SKAM 33
2021 | ANALIS-UM

IIUM KUANTAN
DEPARTMENT OF CHEMISTRY
KULLIYYAH OF SCIENCE

2-Acetylpyrazine Thiosemicarbazone as Multifunctional Food Spoilage Inhibitor: Insights from Tyrosinase Kinetic, Microbial Activity and Computational Approaches

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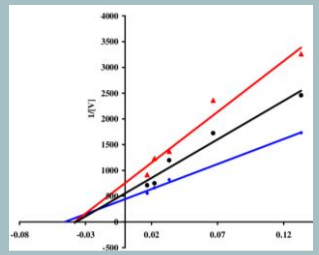
INTRODUCTION

Food spoilage

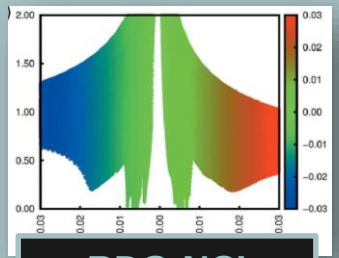
Synthesis

Antimicrobial

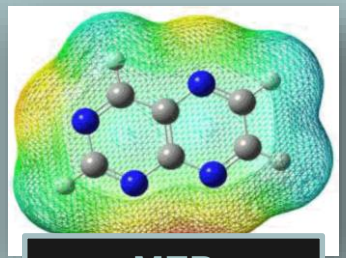


Antityrosinase

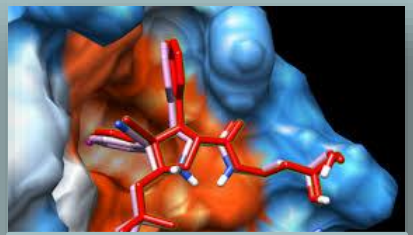
Computational Chemistry



RDG-NCI



MEP



Molecular Docking

PROBLEM STATEMENT

Spoiled food

Biologically and chemically contaminated

Change texture, bad odour, poor taste

Food become toxic and poisonous

FOOD POISONING

AFFECTS 1 OUT OF 6 PEOPLE every year

Better and safer food

RESEARCH OBJECTIVES

01

To synthesis multi-functional food additives.

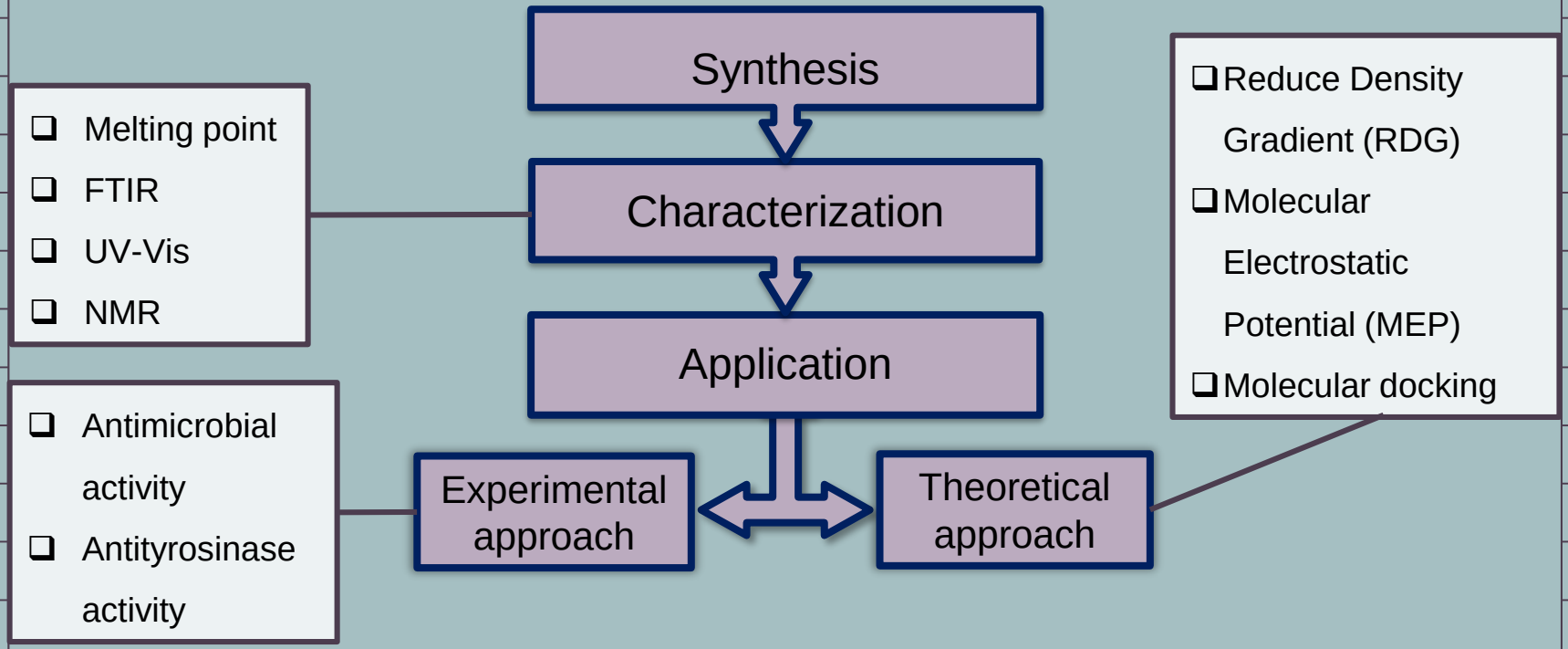
02

To study the potential compounds as food spoilage inhibitor using antimicrobial and antityrosinase experiment.

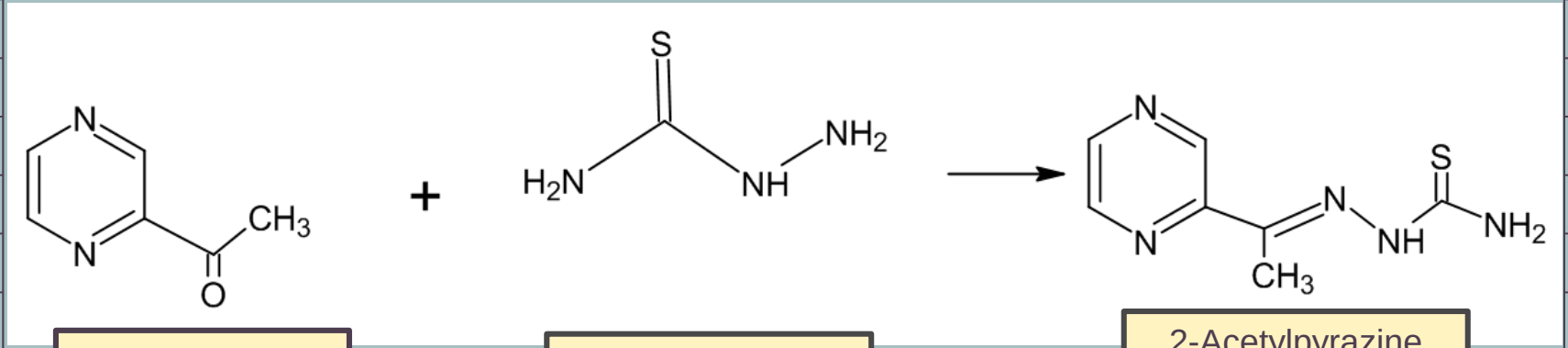
03

To investigate the interaction mechanism of compound by computational approach through RDG-NCI, MEP and molecular docking.

METHODOLOGY



Synthesis of 2-Acetylpyrazine Thiosemicarbazone (2APT)



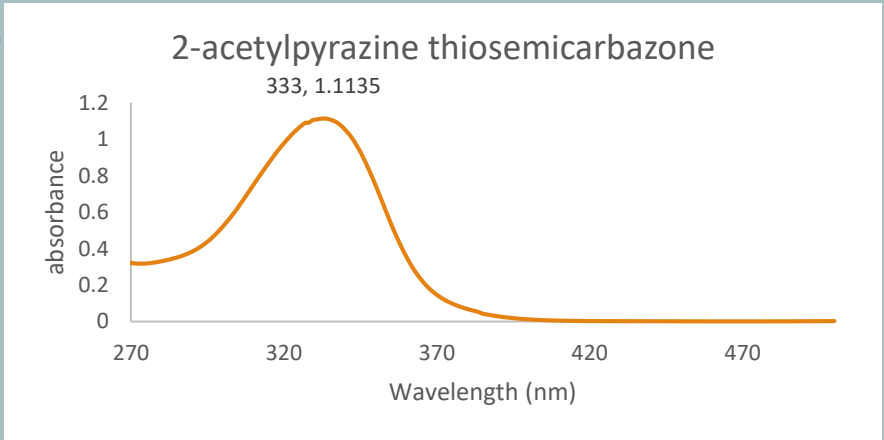
2-Acetylpyrazine

Thiosemicarbazide

2-Acetylpyrazine Thiosemicarbazone

Characterization of 2APT

UV-Vis



MP

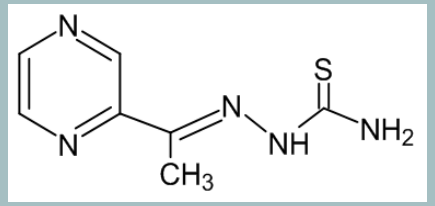
222℃ - 223℃

CHNS

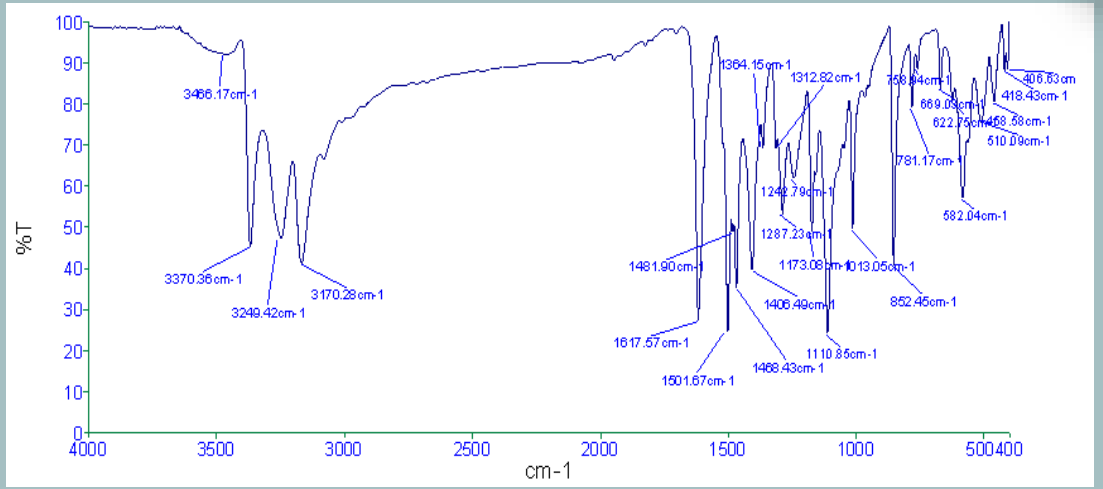
2APT	C	H	N	S
ELEMENTAL ANALYSIS CALCULATION (%)	43.06	4.65	35.87	16.42
FOUND (%)	43.18	4.78	35.54	16.50

FTIR

Characterization of 2APT



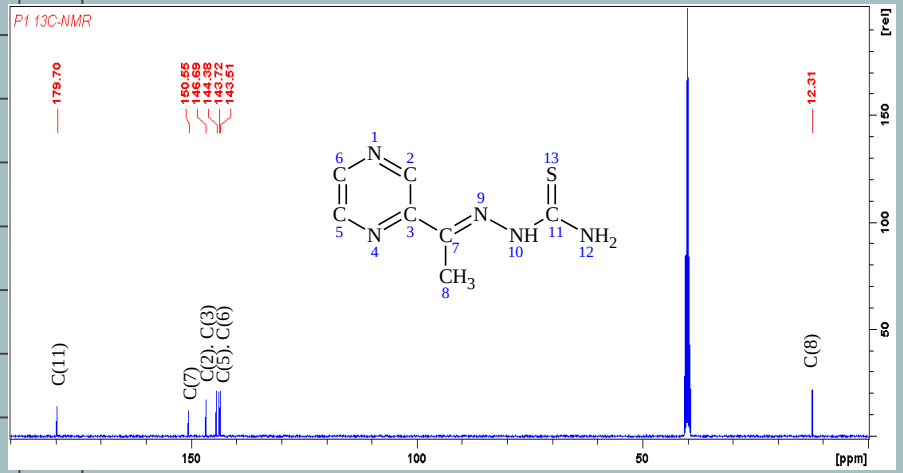
2APT



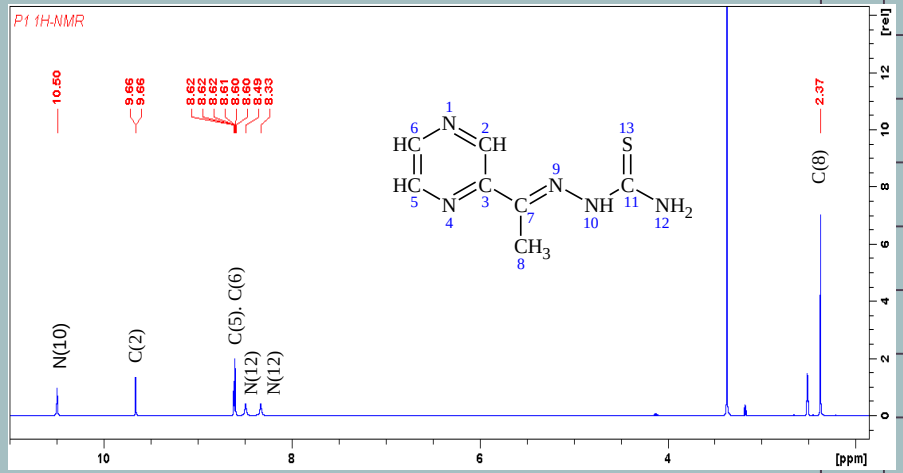
No.	Vibrations	2APT	2APT - Li et al. (2007)
1.	v(N–H)	3249 and 3370 cm^{-1}	3283 and 3410 cm^{-1}
2.	v(N-H_2)	3170 cm^{-1}	3118 cm^{-1}
3.	v(C=N)	1617 cm^{-1}	1595 cm^{-1}
3.	v(C=S)	852 cm^{-1}	850 cm^{-1}

Characterization of 2APT

^{13}C-NMR

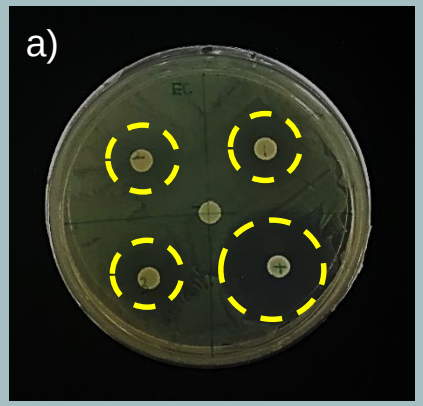


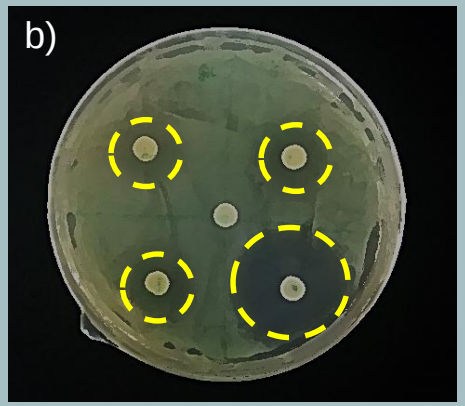
^{1}H-NMR

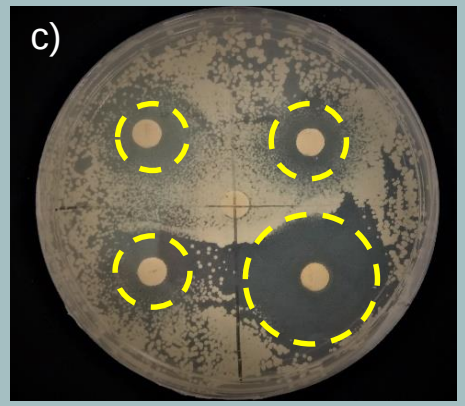


Experimental Section

Antimicrobial Activity (Disc diffusion method)







Bacteria/fungi	a) ***E. coli***	b) ***B. cereus***	c) ***C. albicans***
Zone of inhibition 2APT	1.4 ± 0.1 cm	1.6 ± 0.1 cm	1.2 ± 0.1 cm
Positive control (Streptomycin/ Nystatin)	3.00 ± 0.12 cm		

Enzyme Assay (Tyrosinase)

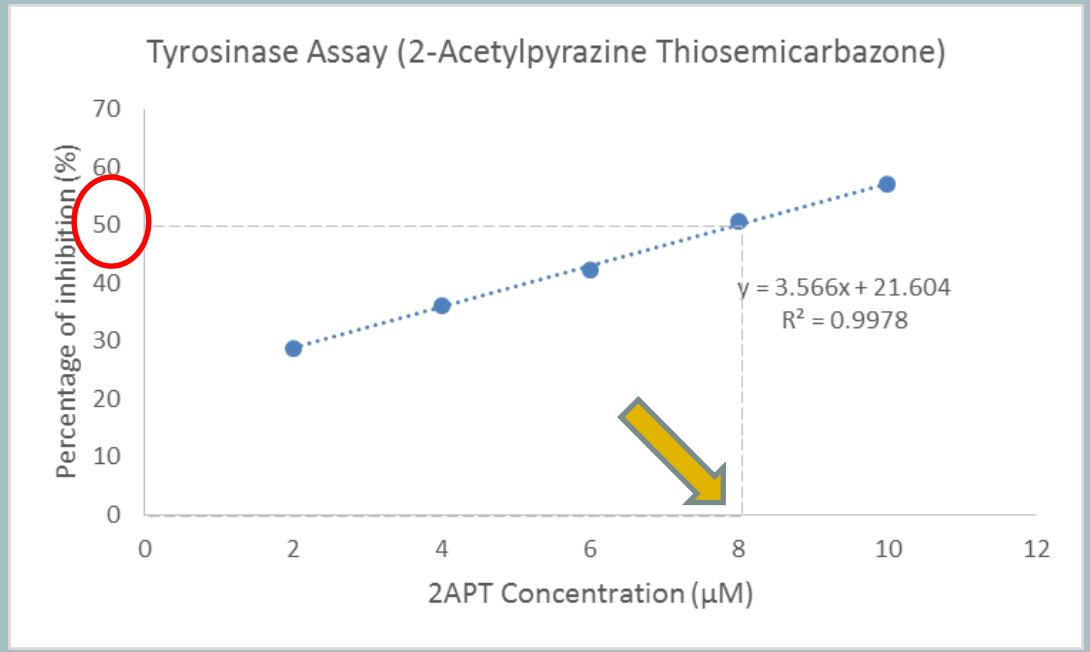


Figure 3 Enzyme assay

Control:
Kojic acid
10 µM
8.68 %

Enzyme Kinetic

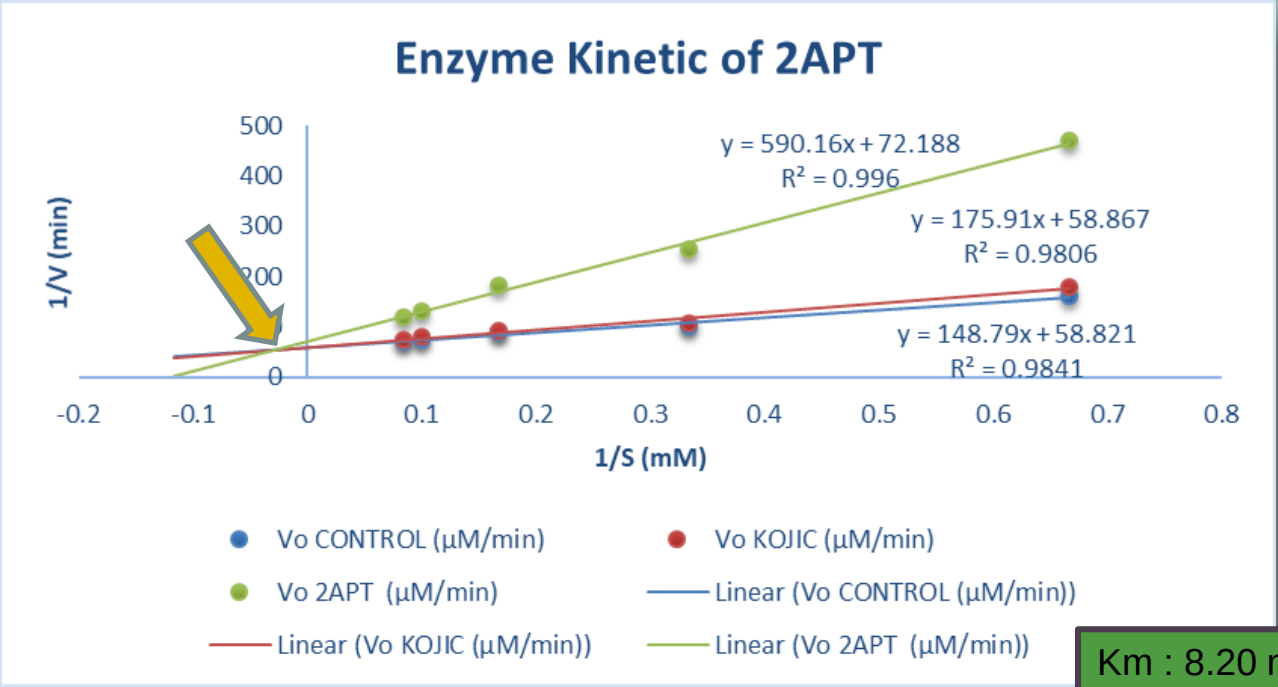


Figure 4 Lineweaver-Burk Plot of Enzyme Kinetic

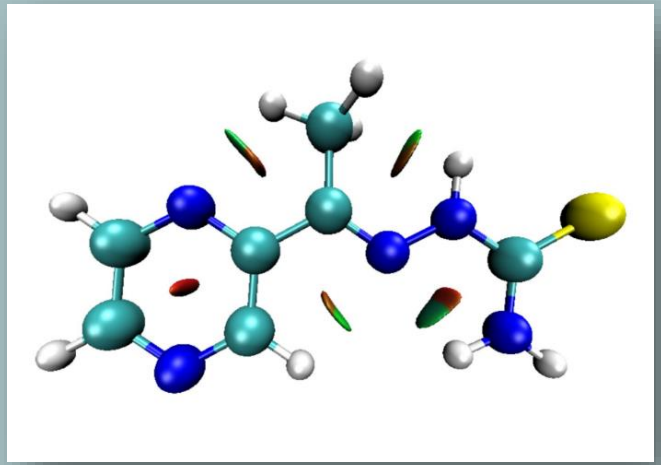
ENZYME KINETIC

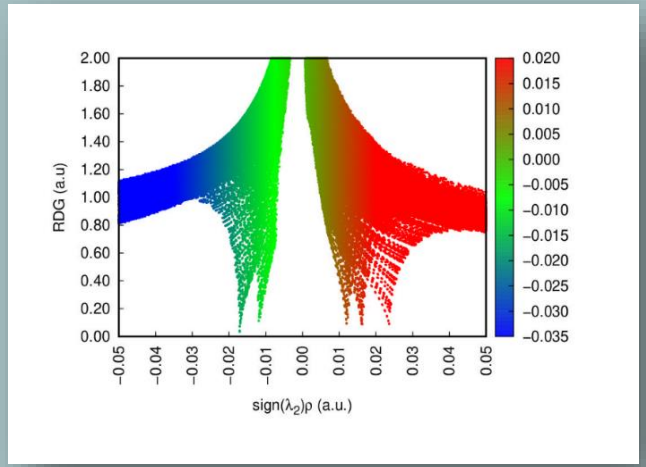
- Vmax and Km, to understand how enzymes work together to control metabolism.
- To explore the inhibition type of compound.

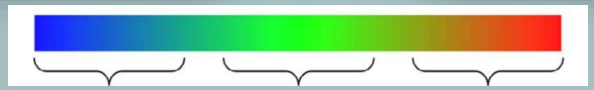
Km : 8.20 mM
Vmax : 0.013 µM/min
Type of inhibitor : mixed-type inhibitor

Theoretical section

REDUCED DENSITY GRADIENT-NON COVALENT INTERACTION (RDG-NCI)



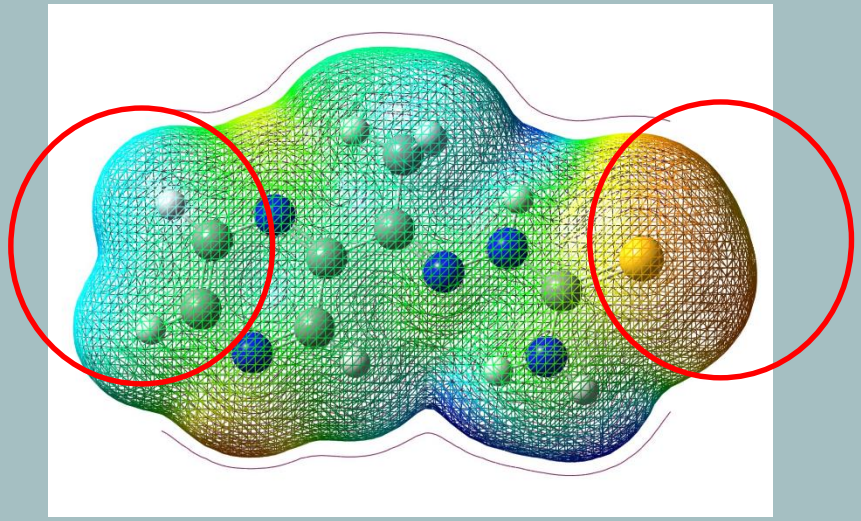




H Bond Van De Waals Steric Effects

Figure 5 Optimization structure and RDG-NCI plot for 2APT

MOLECULAR ELECTROSTATIC POTENTIAL (MEP)



- Different region colour indicated different potential strength.
- **Blue** < **green** < **red**
- **Blue region**: potential binding site for nucleophilic attack
- **Red region**: potential binding site for electrophilic attack

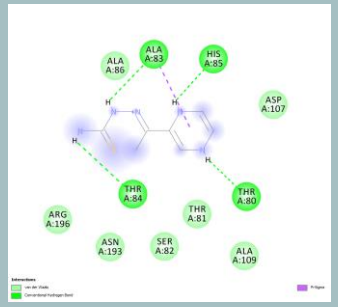
Figure 6 Molecular Electrostatic Potential (MEP) of 2APT

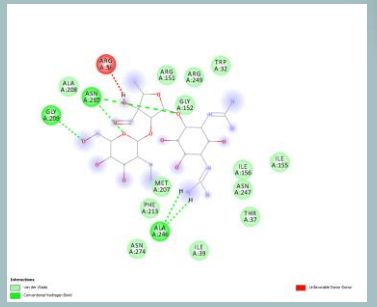
MOLECULAR DOCKING (BACTERIA AND FUNGI)

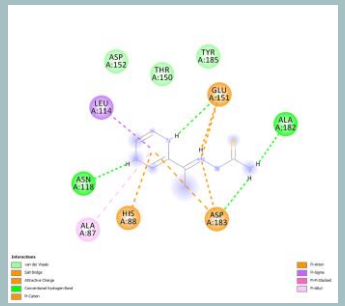
Docking simulation results

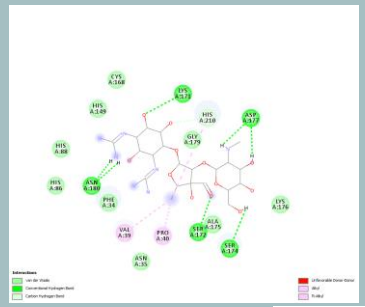
Ligands	Binding Energy (kcal/mol)
2APT (*E. coli*)	-5.2
Streptomycin (*E. coli*)	-6.2
2APT (*B. cereus*)	-5.1
Streptomycin (*B. cereus*)	-6.2
2APT (*C. albican*)	-5.0
Nystatin (*C. albican*)	-7.5

E. coli



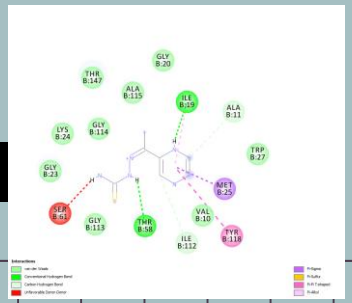


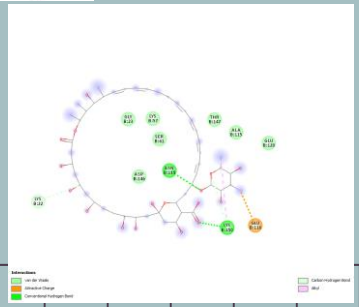




B. cereus

C. albican





MOLECULAR DOCKING

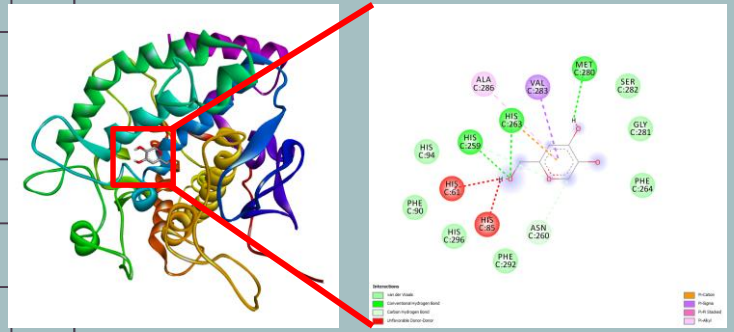


Figure 6 Interaction of 2APT with amino acid residues of tyrosinase

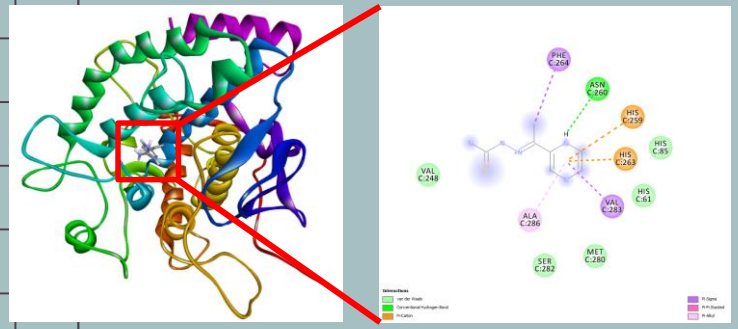


Figure 7 Interaction of Kojic Acid with amino acid residues of tyrosinase

Docking simulation results

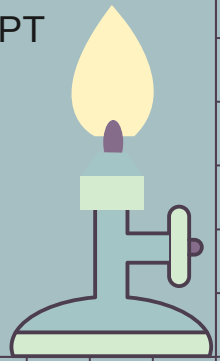
Ligands	Binding Energy (kcal/mol)	Number of Bonds formed		
		Hydrogen bond	Van der Waals	π Bond
2APT	-5.4	2	3	3
Kojic Acid (+)	-4.8	1	0	5

Hydrophobic residues of tyrosinase receptors with control and 2APT

Receptor	Ligand	Number of residues	Interaction residues
Tyrosinase	2APT	15	Asn260, His85, His61, His259, His263, Ala286, Val283, Met280
	Kojic Acid	11	Phe264, Asn260, His259, His263, Val283, Ala286

CONCLUSION

- 2APT compound has been synthesized and characterized by elemental analysis and spectroscopic (FT-IR, UV-visible, $_{1}$H NMR, $_{13}$C NMR) methods.
- 2APT successfully exhibited antibacterial and antityrosinase activities.
- The Lineweaver-Burk plot revealed that 2APT function as a mixed-type inhibitor with Km and Vmax value for 2APT were 8.20 mM and 0.013 µM/min, respectively.
- The molecular Docking result supports the experimental result, showing 2APT inhibits tyrosinase better than kojic acid.



Thanks!

Questions?

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