory assay of the CH_2Cl_2 and MeOH extracts of leaves and rhizomes of the plant by using Kojic acid as standard was determined and the results are shown in (Table 2) CH_2Cl_2 and MeOH extracts of both leaves and rhizomes of the plant showed minute tyrosinase inhibitory activity.

Table 1. Antioxidant activity of CH_2Cl_2 and MeOH extracts of *Asparagus setaceus* kunth leaves and rhizomes.

Extract	Leaves	Rhizome	Standard drug (Ascorbic acid)
Methanolic	37.8±0.10	8.3±1.11	197±2.20
DCM	35.9±0.081	9.3±0.10	

Table 2. Tyrosinase inhibitory activity of CH_2Cl_2 and MeOH extracts of *Asparagus setaceus* kunth leaves and rhizomes.

Extract	Leaves	Rhizome	Standard drug (Kojic acid)
Methanolic	35.1±0.81	8.3±0.51	205±0.98
DCM	34.5±0.081	7.6±0.24	

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases (Bhatia et al., 2010). However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria (Boucher et al.,2009). In the current work antibacterial activity of the CH_2Cl_2 and MeOH extracts of leaves and rhizomes of the plant by using micro-organisms like *Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and ***Ofloxacin*** as standard was determined (Table 3,4) CH_2Cl_2 and MeOH extracts of both leaves and rhizomes of the plant showed minimum antimicrobial activity.

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries (Oyedano et al., 2008). Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required (Anilkumar., 2010). In the present study anti-inflammatory activity of the methanolic and CH_2Cl_2 and MeOH extract of leaves and rhizomes of the plant by using ***Ibuprofen*** as standard was determined (Table 5) Lack of activity was observed in both parts of the plant in both extracts.

Table.3 Antibacterial activity of CH_2Cl_2 and MeOH extracts of *Asparagus setaceus* kunth leaves

Bacterial strain	**DCM Extract Inhibition (%)**	**DCM Extract Inhibition (%)**	**Ofloxacin Inhibition (%)**
Escherichia coli	No inhibition	No inhibition	92.63
Salmonella typhi	No inhibition	No inhibition	92.95
Bacillus subtilis	6.31	5.135	91.41
Staphylococcus aureus	No inhibition	No inhibition	90.93
Pseudomonas aeruginosa	40.5	No inhibition	91.46

Table.4 Antibacterial activity of CH_2Cl_2 and MeOH extracts of *Asparagus setaceus* kunt Rhizome

Bacterial strain	**DCM Extract Inhibition (%)**	**Methanol Extract Inhibition (%)**	**Ofloxacin Inhibition (%)**
Escherichia coli	No inhibition	No inhibition	92.63
Salmonella typhi	No inhibition	4.49	94.85
Bacillus subtilis	1.35	No inhibition	95.91
Staphylococcus aureus	No inhibition	No inhibition	90.93
Pseudomonas aeruginosa	40.5	No inhibition	90.99

Table 5. Anti inflammatory activity of CH_2Cl_2 and MeOH extracts of *Asparagus setaceus* kunth leaves and rhizome

Sample	Extract Inhibition (%)	Ibuprofen Inhibition (%)
Rhizome (CH_2Cl_2)	23.9	73.2
Leaves(CH_2Cl_2)	8.6	
Rhizome(MeOH)	10.6	
Leaves(MeOH)	9	

Table 6. BSLA of CH_2Cl_2 extracts of *Asparagus setaceus* kunth rhizomes and leaves.

Dose(µg/ml)	Rhizome Survivors /30 Shrimps	Leaves Survivors /30 Shrimps	Rhizome Extract LD_{50}(µg/ml)	Leaves Extract LD_{50}(µg/ml)	Etoposide LD_{50}(µg/ml)
10	27	28	-	-	7.4625
100	27	27			
1000	22	24			

Table.7 BSLA of MeOH extracts of *Asparagus setaceus* kunth rhizome and leaves

Dose(µg/ml)	Rhizome Survivors /30 Shrimps	Leaves Survivors /30 Shrimps	Rhizome Extract LD_{50}(µg/ml)	Leaves Extract LD_{50}(µg/ml)	Etoposide LD_{50} (µg/ml)
10	24	23	133.8291	68.6479	7.4625
100	17	13			
1000	07	05			

Brine shrimp (Artemia salina, fairy shrimp or sea monkeys) lethality assay is commonly used to check the cytotoxic effect of bioactive compounds. It is a preliminary toxicity screening of plant extracts (Ghosh et al., 2015; Kibiti and Afolayan, 2016). CH_2Cl_2 and MeOH extracts of leaves and rhizomes of the plant by using ***Etoposide*** as standard were evaluated. CH_2Cl_2 extracts of both leaves and rhizomes showed negligible activity (Table 6) while MeOH extracts of both leaves and rhizomes (Table 7) showed significant activity in which rhizomes extract showed more strong activity as compared to the leaves of plant. Investigated results of the Extracts (Methanolic and DCM) of leaves and rhizome for antioxidant, enzymatic, antibacterial, anti inflammatory and antifungal activity indicate that leaves and rhizome have no antioxidant, melanin inhibitory, antibacterial, and anti-inflammatory activity, although significant cytotoxic potential of the extracts may develop a cytotoxic medical claim of the plant.

4. CONCLUSION

As a result of this research activity,it is concluded that in light of significant toxic potential against Artemia salina methanol extract of both leaves and rhizomes of *Asparagus setaceus* Kunth proved to be a toxic plant.A great deal of work is still required to be done on this plant; as elaboration of different other therapeutic values for which it has been used in the past and search for different new phytochemicals which may be used medicinally for the cure of different diseases is still needed.

5. REFERENCES

1. Alves, T.M.D.A., Silva, A.F., Brandão, M., Grandi, T.S.M., Smânia, E.D.F.A., Smânia Júnior, A. and Zani, C.L., 2000. Biological screening of Brazilian medicinal plants. *Memórias do Instituto Oswaldo Cruz,* 95(3) :367-373.
2. Anilkumar, M., (2010). 10 Ethnomedicinal plants as anti-inflammatory and analgesic agents. *Ethnomedicine: A source of complementary therapeutics, 2* 67-293.
3. KIVÇAK, B., MERT, T. and ÖZTÜRK, RH.T., 2002. Antimicrobial and cytotoxic activities of Ceratonia siliqua L. extracts. *Turkish Journal of Biology* **26**(4):197-200.
4. Bhatia, R. and Narain, J.P., 2010. The growing challenge of antimicrobial resistance in the South-East Asia Region-Are we losing the battle?. *The Indian journal of medical research*, **132**(5): 482.
5. Boucher, H.W., Talbot, G.H., Bradley, J.S., Edward J.E., Gilbert, D., Rice, L.B., Scheld, M., Spellberg, B. and Bartlett, J.(2009). Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseaeses Society of America. *Clinical infectious diseases,48* (1):1-12.
6. Carballo, J.L., Hernández-Inda, Z.L., Pérez, P. and García-Grávalos, M.D. (2002). A comparison b/w two brine shrimp assays to detect invitro cytotoxicity in marine natural products. *BMC biotechnology*, **2**(1):17.
7. de Boer, Hugo & Kool, Anneleen & Broberg, Ander & Mziray, William & Hedberg, Inga & Levenfors, Jolanta. (2005). Antifungal and antibacterial activity of some herbal remedies from Tanzania. Journal of ethnopharmacology. 96. 461-9.
8. Ellof, J.N. (1998) which extractant should be used for the screening and isolation of antimicrobial of components from plants? *J. Ethnopharmacol*. **60**:1-6.
9. Farnsworth, N. R. and Bingel, A. S. (1997). Problems and prospects of discovery new drugs from higher plants by pharmacological screening In: H. Wagner and P.Wolff (eds.), new natural products and plant drugs with pharmacological, biological & therapeutical activity, *Springer Verlag*: 1-22.
10. Finney, D. (1971). Probit analysis, third ed. Cambridge University Press, Cambridge.
11. Garg, A., Darokar, M.P., Sundaresan, V., Faridi, U.Z.M.A., Luqman, S. and Khanuja, S.P.S., (2007). Anticancer activity of some medicinal plants from high altitude evergreen elements of Indian Western Ghats. *J. Res. Educ. Indian Med*, **13**(3):1-6.
12. Ghosh A, Banik S, Islam M. (2015). In vitro thrombolytic, anthelmintic, anti-oxidant and cytotoxic activity with phytochemical screening of methanolic extract of Xanthium indicum leaves Bangladesh J Pharmacol. **10**: 854-59.
13. Gilgun-Sherki, Y., Rosenbaum, Z., Melamed, E. and Offen, D., (2002). Antioxidant therapy in

acute central nervous system injury: current state *Pharmacological reviews*, 54(2):271-284.

14. Helfand, S. L., Werkmeister, J. E. R. O. M. E., & Roder, J. C. (1982). Chemiluminescence response of human natural killer cells. I. The relationship between target cell binding, chemiluminescence, and cytolysis. *The Journal of experimental medicine* 1-56(2):492-505.
15. Kibiti C, Afolayan A. (2016). Antifungal activity and brine shrimp toxicity assessment of Bulbine abyssinica used in the folk medicine in the Eastern Cape Province, South Africa. Bangladesh J. Pharmacol. 11: 469-77.
16. Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen L.B., Nichols, D.E., McLaughlin, J.L. (1982). Brine Shrimp: A convenient general bioassay for active plant constituents. Planta Medica. 45:31-34.
17. Maeda, K.A. and Fukuda, M. (1991). In vitro effectiveness of several whitening cosmetic components in human melanocytes. *Journal of the Society of Cosmetic Chemists*, 42(6):361-368.
18. Negi, Jagmohan & Singh, Pushpa & Joshi, G & Rawat, M.s.M. & Bisht, Vinod. (2010). Chemical constituents of Asparagus. Pharmacognosy reviews 4. 215-20.
19. Oyedapo, O.A., Adewunmi, C.O., Iwalewa, E.O. and Makanju, V.O. (2008). Analgesic, antioxidant and anti-inflammatory related activities of 21- hydroxy-2, 41-dimethoxychalcone and 4-hydroxychalcone in mice. *Journal of Biological Sciences,* 8(1): 131-136.
20. Pettit, R.K., Weber, C.A., Kean, M.J., Hoffmann, H., Pettit, G.R., Tan, R., Franks, K.S. and Horton, M.L. (2005). Microplate Alamar blue assay for Staphylococcus epidermidis biofilm susceptibility testing. *Antimicrobial Agents and Chemotheraphy,* 49(7):2612-2617.
21. Rao, K.V.K., Schwartz, S.A., Nair, H.K., Aalinkeel, R., Mahajan, S., Chawda, R. and Nair, M.P. (2004). Plant derived products as a source of cellular growth inhibitory phytochemicals on PC-3M, DU-145 & and LNCaP prostate cancer cell lines. *Current sci:* 1585-1588.
22. Ratshilivha, N., Awouafack, M.D., Du Toit, E.S. and Eloff, J.N. (2014). The variation in antimicrobial and antioxidant activities of acetone leaf extracts of 12 Moringa oleifera (Moringaceae) trees enables the selection of trees with additional uses. *South African Journal of Botany*, 92: pp.59-64.
23. Rosangkima, G. 1. and Prasad, S.B. (2004). R. Antitumor activity of some plant from Meghalaya and Mizoram against murine ascites Dalton's lymphoma. Indian J. Exp. Biol. 42: 981-988.
24. Santos, P.R.V.; Oliveira, A.C.X.; Tomassini, T.C.B. (1995) Controle microbiógico de produtos fitoterapicos. *Rev. Farm. Bioquím*. 31:35-38.
25. Slominski, A., Tobin, D.J., Shibahara, S. and Wortsman, J.(2004). Melanin pigmentation in mammmammalian skin and its hormonal regulation. Physiological reviews, 84(4):1155-1228.
26. Tyler, V. E., Brady, L. R. and Robbers, M.J.E. (1976). Pharmacognosy, *Lea and Febiger*, Ed: 7th: 1-3, 15-25.
27. Wu, Y.Y., Li, W., Xu, Y., Jin, E.H. and Tu, Y.Y. (2011). Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses. *Journal of Zhejiang University Science B*, 12(9):744.