Antioxidant activity of different crude fractions of *Phlomis bracteosa*

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Abstract: In the present study, the antioxidant potential of different crude extracts of *Phlomis bracteosa*, was evaluated. The extracts were investigated for its antioxidant activity using 2, 2-diphenyl-1-picryal-hydrazyl (DPPH) method. The crude fractions evaluated were *n*-hexane, chloroform, ethyl actate and water. The ethyl acetate extracts from *P. bracteosa* at 500µg/mL exhibited highest 69.01% DPPH activity fallowed by chloroform showing 57.09%. The other extracts of plants also showed significant antioxidant activity.
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Introductions

The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms. This is reported that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others. A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems.. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective. They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions. The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Prakash V etal 2009). On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied extracts of *Phlomis bracteosa*. The free radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was evaluated during the course of work.

2. Materials and methods

2.1 Plant materials

Plant material was collected from Swat, KPK Pakistan in flowering season in March-May 2012. It is identified by plant taxonomist Professor Mehboor ur Rehman Matta College Swat.

2.2 Extraction and fractionations

The whole plants was dried under shade for 10 days and milled into powder with electrical grinder and finally dipped in methanol for one month. It was shacked throughout and finally methanol was evaporated through rotary evaporator. The resulting methanol extract (80 g) was fractionating by separating funnel into *n*-hexane, chloroform, ethyl acetate and water fractions. These fractions were evaluated for antioxidant activity.

2.3 Antioxidant bioassay

DPPH diphenylpicrylhydrazyl method was adopted for antioxidant activities. The molecule of 1, 1-diphenyl-2-picrylhydrazyl is character- ized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 515 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present). Test samples were allowed to react with stable free radical, 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) (from Sigma Aldrich) for half an hour at 37º C. The concentration of DPPH was kept as 300 µM. The test samples were dissolved in DMSO while the DPPH solution was prepared in ethanol. After incubation, decrease in absorption was measured at 515 nm using multiplate reader (Spectra MAX-340). Percent radical scavenging activity by samples was determined in comparison with a DMSO treated

control group [12]. % Radical scavenging activity was calculated by using the following formula: % RSA= 100 – {(OD test compound / OD control) X 100 (Riaz Ullah etal 2013).

3. Results and discussion

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Koleva et al., 2002; Suresh et al., 2008) The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful (Kanatt et al., 2007). Keeping in mind the importance of antioxidant activity, *Phlomis bracteosa* were screened for antioxidant activity.

Results obtained are given in table 1. From Table-1 it is clear that ethyl acetate showed the highest activity 69.01 % fallowed by chloroform showing 57.09%. It is significant in comparing with standard showing result of 90.11 %. Also moderate result observed for *n*-hexane and water fractions. These fractions can be use for the isolation of antioxidantagents.

Table. 1. Antioxidant Activities of Crude Fractions of *Phlomis bracteosa* against DPPH Radical.

S. No	Name of extracts	Concentration	Results	Standard *n*-Propyl gallate
1	***n*-Hexane**	**500µg/mL**	31.14%± 0.01	90.11%± 0.01
2	**Chloroform**	**500µg/mL**	57.09%.±0.01	
3	**Ethyl acetate**	**500µg/mL**	69.01%±0.01	
3	**Water**	**500µg/mL**	37.32%±0.01	

Conclusion

This is preliminary study showed that ethyl acetate is the most active crude fraction as an antioxidants agent. It may be using in future for isolation the antioxidant constituents.

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