Biological potential of *Phlomis bracteosa*

Riaz Ullah [1*], Naser M. AbdEIslam[2], Sultan Ayaz[3], Iqbal Hussain[4], Shabir Ahmad[4]

[1]Department of Chemistry, Sarhad University of Science and Information Technology Peshawar, KPK, Pakistan
[2]Arriyadh Community College, King Saud University, Riyadh, Saudi Arabia
[3]Department of Zoology, Kohat University of Science and Technology Kohat, KPK, 26000, Pakistan
[4]Department of Chemistry, Kohat University of Science and Technology Kohat, KPK, 26000, Pakistan
Corresponding Author Dr Riaz Ullah afridiriaz@yahoo.com

Abstract: The aim of this study was to investigate the biologically active fractions of *Phlomis bracteosa* against Insecticidal Bioassay, cytotoxicity (brine shrimp bioassay) and Phytotoxicity. Methanol, *n*-hexane, chloroform, ethyl acetate and water fractions derived from the aerial parts of *Phlomis bracteosa* were screened for various in vitro biological activities. These fractions did not display any significant results.
[Riaz Ullah, Naser M. AbdEIslam, Sultan Ayaz, Iqbal Hussain, Shabir Ahmad. **Biological potential of *Phlomis bracteosa.*** *Life Sci J* 2013;10(1):2954-2957**]** (ISSN: 1097-8135). http://www.lifesciencesite.com. 360

Key words: *Phlomis bracteosa*, biological activities, crude fractions

1. Introduction

The genus *Phlomis* (Lamiaceae) consists of about 100 species (Albaladejo et al., 2004; Kyriakopoulou et al.,2001). A number of which are employed as stimulant and tonics in Anatolian folk medicine (Calis and Kırmızıbekmez, 2004). *Phlomis* species are explained by Dioscorides as herbal medicines, and are in practice ethno-pharmacologically in herbal drugs for respiratory tract ailments and for local healing of injuries. Some *Phlomis* species are used in folk medicine for their analgesic and antidiarrheal properties, and for the treatment of ulcers and hemorrhoids. There are few reports about the pharmacological and biological effects of *Phlomis.* Some studies have shown various activities such as anti-inflammatory, immuno-suppressive, antimutagenic, anti-nociceptive, antifibriel, free radical scavenging, anti-malarial, and anti-microbial effects (Sarkhail et al., 2006). Different classes of glycosides comprising diterpenoids, iridoids, phenylpropanoids, phenylethanoids and flavonoids have been identified from the genus *Phlomis*. Many of these phenylpropanoids showed significant biological activities, such as cytotoxic, cytostatic, anti-inflammatory, immuno-suppressant and anti-microbial (Kamel et al., 2000).

2. Materials and Methods

2.1. Plant materials

The whole parts of the plant *P. bracteosa were* collected from the Kurram Agency NWFP, Pakistan in June 2005 and were identified by Mr. Naveed Botanist: at the Department of Botany, University of Peshawar NWFP Pakistan. Herbarium specimens were deposited in Department of Botany.

2.2. Extraction

The whole parts of *P. bracteosa* were dried in dark, chopped and ground to coarse powder. The powdered plant (3 Kg) was initially extracted with methanol (7 days × 3) at room temperature. The combined methanol extract was evaporated under reduced pressure leaving behind a greenish, syrup residue (155 g). The methanol extract was partitioned in various fractions through separating funnel. It was partitioned in hexane (45 g), chloroform (60 g), ethylacetate (28 g) and water (22 g) successively

2.3. Brine Shrimp Lethality Bioassay Methodology

Via the protocol of (Meyer et al., 1982), brine shrimp (*Artemia salina* larvae) eggs were hatched in a shallow rectangular plastic dish, filled with artificial seawater, which was prepared by mixing a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, OH, USA) with double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. An approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the smaller compartment was opened to ordinary light. After two days a pipette collected naupil from the lighter side. A sample of the test fraction was prepared by dissolving 20 mg of each fraction in 2 ml of methanol. From this stock solution, 1000,100 and 10 µg/mL was transferred to 12 vials; three for each dilution, and three vials were kept as control having 2 ml of methanol only. The solvent was allowed to evaporate overnight. After two, when shrimp larvae were ready, I ml of sea water was added to each vial along wit 10 shrimps and the volume was adjusted with sea water to 5 ml per vial. After 24 hours, the number of surviving shrimps counted. Data was analysed by a Finney computer program to determine the LD_{50} (Finney, D.J. 1971).

2.4. Phytotoxicity Bioassay Methodology

This test was performed according to the modified protocol of (McLaughlin, J.L, 1988). According to McLaughlin "The test fraction were incorporated with sterilized E-medium at different concentrations i.e. 10, 100, 1000 µg/mL. in methanol. Sterilized conical flasks were inoculated with fractions of desired concentrations prepared from the stock solution and allowed to evaporate overnight. Each flask was inoculated with 20 ml of sterilized E-medium and added ten *Lemna acquinoclialis* Welv, each containing a rosette of three fronds. Other flasks were supplemented with methanol serving as negative control and reference inhibitor.i.e. parquet serving as positive control. Treatment was replicated three times and the flasks incubated at 30°C in Fisons Fi-Totron 600H growth cabinet for seven days, 9000 lux intensity, 56+10 rh (relative humidity) and 12 hours day length. Growth of *Lemna acquinoctialis* in fraction containing flask was determined by counting the number of fronds per dose and growth inhibitor calculated with reference to negative control (McLaughlin, J.L, 1988).

2.5. Insecticidal Bioassay Methodology

Concentration of trial sample (each fraction) (1571.33 µgcm^2) was set. Permethrin (coopexTM) was used as standard drug with 235.71 µg/cm^2 conc. The stored grain pests are nurtured in the laboratory under controlled temperature and humidity, so that the insects of uniform age and size were available for the experiments. Ten pairs of insects are reared in 9.0 diameter and 11.0 cm high plastic bottles containing 250 g of breeding media. Then bottles are covered with muslin cloth tied by means of rubber bands, or small jars or wide mouthed bottles sealed with filter paper (Whatman No. 29, black) and paraffin wax (to prevent contamination) are suitable. The media should be sterilized at 60 C for one hour. The insects are exposed to test sample (each fraction) by contact method using filter paper. 1 ml of different concentration of every fraction is applied by micropipette to 90 mm diameter filter papers and then placed in the petri dishes. After that adult insects of same size and age in each batch are transferred to Petri dishes. A check batch is treated with solvent for determination of solvent effect. A control batch is kept for determination of environmental effects. Another batch supplemented with reference insecticides e.g. coopex and Deeis (synthetic Pyrethroids) are used. All these are kept without food throughout 24 hours exposure period. Mortality counts are done after 24 hours exposure period.LC50 Values then determined by probate mortality curve that is drawn on log-log graph paper. (Majeed, I 1994, Naqvi S. N. H., Parveen, F 1991, Parveen F 1994)

3. Result and Discussion

3.1. Brine-shrimp lethality bioassay

The fractions obtained were determined for cytotoxicity in the brine-shrimp lethality bioassay by using the protocol of Meyer (Meyer, et al 1982) of these fractions were screened at three concentration levels i.e. 1000,100 10 /ml and LD50= values were calculated by using Finny computer program (Finney, D.J. 1971). Standard drug used was etoposide**.** All the tested fractions did not show any significant cytotoxic activity results are given in (Table 1-5).

Table-1 Cytotoxicity of Methanol Fraction

Dose (µg/mL)	No. of Shrimps	No. of Survivors	LD_{50}(µg/mL)	STD. Drug	LD_{50}(µg/mL)
1000	30	21	-	Etoposide	7.4625
100	30	25			
10	30	28			

No. of Replicates: 03

Table-2 Cytotoxicity of Ethyl acetate Fraction

Dose (µg/mL)	No. of Shrimps	No. of Survivors	LD_{50}(µg/mL)	STD. Drug	LD_{50}(µg/mL)
1000	30	24	-	Etoposide	7.4625
100	30	26			
10	30	27			

No. of Replicates: 03

Table-3 Cytotoxicity of Choroform Farction

Dose µg/mL)	No of Shrimps	No. of Survivors	LD_{50}(µg/mL)	STD.Drug	LD_{50}(µg/mL)
1000	30	21	-	Etoposide	7.4625
100	30	23			
10	30	26			

No. of Replicates: 03

Table-4 Cytotoxicity 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	16	-	Etoposide	7.4625
100	30	20			
10	30	21			

No. of Replicates: 03

Table-5 Cytotoxicity of Water Fraction

Dose (µg/mL)	No. of Shrimps	No of Survivors	LD_{50}(µg/mL)	STD.Drug	LD_{50}(µg/mL)
1000	30	20	-	Etoposide	7.4625
100	30	21			
10	30	23			

No. of Replicates: 03

3.2. Phytotoxicity bioassay

The phytotoxicity of all fractions obtained from the crude methanolic extract was carried out against Lemna acquinootialis Welv. And considered by using the procedure of McLauughlin et al. this assay was performed at three different concentrations i.e. 1000,100 and 10 µg/ml.(Table 6-10). It is concluded from table 6-10 the results are non significant

Table-6 Phytotoxicity of Methanolic fraction

Name of Plant	Conc. of Comp(µg/mL)	No. of Fonds		%Growth Regulation	Conc. of Std. Drug (µg/mL)
		Sample	Control		
Lemna Minor	1000	17	16	0	0.015
	100	18		-11.5	
	10	19		-24	

Table-7 Phytotoxicity of Ethyl Actate Fraction

Name of Plant	Conc. Of Compd (µg/mL)	No. of Fonds		% Growth Regulation	Conc. Of Std. Drug(µg/mL)
		Sample	Control		
Lemna Minor	1000	19	16	-3.5	0.015
	100	17		-17.75	
	10	15		-11.5	

Table-8 Phytotoxicity of Chloroform Fraction

Name of Plant	Conc. Of Compd (µg/mL)	No. of Fonds		% Growth Regulation	Conc. Of Std. Drug(µg/mL)
		Sample	Control		
Lemna Minor	1000	14	16	5.25	0.015
	100	17		0	
	10	19		-16.75	

Table-9 Phytotoxicity of n-Haxane Fraction

Name of Plant	Conc. Of Compd (µg/mL)	No. of Fonds		% Growth Regulation	Conc. Of Std. Drug(µg/mL)
		Sample	Control		
Lemna Minor	1000	13	16	5.21	0.015
	100	12		-2.13	
	10	13		-13.65	

Table-10 Phytotoxicity of Water Fraction

Name of Plant	Conc. Of Compd (µg/mL)	No. of Fonds		% Growth Regulation	Conc. Of Std. Drug(µg/mL)
		Sample	Control		
Lemna Minor	1000	16	16	7.45	0.015
	100	17		-12.13	
	10	18		-15.78	

3.3. Insecticidal activity

From table 11-15 it showed that all tested fractions are showed no activities.

Table 11 Insecticidal activity of Methanol fraction

Name of Insects	% Mortality		Sample
Tribolium castaneum	100	0	0
Sitophilus oryzae	100	0	0
Rhyzopertha dominica	100	0	0
Callosbruchus analis	100	0	0
Trogoderma granarium	-	-	-

Table 12 Insecticidal activity of Ethyl acetate fraction

Name of Insects	% Mortality		Sample
Tribolium castaneum	100	0	0
Sitophilus oryzae	100	0	0
Rhyzopertha dominica	100	0	0
Callosbruchus analis	100	0	0
Trogoderma granarium	-	-	-

Table 13 Insecticidal activity of Chloroform Faction

Name of Insects	% Mortality		Sample
Tribolium castaneum	100	0	0
Sitophilus oryzae	100	0	0
Rhyzopertha dominica	100	0	0
Callosbruchus analis	100	0	0
Trogoderma granarium	-	-	-

Table 14 Insecticidal activity of *n*-Hexane fraction

Name of Insects	% Mortality		Sample
Tribolium castaneum	100	0	0
Sitophilus oryzae	100	0	0
Rhyzopertha dominica	100	0	0
Callosbruchus analis	100	0	0
Trogoderma granarium	-	-	-

Table 15 Insecticidal activity of Water fraction

Name of Insects	% Mortality		Sample
Tribolium castaneum	100	0	0
Sitophilus oryzae	100	0	0
Rhyzopertha dominica	100	0	0
Callosbruchus analis	100	0	0
Trogoderma granarium	-	-	-

Acknowledgements: The authors are thankful to the Deanship of Scientific Research, King Saud University Riyadh for funding the work through the research Group project No RGP-VPP- 210. Authors also wish to thanks HEC Pakistan for promoting the activity of science and technology in Pakistan

Corresponding Author:
Dr. Riaz Ullah; Department of Chemistry
Sarhad University of Science and Information Technology Peshawar KPK Pakistan

References

1. Albaladejo RG, Aparicio A, Silvestre S (2004). Variation patterns in the *Phlomis* × *composita* (Lamiaceae) hybrid complex in the Iberian Peninsula. Bot. J. Linn. Soc. 145: 97-108.
2. Calis I, Kırmızıbekmez H (2004). Glycosides from *Phlomis lunariifolia*. Phytochem. 65: 2619-2625.
3. Finney, D.J. (1971) *Probit Analysis*, 3rd ed.,Cambridge University Press, Cambridge.
4. Kamel MS, Mohamed KM, Hassanean HA, Ohtani K, Kasai R, Yamasaki K (2000). Iridoid and megastigmane glycosides from *Phlomis aurea*. Phytochem., 55: 353-357.
5. Kyriakopoulou I, Magiatis P, Skaltsounis AL, Aligiannis N, Harvala C (2001). Samioside, a new phenylethanoid glycoside with free-radical scavenging and antimicrobial activities from *Phlomis samia*. J. Nat. Prod., 64: 1095-1097.
6. Majeed, I., *Proc. Pakistan Congr. Zool*. **14**, 44, 1994
7. McLaughlin, J.L. (1988) *Bioassay For Discovery of Antitumoral, Antiviral Agents from Natural Sources* In: Proceedings of NIH Workshop Bethesda Oct. 18-19, 1988, p.22
8. Meyer, B.N., N.R. Ferrigni, J.E. Putnam & L.B.Jacobson (1982), Brine shrimp: a constituent general bioassay for active plant constituents, *Planta Med.* 45: 31- 41
9. Naqvi S. N. H., Parveen, F., *Pak. J. Entomol*. **6**, 35, 1991.
10. Parveen F., *Proc. Pakistan Congr. Zool*. **14**, 43, 1994
11. Sarkhail P, Monsef EHR, Amin G, Surmaghi MHS, Shafiee A (2006). Phytochemical study of *Phlomis olivieri* Benth. And *Phlomis persica* Boiss. DARU. 14:115-121.

3/2/2013