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Trypsin and Chymotrypsin Inhibitory Activity of Polyphenols from Long Pepper (*Piper longum*)

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ABSTRACT

Indian long pepper (*Piper longum*) belongs to the family of Piperaceae used as herbal medicine and also as a spice in the South Asian continent. Herein we attempted to discover and report for the first time the Trypsin and Chymotrypsin inhibitor activity of Polyphenols. The proximate analysis of the crude extract showed that it was rich in Polyphenols. It exerts protease inhibitor activity up to 49% and 48% at 25µg crude polyphenol dose against Serine proteases Trypsin and Chymotrypsin respectively. The crude polyphenol extract was stable up to a temperature of 100^0C.

INTRODUCTION

Pulses are a rich source of proteins with poor digestibility due to the presence of protease inhibitors. These proteases reduce the nutritional potential of the legume protein.[1] Protease inhibitors are extremely present throughout the plant kingdom and are also involved in the regulation of protein turnover, apoptosis, stress tolerance, and defense against pathogens and pests.[2,3] Polyphenols are the rich source of antioxidant which is present in almost of all the plants. Gallic acid, curcumin etc are a few popular examples of polyphenol.[4]

Generally Trypsin, chymotrypsin protease inhibitors are present legume seeds.[5,6] These leguminous seeds lots of applications in medicine, agriculture, food technology.[7]

Piper longum is popularly known as long pepper in the Indian subcontinent widely used as spice and in herbal medicine. This is also a close associate of *Piper nigrum.*[8,9]

The present study is to highlight the inhibitory action of crude polyphenol extract of *Piper longum* seeds against proteases like trypsin and chymotrypsin.

MATERIALS AND METHODS

The present *in-vitro* study was aimed to study the protease inhibitory efficiency of crude polyphenols from *Piper longum* seeds. Trypsin, Chymotrypsin, and all other chemicals unless otherwise mentioned were of analytical grade procured from Merck (Germany).

Extraction of crude Polyphenols from *Piper longum* seeds: *Piper longum* seeds were procured from a local arcade in consultation with Botanist. Further seeds were desiccated in sunlight and moisture content was removed. Further, it was ground into a fine powder and stored in a dry glass container. *Piper longum* seeds powder (10 g) was extracted with methanol solvent (150 mL) by using Soxhlet extractor for 72 h. After complete extraction, the methanol solvent was evaporated by using rotary evaporator under reduced pressure to obtain methanol crude extract. Further, it was extracted with different organic solvents such as hexane, chloroform, ethyl acetate and butanol to obtain hexane, ethyl acetate, chloroform-butanol and residual methanol fractions, respectively. All crude extracts were mixed, filtered. The particle-free crude extract was evaporated completely by using rotary evaporator under reduced pressure. The combined extracts were concentrated and dried by using rotary evaporator under reduced pressure[13].

Proximate analysis

The polyphenol extract of *Piper longum* (PEPL) was subjected to phytochemical analysis to check the presence of other bioactive compounds by using standard protocols. The protein estimation was carried[11]using BSA as standard and absorbance were read at 535nm. Total phenolics were determined[12] using gallic acid as a standard and absorbance were read at 750 nm. Ascorbic acid estimation was carried out[13] and the absorbance was read against a reagent blank at 540nm. Total sugar estimation was done[14] and the absorbance was read at 520 nm. Flavonoids estimation was done[15] by using Quercetin as a standard and the absorbance was measured at 415 nm. In the above analysis, a standard curve was used to compare.

Trypsin and Chymotrypsin inhibitory activity:

The protease like Trypsin and Chymotrypsin inhibitory activity was assayed[16]. 50 µL aliquot of trypsin and chymotrypsin was preincubated separately with different concentrations of standard protease inhibitor PMSF and crude polyphenols of *Piper longum* (PEPL). To the above-denatured casein was added as the substrate of 0.4 mL (2%) in a final volume of 1 mL using 0.2 M Tris-HCl buffer of pH 8.5 for 2 h at 37^0C. After incubation, the reaction was stopped by adding 1.5 mL of 0.44 M TCA, and the mixture was allowed to stand for 30 min. The reaction mixture was centrifuged at 1500g for 15 min. An aliquot (1 mL) of the supernatant was mixed with 2.5 mL of 0.4 M sodium carbonate and 0.5 mL of Folin–Ciocalteu reagent (1:2 v/v). The color developed was read at 660 nm. The activity was expressed as units/hr. Protease inhibitor activity of the standard and Polyphenols of *Piper longum* was expressed in terms of percent inhibition.

DPPH radical scavenging activity

The DPPH radical scavenging activity was assessed[13]. Different doses of PEPL and a constant amount of proteases like trypsin or chymotrypsin was mixed with 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer. The resulting reaction mixtures were incubated at 37°C for 30 min, and the absorbance was measured at 517 nm. The % DPPH radical scavenging activity was calculated using the following formula.

Thermal and pH stability of Polyphenols extract of Piper longum (PEPL) protease inhibitor:

The effect of temperature on trypsin and chymotrypsin inhibitory activity of extracts from PEPLwere tested by incubating at different temperatures 37, 40, 50, 60, 70, 80, 90, 100^{0}C for 30 min. After cooling the samples to room temperature the residual trypsin and chymotrypsin, inhibitory activity was determined as described earlier.

Statistical analysis

All experiments were carried out in triplicate to check the reproducibility of results. The data presented here are the averages of triplicate determinations and the standard deviations for all the values were < ± 5%.

RESULTS AND DISCUSSION

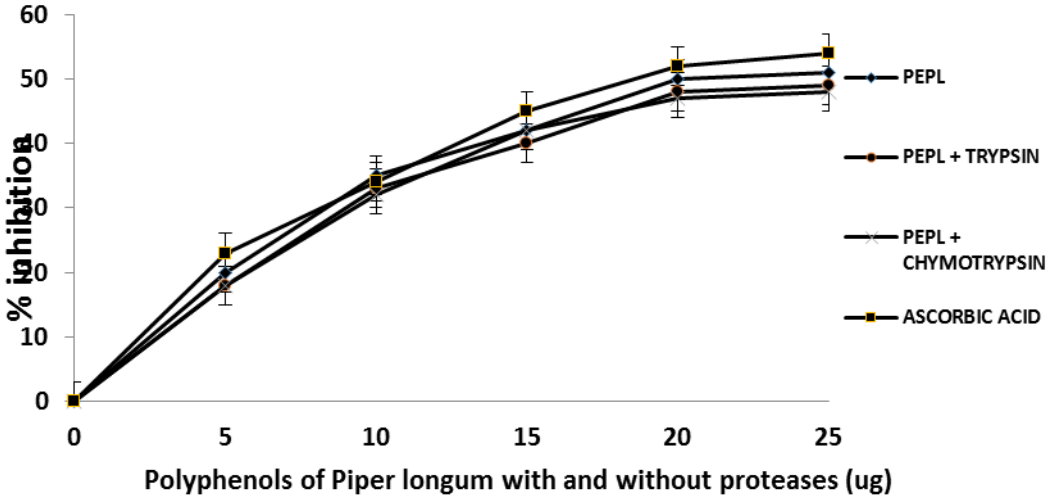

Figure No. 1: DPPH radical scavenging activity of Polyphenols extracts from *Piper longum* (PPEL) and Ascorbic acid

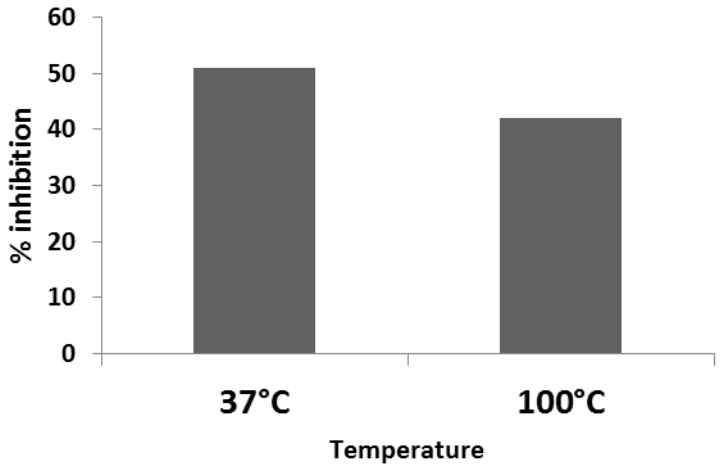

Figure No. 2: Effect of temperature on Polyphenols extract of *Piper longum* in DPPH antioxidant model system

The thick dark brown polyphenol extract of *Piper longum* (PEPL) showed a promising serine protease (Trypsin and Chymotrypsin) inhibitory activity. To analyze the protease inhibitor activity of PEPL, a fixed interval dose-dependent DPPH radical scavenging activity was done, where different doses of Trypsin and Chymotrypsin enzymes are mixed with PEPL incubated at room temperature and subjected to DPPH antioxidant analysis. **"Fig. 1"** shows that no effect of protease enzymes on PEPL and the results compared to PEPL alone. The protease inhibitor PEPL was stable up to 100^0C where a negligible amount of loss in its activity was noticed as shown **"Fig. 2"**. Earlier, the same authors reported that, crude protein extract of *Piper longum* showing antiprotease activity. A similar type of studies was done using spices like Turmeric, Ginger etc.[17, 18]

CONCLUSION:

This is the first report a protease inhibitor crude Polyphenol from *Piper longum* and showed a potent inhibitory activity against both trypsin and chymotrypsin. Therefore, future studies in this direction have to be performed to completely elucidate the characteristic features of Serine type protease inhibitor of the PEPL.

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