International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494

Effect of Isolated Compounds of Smilax China Rhizome on Intestinal Enzyme Activity

Apada Reddy Gangadasu[1*], Rakesh Jat[2]
[1*]*Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan – 333001*
[2]*Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan – 333001*

Submitted: 20-09-2022 Accepted: 30-09-2022

ABSTRACT
Smilax china, also known as Smilacaceae, is a family of plants that is used medicinally and is a naturally occurring substance beneficial to human health. Natural product Smilax china, which was gifted to us by Mother Nature, has been documented as having a wide range of biological activity. This is due to the fact that it contains enriched bioactive molecules that have a diverged chemical nature from hit molecules. In the current study, the ability of the Smilax china (SC) extracts and isolated compounds on intestina enzyme activity was investigated. The proteolytic action of plant chemicals on the gelatin protein results in the formation of enzymatic activities zones upon gelatin plates. After the addition of protein, there was a discernible narrowing of the zone, which indicated that enzyme activity had been suppressed. An enzyme mixture consisting of trypsin, papain, pepsin, and -chymotrypsin was used to incubate plant material in the appropriate buffers before subjecting it to an assay with a gelatin disc diffusing plate. Positive controls in the form of enzymes were used in this experiment, and the zones of activity were compared in order to evaluate the enzyme-inhibiting potential of the compounds. As the concentration of the compound increased, the area of the clearance zone decreased for all papain and chymotrypsin enzymes. This was in relation to the amount of enzyme that was fixed at 5 micrograms. During the course of this research, the proteolytic and esterolytic activities of the SC, SC-I, and SC-II plant extracts were analysed for their levels of activity.

I. INTRODUCTION

Since ancient times, people have been looking for medications that can treat their diseases that can be found in nature. The use of medicinal plants began unconsciously, just as the use of medicinal plants occurs instinctually with animals. Everything was based on experience at the time because there was insufficient information about the causes of the ailments or about which plant could be used as a cure and how it could be used because there was a lack of both types of information. The use of medicinal plants shifted away from an empiric framework or toward one that is based on explicatory facts as the reasons for the use of various medicinal plants for the treatment of specific ailments were identified over the course of time. Before the discovery of iatrochemistry in the 16th century, treatments and preventative measures were derived from the components of plants. The ability of pharmacists and physicians to respond to the challenges that have arisen with the spread of professional services in the facilitation of man's life has increased as knowledge of the development of ideas related to the use of medicinal plants as well as the evolution of awareness has increased. This is because the pharmacists and physicians have become more aware of the evolution of ideas related to the use of medicinal plants.

METHOD OF ISOLATION

ISOLATION OF COMPOUNDS:

PREPARATION OF EXTRACT:

10 gm of course powder extracted with 100 ml of methanol by hot continuous extract method. The extract is filter and dried under vacuum. Column chromatography was used to separate components in dried extract.

Column Chromatography Separation:

Class column chromatography packed with silica for column chromatography up to one third and dried 2 g of extract packed in column and covered with filter paper to avoid disturbance. Column run with 2000 ml of pure HPLC grade methanol and collected 30 fraction of each 50 ml in breaker and 10 fraction of each 50 ml first fraction yellow in colour and combined as single fraction and purity checked by TLC. Second fraction combined as single solution purity checked by TLC. Fraction 1 and 2 concentrated by heating and used for further purification by preparative TLC. Preparative TLC run by using 100 % methanol in

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494

20 x 20 cm plate and collected fraction washed with methanol and dried under vacuum.

SPECTROSCOPICAL ANALYSIS

When it comes to study of how electromagnetic radiation interacts using materials, spectroscopy is appropriate scientific field to turn to. Molecular & Atomic composition may be studied in great detail using spectroscopy, most powerful method currently accessible. More advanced techniques, such as U.V, F.T-I.R, L.C-M.S(Mass spectroscopy, were used to analyze SC extract with extracted chemicals).

UV ANALYSIS

UV spectroscopic analysis had been carried out according to process proposed by Pramila et.al, (2012); Kalaichelvi&Dhivya (2017) and Jain et al., (2016).

UV-visible spectrophotometer (Shimadzhu 1800 series)

Preparation of sample

1. Blank - methanol (1000 µl)
2. Sample

(i) SC ME I (100 µl+1000 µl methanol)
(ii) SC ME II (100 µl+1000 µl methanol)
(iii) Crude material (Crude + 1000 µl methanol)

FT-IR ANALYSIS

FT-IR analysis was performed as per the procedure proposed by Karpagasundari&Kulothungan (2014); Kalaichelvi&Dhivya (2017) and Jain et al., (2016). The F.T-I.R spectra have been documented upon "Shimadzu I.R.TRACER 100F.T-I.R spectrometer-Spectrum". Solids were recorded as KBr pellets and liquids as $C.H.C.l_3$ solution or thin film. Infrared radiation absorption is focus of FT-IR spectroscopy. Data for molecular vibrations and, most specifically, on the transition amongst rotational and vibrational energy levels, is provided by FT-IR spectroscopy. FT-IR spectroscopy is amongst prevailing methods offering possibility of chemical recognition. The optimal region for FT-IR spectroscopy is at 2.5-15 µ.

LC-MS ANALYSIS

LC-MS analysis had been carried out according to process proposed by Florina et al., (2013) and Jain et al., (2016). A.P.I-2000 Applied Biosystem mass spectrometer (Canada) annexation having E.S.I (Electrospray ionisation) source connected containing H.P.L.C system (Shimadzu, Kyoto, Japan) having of binary L.C 20.A series, column oven, auto sampler (S.I.L-H.Tc), gradient pump with solvent degasser. Parting has been accomplished using a reverse phase column (Hiber 250 x 4.6mm, Pure sphere S.T.A.R R.P C.18, 5µparticle size, Merck, Germany) and elution in a linear gradient with mobile phase A of 0.1 percent formic acid into water & a mobile phase B of Acetonitrile (95:5 percent v/v) under isocratic conditions. The column temperature oven has been set at 40°C, while auto sample kept at 20°C and injection volume is 10µl. Separation was obtained later on a 10µl injection utilizing a gradient program that began with 95 percent A mobile phase and 5 percent B mobile phase for 0.5 minutes before shifting to 50 percent mobile phase A within 8.5 minutes. This gradient was held constant for 4.6 minutes before being reset for 3 minutes. Lastly, mobile stage A has been raised till 95% within 0.5 min & sustained at that level till 11.5 min until the end of the experiment. Having rate of flow 0.3 ml/min 130, entire run duration has been 20 minutes.

SAMPLES AND STANDARD PREPARATION

SC extracts and standards Quercetin & Rutin were submitted for L.C-M.S (LC M.S: Agilent Model: 6530 LC/Q–TOF) for this qualitative analytical investigation. Quercetin & Rutin standard primary stock solutions was produced distinctly with methanol for obtaining necessary concentration of 0.5 mg/ml. Serial diluting of primary stock solution with methanol was used to make a working standard solution. In methanol, a stock solution of quercetin and SC extracts was produced at a concentration of 10 mg/ mL. Every sample was prepared & filtered using a 0.45 nylon filter (Spinco Biotech, India) before being transmitted in an auto sampler vial for qualitative LC-M.S analysis. Concentration of every constituent were expressed into μg/mg. Mobile phase pump A containing of buffer (0.1% formic acid into water) & pump B containing of organic solvent (Acetonitrile), Runtime set to 20 min, injection volume as 1µl. The carrier gas utilised was Nitrogen 50 psi pressure & 0.3 ml /min 130.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^1H N.M.R &^{13}C N.M.R spectra was authenticated upon "Jeol JNM M.Y.60 F.T.F.T.N.M.R System" 60 MHz or 300 MHz.^{13}C &^1H N.M.R have been noted in Varian 300 M.Hz instrument. Chemical shift values are expressed into δunits relatively for tetramethylsilane (T.M.S) signal as internal reference standard in $C.C.l_4$ or $C.D.C.l_3$ or D.M.S.O.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494

CHEMICAL SHIFT

The changes in NMR signals (relative to standard reference) caused by electron shielding & de-shielding are known as chemical shifts.

Molecular electrons generate secondary magnetic fields that cause chemical shifts. The constant magnetic field induces what are known as "local diamagnetic currents," which produce a secondary field which might potentially lower or augment the field to which a particular proton reacts, depending on their strength. Relative to aggregate reference resonant frequencies, chemical shift is recorded. (^{1}H, ^{13}C). TMS is commonly used as reference.

In order to calculate chemical shift, such discrepancy among signal and reference frequencies is divided by frequencies of reference signals. As fundamental NMR frequencies is often 100s of MHz, frequency shifts (typically 100s of Hz) are typically reported using ppm.

^{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Naturally abundance of isotope ^{13}C is just 1.10%. Downfield from TMS, ^{13}C resonance occurs in spectrum of 0-200 ppm, with one signal for each carbon type. There is 1H NMR coupling among both signals of neighboring atoms in 13C NMR spectra, however this coupling is weaker. Because of interaction between carbon and proton, this may be utilized to identify spectrum of the signal.

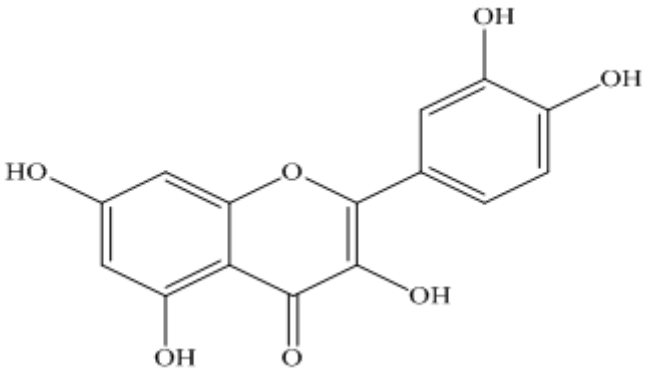


Figure 16: Compound A: Quercetin

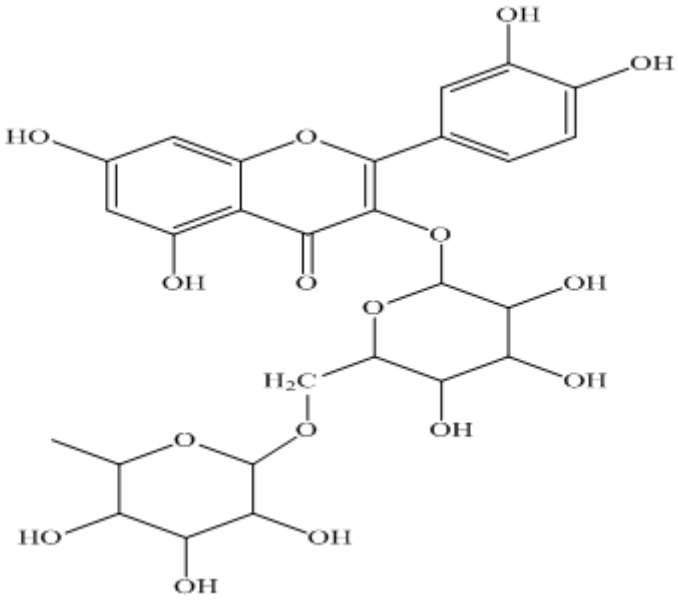


Figure 17: Compound B: Rutin

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494

II. RESULTS

Effect Of Isolated Plant Compounds On Intestinal Enzyme Activity

The proteolytic action of plant chemicals on the gelatin protein results in the formation of enzymatic activities zones upon gelatin plates. After adding protein, width of the zone shrank, indicating that enzyme activity was inhibited. Plant substance was incubated by trypsin, papain, pepsin, & -chymotrypsin enzyme into appropriate buffers and then tested using a gelatin disc diffusing plate assay. Enzymes were utilized as positive controls, and the zones of activity were compared to determine the compounds' enzyme inhibitory activity. The area of clearance zone of all papain & -chymotrypsin enzyme was reduced as compound concentration increased in relation for fixing quantity of enzyme (5µg) (Vikram et al., 2021).

Preparation Of In Vitro Plant Compounds Digests With Different Enzymes

To imitate the digestive actions of different parts of the gastrointestinal tract in vitro, assortment of fluids having singular or numerous enzymes have been created. The antioxidative efficiency of several enzyme digests was tested utilising animal & plant digestion enzymes. Papain and -chymotrypsin enzymes were utilised to hydrolyze the proteins separately to replicate in vitro stomach digesting. 5 mg lyophilized powder of Smilax china stem extraction was treated into 1ml phosphate buffer for papain digestion (50 mM; pH 6.8) (Vikram et al., 2021).

PAPAIN HYDROLYSATES

Papain digestion were carried out by dissolving 1.5 mg of precipitating proteins in callus tissue in 1 ml of 50 mM phosphate buffer (pH 6.8). Activating papain was accomplished by adding 4 mg enzyme, 20 mM cysteine, & 50mM E.D.T.A to 20 ml of phosphate buffer (p.H6.8). After dissolving 50 mg of papain in 250µl of phosphate buffer & treating it by protein solution, enzyme/substrate proportions were 1:100, having 1500U papain / mg protein. Using an orbital shaker, digestion mixture was shook about 120 min at 37°C before reaction liquid was boiled for 6 min to irreversibly denature papain enzyme. (Vikram et al., 2021).

A-CHYMOTRYPSIN HYDROLYSATES

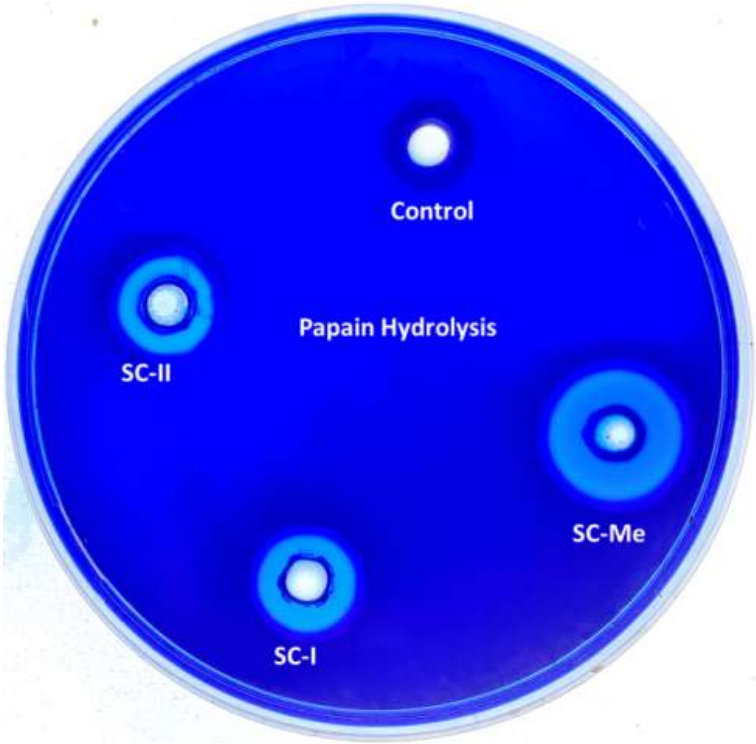
Proteins were hydrolyzed with help of trypsin and α-chymotrypsin enzymes. To get final concentration of 5 mg/ml, precipitated proteins using callus tissue have been produced in Tris-HCl buffers of pH 7.8 & 8.0, accordingly. Protein solutions with Tris-HCl buffer at pH 7.8 or 8.0 were treated with α-chymotrypsin & porcine trypsin enzyme solutions to give 8U of bovine -chymotrypsin & 2000U of porcine trypsin / mg of protein, correspondingly. (Karthikeyan, Shanthi &Nagasathaya, 2009 and Moonmun et al., 2017). When 5 mg of protein were incubated using 1 mg of enzyme, 1:5 (w/w) enzyme/substrate ratio was obtained for α -chymotrypsin. For further analysis, digestions were performed in a 37°C shaking incubator, and aliquots (100µl) were obtained after 180 minutes. In order to halt digestion process, digestion mixtures were boiled at 100°C for six minutes to permanently denature -chymotrypsin & trypsin present. (Vikram et al., 2021).

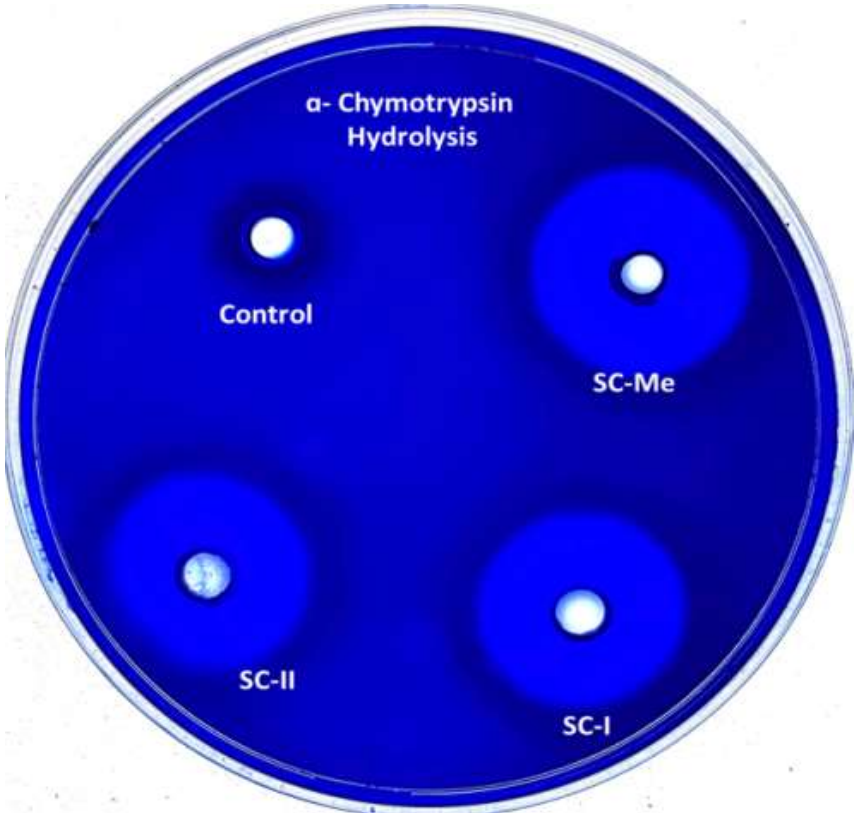
Enzymatic Hydrolysis Assay (Papain Hydrolysates & A-Chymotrypsin Hydrolysates

The enzyme hydrolysis of α-chymotrypsin and papain is a protease with proteolytic as well as esterolytic activity of the SC, SC-I, SC-II plant extracts samples in the cellular activity. Besides processes based on stereospecific hydrolysis, α-chymotrypsin and papain has been used for the synthesis of peptides during the metabolic activity. In this conclusion of SC, SC-I, SC-II is very active compounds was found in the samples for both enzyme were hydrolysis.

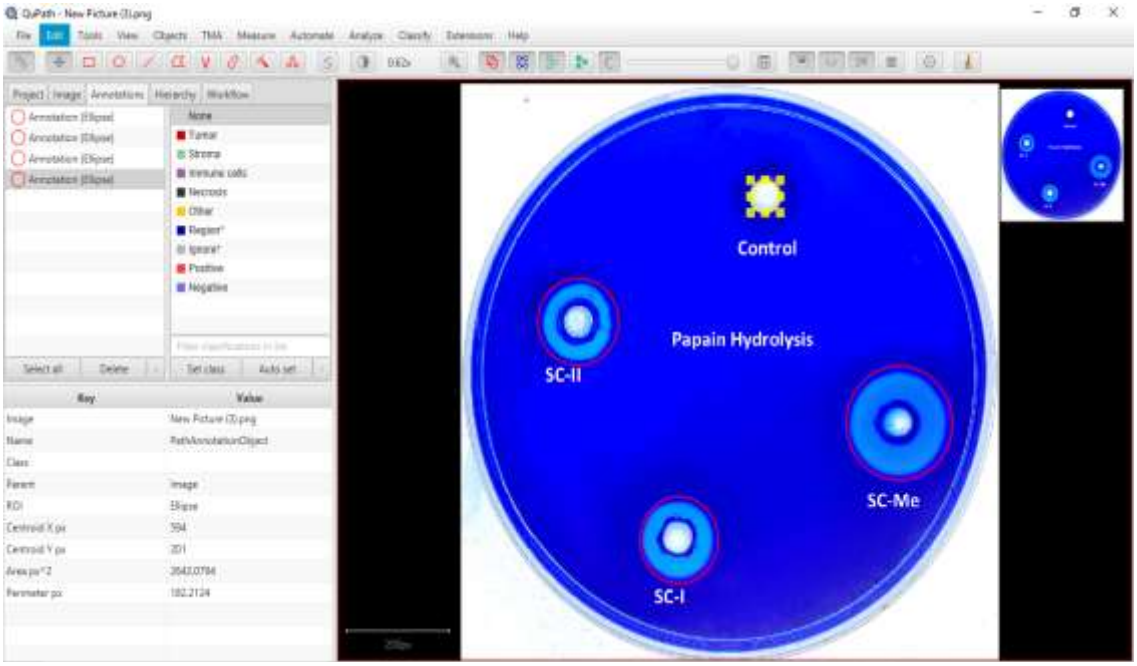
International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494

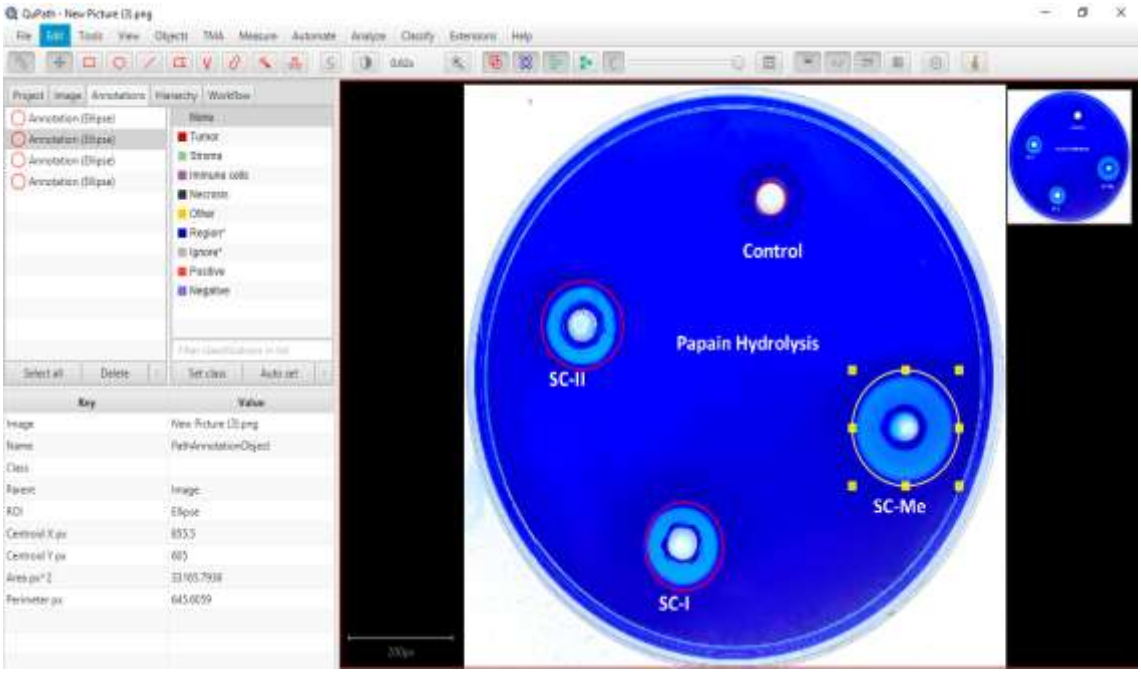
Figures: Photographs showing enzymatic hydrolysis assay of SC, SC-I, SC-II plant extracts



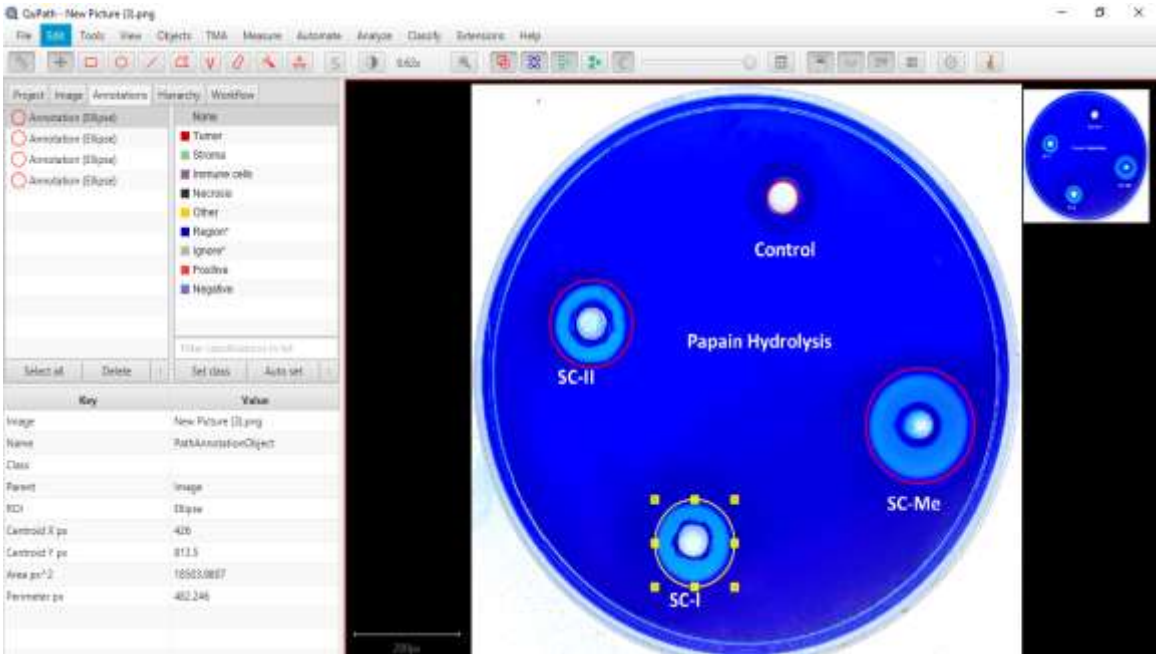


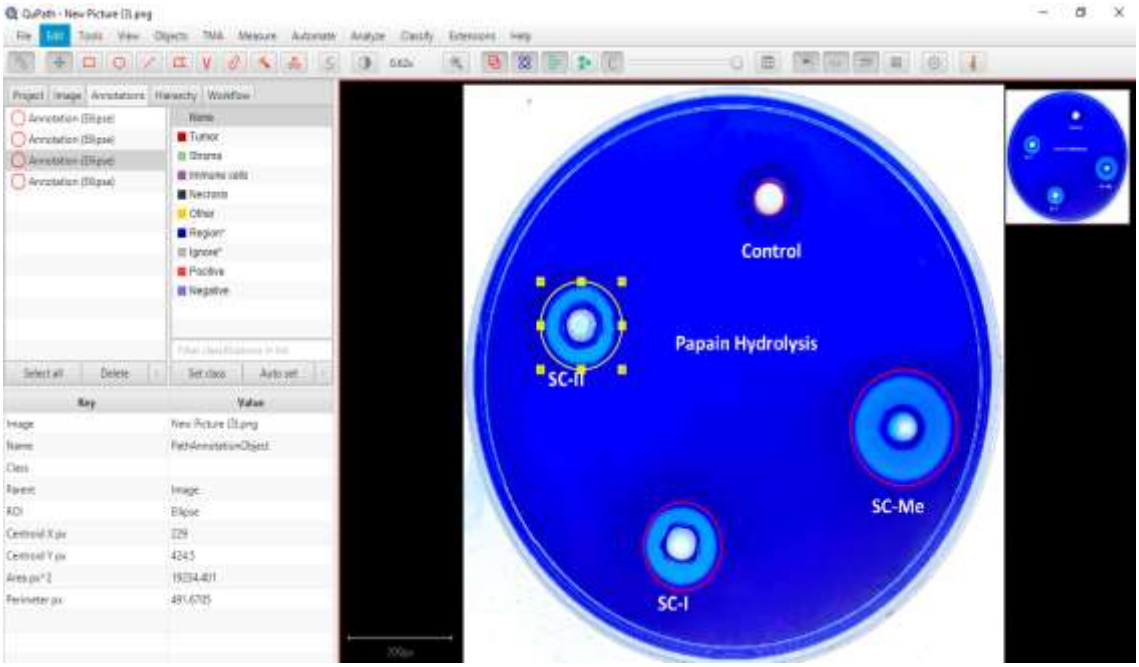
International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494



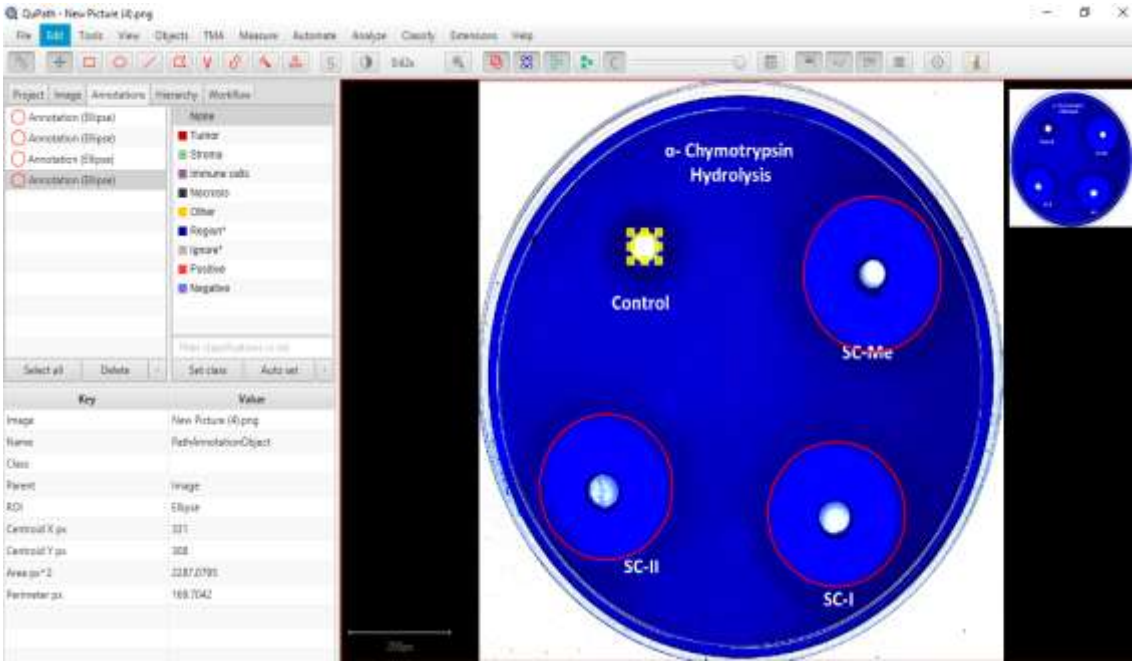


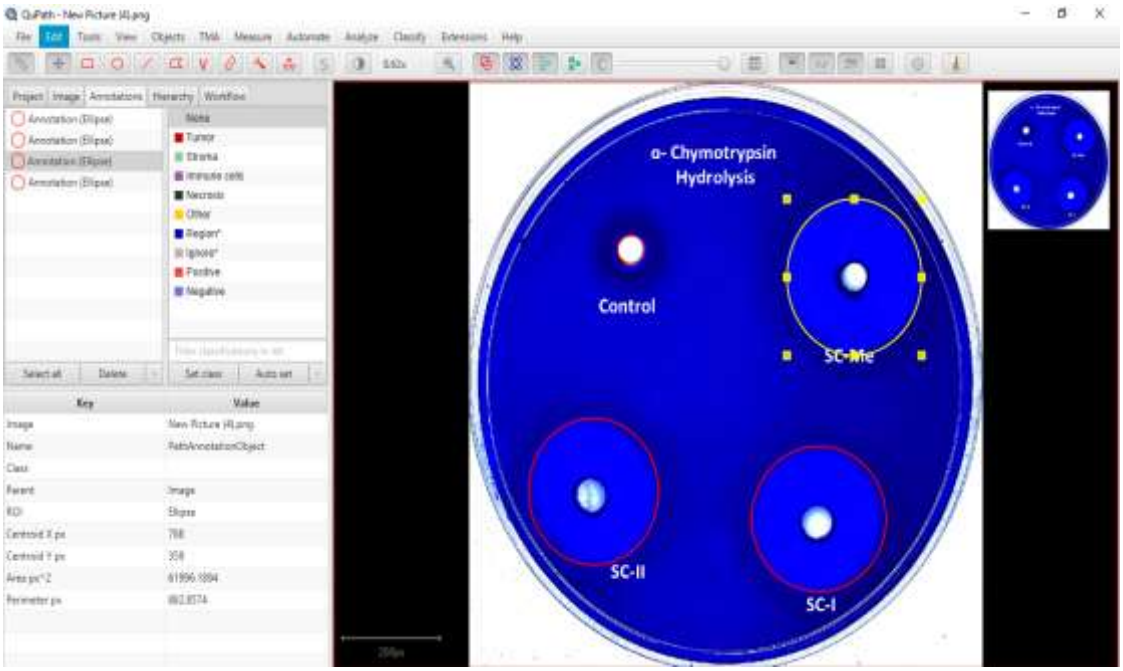
International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494



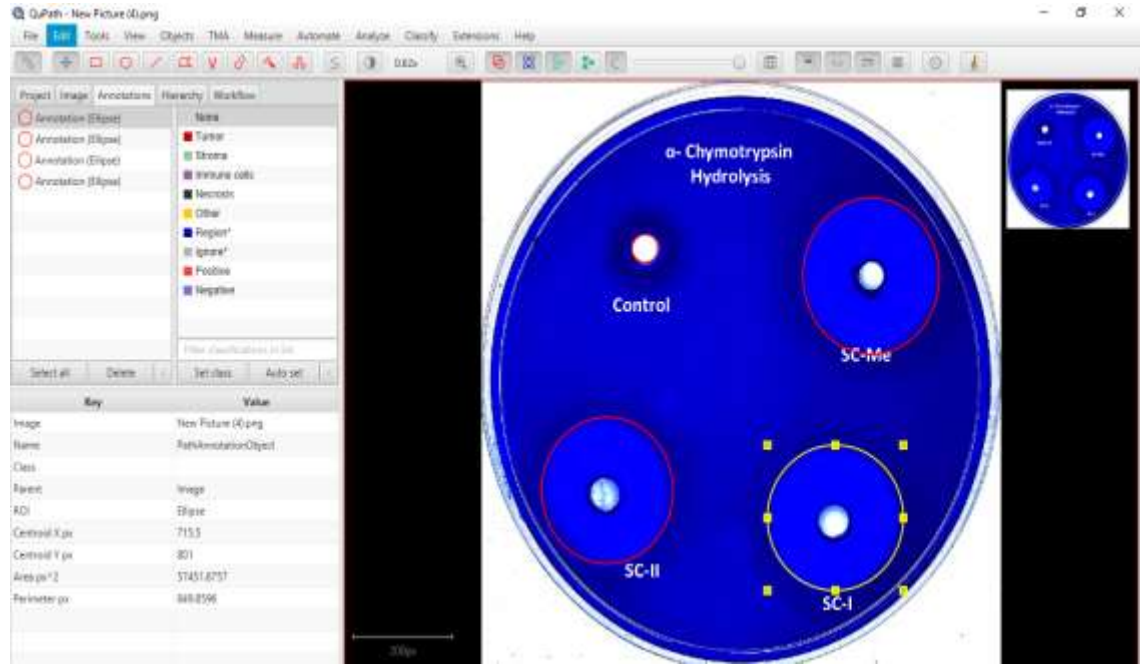


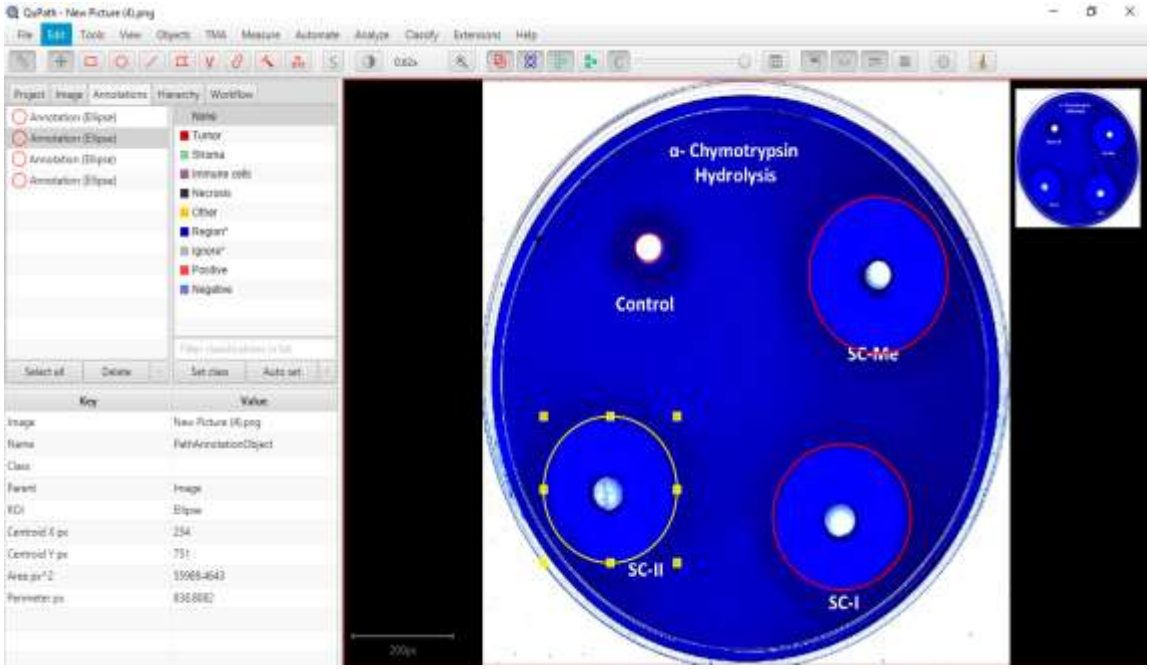
International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494





International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494





III. DISCUSSION

The proteolytic action of plant chemicals on the gelatin protein results in the formation of enzymatic activity zones upon gelatin plates, as shown by the isolated phytochemicals of the smilax china extract. After the addition of protein, there was a discernible narrowing of the zone, which suggested that the isolated compounds had an inhibitory effect on the enzyme activity.

IV. CONCLUSION

Proteolytic effect of plant chemicals on gelatin protein leads in the creation of enzymatic activity zones on gelatin plates, as demonstrated by the separated phytochemicals of smilax china extract. The addition of protein caused a reduction in the zone's breadth, showing that the separated chemicals had suppressed enzyme activity.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 811-820 www.ijprajournal.com ISSN: 2456-4494

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgements

The authors acknowledge "This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors."

REFERENCES

[1]. Arif M, Fareed S. (2011). Pharmacognostical and preliminary phytochemical analysis of Carissa carandas fruits. Journal of Medicinal and Aromatic Plant Sciences; 33 (1): 53-58.

[2]. Bruce So, Onyegbule FA and Ezugwu Co (2019). Pharmacognostic, physicochemical and phytochemical evaluation of the leaves of FadogiaclenkowskiSchweinf (Rubiaceae). J of Pharma and Phytothe; 11(3): 52-60

[3]. Cheng Z, Deng H, Lian-Bing H, Lu-yao S, Ying-Jun Z and Yang X (2017). Phenolic compounds from the rhizomes of Smilax china L. and their anti inflammatory activity. Molecules; 22: 515.

[4]. Cheng Zhong et al., (2017). Molecules. 22, 515; doi:10.3390/molecules22040515

[5]. Feng F, Liu WY, Chen YS, Liu JH, Zhao SX (2003). Flavonoids and stilbenes from Smilax China. Journal of China Pharmaceutical University 34(2):119-121.

[6]. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. (2015). GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of Evolvulusalsinoides (L.) L. Journal of Food Science Technology; 52 (2): 1212-1217.

[7]. Huang, H.L., R.H. Liu and F. Shao, (2009). Structural determination of two new steroidal saponins from Smilax china. Magn. Resonance Chem., 47: 741-745

[8]. Kelly K. (2009). History of medicine. New York: Facts on file. pp. 29–50.

[9]. Mahendra Jain, Rakhee Kapadia, Susy Albert & Shri Hari Mishra (2011). Standardization of Feronia limonia L. leaves by HPLC, HPTLC, physico-chemical and histological parameters. BoletínLatinoamericano y del Caribe de PlantasMedicinales y Aromáticas, 10: 525-535.

[10]. Sheila John, Priyadarshini S, Sarah Jane Monica, Arumugam P. (2017a). Phytochemical profile and thin layer chromatographic studies of Daucus Carota peel extracts. Inter J Food Sci Nutri.; 2(1); 23–26.

[11]. Stojanoski N. (1999). Development of health culture in veles and its region from the past to the end of the 20th century. Veles: Society of science and art. 13–34.

[12]. Tinospora cordifolia (willd.) miers exposed to cyclodextrin, IJPSR; Vol. 12(1): 524-535.

[13]. Vikram V., Ram Kishore, A. Kalaiyarasan and N. Hariram (2021). Protein profiling and isolation of bioactive hydrolysates with antioxidant activity from stem callus tissue of