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Antiglycation and Antimicrobial Activities of the Crude Extract *of Phlomis bracteosa*

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Abstract: In this study, the antiglycation, antibacterial and antifungal activities of *Phlomis bracteosa* (Labiatae) were investigated. Ethyl acetate (EtOAc) fraction showed 57.90% antiglycation activity against the protein glycation and chloroform ($CHCl_3$) fraction showed 54.25% inhibitory potential, while an aqueous (H_2O) and *n*-hexane fractions showed less than 50% inhibitory potential. The antibacterial effect of this plant was tested by agar well diffusion method and tested fractions (H_2O, EtOAc, $CHCl_3$ and *n*-hexane) showed activity bacteria *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureu*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Surprising tested fractions didn't show any antifungal activity.

Key words: Labiatae · *Phlomis bracteosa* · Antiglycation and antimicrobial activities

INTRODUCTION

The genus *Phlomis* belongs to family labiatae comprising of approximately 100 species in the world. Some *Phlomis* species are used in Anatolian folk medicine as tonics, stimulants, analgesic, anti-diarrhea tic, ulcers, hemorrhoids immunosuppressive anti-inflammatory, anti mutagenic, anti-nociceptive, antifibriel, free radical scavenging, anti-microbial and anti-malarial effects [1,2]. Different classes of glycosides consist of diterpenoids, phenylpropanoids, iridoids, phenylethanoids and flavonoids had been reported from genus *Phlomis*. [3]. Several *Phlomis* species are used in herbal medicine, for diseases of the respiratory tract or externally for treatment of wounds. Analysis of the methanol fraction of medicinal plant *P. armeniaca* showed cytostatic and cytotoxic activities. Also the essential oil and ethanol fraction of *P. fruticosa* showed antimicrobial activities [4]. The aim of our present study was to screen out the active fractions of *P. bracteosa* and to see possibility of utilization of the samples in future, for isolation of targeted compounds. Antiglycation, antibacterial and antifungal were performed for various fractions of methanolic extract of the plant *P. bracteosa*.

MATERIALS AND METHODS

Plant Material: 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 the herbaria of Department of Botany, University of Peshawar, NWFP Pakistan.

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 x 3) at room temp. 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 into Hexane (45 g) chloroform (60 g), ethylacetate (28 g) and water fractions (22 g) successively.

Methodology

Antiglycation: BSA (Bovine Serum Albumin) was used as 10mg/mL, dissolved in 67 mM phosphate buffer (pH: 7.4). Glucose as 50 mg/mL, dissolved in 67 mM phosphate buffer (pH: 7.4). 3mM Sodium azide was added in required quantity of phosphate buffer to inhibit bacterial growth. 1mg/1000µL concentration of each fraction was used to calculate antiglycation activity along with standard inhibitor. The dissolved sample (60 µL in each well of 96-well plate) was incubated for a week at 37°C. After a week, the samples were taken out and cooled at room temperature. Then 6 µL of 100% TCA (Trichloroacetic

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acid) was added to each of the well, supernatants containing unbounded glucose, inhibitor and interfering substances were removed after centrifugation at 14,000 rpm for 4 minutes. Pellets were obtained at the bottom of the wells, supernatant were removed from each well and 60 µL of PBS (Phosphate Buffer Saline) pH: 10, was added to dissolve the pellets. The comparison of fluorescence intensity at 370 nm excitations and emission at 440 nm was obtained by using Spectrofluorimeter [5,6,7]. Rutin was used as the standard inhibitor [8]. Percentage Inhibition was calculated by the following equation. % inhibition = 100-(OD (sample) /OD (blank) x 100.

Antibacterial Activity: The antibacterial activity was determined by agar well diffusion method. A loopful of a 10^4-10^6 suspension of 24 h old broth of each bacterium was streaked on the surface of Mueller-Hinton agar (BBl-USA) plates. Wells were dug in the agar with the help of sterile dimethyl sulfoxide (DMSO). Dilutions of the stock solution containing 50,100, 150 and 200 µg were prepared in DMSO and 100 µl of each dilution was added in the respective wells. The plates were then incubated at 37°C for 24 h and zone of inhibitions were measured in millimeters (mm) and compared with the control [9]. Antibacterial activity was studied against *Eschericha coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi.* Ampicillin, Tobramycin and Amoxacilline were used as standard drugs.

Antifungal Activity: For antifungal study each extract was resuspended in DMSO at a concentration of 100mg/mL and stored in a refrigerator till further used. Antifungal activities of the extracts were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of [10]. Sabouraud dextrose agar (Difco) was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose agar and distilled water and autoclaved at 121°C for 15 minutes. Twenty ml of molten (45 °C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish. All these dishes were inoculated with 4mm diameter piece of inoculums removed from a seven days old culture of fungus [11]. For counting of colonies another 4mm culture (fungi) were suspended in normal saline to make volume up to 1ml and then counted with help of heamocytometer (neubar chamber). Once the agar was hardened, 11mm wells were bored using a sterile cork borer. Then 0.1mL (100µL) from each stock solution of the extracts having final concentration of 100mg/mL was placed in each the well and the plates were incubated at 27-29°C for 7-10 days. Two wells in each Petri dish were supplemented with DMSO and reference antifungal drug Amphotericin B (0.2mg/mL) dissolved in DMSO (sigma) serve as negative and positive control respectively. The tests were carried out in triplicate. The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition. The humidity in incubation room should be maintained from 40% to 50 % [12]

RESULTS AND DISCUSSION

During the studies we found that all the screened samples were positive showing inhibition against the formation of Advanced Glycation Endproducts (AGEs). However antiglycation activity of *n*-hexane and aqueous fraction are less than that of ethyl acetate and chloroform fraction. Under the same conditions studied, we found that the ethyl acetate fraction showed 57.90 % inhibition at concentration of 1mg/100µL while chloroform fraction has also shown reasonably good inhibition i.e 54.25 % at concentration of 1mg/100µL while *n*-hexane and aqueous fractions showed less than 50 % inhibition at same concentration as shown in Table 1. From the results stated above we can conclude that ethyl acetate and chloroform fraction may be the active and potential fractions for further study of biologically active constituents. Similar kind of results were also reported by Choi *et al.* [13], who observed butanolic fraction of *Plantago asiatica* with higher antiglycation activity (75%) which later on lead to the identification of a new compound, plantamajoside.

The antibacterial effect of *P. bracteosa* was also tested by agar well diffusion method and different fractions (H_2O, EtOAc, $CHCl_3$ and *n*-hexane) were tested against bacteria *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureu*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Table 2). EtOAc and H_2O fractions showed promising activity against bacteria *S. typhi* and *B. subtilis*. Similarly $CHCl_3$ and *n*-hexane fractions showed good activity against bacterium *P. aeruginosa* and *n*-hexane also showed good activity against *B. subtilis*. Only H_2O fractions showed good activity against *E. coli*.

In vitro antifungal study was also performed by subjecting different micro organisms to various fractions of *P. bracteosa* (H_2O, EtOAc, $CHCl_3$ and *n*-hexane). All these fractions were inactive against the various fungi used namely *Trichphyton longifusus*, *Aspergillus flavus*, *Candida albicans*, *Fusarium solani*, *Candida glabrata* and *Microsporum canis*.

Table 1: Antiglycation bioassay of tested fractions of *P. bracteosa*

Tested fractions of *P. bracteosa*	Concentration	% Inhibition
EtOAc	1mg/100µl	57.90
$CHCl_3$	1mg/100µl	54.25
H_2O	1mg/100µl	29.80
n-Hexane	1mg/100µl	2.25

Table 2: Antibacterial activity of tested fractions of *P. bracteosa*

Name of Bacteria	EtOAc[a]	$CHCl_3$[a]	n-Hexane[a]	H_2O[a]	Std. Drug
E. coli	-	3	-	7	30
B. subtilis	9	-	9	6	37
S. flexenari	-	-	5	-	36
S. aureu	5	-	-	-	26
P. aeruginosa	-	9	11	-	32
S. typhi	12	-	4	10	30

[a] Zone of inhibition in mm

CONCLUSION

From the results of the present study it was concluded that EtOAc and $CHCl_3$ fractions of the whole parts of *P. bracteosa* have significant antiglycation activity. In order to further exploit the antiglycation activities of this indigenous medicinal plant and to come up with a potent, safe and economically affordable formulation, further investigations are to be required.

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