Pak. J. Bot., 47(3): 1195-1197, 2015.

LIPID PEROXIDATION, ANTIGLYCATION, CYTOTOXIC, PHYTOTOXIC, ANTIOXIDANT, ANTIPLATELET AND ANTIMICROBIAL ACTIVITIES OF *AJUGA BRACTEOSA* AGAINST VARIOUS PATHOGENS

NAJEEB UR REHMAN[1,2], NAEEMA BEGUM[1], LIAQAT ALI[2], AHMED AL-HARRASI[2,3], GHULAM ABBAS[3], SAGHEER AHMAD[1], ABDUL LATIF KHAN[2], ZABTA KHAN SHINWARI[4*] AND JAVID HUSSAIN[2,3*]

[1]*Department of Chemistry, Kohat University of Science and Technology Kohat-26000, Khyber Pakhtunkhwa, Pakistan*
[2]*UoN Chair of Oman's Medicinal Plants and marine Natural products, University of Nizwa, Nizwa-616, Oman*
[3]*Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, Nizwa-616, Oman*
[4]*Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan*
[*]*Corresponding author e-mail: shinwari2008@gmail.com & javidhej@yahoo.com*

Abstract

This study was carried out to evaluate the Brine shrimp lethality (cytotoxicity), antiglycation, phytotoxicity, antiplatelet, antifungal, and antibacterial activities of *Ajuga bracteosa*. The *n*-hexane fraction of *Ajuga bracteosa* was investigated for various biological activities *in vitro*. Hexane extract showed promising phytotoxicity (100%) at the highest dose only. Interestingly, *n*-hexane fraction showed 100% antiplatelet activity against AA (48 μg/mL) and PAF (15 μg/mL) but it was inactive against pathogenic bacteria and fungi used in the antibacterial and antifungal assays. The antioxidant potential of *n*-hexane fraction was evaluated by its potential to scaveng superoxide anion radical, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, and the lipid peroxidation assays. The results showed that *n*-hexane fraction exhibit moderate activity against lipid peroxidation, DPPH, and superoxide assays. In conclusion, the *n*-hexane fraction of *A. Bracteosa* possess a strong biological role, which suggest the presence of bioactive metabolites.

Key words: *Ajuga bracteosa*, Biological activities, Medicinal plant, Labiateae.

Introduction

Ajuga bracteosa Wall ex Benth. (Labiatae) is distributed in Kashmir, Nepal, and sub-Himalayan tract, with diffused branching system of a perennial herb (Kaithwas *et al.*, 2102). The herb is recommended in ayurvedic medicine system for the treatment of gout, rheumatism, amenorrhea, and palsy. The astringent, diuretic, tonic, stimulant, and febrifugal properties of the herb are also reported in literature (Kaithwas *et al.*, 2102). The previous pharmacological investigations on *A. bracteosa* have reported the antifeedant activity against *Spodoptera littoralis* larvae (Castro *et al.*, 2011), antiplasmodial activities (Chandel & Bagai, 2010), and chemopreventive activities against various cancers (Ghufran *et al.*, 2009). *A. bracteosa* was also reported to inhibit the acetylcholinesterase, butylrycholinesterase and lipoxygenase activity (Riaz *et al.*, 2007). The medicinal importance of *A. bracteosa* inspired us to investigate lipid peroxidation, antiglycation, cytotoxic, phytotoxic, antioxidant, antiplatelet, and antimicrobial activities of this important medicinal plant.

Materials and Methods

Plants collection: The whole plant of *A. bracteosa* was collected at Kurram Agency (Parachinar), Khyber Pakhtunkhwa, Pakistan, in May 2005, and the plant taxonomist at the Department of Botany, Kohat University of Science and technology, Pakistan, identified the plant. A specimen of the plant was deposited in Herbarium of the Department.

The whole plants (7 kg) of *A. bracteosa* were air-dried and then extracted exhaustively at room temperature, with MeOH. The crude extract obtained, was evaporated to get 80 g of the residue. This crude extract was then exhaustively and sequentially extracted with *n*-hexane, chloroform, ethyl acetate, and MeOH fractions. These extracts were then concentrated in *vacuo* to get various polarity organic fractions: *n*-hexane, chloroform, ethyl acetate, and methanol respectively.

Phytotoxicity bioassay, brine shrimp lethality bioassay, antiglycation, and inhibition of platelet aggregation was determined according to the procedure of Hussain *et al.* (2003), whereas the antioxidant assays including lipid peroxidation, DPPH scavenging, and superoxide scavenging activities were performed according to Dubey & Batra, (2009).

Results and Discussions

Biological activities of extracts of *A. bracteosa*: The most of the rural population of Pakistan mainly depends on indigenous medicine system for the health problems, as the traditional ways of remedies are still reliable and very common to the people living in far off villages in many developing countries (Khattak *et al.*, 1985; Jan *et al*., 2015; Hussain *et al*., 2014). Therefore, the present study was started to investigate the aerial parts of an indigenous medicinal plant, *A. bracteosa*, being used by the traditional practitioners commonly.

Phytotoxic bioassay: The *n*-hexane fraction showed potent phytotoxicity at the highest concentration (1000 μg /mL) and complete inhibition (100%) of growth of *L. minor* (Table 1) was observed. There is a dire need to discover more and more herbicides. It is due to the continuous increase in the number of herbicide-resistant

weeds and the decrease in effectiveness of the conventional synthetic herbicides. In addition to this the environmental status and the health related concerns are also appealing for the new herbicides (Bhowmik & Inderjit, 2003). The new herbicides isolated from the natural sources are, therefore, gaining much attention of the scientific community. These natural herbicides are more appropriate and less hazardous as compared to the currently available synthetic agrochemicals. In addition, the natural products are generally considered as the more effective, more biodegradable and thus less threatening to the environment and the society. The present study indicated that *A. bracteosa n*-hexane is a useful natural herbicide, and thus considered as a natural source of bioactive agrochemicals (Table 1).

Brine shrimp lethality: Brine shrimp lethality is the simple bioassay useful for screening large number of extracts in the drug discovery process from the medicinal plants. *n*-Hexane extract was found to be non-toxic, when observed at the test concentrations of 10, 100 and 1000 μg/mL in the brine shrimp lethality assay. The LD_{50} was found to be lower than 1000 μg/mL value (Table 2).

Thus, the present study was helpful in the toxicity evaluation of the extract, and it was declared as safe, based on the toxicity profile. According to Hussain *et al.* (2009), *n*-hexane fraction of *Nepeta juncae* did not show any significant cytotoxic activity. The extracts with LD_{50} values higher than 200 mg/mL in the brine shrimp test are considered inactive (Anderson *et al.*, 1991).

Antiglycation activity: The *n*-hexane fraction was also tested for its potential inhibitory effects against AGEs (advanced glycation endproducts) formation *in vitro*. This fraction showed 53.0 % inhibition at concentration of 0.5 mg/100 μL (Table 3).

Table 1. Phytotoxicity of *n*-hexane fraction.

Name of plant	Conc. μg/ml	No. of fronds		% Growth regulation	Conc. of std. drug (μg/ml)
		Sample	Control		
Lemna minor	1000	0	19.6	100	0.015
	100	3		13.5	
	10	5		1.72	

Table 2. Brine shrimp lethality of *n*-hexane fraction.

Dose (μg/ml)	No of shrimps	No of survivors	LD_{50} (μg/ml)	Std. drug	LD_{50} (μg/ml)
1000	30	12	370.6	Etoposide	7.6425
100	30	19			
10	30	25			

In this study *n*-hexane fraction was found to be moderate inhibitor against protein glycation, which perhaps reflected the presence of antiglycation agents in this fraction and further studies can be conducted on this fraction to identify and isolate biologically active agents.

Antimicrobial activity (antibacterial/antifungal): Antbacterial activity of the *n*-hexane extract of *A. bracteosa* was tested against six bacteria (*Bacillus subtilis, Eschericha coli, Shigella flexneri, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi*).

The *n*-hexane fraction was found inactive against the tested bacteria (Table 4). The antifungal activity of the *n*-hexane extract of *A. bracteosa* was also tested against six fungi (*Candida albicans, Fusarium solani, Trichphyton longifusus, Aspergillus flavus, Candida glabrata,* and *Microsporum canis*). However, the *n*-hexane extract was found to be inactive against all the fungi except *Microsporum canis* which showed low activity with 30 % inhibtion (Table 5).

Table 3. Antiglycation activity of n-hexane fraction.

Sample	Conc. (mM)	% Inhibition	IC_{50} + SEM [mM]
Hex Fr	0.5 mg/100µl	53.0	-
Rutin (st. drug)	-	82%	-

Table 4. Antibacterial activity of *n*-hexane fraction.

Name of bacteria	% inhibition	Zone of inhibition of std. drug (mm)
Eschericha coli	0	35
Bacillus subtilis	0	36
Shigella flexenari	0	36
Staphylococcus aureu	0	43
Pseudomonas aeruginosa	0	32
Salmonella typhi	0	40

Table 5. Antifungal activity of *n*-hexane fraction.

Name of fungus	Linear growth (mm)		% inhibition	Miconazole MIC (μg/ml)
	Sample	Control		
Trichphyton longifusus	-	100	-	-
Candida albicans	100	100	0	110.8
Aspergillus flavus	100	100	0	20
Microsporum canis	70	100	30	98.4
Fusarium solani	100	100	0	73.25
Candida glabrata	100	100	0	110.8

Antiplatelet activity: The *n*-hexane extract of *A. bracteosa* was also investigated for anti-human platelet aggregation activity. The agonists, epinephrine, AA, and PAF, were used for the induction of platelet aggregation. Anti-platelet activity was observed when aggregation was induced by epinephrine. However, the effect of *n*-hexane fraction on the platelet aggregation induced by AA and PAF was dose dependant. 15 μg/mL and 48 μg/mL of the *n*-hexane extract were required to inhibit PAF and AA induced human platelet aggregation respectively by 100%.

Antioxidant activity

Assay of lipid peroxidation: Lipid peroxidation was induced by $FeSO_4$, and the results of this assay indicated that the *n*-hexane extract of *A. bracteosa* inhibition was dose dependent. The maximum inhibition by the *n*-hexane extract was only 20.7 % at 300 μg/ml dose. Thus it was considered as mild inhibitor when compared with standard drug quercetin (85%).

DPPH scavenging activity: DPPH free radical is stable enough and it has the ability to accept an electron or a hydrogen radical, and then become a diamagnetic molecule. The methanolic solution of DPPH free radical absorbs strongly at 517nm. However, after reacting with a suitable reducing agent, it becomes diamagnetic by pairing up of electrons, and the solution then loses color stoicheometrically. Thus the free radical scavenging ability of any compound or the plant extract can be tested by using this principle. The decrease in absorbance of solution at 517nm indicates the reduction of the DPPH radicals, which is then correlated with the ability of the radical scavengers. The DPPH free radical scavenging activity of the *n*-hexane extract of *A. bracteosa* was found to be 17.8% at 300 μg/ml. The propyl gallate with 90.3% scavenging ability was used as the standard drug during this study.

Superoxide scavenging activity: The superoxide free radical (O_2) is generated by various photochemical and biological reactions, and is considered as a highly toxic species. The breakdown of superoxide radical is catalyzed by the enzyme, superoxide dismutase, which is possessed both by the aerobic and anaerobic organisms. To measure the superoxide dismutase activity of EFTO, the reduced phenazine methosulfate assay was used, and the results of this assay indicated that the radical scavenging activity of the *n*-hexane extract was only 11% at 300 μg/ml dose, whereas, the standard used in the present study (propyl gallate) has scavenging activity 92.5%. Thus our tested sample was considered as poor scavenger for superoxide free radical.

References

Anderson, J.E., C.M. Goetz, J.L. McLaughlin and M.A. Suffness. 1991. A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. *Phytochem. Anal*., 2: 107.

Bhowmik, P.C. and Inderjit. 2003. Challenges and opportunities in implementing allelopathy for natural weed management. *Crop. Prot*., 22: 661-671.

Castro, A., J. Coll and M. Arfan. 2011. neo-Clerodane diterpenoid from *Ajuga bracteosa*. *J. Nat. Prod*., 74: 1036-1041.

Chandel, S. and U. Bagai. 2010. Antiplasmodial activity of *Ajuga bracteosa* against *Plasmodium berghei* infected BALB/c mice. *Ind. J. Med. Res*., 131: 440-444.

Dubey, S.K. and A. Batra. 2009. Antioxidant activities of *Thuja occidentalis* linn. *Asian J. Pharm. Clin. Res*., 2: 73-76.

Ghufran, M.A., R.A. Qureshi, A. Batool, T.P. Kondratyuk, J.M. Guilford and L.E. Marler. 2009. Evaluation of selected indigenous medicinal plants from the western Himalayas for cytotoxicity and as potential cancer chemopreventive agents. *Pharm. Biol*., 47: 533-538.

Hussain, J., A. Al-Harrasi, A.L. Khan, N.U. Rehman, L. Ali, J.S. Kim, A. Zakarova and Z.K. Shinwari. 2014. Phytochemical and Biological Assessment of Medicinally Important Plant *Ochradenus arabicus*. *Pak. J. Bot*., 46(6): 2027-2034.

Hussain, J., F.U. Khan, S.A. Gillani, G. Abbas, S. Ahmed, A.U. Khan, W. Ullah, and M.I. Choudhary. 2003. Antiglycation, antiplatelets aggregation, cytotoxic and phytotoxic activities of *Nepeta suavis*. *Lat. Am. J. Pharm*., 29: 573-578.

Hussain, J., N. Jamila, S.A. Gilani, G. Abbas and S. Ahmed. 2009. Platelet aggregation, antiglycation, cytotoxic, phytotoxic and antimicrobial activities of extracts of *Nepeta juncea*. *Afr. J. Biotech*., 8: 935-940.

Jan, SA; Z.K. Shinwari, A. Zeb, A.T. Khalil and S.H. Shah. 2015. Ethnobotany and research trends in *Trachyspermum ammi* L. (Ajowan); A popular folklore remedy. *American-Eurasian J. Agric. & Environ. Sci*., 15(1): 68-73.

Kaithwas, G., R. Gautam, S.M. Jachak and A. Saklani. 2012. Antiarthritic effects of *Ajuga bracteosa* Wall ex Benth. in acute and chronic models of arthritis in albino rats. *Asian Pac. J. Trop. Biomed*., 2(3): 185-188.

Khattak, S.G., S.N. Gilani and M. Ikram. 1985. Antipyretic studies on some indigenous Pakistani medicinal plants. *J. Ethnopharmacol*., 14: 45-51.

Riaz, N., S.A. Nawaz, N. Mukhtar, A. Malik, N. Afza, S. Ali, S. Ullah, P. Muhammad and M.I. Choudhary. 2007. Isolation and enzyme-inhibition studies of the chemical constituents from *Ajuga bracteosa*. *Chem. Biodivers*., 4: 72-83.

(Received for publication 17 April 2014)