ISSN No:-2456-2165

Evaluation of Ficain Activity in Different Fruit Samples of *Ficus carica Linn*

N.Ranjini, Kusuma.K, Srilakshmi.S, Sharu Raj K M*
Department of PG studies in Biotechnology, Government Science College (Autonomous) Bengaluru-560001

Abstract:- Protease enzymes have increased commercial applications, but there are limited available known natural sources of these enzymes. This study was done to identify other sources of proteases, for which different stages of fig fruits (raw, ripened, and dry) were used, as they are known sources of ficain enzyme. Ficain extracted from different fruit samples (raw, ripened,and dry) was partially purified by chromatography on DEAE-cellulose. Caseinolytic activity studies were also carried out on the enzyme sample. Extraction of ficain from fig fruits is a novel approach.

Keywords:- *Ficain, Fig Fruit, Protease, DEAE-Cellulose, Caseinolytic.*

I. INTRODUCTION

Ficus carica Linn.(Family: Moraceae) is a small tree grown throughout India and some other tropical countries. It is the source of fig fruits. Ficain, a cysteine protease present in the fig latex. Ficain has an effective range of pH(6-6.5) and has optimum temperature ranges from 45 to 55ºC. Ficain mainly extracted from the latex of plant is widely used in industries and pharmaceuticals. The present investigation deals with Ficain extraction from three different fruit samples(raw, ripened, dry) of *Ficus carica* L. ; partial purification by ion exchange column chromatography using DEAE cellulose , synthesis of silver nanoparticles using the purified ficain enzyme and enzyme study.

II. MATERIALS AND METHODS

2.1. Collection and identification of sample:

The fruit samples (raw, ripened, and dry) of fig were collected in the month of January in 2020 from KR market, Bangalore and identified as *Ficus carica* L. (UASB4607).

2.2. Extraction:

Three different samples of fig fruits (raw, ripened, dry) were used for extraction of enzyme ficain. Fruits were homogenized using 0.9% NaCl containing 5mM EDTA and 5mM cysteine, soaked for 3 hours and filtered. The filtrate was centrifuged at 6000rpm for 10 minutes. To the supernatant, tannic acid was added and concentration made up to 0.2%. It was then incubated at 4oC for 2 hours and centrifuged at 6000rpm for 10minutes. To the supernatant, phosphate buffer containing 5mM EDTA and 5mM cysteine was added and centrifuged at 6000rpm for 10minutes. It was repeated twice. To the precipitate, phosphate buffer containing 5mM EDTA and 5mM cysteine was added and freeze dried.

2.3. Qualitative analysis:

Qualitative test for protein

To identify the crude extract of (raw, ripened, dry) fruit samples of *Ficus carica* L.as protein, some qualitative test were carried out such as Ninhydrin test and Xanthoprotein test. This test gives color reactions on the basis of amino acid present in them. (Deb A C, 1996).

2.4. Caseinolytic assay:

Assay was carried out to determine the enzyme activity, by using casein as a substrate. 2% casein was prepared by using 1% sodium hydroxide solution. The reaction was carried out on 0.25ml of enzyme with 0.2ml of 2% casein. The reaction was stopped by adding 0.75ml of 0.44M trichloroacetic acid, incubated at room temperature for 30 minutes and centrifuged for 15 minutes at 1500rpm. Absorbance was measured at 660nm in UV-spectrophotometer. The enzyme concentration was found using tyrosin standard.

2.5. Purification:

Extracted enzyme was partially purified by using ion-exchange column chromatography, using NaCl as a solvent. Sample was loaded into column upon the pre-equilibrated resin of DEAE-cellulose. Purification was carried out in the presence of phosphate buffer (pH-6). The purified enzyme was collected in different fractions by adding solvent in the fractions of (0.2, 0.4, 0.6, 0.8,1.0 & 1.2M) of Nacl. Enzyme assay was performed for all the collected fractions.

2.6. Synthesis of silver nanoparticles:

The purified enzyme of raw, ripened, dry fig fruits which showed the highest enzyme concentration was used to prepare silver nanoparticles. 0.25ml of enzyme was taken and made up to 1ml using 0.01M silver nitrate solution. The mixture was incubated at room temperature for 24 hours for color change.

Analysis: Silver nanoparticles of ficain were analyzed by observing the color change from colorless to purple brown. Caseinolytic activity was performed after the synthesis of ficain silver nanoparticles.

2.7. Evaluation:

Evaluation of ficain was done by comparing the enzyme concentration of crude, purified and ficain silver nanoparticle of different fruits (raw, ripened, dry) of Ficus *carica* L.

ISSN No:-2456-2165

III. RESULTS AND DISCUSSION

Crude extract was obtained from all three fruit samples (raw, ripened and dry) of *Ficus carica* L. Qualitative analysis confirmed the presence of protein in the crude extract.

Qualitative analysis:

Ninhydrin and xanthoproteic test showed positive for all the three samples of fig fruit (raw, ripened, dry)

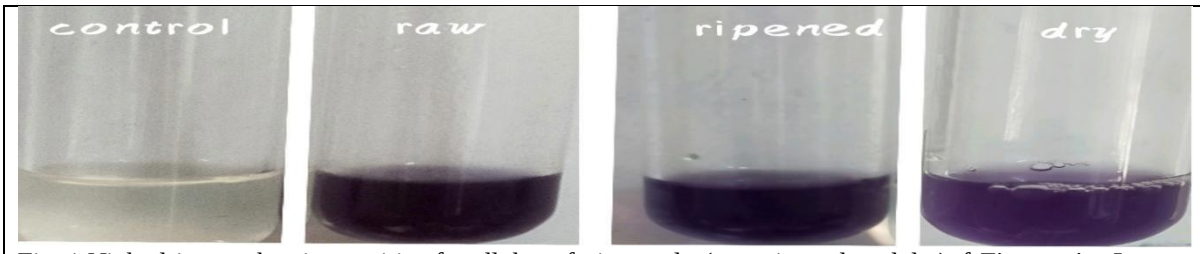


Fig. 1 Ninhydrin test showing positive for all three fruit samples(raw, ripened and dry)of *Ficuscarica* L.

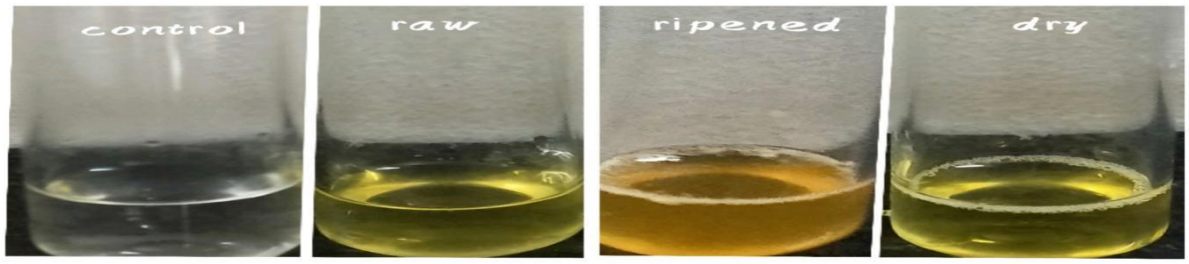


Figure. 2 Xanthoproteic test showing positive for all three fruit samples (raw, ripened and dry) of *Ficus carica* L.

Caseinolytic activity of crude extract

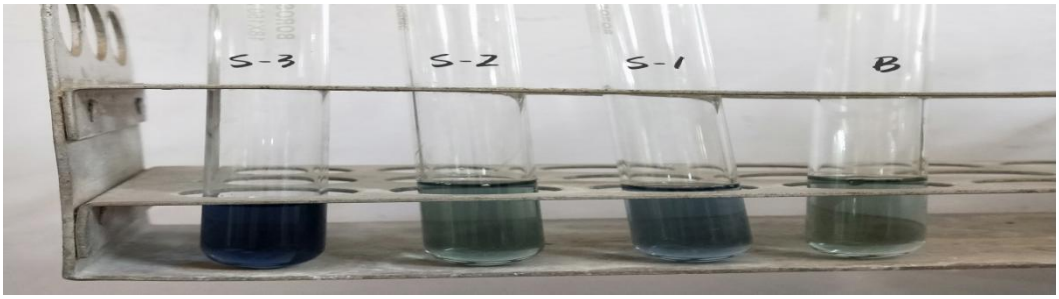


Fig. 3 Caseinolytic assay of crude extracts of S-1 raw, S-2 ripened, S-3 dry of *Ficus carica* L.

Table: 1 Enzyme assays and enzyme concentration of crude extracts of raw, ripened and dry.

Test tube number	Volume of casein (ml)	Volume of enzyme (ml)	TCA (ml)	Incubate at room temperature for 30minutes	Centrifuge for 15minutes at 1500rpm and take the supernatent	Sodium carbonate (ml)	FC reagent (ml)	Absorbance at 660nm	Enzyme concentration (micrograms/ml)
Blank	0.2	0.00	0.75			1.25	0.25	0.00	00
Raw	0.2	0.25	0.75			1.25	0.25	0.32	32
Ripened	0.2	0.25	0.75			1.25	0.25	0.18	20
Dry	0.2	0.25	0.75			1.25	0.25	1.09	120

Presence of ficain was confirmed by performing caseinolytic activity using casein as substrate. Since casein acts as substrate for ficain.

Ion exchange chromatography

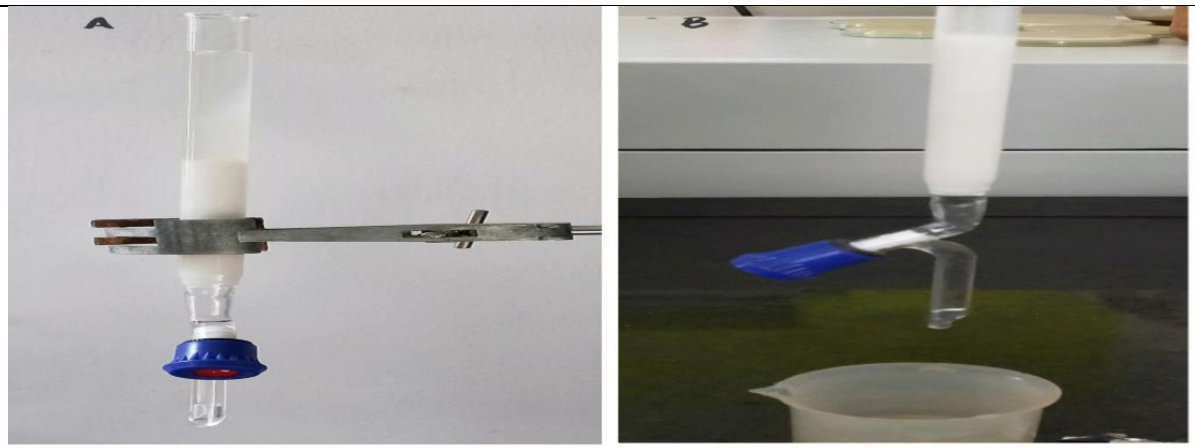
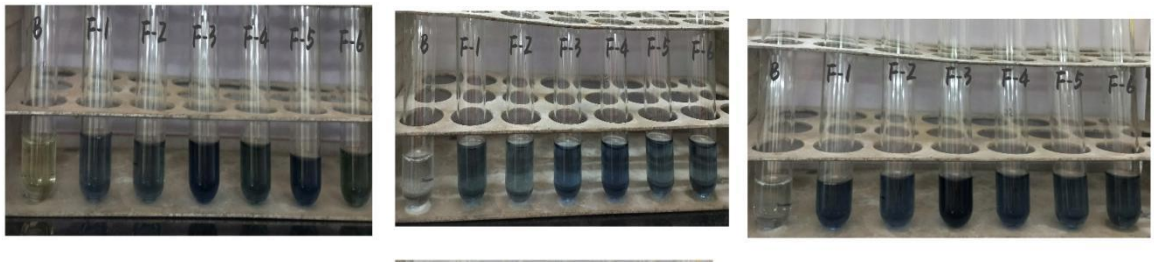


Fig. 4 (A) Column loaded with crude enzyme upon DEAE-cellulose matrix, (B) elution of purified enzyme using different molar con. of NaCl in fractions

Caseinolytic activity of purified ficain



(A) (B) (C)

Fig. 5 Caseinolytic assay of purified fractions of(A-Raw, B-Ripened, C-Dry) of *Ficus carica* L.

Table: 2 Elution and caseinolytic activity of ficain with different concentrations of NaCl from raw, ripened and dry.

Test tube number	Concentration of NaCl(M)	Volume of enzyme eluted (ml)	Absorbance of enzyme assay at 660nm(**raw**)	Absorbance of enzyme assay at 660nm(**ripened**)	Absorbance of enzyme assay at 660nm(**dry**)
Fraction 1	0.2	2	0.522	0.384	0.392
Fraction 2	0.4	2	0.461	0.248	0.398
Fraction 3	0.6	2	**1.398**	0.415	**0.932**
Fraction 4	0.8	2	0.511	**0.479**	0.560
Fraction 5	1.0	2	0.384	0.298	0.768
Fraction 6	1.2	2	0.381	0.320	0.374

Purified ficain enzyme was collected in different fractions using varied concentrations of NaCl.

Concentration of purified ficain

Table:3 Concentration of purified ficain of raw, ripened and dry.

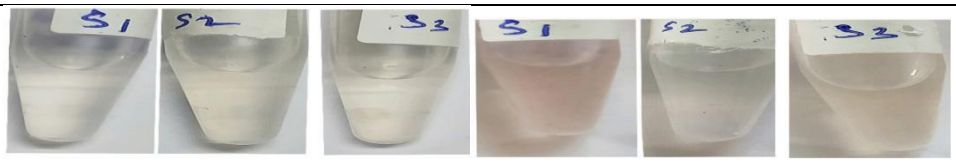
Samples	Purified enzyme(ml)	Absorbance at 660nm	Enzyme concentration (micrograms/ml)
Raw	**0.25**	1.398	**154**
Ripened	0.25	0.479	52
Dry	0.25	0.932	104

Fraction (3) of raw fig eluted with 0.6M NaCl, fraction (4) of ripened fig eluted with 0.8M NaCl and fraction (3) of dry fig eluted with 0.6M NaCl (Table: 2) showed highest enzyme activity and were selected for silver nanoparticle formation.

Synthesis of silver nanoparticles

Synthesis of ficain silver nanoparticle was confirmed by the formation of purple brown colour after 6hours of incubation at room temperature.

ISSN No:-2456-2165



(a) (b)

Fig. 6 (a) Tubes loaded with purified ficain and silver nitrate solution, before incubation. (b) Color change indicates the formation of silver nanoparticle. (S1-raw, S2-ripened, S3-dry)

Caseinolytic activity of ficain silver nanoparticles

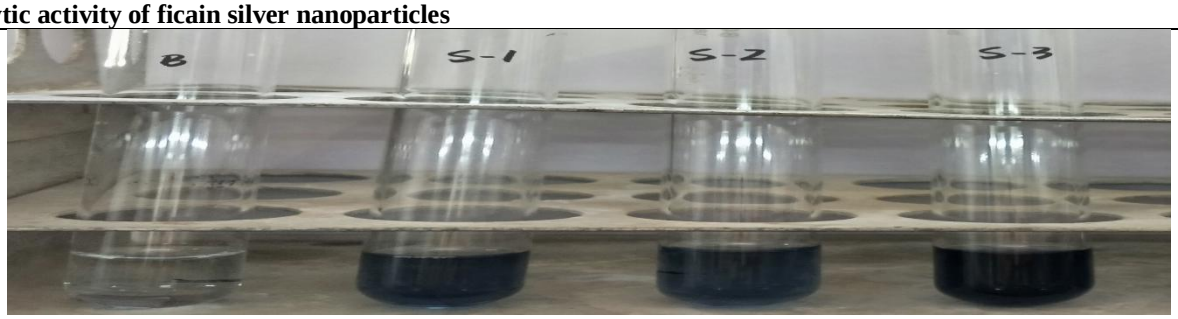


Fig.8 Caseinolytic assay of ficain silver nanoparticle S-1 raw, S-2 ripened, S-3 dry of *Ficus carica* L.

Concentration of ficain silver nanoparticles

Table:4 Enzyme assay of ficain silver nanoparticles of raw, ripened and dry.

Samples	Ficain nanoparticle(ml)	Absorbance at 660nm	Concentration of enzyme(micrograms/ml)
Raw	**0.25**	0.14	**38**
Ripened	0.25	0.05	6
Dry	0.25	0.33	16

The enzyme was active after the formation of silver nanoparticle.

Evaluation

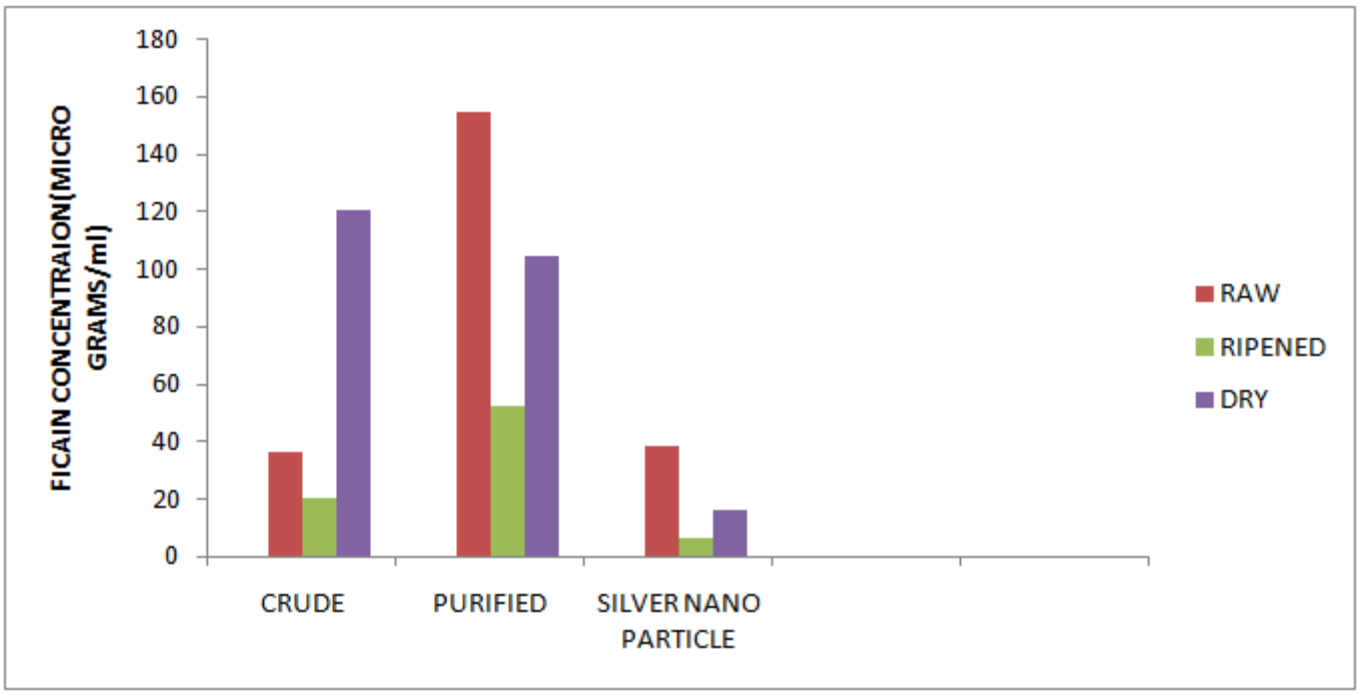


Fig 9: Graphical representation of ficain concentration between crude, purified and in nanoparticle form at different fruit stages of *Ficus species.*

Above graph represents the concentration of ficain varies in all the fruit samples at different stages(raw, ripened and dry). There was an increased concentration of ficain from crude to purified of raw and ripen whereas, ficain obtained from dry decreased. Similarly, the ficain concentration decreased when it is in silver nanoparticle form. The concentration of ficain was found through caseinolytic assay using Tyrosine standard. When the ficain was in crude form the concentration was found to be high in dry sample whereas when it is purified the ficain concentration was found to be high in raw sample. When ficain was in silver nanoparticle form the concentration was found be high in raw sample.

IV. CONCLUSION

Presence of proteolytic activity was detected in fruit samples (raw, ripened, dry) of *Ficus carica* L. The concentrations of ficain varied in all three fruit stages. There was an increase in the concentration of ficain extracted from fruits samples compared to latex which was previously reported. The ficain was active in all the three forms (crude, purified and in nanoparticle). The highest concentration of ficain was obtained in purified raw fig i.e. 154 micrograms/ml. It was observed that the concentration of ficain decreased after the formation of nanoparticles. Ficain is a cystein protease and can be widely used in food industry and health care.

REFERENCES

[1]. Bandana Chatterjee & Anshuman Sharma. Fruit Enzyme and their application:A review. *International Journal of Clinical & Biomedical Research*,**4**(2): 84-88 (2018).
[2]. Brankia Raskovic, et al. Identification, purification and characterization of a novel collagenolytic serine protease from fig (*Ficus carica* var. Brown Turkey) latex. *J Biosci Bioeng*. **118**(6): 622-7 (2014 Dec).
[3]. Donald E. Kramer and John .R Whitaker. Multiple Molecular Forms of Ficin- Evidence Against Autolysis as Explanation[1,2]. *Plant physiol*,**44**(11); 1560-1565 (1969 Nov).
[4]. D. Chetia[1], L.K.Nath[1], & S.K. Dutta[2] , Extraction, Purification and physio chemical Properties of A proteolytic Enzyme from The Latex of *Ficus Hispida* Linn. *Indian J-Pharm. Science*,**61**(1) 29-33 (1999).
[5]. H.P, Borase[1], C.D. Patil[1], et al. Mosquito, larivicidal andsilver nanoparticles synthesis potential of plant latex. *Journal of entomological & Acarological Research*, 46: 1920 (2014).
[6]. K.M. De Angelis. Phosphate Buffer (2007).
[7]. Mario Perello, Maria Cecilia Arribere, et al. ProteolyticEnzymes from the latex of *Ficus pumila* L. (Moraceae). *Acta Farm Bonaerense* **19**(4): 257-62 (2000).
[8]. Melisa l Barolo et al.*Ficuscarica* L.(Moraceae):an ancient sources of food and health. *Food Chem*, 1;164: 119-27 (2014 Dec).
[9]. Mohammed Gagaoua, et al. *Separation & Purification Technology*.**132**(2): 461-467 (2014 August).
[10]. Muthuswamy Chandrashekaran et al. Enzymes in food and beverage production:An overview.*Enzymes in food & Beverage Processing*,pp.117-137 (2015 Oct).
[11]. N Nyoman Rupiasih, etal. Green synthesis of silver nanoparticles using latex extract of Thevetia peruviana :a noval approach towards Poisonous plant utilization.*Journal of physics: conference series*.**423,** 012032(2013).
[12]. Rajmund Michalski,Ion chromatography applications in wastewater analysis.*Separation*. 5, 16 (2018 Feb).
[13]. Ram Prasad, Synthesis of silver nanoparticles in photosynthic plants. *Journal of Nanoparticles*. 963961 (2014).
[14]. Satish V. Patil, et al. Biosynthesisof silver nanoparticles using latex from few Euphorbian plants and their antimicrobial potential. *Appl Biochem Biotechnology*. **167**(4) : 776-90 (2012 June).
[15]. Tao Cai, et al. Extraction and process condition optimization of ficain by ionic liquid aqueous two phase system. (2015).
[16]. W. M. Salem, et al. Antimicrobial activity of silver nanoparticles synthesized from latex and leaf extracts of Ficus Sycomous. *Industrial Crops & Products*. **62**, 228-234 (2014).