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# Study of proteins with protease activity from some plants groving in Georgia and preparation of highly active preparations

**T. Ninua*, T. Khobelia, K. Museliani, E. Kvesitadze, T. Buachidze**

Faculty of Chemical Technology and Metallurgy, Georgian Technical University, Tbilisi, Georgia

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A B S T R A C T

The purpose of this paper was to identify proteins with protease activity from some young plants in Georgia and to study them in order to obtain highly active preparations. According to the methodology developed in "Biology", several of them were selected for further work.

**Key words:** Vegetative organs of plants, protease activity, extraction, EWMP

*Corresponding Author: T. Ninua; Email: ninuatea@gmail.com

## Introduction

Interest in proteolytic enzymes is great. Peptidohydrolases are characterized by selective action: they cleave only specific bonds for them. For example, pepsin acts only on the bonds established between aromatic amino acids, trypsin removes the bond between arginine and lysine, etc. The spectrum of use of proteases is very wide: in the production of dairy products - making cottage cheese, cheese, in the production of meat and meat products - softening of meat, in perfumery - bioactive additive in ointments, toothpastes, lotions; In the production of synthetic detergents - washing additive for proteinaceous contaminants, in medicine - treatment of burns, thrombosis, inflammatory processes, etc. [1-6].

From this point of view, there is a sustainable trend of interest in products of plant origin. The flora of Georgia is distinguished by the wealth of medicinal and edible plants, most of them attract attention with their chemical composition.[7-8]

Based on the above, we aimed to obtain proteins with protease activity from the raw materials selected by us for their further use.

## Materials and Methods

**Materials:** for research Necessary reagents $Na_2HPO_4$, $KH_2PO_4$ purchased Alfa Chemical (India), Folin & Chiocalteu 's phenolic reagent and EWMP substrate "Biologica" LLC were used, Chimoral was used as a commercial protease ( **Manufacturer:** Gelenica A. d. Serbia, Belgrade). The following plants were taken for research: Rubia and yellow bedstraw (Rubiaceae), yellow nanny and licorice (Fabaceae), rosebay willowherb (Onagraceae); Thyme (Lamiaceae) ; Isabella (Vitaceae). It should be noted, that all of these plants are wild, except Isabella.The Isabella grape is a cultivar derived from the grape species *Vitis labrusca* or 'fox grape,' which is used for table, juice and wine production.

## Preparation of samples:

As material we took vegetative organs of plants (root, stem, leaf) in a dry state. We crushed the material, for protein extraction we added 0.1M phosphate buffer (PBS pH=7.4) at a ratio of 1/10 (mass/volume).

## Determination of enzyme-substrate interaction:

The method for determining the protease activity of the enzyme is based on the effect of the enzyme on the insoluble substrate: 3 ml of 0.1 M phosphate buffer (PBS pH=7.4) and 30 µl of the enzyme solution were added to the EWMP substrate (30 mg/ml) and incubated at $27^0$ C for 20 minutes . After the delay time, the substrate conversion process was stopped by placing the samples on an ice bath. After that, we centrifuged at 1500 rpm for 10 minutes.[9]. For analysis, we took the supernatant, $Na_2CO_3$ and so-called Folin's reagent in a ratio of 2: 5: 1. We delayed for 30 minutes. And we measured the light absorption rate spectrophotometrically (λ=660 nm). [10-11].As a control for the samples, we used the substrate solution without the enzyme, which was similarly delayed for 30 minutes, then we added the enzyme and measured immediately. The control solution prepared in this way excludes the influence of the enzyme and the substrate on absorption.

## Determination of enzyme activity in various biological extracts:

To determine enzyme activity in units, units/mL and units/mg, we used the following formulas:

$$A(U)=\frac{[PR]\mu m*[Vt]ml}{[Rt]min*[Vm]ml}$$

$$A(U/ml)=\frac{[PR]\mu m*[Vt]ml}{[Rt]min*[Vm]ml*[VE]ml}=A(U)/[V_E]_{ml}$$

$$A(U/mg)=\frac{A(\frac{U}{ml})}{E(\frac{mg}{ml})}$$

$[PR]\mu ml$ Product in micromoles

$[Vt]ml$ Reaction total volume in milliliters

$[Rt]min-$ Reaction time in minutes

$[Vm]ml$ Measurement volume of the cuvette in milliliters

$[V_E]_{ml}$ Volume of Enzyme in milliliters

E(mg/ml)-Enzyme(mg.ml)

## Results:

During incubation with the substrate, the proteases present in the extract begin to break down the substrate - soluble proteins and peptide fragments appear in the solution. Accordingly, the light absorption index changes. The degree of dilution depended on the rate of light absorption and the degree of its change during incubation with the substrate. We made the dilution so,that the light absorption of the extract and the substrate-enzyme complex was minimal at the initial stage, and at equal intervals of time - reflecting the kinetics of activity. We judged the intensity of the reaction by the difference between the maximum and minimum absorbance values. The proteolytic conversion of the substrate was indicated by the change in the absorbance index. According to the protein calibration curve measured by the Bradford method, we have calculated the coefficient that connects the absorption rate and the amount of protein in mg/ml (OD 1.52-0.75mg/ml). Enzyme activity was calculated as the increase in protein concentration in mg/mL/s, which indicated the accumulation of reaction product over time. The results of the study are presented in the table below:

| Plants | Protein mg/ml | µg /ml/sec | U/mg |
|---|---|---|---|
| **Yellow nanny** | 0.0064 | 0.26 | 40.63 |
| **Yellow bedstraw** | 0.043 | 1.74 | 40.47 |
| **Liquorice** | 0.049 | 1.54 | 31.43 |
| **Rosebay willowherb** | 0.077 | 1.1424 | 14.84 |
| **Isabella** | 0.127 | 0.17 | 1.34 |
| **Rubia** | 0.156 | 0.13 | 0.83 |
| **Thyme** | 0.086 | 0.0175 | 0.20 |

## Conclusion

It can be seen from the table that the total protease activity is high in field needle, barberry and licorice, as for Isabella, Endros and Begkondara, their rate is relatively low, which does not exclude the fact that there may be proteases in these plants, the activity of which at pH≠7.4 is more low (alkaline proteases) or high (acidic proteases). This prompts us to obtain extracts from them with different extraction buffers. Based on all of the above, the biochemical research of enzymes obtained from plants with high protease activity for their further use is not devoid of interest.

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