

Short communication

Phytochemical Modulation of Immune Response by *Phlomis bracteosa*

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The present study reports the immunomodulatory activities of different crude fractions of *Phlomis bracteosa*. In case of immunomodulatory study, the ethylacetate fraction demonstrated significant modulatory effect for oxidative burst of polymorphonuclear neutrophils and CHCl_3 crude fraction for the whole blood, while the aqueous fraction demonstrated reasonable inhibition. Hence, both the chloroform and ethylacetate fractions of *P. bracteosa* are suggested further for the isolation and identification of active constituents responsible for the observed effects.

Key words: *Phlomis bracteosa*, lamiaceae, immunomodulatory potential.

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, anti-mutagenic, anti-nociceptive, antifibrotic, 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).

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 Department of Botany, University of Peshawar, NWFP Pakistan.

Extraction

The whole parts of *Phlomis 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.

METHODOLOGY

Immunomodulatory activity

Luminol-enhanced chemiluminescence assay was performed as described by Helfand et al. (1982). Briefly, whole blood (diluted 1:200) neutrophils (1×10^7) and PMNs (1×10^6), were suspended in Hank's balance salt solution with calcium and magnesium (HBSS) and incubated with 50 μ l of test compounds concentrations

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Table 1. Immunomodulatory activity of *Phlomis bracteosa* various fractions and the control drug, Ibuprofen.

Extracts fractioned	With whole blood ($\mu\text{g/ml}$)	with PMNs ($\mu\text{g/ml}$)
Ethyl acetate	113.0 \pm 2.6	53.5 \pm 4.8
Chloroform	31.8 \pm 5.9	15.8 \pm 0.3
Aqueous	195 \pm 1.5	197 \pm 2.3
Ibuprofen	11.2 \pm 1.9	2.88

PMNs = Polymorphoneutrophils

(1.6-50 $\mu\text{g/ml}$) for 30 min. To each well, 50 μl (20 mg/ml) zymosan (Sigma Chemical Co. USA), followed by the addition of 50 μL (7×10^6 M) luminol (G-9382 Sigma Chemical Co.) and then HBSS were added to adjust the final volume to 0.2 ml. HBSS was used as a control. Chemiluminescence's peaks were recorded with a Luminometer (Luminoskan RS Lab Finland).

Statistical analysis

The data expressed are median inhibitory concentrations (IC) with \pm standard error of mean.

RESULTS AND DISCUSSION

The oxidative burst of polymorphoneutrophils (PMNs) and their ability to inhibit reactive oxygen species (ROS) were analyzed for the various fractions of *P. bracteosa* including ethyl acetate, aqueous and chloroform fractions. Phagocytic cells on activation induce release of reactive oxygen free radicals (oxidative burst), which is then quantified by a luminol enhanced chemiluminescence assay. A measurement of chemiluminescence is an efficient and highly sensitive to investigate the different kinds of reactive oxygen species (HO, O₂ and H₂O). Luminol dependent chemiluminescence is a convenient method for detection of super oxide radicals anion in a biological system. Various concentrations of the crude extract of *P. bracteosa* were incubated with PMNs for 30 min. After the addition of serum treated zymosan and luminol phagocytic cells were scanned at 37°C for their chemiluminescence's activity. Ibuprofen was used as positive control. Ethyl acetate, aqueous and chloroform fractions from *P. bracteosa* were screened over a wide range of concentration (6.25-200 $\mu\text{g/ml}$) for their possible modulatory effect on the oxidative burst in whole blood and PMNs, using a luminol based chemiluminescence assay (Hadjimitova, 2002). The result of different assays employed in this study showed that ethylacetate fraction has a potential suppressive effect and clear inhibitory activity for oxidative burst of PMNs at a concentration of 15.8 $\mu\text{g/ml}$ as compared to chloroform and aqueous fraction, while CHCl₃ has a significant potential suppressive effect in whole blood at a concentration of 31.8 $\mu\text{g/ml}$ in this assay as shown in the Table 1. This exhibited a clear suppressive effect on

phagocytosis response upon activation with serum opsonized zymosan in a dose dependent manner. Proposed implications of the immunomodulatory activity are inhibitors for ROS inflammation control or other immunomodulatory uses.

Conclusion

In conclusion the results of the present study indicate that fractionated samples of *P. bracteosa* possess significant immunomodulatory activities. In order to further exploit the immunomodulatory activities of this indigenous medicinal plant and to come up with a potent, safe and economically affordable formulation, further investigations are required.

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