



Full Length Article

Comparison of Enzymatic Characteristics in the Fruits and Latex of Three *Ficus* Species

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Abstract

Three *Ficus* species, *Ficus padana* Burm.f., *Ficus aurata* (Miq) Miq. and *Ficus racemosa* L. were analyzed concerning total activity of crude enzymes, total protein and specific activity in their fruits and latex. To identify the primary proteins, SDS-PAGE characterized the crude extracts in terms of protein bands of different molecular weights. *F. aurata* displayed the highest total activity from the set of fruit samples at 9.02 U with a total of 4.830 mg of proteins and a specific of 1.870 U/mg. *F. padana* and *F. racemosa* had lower total activities (8.49 U and 8.56 U), with specific activities being 2.03 U/mg and 1.99 U/mg, respectively. *F. racemosa* latex had a two-fold increase in total activity of 24.01 U compared to *F. aurata* (7.35 U) and *F. padana* (8.67 U). The results showed a higher specific activity for *F. racemosa* latex, followed by *F. aurata* and then *F. padana* at 5.41 U/mg, 4.62 U/mg and 4.37 U/mg, respectively. Bands of around 35 kDa were visible in the latex preparations, indicating that the above-mentioned fraction of SDS-PAGE is enriched in proteolytic enzymes. The elevated levels of these particular enzymes in the latex correlated with the increased enzyme activity in *F. racemosa*. The results of this study contribute to knowledge of the enzymatic potential of species of the genus *Ficus* and its potential applications in biotechnology and the industry.

Keywords: *Ficus* species; Crude enzymes; Enzymatic activity; SDS-PAGE; Proteolytic

Introduction

Plants are a source of active compounds, such as enzymes, essential in numerous physical and biological processes (Shu *et al.* 2018; Srisai *et al.* 2022). *Ficus* species of the family *Moraceae* found in tropical and subtropical areas and they have great role as important species in the ecosystems (Rocchetti *et al.* 2018; Gupta *et al.* 2021). Consideration has also been given to the genetic resources of *Ficus* species, especially their medicinal properties and potential usefulness as food or industrial products (Swargiary *et al.* 2020).

Based on the numerous application domains, the global importance of ensuring environmental compliance, and ready-to-commercialize items, plant-based proteolytic enzymes have excited interest as a bio-active protective agent (Murthy and Rao 2018; Debbarma *et al.* 2020; Obed *et al.* 2021; Abdoulaye *et al.* 2023). These enzymes nowadays are extensively in food and pharma industries and

environmental biotechnological applications and are generally present in crude extracts (Balakireva *et al.* 2018; Doneva *et al.* 2018; Boudreau *et al.* 2019; Moshobane *et al.* 2022; Pawar 2023).

Proteases are widely used for medical and food-related activities such as protein hydrolysis (Obed *et al.* 2021; Abdoulaye *et al.* 2023). Furthermore, ethnomedicinal use of latex from *Ficus* species has been reported for centuries, demonstrating the bioactivity of its enzymatic compounds with therapeutic applications as well (Doneva *et al.* 2018; Brito *et al.* 2021; Obed *et al.* 2021; Yadav *et al.* 2022). Furthermore, numerous researchers have noted the occurrence of proteolytic enzymes in the latex of several *Ficus* spp. along with their high proteolytic activities and possibilities for industrial and medical use (Das *et al.* 2018; Brito *et al.* 2021; Nour *et al.* 2021; Wen *et al.* 2022).

In comparison to fruit, there is more availability of the enzymes obtained from latex are generally increased

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availability. However, limited progress has been achieved to compare the interspecies differences in enzyme activity and protein content in the fruit and other parts of various *Ficus* species (Vaou *et al.* 2022; Nawfetriyas 2023). It must also be noted that the specific activity which determines the effectiveness of the enzyme along with other sufficient parameters, which is essential in the evaluation of the enzyme potential has not yet been done on the *Ficus* fruits and latex (Srisai *et al.* 2022). This makes the knowledge gaps critical to the quest of searching for the best-suited plant-based sources of enzymes for several biotechnological processes (Beltrán-Espinoza *et al.* 2021; Ramli *et al.* 2021; Anand *et al.* 2023).

We selected *Ficus aurata* (Miq) Miq., *Ficus padana* Burm f. and *Ficus racemosa* L. as subjects for our study due to their abundance and conventional utilisation. In this particular study, an effort was made to obtain a comparative enzymatic profile of the fruits and latex of the selected species by comparing the values of total enzymatic activity, and total protein content of crude enzyme extracts from the fruits and latex of both species and locate plant parts and species which have high enzyme activities for possible utilization in several sectors (Aktayeva *et al.* 2018; García-Magaña *et al.* 2018; Bhuyar *et al.* 2020; Gopu *et al.* 2021; Teplova *et al.* 2021; Mangena 2022). These outcomes would be useful for understanding the possible industrial and biotechnological applications of *Ficus* species (García-Magaña *et al.* 2018; Bhuyar *et al.* 2020; Teplova *et al.* 2021; Mangena 2022). In addition, this study also aimed to bridge the knowledge gaps in comparative enzymatic investigation of pulp and latex of some *Ficus* species which would allow further evaluation of the bioactive potential of the genus *Ficus* (Campos *et al.* 2019; Loi *et al.* 2020; Mangena 2022; Lakkannavar 2024).

This study aimed to compare the protein content and specific activities of latex from *F. padana*, *F. racemosa* and *F. aurata* highlighting the differences among these species. It seeks to identify and analyze variations in the enzymatic properties of latex from different *Ficus* species, providing insights into their potential applications in biotechnology. Additionally, the research will evaluate the potential of specific *Ficus* species latex (and possibly other plant parts) for enzymatic applications, suggesting which species and plant parts are more suitable for biotechnological purposes. Ultimately, the study aimed to provide foundational data for future research on the biotechnological uses of *Ficus* species, with a focus on enzyme-based applications, protein characteristics, and potential industrial or medicinal uses.

Materials and Methods

Experimental details

The location of this research was Limau Manis, Pauh, West Sumatera, Indonesia. This study is descriptive qualitative research aimed at examining the enzyme characteristics of

three different species of *Ficus* specifically *F. padana*, *F. racemosa* and *F. aurata*.

Research implementation

Enzyme extraction procedure: The main resources utilized in this study were sap or latex and the fruits of *F. padana*, *F. racemosa* and *F. aurata*. The latex extracted from the stems is subsequently placed in a tube containing 0.05% NaN₃ and preserved at -20°C. Frozen latex was thawed at 4°C and diluted with distilled water in a 1.0:0.5 ratio, mixed, then centrifuged at 5000 rpm for 15 min at 4°C to exclude grit and other insoluble substances. The supernatant was filtered using Whatman paper. Clarified juice is termed "crude extract" (Gagaoua *et al.* 2014). The raw material for fruit is chosen to be green and fresh, weighing up to 200 g, then washed and sliced to a thickness of around 2.5 mm. The fruit was subsequently chopped with a blender, utilizing 450 mL of 50 mM sodium phosphate buffer (pH 7.0) treated with 10 mM L-cysteine. The chopped and homogenized fruit was centrifuged at 150 rpm for 45 min at 4°C. The sample followed filtration with a filter cloth, insoluble impurities were eliminated, and the supernatant was then filtered through Whatman paper (Gagaoua *et al.* 2015).

Enzyme activity: The method is used to determine the activity level of the enzyme ficin. A 0.5 mL solution of 1% (w/v) casein was mixed with the ficin enzyme and incubated for 20 min. After that, 3 mL of 5% TCA was added to stop the enzyme hydrolysis reaction. The solution was then left to stand for 10 min. The mixture was centrifuged at 3000 rpm for 10 min, and the supernatant was collected. It was then reacted with 3 mL sodium carbonate and 1 mL of 25% Folin-Ciocalteu reagent and incubated for 10 min. The absorbance of the solution was then measured using a UV-Vis spectrophotometer at a maximum wavelength. The standard curve was created using tyrosine at varying concentrations. A 2 mL of the tyrosine solution was added to 3 mL of 0.5 M sodium carbonate and 1 mL of 25% Folin-Ciocalteu reagent. Total enzyme activity and specific enzyme activity were then measured.

Protein analysis: The protein concentration was tested using the Bradford method. The Bradford reagent was prepared by diluting it to 20% with distilled water. A 0.05 mL of the sample solution was placed into a test tube, and 2.5 mL of Bradford reagent was added. Then, the sample was incubated in the dark for 5 min. The absorbance was measured at a wavelength of 595 nm. A protein standard curve was created by varying the BSA concentrations, and its absorbance was measured at a wavelength of 595 nm (Bradford 1976).

Data Analysis

Data analysis was performed by calculating the mean and standard deviation using Microsoft Excel.

Results

The enzymatic activity of crude extracts obtained from the fruits and latex of *F. padana*, *F. racemosa* and *F. aurata* demonstrated distinct patterns across different plant parts. The fruit of *F. aurata* showed the highest total activity (9.02 U), followed by *F. racemosa* (8.56 U) and *F. padana* (8.49 U), reflecting similar enzymatic potentials across all three species. In contrast, significant variation was observed in the latex samples, where *F. racemosa* showed a markedly higher total activity (24.01 U) compared to *F. aurata* (7.35 U) and *F. padana* (8.67 U). The total activity, total protein and specific activity of crude enzymes in fruits and latex of *Ficus* species are displayed in Table 1. Three species of *Ficus* fruits namely *F. padana*, *F. racemosa* and *F. aurata* are displayed in Fig. 1.

SDS-PAGE analysis of crude enzyme extracts from latex and fruits of *Ficus* species

The analysis of crude enzyme extracts from latex and fruits of *Ficus* species are displayed in the Fig. 2. The SDS-PAGE results presented here for the protein profiles of fig extracts provide fundamental data on the molecular spread of the protein extracts. Determinations of the molecular weights of the bands were carried out using molecular weight sails on protein marker lanes, and bands were observed in the range of 10 to 180 kDa.

Discussion

According to Mutungi *et al.* (2021), increased activity of the enzymes in the latex of *F. racemosa* may be related to the anatomical or physiological adaptations of the species as ecological strategies that enhance the effectiveness of the more robust enzymatic defense system. Murthy and Rao (2018) and Swargiary *et al.* (2020) emphasized on the function of plant exudates, such as latex, in providing defense due to the presence of proteolytic enzymes and other bioactive substances that can repel herbivores and pathogens. The latex of *F. racemosa* is identified as a source of enzymes, supported by its various biological activities, such as antioxidant and antibacterial capabilities, which are frequently linked to enzymatic activity (Yadav *et al.* 2022). To support this, several studies showed that latex has more enzymatic activity than fruit extract (Boudreau *et al.* 2019; Yadav *et al.* 2022). The protein content of the fruit extracts was maximally found in *F. aurata* (4.83 mg), followed by the fruits of *F. racemosa* (4.31 mg) and *F. padana* (4.19 mg). This suggests that fruits behave more as secondary reserve tissues of the enzyme and hence have lower protein content than latex (Shinde *et al.* 2019). The protein content in the latex extracts was quite significantly different. Latex of *F. racemosa* recorded the highest amount of protein (4.45 mg) followed by *F. padana* (2.14 mg) and *F. aurata* (1.64 mg). This trend corresponded to the estimated enzymatic

Table 1: Total activity, total protein, and specific activity of enzymes

Plant Part	Total activity (U)	Total protein (mg)	Specific activity (U/mg)
Fruits			
<i>Ficus aurata</i> (Miq) Miq.	9.02 ± 0.08	4.83 ± 0.09	1.87 ± 0.02
<i>Ficus padana</i> Burm.f.	8.49 ± 0.18	4.19 ± 0.18	2.03 ± 0.05
<i>Ficus racemosa</i> L.	8.56 ± 0.05	4.31 ± 0.05	1.99 ± 0.01
Latexs			
<i>Ficus aurata</i> (Miq.)	7.35 ± 0.30	1.64 ± 0.30	4.62 ± 0.69
<i>Ficus padana</i> Burm.f.	8.67 ± 0.74	2.14 ± 0.74	4.37 ± 0.93
<i>Ficus racemosa</i> L.	24.01 ± 0.16	4.45 ± 0.16	5.41 ± 0.16



Fig. 1: Three species of *Ficus* fruits

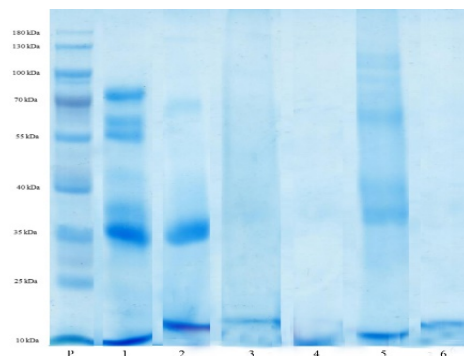


Fig. 2: SDS-PAGE analysis of crude enzyme extracts from latex and fruits of *Ficus* species

Note: P: Marker; 1: *Ficus aurata* (Miq) Miq. latex; 2: *Ficus padana* Burm. f. latex; 3: *Ficus racemosa* L. latex; 4: *Ficus aurata* (Miq) Miq. fruit; 5: *Ficus padana* Burm. f. fruit; 6: *Ficus racemosa* L. fruit

activity, which correlated well with the conclusion that *F. racemosa* latex is a protein source with abundant nutrients and enzymes (Fenclová *et al.* 2019; Singh *et al.* 2019). A decrease in the protein content of *F. aurata* latex implies that the latex carries a large number of enzyme molecules that are potentially less active in this species (Wang *et al.* 2019).

Specific enzyme activity can provide additional insight into the quality and how the enzyme works (Boudreau *et al.* 2019; Liu *et al.* 2022; Yadav *et al.* 2022). The highest specific activity for fruit extracts was recorded for *F. padana* (2.03 U/mg), followed by *F. racemosa* (1.99 U/mg), and *F. aurata* (1.87 U/mg). This implies that these three species of fig fruit have remarkable enzymatic efficiency in the same range (Mohiuddin and Lia 2020; Wen *et al.* 2022). However, observed specific activity in latex extracts was higher by a very significant gap, which again points out the higher enzymatic activity of latex. Data exhibited efficacy in latex, with *F. racemosa* showing the most effectiveness (5.41 U/mg), subsequently, *F. aurata* and *F. padana* were included (4.62 U/mg and 4.37 U/mg, respectively). A higher

specific activity of latex of *F. racemosa* indicated a highly active enzymatic system that possesses an intense activity at a very low level of protein concentrations (Shrivastava and Mishra 2019; Vaou *et al.* 2022). This means that one should expect that *F. racemosa* latex is extraordinary for purposes that require very high specificity and efficiency in enzymatic catalysis (Birusanti *et al.* 2018; Zanzabil 2023).

The presence of differences in the activity of the enzymes among the extracts of both fruit and latex suggests their potential biological roles. Latex in all of the investigated species, total activity and specific activity, were, in most cases, higher when compared to fruits, thus confirming their defensive role against herbivores and pathogens (Rajesh *et al.* 2022; Anand *et al.* 2023). In contrast with other organs, fruits may have a less important function, including seed dispersal or fruit decomposition (Wang *et al.* 2018; Pawar 2023), which explains the lower activity of the enzymes. However, despite the differences in the protein contents in fruit and latex, the specific activity of latex consistently exceeded that of the respective fruit. This indicates that specialized proteolytic enzymes will likely extensively catalyze the relatively high latex enzymes (Choudhury 2022; Nawfetrias 2023). Such differences indicate that choosing a particular plant part for specific enzymatic purposes is very important (Boudreau *et al.* 2019; Yadav *et al.* 2022).

Overall, all three species exhibited comparable trends, with the best results reported for all three enzymatic parameters, particularly regarding all the standard *F. racemosa* and its latex extracts. The activity, protein content, and specific activity of *F. racemosa* latex are very high, making it superior to other species, as clearly supported by the results. The result is also consistent with the conclusions of other researchers. *F. racemosa* fruit is rich in proteolytic enzymes with significant industrial and medicinal relevance. On the other hand, *F. aurata* and *F. padana* had low activity. These had low protein content, indicating possibly decreased concentrations of enzymes or less reactive enzymes. However, such species may also be acceptable for biotechnological processes, which do not require very high enzyme activity. The latex from *F. racemosa* tree is the most active source of enzymes. It uses an economical and sensitive purification technique for various industrial and biotechnological uses. This comparison sets the stage for considering the use of the different *Ficus* species for biotechnological work in the future. Also, it emphasizes the importance of choosing the appropriate plant part for the desired enzymatic application.

The significant three bands at the approximate molecular weight of 35 kDa for certain enzymes suggest the presence of certain key proteolytic enzymes or enzyme subunits (Al-Ghanim *et al.* 2020; Julia 2020). Enzymatically, these are usually associated with proteases such as papain or bromelain. Also, one peak in the band, which is not specified (1 - 2) and another significant (3 - 6) indicate that the amounts of the particular protein differed by a large margin in samples that showed the highest

enzyme activity (Wenzel *et al.* 2018). Loss or decrease in these bands in the lanes 3 – 6 may reflect high values of protein that was extracted or the degree of protein breakdown at the sample preparation stage.

Conclusion

This research demonstrated that *F. aurata*, *F. padana* and *F. racemosa* are differentiated based on the enzymatic activity, protein concentration and protein molecular mass distribution of their fruits and latex. Among the tested fruit materials, the highest total enzymatic activity (9.02 U) was obtained from *F. aurata*, which was followed by protein content (4.83 mg) with specific activity of 1.87 U. *F. padana* and *F. racemosa* showed relatively low total activities *i.e.*, 8.49 U and 8.56 U, respectively, but their specific activities were higher at 2.03 U/mg and 1.99 U/mg. *F. racemosa* showed the highest total enzymatic activity (24.01 U) and specific activity (5.41 U/mg) of the latex samples among the evaluated species. *F. aurata* described a total activity of 7.35 U (4.62 U/mg specific activity) and *F. padana* described a total activity of 8.67 U (4.37 U/mg specific activity). The latex samples on SDS-PAGE showed a visible band corresponding to the molecular weight of about 35 kDa. The research results showed that *F. racemosa* showed more enzymatic activity and furthermore, it is beneficial for biotechnology and industrial applications.

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Author contributions

I and RY planned the experiment design and conducted laboratory work; I and H conducted lab work and interpreted the results; I, RY, H, and DS wrote the original manuscript and revised one; I, RY, and Y performed statistically analyzed the data and made illustrations, and I reviewed final manuscript.

Conflicts of Interest

The authors declare no conflict of interest among themselves.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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