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# *IN VITRO* INHIBITION OF UREASE AND a-CHYMOTRYPSIN BY SOME SELECTED INDIGENOUS MEDICINAL PLANTS

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ABSTRACT: The ethanol extracts derived from selected medicinal plants of Pakistan including *Trichodesma indicum* (whole plant), *Paeonia emodi* (Aerial parts), *Aconitum leave* (aerial parts) and *Sauromatum gattatum* (rhizomes, leaves and fruits) were screened' for enzyme inhibition activities against Urease (J.B. and B.P.) and a-Chymotrypsin enzymes. Only extract derived from *Paeonia emodi* (Aerial parts) showed significant activity again Jack bean and B.P. Urease by inhibiting them by 74% and 80% respectively. On the basis of the significant inhibition of the.said enzyme from the two respective sources, the ethanolic extract of *Paeonia emodi* was further fractionated into n-hexane, CHCl" EtOAc, BuOH and H2O fractions and were tested for urease enzyme from both the sources. Significant inhibitory activity was observed in EtOAc, BuOH and H2O fractions while the n-hexane and CHCI3 fractions were devoid of any such activity. During a-Chymotrypsin enzyme inhibition studies by the selected medicinal plants the extracts derived from *Trichodesma indicum* (whole plant), *Paeonia emodi* (Aerial parts) and *Sauromatum gattatum* (leaves) showed inhibitory activity of the said enzyme. The derived fractions from *Paeonia emodi* (Aerial parts) where tested for this enzyme and the maximum activity was concentrated into EtOAc fraction.

KEYWORDS: *Trichodesma indicum, Paeonia emodi, Aconitum laeve, Sauromatum gattatum,* Urease, a-Chymotrypsin

## INTRODUCTION

From pre-historic time, plants have been used extensively as medicine for the treatment of various ailments. Several hundred-plant species, in the form of whole plant, crude extracts or purified constituents were used in indigenous system of medicines, which had ultimately evoluted into modern therapeutic sciences (Hamayun *et at.,* 2004; Zaman and Khan, 1970). Pakistan has a variety of medicinal plants. It has a rich tradition of herbal medicines and like most developing countries; its rural population relies mainly on the indigenous system of medicine for their health related matters (Khattak *et aI.,* 1985). Crude medicinal plant materials worth more than Rs.150 million (:::: 3 million US$) are used in Pakistan per year while large quantities of these materials are also exported to the international market (:::: 6 million US$) although at cheaper prices (Atta-ur-Rehman and Choudhary, 1999). Therefore the value-addition in to these currently valueless but potentially worthy products is the utmost need of the time. There are several ways for the economic value to such products including physical, chemical and biological standardizations. In continuation to our biological standardization of some selected medicinal plants of Pakistan (Khan *et at.,* 2002 & 2005).

## MATERIALS AND METHODS

### *Plant Material and Preparation of Extracts*

*Trichodesma indicum* (aerial parts), *Paeonia emodi* (aerial parts), and *Aconitum taeve* (aerial parts) were collected from Swat, while *Sauromatum guttatum* (rhizomes, fruits and leaves) were collected from Mohmund Agency, N.W.F.P., Pakistan. These plants were identified by Prof. Dr. Abdur Rashid Department of Botany, University of Peshawar, Peshawar, Pakistan. Voucher specimens were deposited at the Herbarium of Department of Botany, in the said University. Air-dried and ground plant materials were extracted at room temperature with ethanol (three weaks x 3 times). The residue was filtered with filter paper and was evaporated to dryness under reduced pressure at 40°C. For fractionation of crude extract from *Paeonia emodi,* it was dispersed in purified water and shaken with n-hexane, chloroform, ethyl acetate, n-butanol, consecutively. All the fractions were evaporated under reduced pressure at 40°C.

### *Testfor Urease Inhibtion*

Reaction mixtures comprising 25 ,ul of enzyme (Jack bean Urease) solution and 55 ,ul of buffers containing 100 mM urea were incubated with 5 ,ul of test compounds (l mM concentration) at 30°C for 15 min

**Fig. Invitro Urease Inhibition by Selected Medicinal Plants**

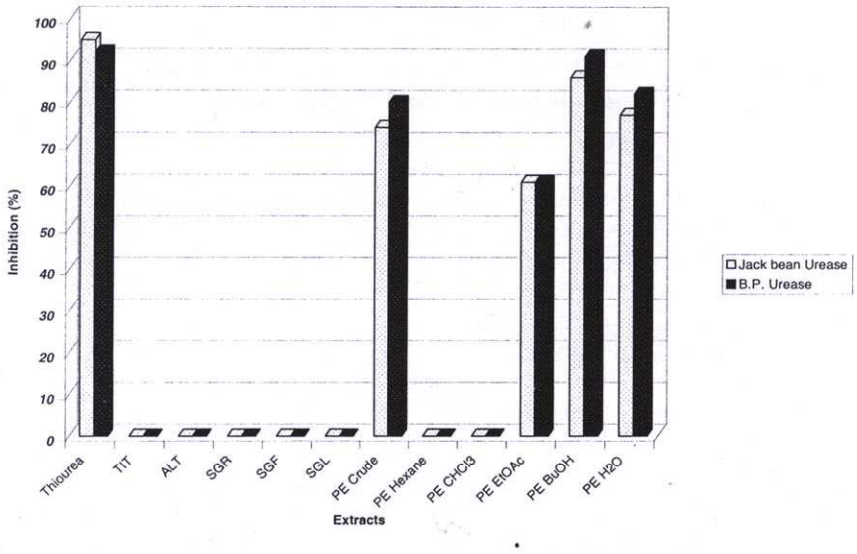
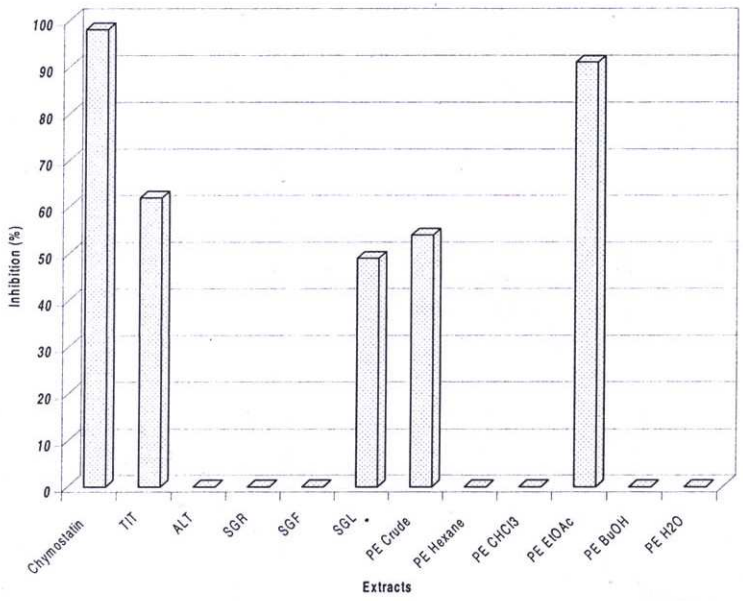



**Fig.2. Invitro α-Chymotrypsin Inhibition by Selected Medicinal Plants**




TIT = Ethanolic extract from *Trichodesma indicum* (whole plant)
SGR = Ethanolic extract from *Sauromatum gattatum* (rhizomes)
SGL = Ethanolic extract from *Sauromatum gattatum* (leaves)
PE Hexane = n-Hexane fraction of PE Crude
PE EtOAc = Ethyl acetate fraction of PE Crude
PE $H_2O$ = Water fraction of PE Crude
ALT = Ethanolic extract from *Aconitum leave* (aerial parts)
SGF = Ethanolic extract from *Sauromatum gattatum* (fruits)
PE Crude = Ethanolic extract from *Paeonia emodi* (Aerial parts)
PE $CHCl_3$ = Chloroform fraction of PE Crude
PE BuOH = Butanol fraction of PE Crude

in 96-well plate. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn (1967). This method consisted of 45 $\mu$l each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 $\mu$l of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 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 pI. The results (change in absorbance per min.) were processed by using SoftMax Pro software (Molecular Device, USA). All assays were performed at pH 8.2 (0.01 M K2HP043H2O, ImM EDTA and 0.01 M LiCb). Percentage inhibitions were calculated as 100-(ODteslwellODcontrol) X 100. Thiourea was used as the standard inhibitor of urease.

*Chymotrypsin Assay*

The a-chymotrypsin inhibitory activity of compounds were performed by method described by Cannel *et aZ.* (1988). Chymotrypsin (9 units/ ml of 50mM Tris-HCI buffer pH 7.6; Sigma Chemical Co. USA) was pre incubated with the compounds for 20 min at25°C. 100 pJ of substrate solution (N-succinyl-phenyla1anine-pnitroanilide, Img/ml of 50 mM Tris-HCI buffer pH 7.6) were added to start the enzyme reaction. The absorbance of released p-nitroaniline was continuosly monitored at 410 nm until a significant color change had achieved. The final DMSO concentration in the reaction mixture was 7%.

## RESULTS AND DISCUSSION

The results are shown in Fig. 1 and Fig. 2. All the crude extracts from the selected medicinal plants showed some antioxidant activities. However, the most promising (83 %) activity was found in the extract derived from *Paeonia emodi* (Aerial parts). The extracts obtained from *Trichodesma indicum* (aerial parts), *Aconitum Zaeve* (aerial parts) and *Sauromatum guttatum* (rhizomes, fruits and leaves) displayed weak free radical scavenging activity and caused 33%, 33%, 36%, 28% and 25% inhibition of the free radical.

Considering the antioxidant potentials of the extract derived from *Paeonia emodi* (Aerial parts), this extract was fractionated into n-hexane, chloroform, ethyl acetate, n-butanol and water fractions and these fractions were tested for antioxidant activities. The results showed all the fractions possess significant (::::50%) antioxidant activity. The highest of the antioxidant activity was concentrated in CHCh fraction, which displayed the highest (85%) activity among all the tested extracts. The other fractions also displayed good antioxidant activity and n-hexane, ethyl acetate, n-butanol and water fractions caused inhibition of the free radical by 60%, 65%, 78% and 63 % respectively.

The significant (::::50%) results obtained with the crude extracts of the above plants indicate the need for further work on the isolation, purification and investigation of the active principles responsible for the enzyme inhibition activity. The mechanism of action has yet to be delineated. Long-term toxicity studies will also be needed to document any cumulative adverse effects.

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