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Enzyme inhibition and radical scavenging activities of aerial parts of *Paeonia emodi* Wall. (Paeoniaceae)

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Abstract

The ethanolic extract derived from aerial parts of an indigenous medicinal plant *Paeonia emodi* was screened for enzyme inhibition activities against Urease (jack bean and *Bacillus pasteurii*) and α-Chymotrypsin. The extract was also investigated for its radical scavenging activity using DPPH assay. The crude extract was found to possess significant enzyme inhibition activities against jack bean (74%) and *Bacillus pasteurii* (80%) urease and a moderate activity (54%) against α-Chymotrypsin. The extract also displayed excellent (83%) radical scavenging activity. On the basis of these results, the crude extract was subsequently fractionated into n-hexane, chloroform, ethyl acetate, n-butanol and water fractions and tested independently for the aforesaid activities. Significant inhibitory activity against urease enzyme was observed for the ethyl acetate, n-butanol and water fractions while the n-hexane and chloroform fractions were devoid of any such activity. In the α-Chymotrypsin enzyme inhibition studies the activity was concentrated into the ethyl acetate fraction. All the fractions displayed potent radical scavenging activity. The crude extract and fractions thereof were also subjected to total phenolic content determination. A correlation between radical scavenging capacities of extracts and total phenolic content was observed in the majority of cases.

Keywords: *Paeonia emodi, enzyme inhibition, urease, α-chymotrypsin, free radical scavenging, total phenolic content*

Introduction

The plant *Paeonia* is also known as 'the queen of herbs' a title deserved on account of the beauty of its flowers and medicinal repute. *Paeonia* plants especially their roots have been known for their medicinal properties since antiquity [1]. *Paeonia emodi* Wall. (Paeoniaceae), commonly known as Mamekh (Urdu), Peony rose Himalayan (English) [2], is widely distributed in N Pakistan, NW India, W Nepal and is known from a single locality in China [3]. It is an erect perennial herb, 50 cm long, glabrous, leaves biternate or ternate, lamina pale, with flowers solitary, axillary [4]. The carpels are densely pubescent; with 3 flowers on a stem [3]. *P. emodi* finds several applications in the indigenous system of medicine. For example, the rhizomes of *P. emodi* are dug out, washed, dried, ground into fine powder and wheat flour, sugar and desi ghee is added to form Halwa which is used as a tonic to cure backache [5]. The roots and rhizomes are used to cure backache, dropsy and epilepsy. It is also a tonic; emetic, cathartic, blood purifier and colic while the seeds are purgative [2]. The roots are used for the treatment of headache, dizziness, vomiting and to aid pregnancy [6].

A novel β-glucuronidase inhibiting triterpene, 1β,3β,5α,23,24-pentahydroxy-30-12,20(29)-dien-28-oic acid along with oleanolic acid, betulinic

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acid, ethyl gallate, methyl grevillate and 1,5-dihydroxy-3-methylanthraquinone have been isolated from *P. emodi* [7]. Two new monoterpene glycosides, wurdin and benzoylwurdin along with known compounds, paeoniflorin, lactiflorin and oxypaeoniflorin have also been isolated from this species [8]. Emodinol, a new oleane type triterpene, isolated from *Paeonia emodi* showed significant β-glucuronidase inhibitory activity. In addition benzoic acid and 3-hydroxybenzoic acids have also been reported for the first time from this species [9]. Paeonins A and B, new monoterpene galactosides, isolated from the roots of *P. emodi* showed potent lipoxygenase inhibitory activity [10].

In the current study, we present the results of investigations into the enzyme inhibition and radical scavenging activities of the aerial parts, in contrast to roots, of *Paeonia emodi*. This study was designed with a view to explore some new pharmacological properties of this genus in general and of the aerial parts of *Paeonia emodi* Wall. in particular. Therefore, the ethanol extract and fractions thereof derived from the aerial parts of *Paeonia emodi* were screened for enzyme inhibition against urease (jack bean and *Bacillus pasteurii* urease) and α-Chymotrypsin and radical scavenging activities. Studies into the determination of the total phenol contents were also undertaken to investigate the possible correlation between the antioxidant activity and phenolic content.

Materials and methods

Plant material

The plant *Paeonia emodi* (aerial parts) was collected from Swat, Pakistan and identified by Mehboob-ur-Rehman, plant taxonomist, Department of Botany, Government Degree College Matta, Swat, Pakistan. A voucher specimen was deposited at the Herbarium Post Graduate Jehanzeb College Swat, Pakistan.

Preparation of extract and fractionation

Air-dried and ground plant material (2.1 kg) was extracted at room temperature with ethanol (6.0 L, three weeks × 3 times). The residue was filtered and the extract evaporated to dryness under reduced pressure at 45°C to yield the gummy crude extract (355 g). For fractionation, a part of the crude extract (250 g) was dispersed in purified water (500 ml) and shaken with 500 ml each of n-hexane, chloroform, ethyl acetate, and n-butanol consecutively. All the fractions were evaporated under reduced pressure to obtain the n-hexane fraction (30.4 g), chloroform fraction (16.4 g), ethyl acetate fraction (83.3 g), n-butanol fraction (36.58 g) and water fraction (73.23 g).

Urease inhibition

Urease (jack bean or *Bacillus pasteurii* urease) solution (25 μl) was mixed with the extracts (each was 5 μl, 0.5 mg/ml) and the mixture was incubated at 30°C. Aliquots were taken after 15 min and immediately transferred to assay mixtures containing urea (100 mM) in buffers (55 μl) and again incubated for 30 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn [11]. Briefly, 45 μl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at λ 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μl. The results (change in absorbance per min.) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4 \cdot 3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula $100-(OD_{testwell}/OD_{control}) \times 100$. Thiourea was used as the standard inhibitor of urease.

α-Chymotrypsin inhibition

The α-chymotrypsin inhibitory activity of the extracts was performed by the method of Cannel et al. (1988) [12]. Chymotrypsin (9 units/ ml of 50 mM Tris-HCl buffer pH 7.6; Sigma Chemical Co. USA) was pre-incubated with the extracts for 20 min at 25°C. 100 μl of substrate solution (N-succinyl-phenylalanine-*p*-nitroanilide, 1 mg/ ml of 50 mM Tris-HCl buffer pH 7.6) was added to start the enzyme reaction. The absorbance of the released *p*-nitroaniline was continuously monitored at λ 410 nm until a significant color change had occurred. The final DMSO concentration in the reaction mixture was 7%.

Free radical scavenging activity

The potential antioxidant activity of the plant extracts was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhidrazyl (DPPH) (Wako Ltd. Japan) free radical [13]. A solution of DPPH was prepared by dissolving DPPH (5 mg) in methanol (2 ml), and the solution was kept in the dark at room temperature. Different concentrations of the test samples were prepared in 96 micro titer plates. Then, 5 μl of the methanol DPPH solution (final concentration 300 μM) was added to each well. The plate was shaken to ensure thorough mixing before being placed in the dark and wrapped with aluminum foil. After 30 min, the optical density of the solution was read using ELISA reader Spectromax Plus

(Molecular Devices) at λ 517 nm. Percentage inhibition by the sample was determined by comparison with the methanol treated control group. All test analyses were run in triplicate and averaged. Quercetin (Sigma, USA) and ascorbic acid (Sigma, USA) were used as positive controls.

Total phenol determination

The total phenol content of in crude extract and fractions were analyzed by the method of Folin and Denis [14,15], reading samples at 770 nm. Each extract (10 mg) was mixed with Folin-Denis phenol reagent (5 ml), Na_2CO_3 (20%, 10 ml) and diluted by a factor 100 with distilled water. The resulting mixture was left at room temperature for 10 min after filtration and the absorbance was read at 770 nm against the blank using Spectronic 20D (Milton Roy). The total phenol content of each extract was estimated by comparison with a standard curve generated using of tannic acid.

Results and discussion

Pakistan is endowed with a wealth of medicinal plants. It has a valuable heritage of herbal remedies and, like most developing countries, its rural population still relies on the indigenous system of medicine to a great extent [16]. It was, therefore, considered to be of interest to scientifically evaluate and determine the efficacy of an indigenous medicinal plant *Paeonia emodi*, commonly used by traditional practitioners for its effects [17–21]. Here, we present investigations into the enzyme inhibition and radical scavenging activities of the aerial parts of *Paeonia emodi*.

Urease inhibition

The results obtained with the crude extract and its subsequent fractions of *Paeonia emodi* for enzyme inhibition activities against jack bean and *Bacillus pasteurii* urease are shown in Figure 1. The crude extract displayed a significant urease inhibitory activity causing 74% and 80% inhibition of jack bean and *Bacillus pasteurii* urease respectively. Following these significant activities exhibited by the crude extract of *P. emodi*, the subsequent fractions (n-hexane, chloroform, ethyl acetate, n-butanol and water) were examined for urease inhibition activity. As shown in Figure 1, the highest activity was contained in the n-butanol fraction with 86% and 91% inhibition against the jack bean and *Bacillus pasteurii* urease respectively. Similarly, the other polar fractions such as ethyl acetate and water, also exhibited good to significant inhibitory activities against the two sources of the urease enzyme (Figure 1). The least polar fractions (n-hexane and chloroform), however, were devoid of any inhibitory activity. Urease inhibitors have recently attracted much attention as potential new anti-ulcer drugs [22]. Activity of urease has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which is detrimental for human and animal health as well as for agriculture [23–25]. Therefore, strategies based on urease inhibition are now considered as the first line treatment for infections caused by urease-producing bacteria. The crude ethanol extract and the fractions thereof derived from the aerial parts of *Paeonia emodi* screened for urease inhibition activities during this study were found to be a source of natural urease inhibitory chemical constituent(s).

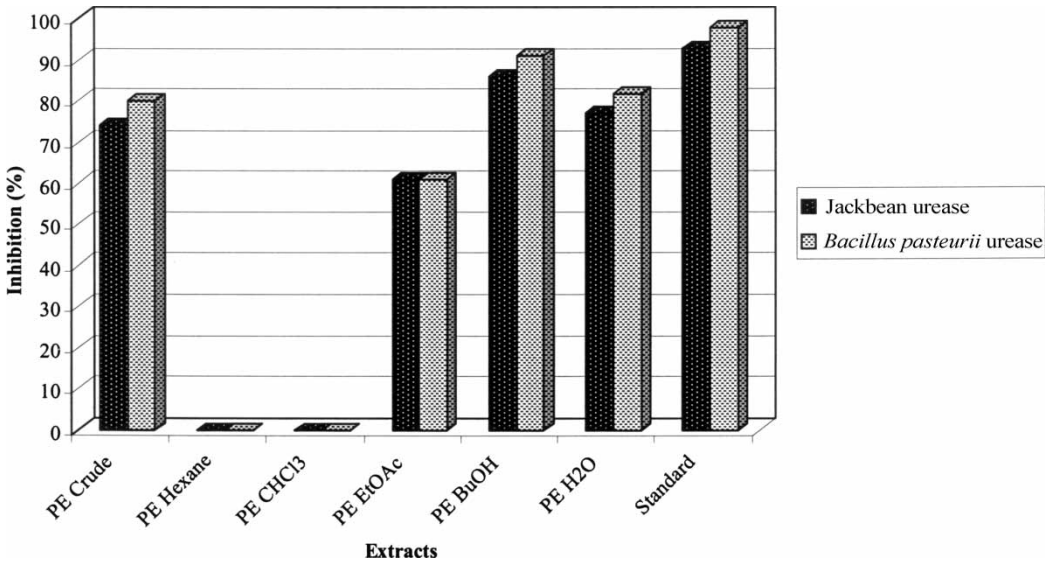

Figure 1. Urease Inhibititory activity of Crude Extract and Fractions of *P. emodi* Wall.

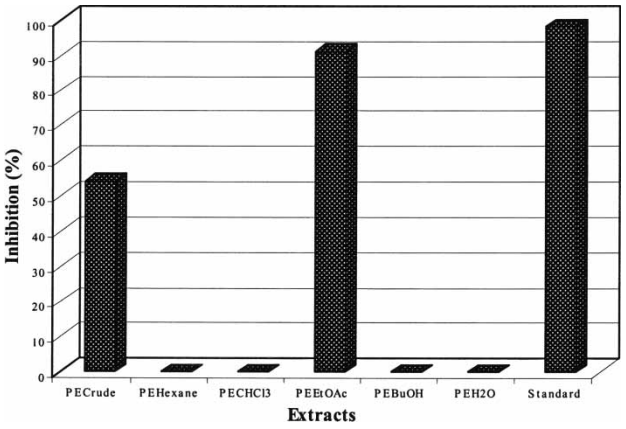

Figure 2. α-Chymotrypsin Inhibitory activity of Crude Extract and Fractions of *P. emodi* Wall.

α-Chymotrypsin inhibition

α-Chymotrypsin inhibition activities found in the current investigations are displayed in Figure 2. The crude extract showed reasonable inhibitory activity (54%) against α-chymotrypsin. When the fractions were screened, the maximum inhibitory activity concentrated into the ethyl acetate fraction, leaving the others completely inactive towards this enzyme (Figure 2). The ethyl acetate fraction caused the inhibition of α-chymotrypsin by 91%, suggesting that the chemical principal(s) of this fraction are responsible for the α-chymotrypsin inhibition of the ethanol extract of this plant. Serine proteases such as chymotrypsin and trypsin are involved in the destruction of certain fibrous proteins [26]. Chronic infection by hepatitis C virus can lead to the progressive liver injury, cirrhosis, and liver cancer. A chymotrypsin-like serine protease known as NS3 protease is thought to be essential for viral replication and has become a target for anti-HCV drugs [27]. Search for new effective inhibitors of serine proteases is an urgent need for drug development and the results with *P. emodi* (aerial parts), especially the ethyl acetate fraction of the ethanolic crude extract, have revealed an effective natural inhibitor of this enzyme.

Radical scavenging activity (RSA) and total phenolic contents

The free radical scavenging activity (RSA) values of the crude ethanolic extract from *P. emodi* and fractions thereof were examined and compared (Figure 3). The ethanolic extract from *P. emodi* afforded a promising (83%) RSA on the stable DPPH free radical. The results also showed that all the fractions possessed good to significant antioxidant activity. However, fractionation could not improve the antioxidant activity except the chloroform fraction, which showed the highest activity (85%) among all the tested fractions. The highest activity of chloroform fraction was followed by n-butanol, ethyl acetate, water and hexane fractions with 78, 65, 63 and 60% activity respectively, but these values were lower compared to the crude extract (83%). Different results on the relation between phenolic content and antioxidant activity have been reported; some authors have found a correlation between the polyphenol content and the antioxidant activity, others found no such relationship [28–32]. When the current data was analyzed for any possible correlation between the antioxidant activity and the total phenolic contents (Figure 3), then a relationship between the two parameters was observed with the exception of the ethyl acetate fraction that contains the highest total phenol content (22.48%) but an intermediate antioxidant activity (65%). For the rest of the extracts a somewhat direct relationship was observed between the antioxidant activity and the total phenolic contents. Reactive oxygenic species in

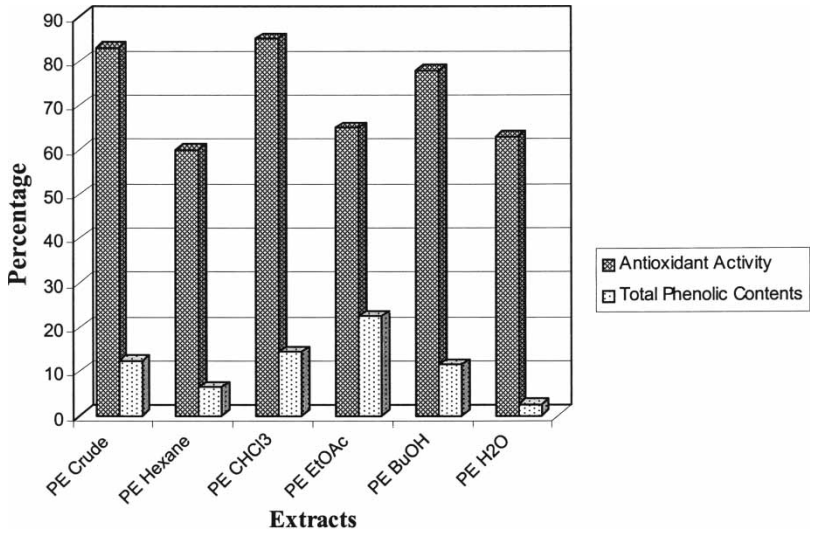

Figure 3. Free Radical Scavenging activity and Total Phenolic content of Crude Extract and Fractions of *P. emodi* Wall.

the form of superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) are natural by-products of the body's metabolism but when present in excess, these can attack biological molecules leading to cell or tissue injury associated with degenerative diseases [33–35]. The antioxidant activity of the plant phenolics is mainly due to their redox properties. Thus, natural antioxidants function as free-radical scavengers and chain breakers, complexers of pro-oxidant metal ions and quenchers of singlet-oxygen formation [36]. During this study *Paeonia emodi* (aerial parts) was evaluated for its radical-scavenging properties and it was assessed that this species could be a source of natural antioxidants for various applications.

The significant results obtained with the crude extract and its subsequent fractions from one of 'the queen of herbs' plant, *Paeonia emodi*, indicates the need for further work on the isolation, purification and investigation of the active principles responsible for the enzyme inhibition and free radical-scavenging activity. The mechanism of action has yet to be ascertained. Long-term toxicity studies will also be required to document any cumulative adverse effects.

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