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Thesis

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Subject

Analysis *in silico* and prediction of mode of action of potential inhibitors of *H. pylori* urease extracted from medicinal plants

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Summary

Helicobacter pylori infection is a major cause of gastrointestinal diseases like gastritis, peptic ulcers, and gastric cancer. The bacterium survives in the stomach's acidic environment due to its urease enzyme, which neutralizes stomach acid. Our research explores traditional medicinal plants for potential urease inhibitors. We selected five plants*: Salvia officinalis, Nigella sativa, Eucalyptus globulus, Zingiber officinale, and Trigonella foenum-graecum*. We conducted molecular docking studies to examine the interaction of natural compounds from these plants with the urease enzyme. Compounds like quercetin, gallic acid, and vanillic acid showed significant urease inhibitory activity. Quercetin, in particular, demonstrated strong non-competitive inhibition by binding to the enzyme's flap region, preventing its active conformation. Gallic acid and vanillic acid also formed stable interactions with key residues in the enzyme's active site. Further *in silico* analysis revealed that these compounds have favorable pharmacokinetic properties and low toxicity, making them promising therapeutic candidates. Our findings suggest that these natural inhibitors could provide viable alternatives to current treatments, addressing antibiotic resistance and offering safer therapeutic options.

Key words : *Helicobacter pylori* , Gastrointestinal diseasesGastritis , Gastric cancer, Urease enzyme , Traditional medicinal plants , Urease inhibitors ,Molecular docking studies, Quercetin ,Gallic acid ,Vanillic acid ,Natural inhibitors.

ملخص

تعد عدوى الملوية البوابية سببًا رئيسيًا لأمراض الجهاز الهضمي مثل التهاب المعدة والقرحة الهضمية وسرطان المعدة. تتمكن البكتيريا من البقاء في البيئة الحمضية للمعدة بفضل إنزيم اليورياز الذي يعادل حمض المعدة. مع تزايد مقاومة المضادات الحيوية، يصبح من الضروري إيجاد علاجات بديلة. تستكشف أبحاثنا النباتات الطبية التقليدية كمثبطات محتملة لإنزيم اليورياز. اخترنا خمسة نباتات: *القصعين الطبي، الحبة السوداء، الأوكالبتوس، الزنجبيل، والحلبة*.

قمنا بدراسات التحام جزيئي لفحص تفاعل المركبات الطبيعية من هذه النباتات مع إنزيم اليورياز. أظهرت مركبات مثل الكيرسيتين وحمض الجاليك وحمض الفانيلك نشاطًا مثبطًا كبيرًا لإنزيم اليورياز. أظهر الكيرسيتين بشكل خاص تثبيطًا غير تنافسي قويًا عن طريق الارتباط بمنطقة الغطاء للإنزيم، مما يمنع هيئته النشطة. كما شكل حمض الجاليك وحمض الفانيلك تفاعلات مستقرة مع البقايا الرئيسية في الموقع النشط للإنزيم.

كشفت التحليلات الحاسوبية الإضافية أن هذه المركبات لها خصائص دوائية مواتية وسمية منخفضة، مما يجعلها مرشحة واعدة للعلاج. تشير نتائجنا إلى أن هذه المثبطات الطبيعية يمكن أن توفر بدائل قابلة للتطبيق للعلاجات الحالية، مما يعالج مقاومة المضادات الحيوية ويوفر خيارات علاجية أكثر أمان

الكلمات المفتاحية : البكتيريا الملوية البوابية، أمراض الجهاز الهضمي، التهاب المعدة، سرطان المعدة، إنزيم اليورياز، النباتات الطبية التقليدية، مثبطات اليورياز، دراسات الالتحام الجزيئي، كيرسيتين، حمض الغاليك، حمض الفانيليك، مثبطات طبيعية.

Résumé

L'infection à *Helicobacter pylori* est une cause majeure de maladies gastro-intestinales comme la gastrite, les ulcères peptiques et le cancer gastrique. La bactérie survit dans l'environnement acide de l'estomac grâce à son enzyme uréase, qui neutralise l'acide gastrique. Avec la résistance croissante aux antibiotiques, des traitements alternatifs sont nécessaires. Nos recherches explorent les plantes médicinales traditionnelles comme inhibiteurs potentiels de l'uréase. Nous avons sélectionné cinq plantes : *Salvia officinalis, Nigella sativa, Eucalyptus globulus, Zingiber officinale, et Trigonella foenum-graecum.*Nous avons mené des études de docking moléculaire pour examiner l'interaction des composés naturels de ces plantes avec l'enzyme uréase. Des composés comme la quercétine, l'acide gallique et l'acide vanillique ont montré une activité inhibitrice significative de l'uréase. La quercétine, en particulier, a montré une inhibition non compétitive forte en se liant à la région de la boucle de l'enzyme, empêchant sa conformation active. L'acide gallique et l'acide vanillique ont également formé des interactions stables avec les résidus clés du site actif de l'enzyme. Les analyses *in silico* supplémentaires ont révélé que ces composés ont des propriétés pharmacocinétiques favorables et une faible toxicité, ce qui en fait des candidats thérapeutiques prometteurs. Nos résultats suggèrent que ces inhibiteurs naturels pourraient fournir des alternatives viables aux traitements actuels, répondant à la résistance aux antibiotiques et offrant des options thérapeutiques plus sûres.

Mots Clés : *Helicobacter pylori* , Maladies gastro-intestinalesGastrite , Cancer gastrique, Enzyme uréase , Plantes médicinales traditionnelles , Inhibiteurs de l'uréase ,Études d'amarrage moléculaire, Quercétine ,Acide gallique ,Acide vanillique ,Inhibiteurs naturels.

Thanks

First of all, we thank ALLAH, our creator, for giving us the strength, will and courage to accomplish this modest work.

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Finally, we would also like to thank all those who participated directly or indirectly in the realisation of this work.

.

Dedication

In reaching this milestone, I am profoundly grateful for the invaluable support and unwavering love that have illuminated my path. First and foremost, I thank God for His boundless grace and guidance throughout this journey, granting me strength and wisdom beyond measure.

Mom, your endless sacrifices and unconditional love have been my rock and inspiration. You believed in me when I faltered and nurtured my dreams with unwavering devotion. Dad, your steady encouragement and wisdom have been the beacon lighting my way forward.

To my cherished sisters, who have been my confidantes, champions, and partners in laughter and tears—your unwavering support and shared dreams have been a constant source of strength and inspiration. Friends, your camaraderie and understanding have brought joy to the toughest days and made every achievement more meaningful.

And to myself, who dared to dream, persevered through challenges, and embraced growth—thank you for your resilience, courage, and belief in what lies ahead.

With deepest gratitude and humility, I dedicate this thesis to each of you who have shaped my journey and enriched my life.

BOUCHRA

Dedication

All praise and thanks be to Allah, the Most Merciful and the Most Compassionate, for His endless blessings and guidance. Without His grace, none of this would have been possible. I am eternally grateful for His support and for surrounding me with such wonderful people.

To my sweetheart who watches me from the sky, who showered me with prayers full of love, and whose river of prayers still overflows to this day : Thank you. Your spirit accompanied me through these long days and sleepless nights. I felt you in the front rows, clapping for me. Thank you, Mom. I hope you are proud of me and that I become the daughter you always wanted

To my father, the light of my eyes, who has been my support, wrapping me in his warm embrace whenever anxiety grips me : Thank you for giving me wings to fly.

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To my beloved grandmother.

To Fatima Zahra and Manouna

To my cousins malek, Nour Al Huda and Afaf.

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SORAYA

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Abbreviation

- **ADME** - Absorption, Distribution, Metabolism, and Excretion
- **ADMET** - Absorption, Distribution, Metabolism, Excretion, and Toxicity
- **BCS** - Biopharmaceutics Classification System
- **CAM** - Complementary and Alternative Medicine
- **CAM** - Complementary and Alternative Medicine
- **GI** – Gastrointestinal
- **GPF** - Grid Parameter File
- ***H. pylori*** - *Helicobacter pylori*
- **HPLC** - High-Performance Liquid Chromatography
- **IC_{50}** - Half Maximal Inhibitory Concentration
- **MBC** - Minimum Bactericidal Concentration
- **MGL** - Molecular Graphics Laboratory
- **MIC** - Minimum Inhibitory Concentration
- **NHI** - National Institutes of Health
- **NMR** - Nuclear Magnetic Resonance
- **PDB** - Protein Data Bank
- **PDBQT** - Protein Data Bank, Partial Charge (Q), and Atom Type (T)
- **PPI -** Proton Pump Inhibitor**.**
- **RCSB** - Research Collaboratory for Structural Bioinformatics
- **UV** - Ultraviolet

Introduction

Introduction

In the realm of combating *Helicobacter pylori* infections, the quest for effective treatments has led to a fascinating intersection of traditional medicine and modern scientific advancements. The intricate interplay between the bacterium and its virulence factors, particularly urease enzymes, has spurred a wave of research aimed at harnessing the therapeutic potential of natural compounds derived from medicinal plants **(Ferreira *et al.*, 2014).**

The historical backdrop of *H. Pylori* infection sets the stage for understanding the significance of its urease enzymes in the pathogenesis of gastrointestinal disorders. Since its discovery, *H. pylori* has been recognized as one of the most common infections worldwide, affecting more than half of the global population. The bacterium's ability to survive in the highly acidic environment of the stomach is largely attributed to the activity of its urease enzyme. This enzyme hydrolyzes urea to produce ammonia and carbon dioxide, which neutralizes gastric acid, creating a more favorable environment for bacterial survival and colonization **(Sonnenberg, 2013).**

From the structural intricacies to the functional implications, the role of *H. pylori* urease in neutralizing gastric acid and promoting bacterial survival underscores its importance as a therapeutic target. The enzyme is a nickel-containing metalloenzyme, and its activity is crucial for the pathogen's ability to colonize the stomach and cause disease. Inhibiting urease activity, therefore, can significantly impair the bacterium's survival and its ability to cause infection **(Amieva and El-Omar, 2008).**

H. pylori infection is widespread, with higher prevalence rates observed in developing countries due to factors such as overcrowding, poor sanitation, and lack of clean water, often occurring in childhood and persisting throughout life if untreated. Regions with high prevalence include parts of Africa, South America, and Asia, where infection rates can exceed 70% of the population. In contrast, developed countries like those in North America and Western Europe report lower prevalence rates, typically below 40%. This geographic variation in prevalence underscores the need for tailored public health strategies and interventions **(Backert and Yamaoka, 2016).**

Drawing inspiration from nature, the exploration of phytotherapy unveils a treasure trove of medicinal plants traditionally revered for their gastrointestinal healing properties. Within the rich botanical landscape of Algeria lie potential extracts harboring urease inhibitory activity, offering a promising avenue for natural-based interventions. Phytotherapy, the use of plant-based compounds for medicinal purposes, has a long history and is known for its broad range of bioactive secondary metabolites, such as flavonoids, alkaloids, and terpenoids. These compounds have demonstrated various biological activities, including antimicrobial, anti-inflammatory, and anti-urease effects **(Liu *et al.*, 2024).**

The identification of secondary metabolites, the elucidation of their biological activities, and the discovery of compounds with anti-urease effects pave the way for understanding the intricate mechanisms through which these plant-derived molecules interact *with H. pylori* urease. Secondary metabolites play a crucial role in the plants' defense mechanisms and have been found to exhibit strong inhibitory activity against various enzymes, including urease. Understanding the specific interactions between these compounds and the urease enzyme can help in identifying potent inhibitors that can be used for therapeutic purposes (**Deng *et al.*, 2024).**

The overarching objective of this work is to elucidate the inhibitory effects of natural compounds from traditional used plants on *H. pylori* urease through *in silico* analysis and molecular docking studies. This research aims to decipher the molecular intricacies underlying the inhibition of *H. pylori* urease by plant-derived compounds by conducting comprehensive docking studies. These studies will involve selecting natural compounds based on their reported anti-urease activity, preparing protein and ligand structures, and performing docking simulations to predict their binding affinities and modes of interaction. The results will be analyzed to identify the most promising inhibitors, followed by a detailed evaluation of their pharmacological and pharmacokinetic properties, as well as their potential toxicity. By unraveling the mode of action of these potential inhibitors, this research seeks to contribute to the development of novel therapeutic strategies for combating *H. pylori* infections.

For the presentation of our work, we have opted for the following plan: starting with a general introduction, followed by bibliographic reminders. Then, we presented the material and the methods as well as the discussion of the results obtained and at the end, we close this manuscript with a general conclusion and perspectives.

Bibliographic synthesis

1. *Helicobacter pylori* and their ureases enzymes

1.1 *Helicobacter pylori* (*H. pylori*) bacteria infection

Helicobacter pylori is a type of bacterium that lives primarily in your stomach and the upper part of your small intestine (the duodenum) , *H. pylori* are spiral-shaped gram-negative bacteria with polar flagella that live near the surface of the human gastric mucosa (figures 01 and 02). They have evolved intricate mechanisms to avoid the bactericidal acid in the gastric lumen, and to survive near, to attach to, and to communicate with the human gastric epithelium and host immune system. This interaction sometimes results in severe gastric pathology. *H pylori* infection is the strongest known risk factor for the development of gastroduodenal ulcers, with infection being present in 60%–80% of gastric and 95% of duodenal ulcers. *H pylori* is also the first bacterium to be classified as a definite carcinogen by the World Health Organization's International Agency for Research on Cancer because of its epidemiologic relationship to gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma. In the last 25 years, since H pylori was first described and cultured, a complete paradigm shift has occurred in our clinical approach to these gastric diseases,From the medical point of view, *H pylori* is a formidable pathogen responsible for much morbidity and mortality worldwide. *However,* H pylori infection occurs in approximately half of the world population, with disease being an exception rather than the rule. **(Amieva & El-Omar, 2008)**

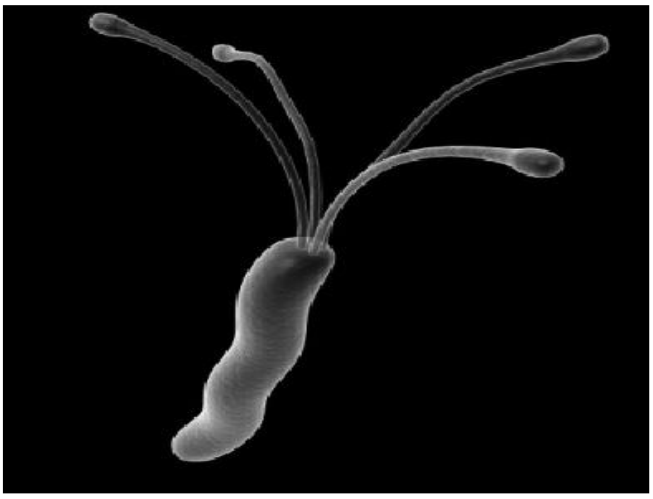


Figure 1: *Helicobacter pylori* **(Hompes, 2011)**

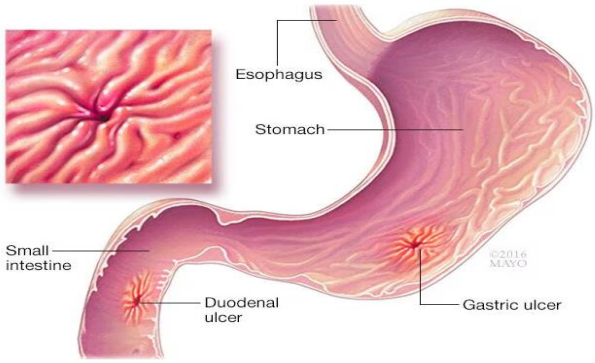


Figure 2:*H pylori* ulcers in the stomach and the upper part of your small intestine (The duodenum) **(Hompes, 2011)**

Understanding how this organism interacts with its host is essential for formulating an intelligent strategy for dealing with its most important clinical consequences **(Go, 2002).**

Host factors such as cytokine promoter polymorphisms, particularly IL-1b and IL-10, play a significant role in determining the outcome of *H. pylori* infection, gastric acid secretion, and the development of premalignant histological features.

These points highlight the intricate relationship between H. pylori and its human host, emphasizing the importance of understanding these interactions in the context of disease pathogenesis and clinical manifestations **(Liu *et al.*, 2024).**

The process of *Helicobacter pylori* infection can be resumed inthese steps:

First, *Helicobacter pylori* enters the lumen of the host stomach, then releases urease, decomposes the metabolites of other microorganisms (urea) into ammonia, changes the acidic environment of the stomach, which is conducive to the growth of *H. pylori*. In the next step, *Helicobacter pylori* releases adhesins (such as BabA, SabA, AlpA, HopQ, HopZ and OipA) that bind to specific receptors on gastric epithelial cells. *Helicobacter pylori* also releases CagA and VacA, which invade gastric epithelial cells and cause inflammatory reactions (see figure 03 below) **(Liu *et al.*, 2024).**

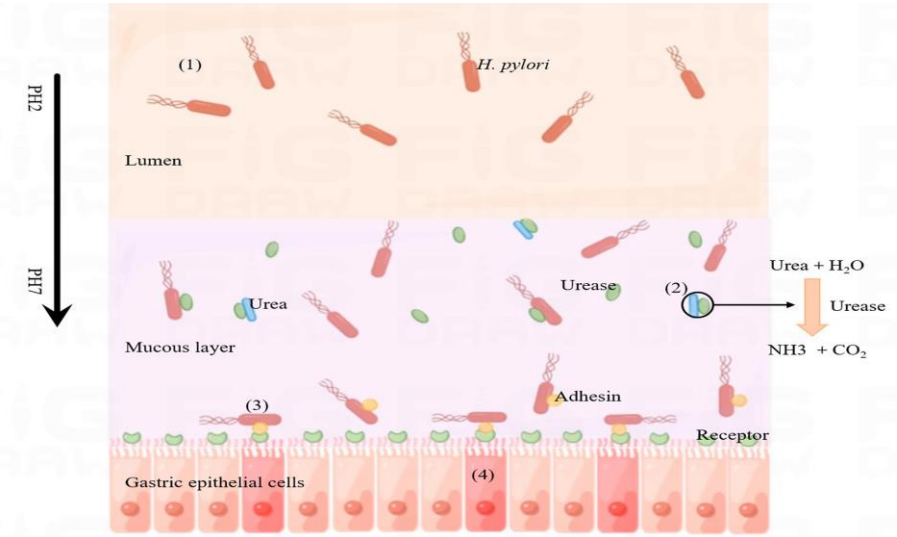


Figure 3: Process of *Helicobacter pylori* infection **(Liu *et al.*, 2024).**

1.2 *H. pylori* Diagnosis

1.2.1 *H. pylori* tests

A *Helicobacter pylori* infection can be identified using noninvasive methods such as serology, urea breath test, urine or blood, and the detection of *H. pylori* antigen in stool specimen, as well as invasive methods such as endoscopy and biopsy (histological examination, culture, polymerase chain reaction). Currently, no single test can be completely trusted to identify *H. pylori* colonization; if possible, a combination of two tests is advised. The tests that are employed should be determined by the clinical situation, the likelihood ratio of positive and negative tests, the testing strategy's cost-effectiveness, and the tests' accessibility **(Rautelin *et al.*, 2003).**

The current symptomatic choices accessible for identifying *Helicobacter pylori* contamination incorporate both obtrusive and non-invasive strategies **(Wang *et al.*, (2015).**

- **<u>Obtrusive Symptomatic Tests:</u>**

➢ Endoscopic Biopsy:

Getting gastric mucosa tests amid endoscopy for advance investigation utilizing different tests like fast urease test, histology, culture, and atomic strategies.

➢ Histology:

Minuscule examination of tissue tests for the nearness of *H. pylori* microbes.

➢ Quick Urease Test:

Identifying the nearness of urease chemical delivered by *H. pylori* in biopsy tests.

- Culture:

Developing *H. pylori* microscopic organisms from biopsy tests in a research facility setting.

- Atomic Strategies:

Utilizing procedures like real-time PCR for exact location of *H. pylori* DNA)**Wang *et al,*. (2015).**

- **Non-invasive Demonstrative Tests:**

- Urea Breath Test:

Persistent ingests labeled urea, and breathed out breath is analyzed for labeled carbon dioxide, showing *H. pylori* disease.

- Stool Antigen Test:

Identifying H. pylori antigens in stool tests.

- Serological Tests:

Identifying antibodies against *H. pylori* in blood tests.

- Atomic Examinations:

Utilizing atomic strategies for non-invasive location of *H. pylori* disease.

These symptomatic strategies offer a run of choices for healthcare suppliers to precisely analyze *Helicobacter pylori* disease based on the patient's clinical condition and the accessibility of assets (**Wang *et al,* (2015).**

1.3 Routes of transmission of *H. pylori* bacteria infection

H. pylori is a common bacterium, estimated to infect approximately 50% of the world's population, Humans are the main reservoir. The prevalence of *Helicobacter pylori* infection varies widely by geographic region, age, race, ethnicity, and SES. Infection rates appear to be higher in developing countries than in developed countries, with most infections occurring during childhood, understanding how *H. pylori* is transmitted is

important when implementing public health measures to prevent the spread of *H. pylori* **(Brown, 2000).**

The main causes of transmition *H. pylori* infection include:

- **Person-to-Person Transmission:** Close contact with an infected individual, such as family members, may lead to the transmission of H. pylori bacteria. This can occur through activities like sharing utensils or food **(Brown, 2000).**
- Contaminated Food and Water: Consuming food or water contaminated with *H. pylori* bacteria can also lead to infection. Poor sanitation practices and contaminated water sources can contribute to the spread of the bacteria **(Brown, 2000).**
- **Poor Hygiene:** Inadequate handwashing and hygiene practices can facilitate the transmission of *H. pylori*. This is particularly relevant in settings where sanitation standards are low **(Brown, 2000).**
- **Medical Procedures**: In rare cases, *H. pylori* infection can be transmitted through medical procedures involving the upper gastrointestinal tract, such as endoscopy or gastrointestinal surgery, if proper sterilization protocols are not followed **(Brown, 2000).**

Other Potential Sources:

Domestic animals like cats and sheep have been suggested as possible sources of transmission, also Houseflies have been proposed as a reservoir, but evidence of transmission to humans is lacking These hypothesized modes of transmission highlight the importance of understanding how *H. pylori* spreads to implement effective public health measures to prevent its transmission **(Liu *et al.*, 2024).**

1.4 Structure and function of *H. pylori* urease

1.4.1 Definition of urease enzyme

Urease (urea amidohydrolase; EC 3.5.1.5) was the first enzyme to be crystallized in 1926. It was also the first enzymatic protein in which nickel ions were detected. Since then, an extensive investigation of urease has been done, resulting in the identification of urease's function in the circulation of nitrogen molecules. It has also been demonstrated that urease may be a virulence factor required in a variety of disorders, including chronic diseases **(Sharaf *et al.*, 2021).**

Urease may hydrolyze urea. This chemical is common; it may be found in the natural environment (water and soil) as well as in the human body, where it is associated with protein breakdown. In humans, urea contributes to normal kidney function. A healthy adult excretes around 30 grams of urea per day. However, it is found not only in urine, but also in blood serum, perspiration, and even the stomach. Urease-mediated urea hydrolysis is a complicated process (figure 4). In the first stage, one molecule of ammonia and one molecule of carbamate emerge. In water, carbamate spontaneously transforms to the second ammonia molecule and carbonic acid. Next, ammonia is protonated. This mechanism causes a pH rise. **(Sharaf *et al.*, 2021)**

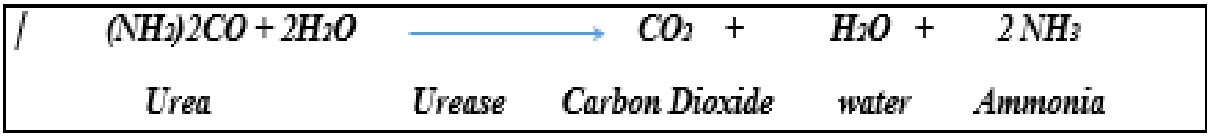


Figure 4: Overall reaction of urea hydrolysis

1.4.2 Structure of urease *H. pylori*

The *helicobacter pylori* urease (fig 05) has a complex and unique structure that is critical for its function. This allows the bacteria to survive under harsh acidic conditions in the stomach. It has a molecular mass of 1.1 MDa and comprises a spherical dodecameric complex (Figure 05) made up of 12 UreA and 12 UreB subunits and five accessory proteins – UreI, UreE, UreF, UreG and UreH organized in a tetrahedral structure. The enzyme has a 160 Å diameter and contains twelve active sites. A cluster of 12 active sites is thought to be important for the acid resistance of the enzyme, which enables it to be active at a pH value of 3 **(Cunha *et al.*, 2021).**

UreA (figure 06) is of a mass 26.5kDa and has a sequence length of 238 amino acids that contains the catalytic site which must bind nickel ions (Ni^{2+}) to activate urease. The other half called UreB (figure 07) weighing 60.3 kDa with 569 residues further helps incorporate nickel and stabilize the structure of urease. They act like essential cofactors in that they hold certain select parts of the enzyme's active site through some nickel atoms bound via them to such sites along with specific amino acid residues, which bring about control over some enzyme catalytic activities. Additional genes, namely ureE, ureF, ureG, and ureD, encode accessory proteins pivotal for inserting nickel ions into the enzyme's active site, although the exact mechanism remains unclear. These accessory polypeptides interact with the apoenzyme, forming complexes necessary for the creation of a catalytically active enzyme. UreI, while not essential for urease activity and lacking apparent homologs in other urease gene clusters, remains enigmatic in its role within the urease system **(Cunha *et al.*, 2021).**

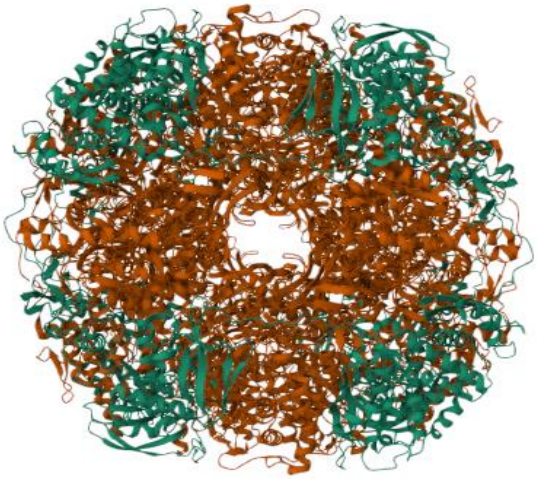


Figure 5: Crystal structure of *Helicobacter pylori* urease

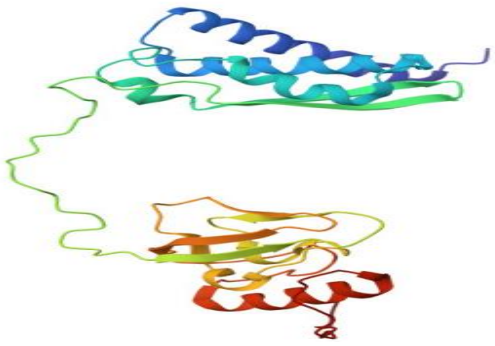


Figure 6: UreA(subunit ALPHA urease)

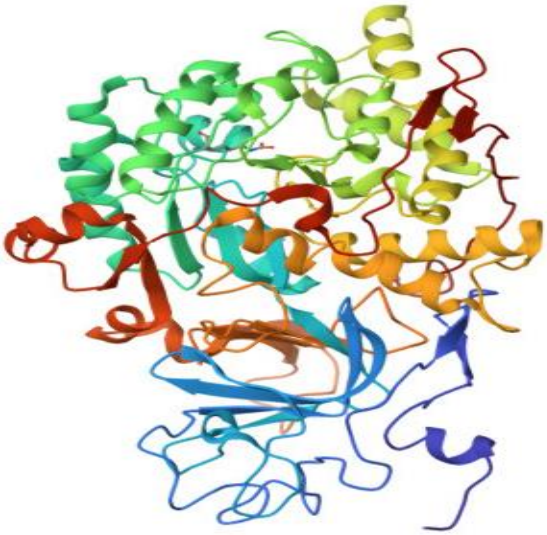


Figure 7: UreB(urease subunit BETA)

1.4.3 The role of *Helicobacter pylori* urease

Helicobacter pylori urease plays a crucial role in the pathogenesis and survival of this bacterium:

- **Acid Neutralization** is a process that helps in making the stomach acid base by dissolving urea into ammonia and carbon dioxide through urease, a process that allows the bacteria to survive in a very acidic environment of the **s**tomach **(Olivera-Severo *et al*., 2017).**
- **Colonization:** The colonization of the gastric mucosa by *H. pylori* depends on urease. Urease-negative mutants have been found to colonize the stomach with difficulty **(Olivera-Severo *et al*., 2017) .**
- **Host Defense Avoidance:** Urease may play a role in avoiding host defense mechanisms by shedding from the bacterial surface, potentially interfering with the host immune response **(Olivera-Severo *et al*., 2017).**
- **Inflammatory Signaling:** Urease has been implicated in causing cellular inflammatory signaling, which can contribute to the development of gastritis and ulcers **(Olivera-Severo *et al*., 2017) .**
- **Chemotactic Activity:** Urease or urease-containing fractions from *H. pylori* can act as chemotactic factors for leukocytes, leading to local inflammation and recruitment of immune cells **(Olivera-Severo *et al*., 2017).**

2. Traitement of *Helicobacter pylori* infection

2.1 Chemical traitement

Treatment for *Helicobacter* pylori infection usually includes a combination of antibiotics and acid-suppressing medicines. The most prevalent antibiotic regimen is:

- **Triple Therapy:** This regimen comprises of a proton pump inhibitor (PPI) and two antibiotics. Clarithromycin, amoxicillin, metronidazole, and tetracycline are examples of common antibiotics used in triple treatment. For example, one regimen could contain a PPI (such as omeprazole or lansoprazole) in addition to clarithromycin and either amoxicillin or metronidazole. **(Chey *et al*., 2017)**

- **Quadruple therapy:** may be chosen in locations where clarithromycin resistance is high, or if a patient has already failed clarithromycin-based therapy. This therapy usually contains a PPI, bismuth subsalicylate (or another bismuth molecule), and two antibiotics (metronidazole, tetracycline, or amoxicillin). **(Chey *et al*., 2017)**

- **Sequential Therapy:** This regimen consists of giving a PPI and amoxicillin for the first 5 days, followed by a PPI, clarithromycin, and metronidazole for the next 5 days. **(Chey *et al*., 2017)**

- **Concomitant Therapy:** This regimen includes a PPI, clarithromycin, amoxicillin, and metronidazole, all administered concurrently. **(Chey *et al*., 2017)**

2.2 Naturel traitement with medicinals plants

In order to address the issue of rising antibiotic resistance rates, alternative treatments for *H. pylori* infection have arisen, including phytotherapy, probiotic therapies, and some developing therapeutics. Probiotics reduce *H. pylori* infection through both immunological and non-immune mechanisms, whereas plant extracts mainly target urease activity and adhesion activity to cure *H. pylori* **(Liu *et al,* 2024).**

The antibacterial mechanisms of phytotherapy include inhibition of urease activity, anti-adhesion activity, DNA damage, inhibition of protein synthesis and oxidative stress **(Liu *et al*, 2024).**

2.2.1 Medicinal plants used traditionaly in Algeria for gastrointestinal disorders:

Algeria's distinct geographical zones, encompassing the arid Sahara and the temperate Mediterranean coast, create a unique phytochemical landscape. This rich tapestry of plant life offers a treasure trove of potential bioactive compounds with therapeutic applications. Ethnobotanical studies reveal a deep understanding within the Algerian population regarding the medicinal properties of these plants **(Rocha *et al*, 2015).**

One prominent family used in Algerian traditional medicine is Lamiaceae, also known as the mint family. Lamiaceae plants are characterized by the presence of volatile terpenes and phenolic compounds. These compounds, including rosmarinic acid in rosemary (*Rosmarinus officinalis*) and thymol in thyme (*Thymus vulgaris*), exhibit anti-inflammatory, antioxidant, and antimicrobial properties Traditionally brewed as teas, these herbs may alleviate digestive discomfort and respiratory problems by reducing inflammation in the gut and airways **(Rocha *et al*, 2015).**

Another key family, *Apiaceae*, encompasses various plants like fennel (*Foeniculum vulgare*) and cumin (*Cuminum cyminum*). These plants are rich in essential oils containing carvone and limonene, known for their carminative properties. Carminative effects help reduce gas and bloating, making *Apiacea*e-derived remedies beneficial for digestive complaints. The *Asteraceae* family, also known as the *daisy* family, offers chamomile (*Matricaria chamomilla*) and dandelion (*Taraxacum officinale*) with well-documented scientific applications. Chamomile flowers contain apigenin, a flavonoid with anti-inflammatory and spasmolytic properties, which contribute to its calming effects and use for digestive upset. Dandelion root is a potential hepatoprotective agent due to its content of chicoric acid and other phenolic compounds, aligning with its traditional use in supporting liver health (**Rúa *et al.*, 2019).**

Beyond these families, Algerian medicine incorporates various other plants with intriguing scientific profiles. The prickly pear cactus *(Opuntia ficus-indica*) contains polysaccharides with anti-inflammatory and wound healing properties, justifying its use for soothing skin conditions like sunburns. The fragrant myrtle (*Myrtus communis*) possesses essential oils rich in cineole and myrtenol, with potential antimicrobial and digestive benefits supporting its use as a digestive tea. The ubiquitous eucalyptus tree (Eucalyptus globulus) is a source of potent essential oil containing eucalyptol, which exhibits antimicrobial and decongestant properties, explaining its application in steam inhalation for respiratory ailments **(Ben**

Hsouna *et al*,. 2014) there is Black Seed (*Nigella sativa*) oil, derived from *Nigella sativa* seeds, is rich in thymoquinone, a bioactive compound with anti-inflammatory, antioxidant, and potential gastroprotective properties, supporting its use for various digestive issues Ginger (*Zingiber officinale*) contains gingerols, which exhibit anti-inflammatory and antiemetic properties. Ginger tea is a popular remedy for nausea, vomiting, and indigestion due to these properties Fenugreek (*Trigonella foenum-graecum L*.) seeds are a good source of fiber and galactomannan gums, which have prebiotic properties. Prebiotics can promote gut health and potentially alleviate digestive discomfort like heartburn and constipation

Algerian traditional medicine offers a fascinating glimpse into the power of plants and their potential health benefits. Understanding the scientific basis behind these practices allows for a deeper appreciation of this rich cultural heritage **(Djidel *et al*., 2010).**

Table 1: Some medicinal plants reported to beused for the treatment of gastrointestinal disorders **(Djidel *et al*., 2010).**

Plant Name (Scientific Name)	Plant Part Used	Traditional Uses	Form of Consumption
Sage (Salvia officinalis)	Leaves	Soothes stomach aches, diarrhea, inflammation	Tea
Black Seed (Nigella sativa)	Seeds	Digestive problems (indigestion, ulcers), anti-inflammatory	Oil
Ginger (Zingiber officinale)	Rhizome (root)	Nausea, vomiting, indigestion	Tea
Fenugreek (Trigonella foenum-graecum L.)	Seeds	Eases digestive discomfort (heartburn, constipation)	Seeds, flour, paste
Rosemary (Rosmarinus officinalis)	Leaves	Digestive issues, respiratory problems	Tea
Thyme (Thymus vulgaris)	Leaves	Digestive issues, respiratory problems	Tea
Fennel (Foeniculum vulgare)	Seeds	Reduces gas and bloating	Chewed seeds, tea
Cumin (Cuminum cyminum)	Seeds	Reduces gas and bloating	Whole seeds in dishes, tea
Chamomile (Matricaria chamomilla)	Flowers	Calming effects, digestive upset	Tea
Dandelion (Taraxacum officinale)	Root	Supports liver health	Tea, tincture (liquid extract)
Prickly Pear Cactus (Opuntia ficus-indica)	Pads	Soothes skin conditions (sunburns)	Topical gel from crushed pads
Myrtle (Myrtus communis)	Leaves	Digestive tea	Tea
Eucalyptus (Eucalyptus globulus)	Leaves	Respiratory ailments (steam inhalation)	Essential oil for steam inhalation

2.2.2 General information on secondary metabolites:

Secondary metabolites (SM) are organic compounds that are not necessary for a cell (organism) to live, but play a role in the interaction of the cell (organism) with its environment which produced by plants through various metabolic pathways, distinct from primary metabolites essential for growth and development(Figure 8). These compounds are often involved in plants protection against biotic or abiotic stres sessuch as defense against herbivores, attraction of pollinators, or allelopathy, and many possess pharmacological properties that make them valuable in traditional and modern medicine. Secondary metabolites are from different metabolites families that can be highly inducible in response to stresses. These new technologies will serve to extend and enhance the continued usefulness of the higher plants as renewal sources of chemicals, especially medicinal compounds **(Pagare *et al*., (2015).**

Numerous natural products and traditional remedies have exhibited antimicrobial properties, supported by both scientific research and generations of medical practice. Even predating the discovery of *H. pylori*, a wide array of plants and substances have been utilized to alleviate gastric symptoms now recognized to be associated with this pathogen's infection Presently, with enhanced understanding of *H. pylori* following decades of investigation, ethnomedicine continues to serve as a valuable resource in the quest for effective natural products and therapeutic strategies to combat the infection (**Deng *et al*., (2024).**

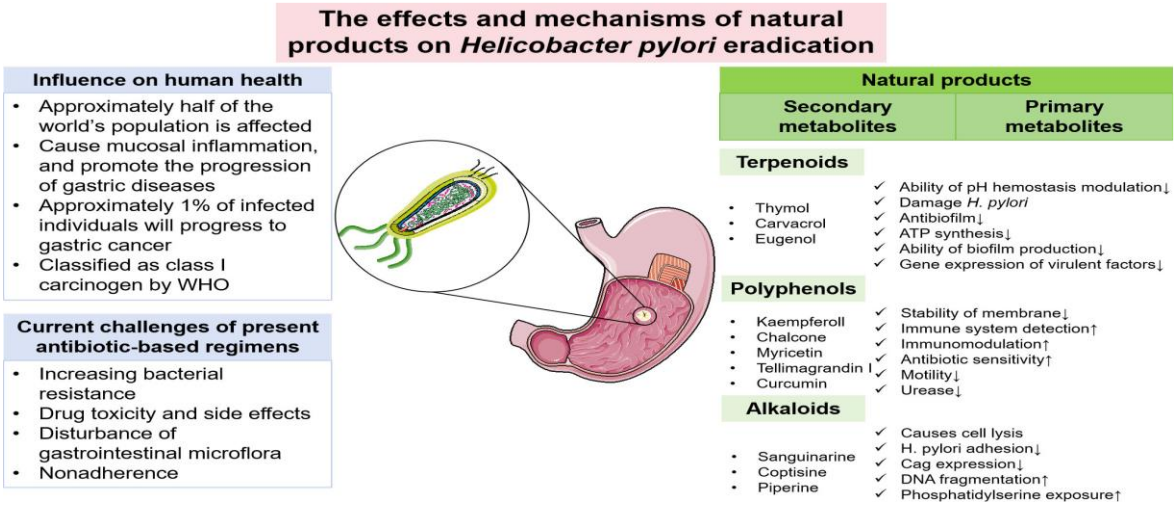
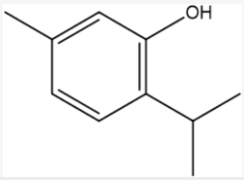
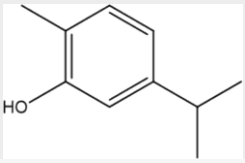
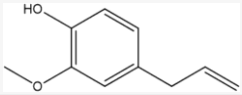
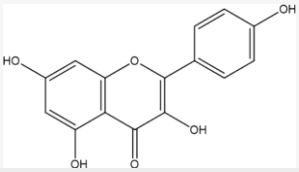
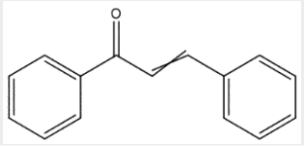
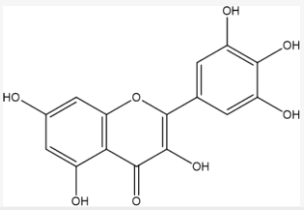
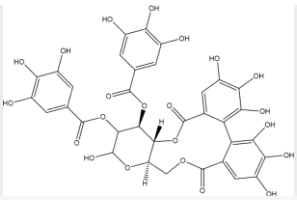
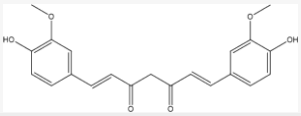
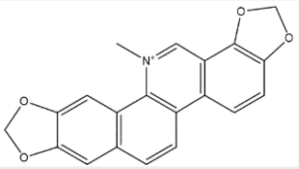
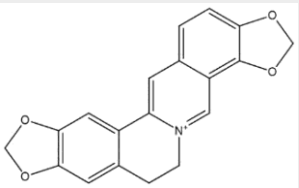
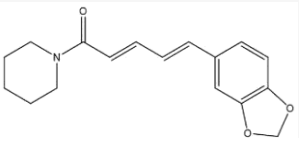


Figure 9: current challenges of *H.pylori* eradication and the potential role of natural products in eradicating *H .pylori* **(Deng *et al*. (2024).**

Some examples of secondary metabolites with inhibitory effects against *H. pylori* are resumed in the table below:

Table 2: secondary metabolites with inhibitory effects against *H pylori* secondary metabolites with inhibitory efeects againts *H pylori* **(Deng *et al.*, (2024)**

Compounds name	Reported from (plant name)	Mechanisms
Terpenoids		
Thymol 	*Thymus kotschyanus*	Ability of pH hemostasis modulation↓; damage *H. pylori*; membrane integrity; ATP synthesis↓; ability of biofilm production↓.
carvacrol 	*Origanum vulgare*	Ability of pH hemostasis modulation↓; damage *H. pylori*; membrane integrity; ATP synthesis↓; ability of biofilm production↓.
Eugenol 	*Eugenia caryophillis*	Antibiofilm↓; gene expression of virulent factors↓;
Polyphenols		
kaempferol 	*kaempferol galanga*	Stability of membrane↓.
chalcone 	species of the *Leguminosae, Asteraceae and Moraceae* families	Interactions between *H. pylori* and gastric epithelium↓; motility↓;urease↓;
myricetin 	*Myrica*	Immune system detection↑; antibiotic sensitivity↑; biofilm↓.
Tellimagrandin I	*Cornus canadensis*	damage *H. pylori*; membrane integrity;

		
Curcumin	*Turmeric*	Urease↓; immunomodulation↑.
		
Alkaloids		
sanguinarine	*Zanthoxylum nitidum*	Urease↓;cell lysis.
		
coptisine	*Rhizoma Coptidis*	Urease↓; Cag expression↓; DNA fragmentation↑; phosphatidylserine exposure↑; membrane stability↓.
		
Piperine	*black pepper*	Motility↓; *H. pylori* adhesion↓.
		

2.2.3 Mode of inhibition of molecules

Urease inhibitors are categorized into two primary classes based on their binding mechanisms:

- substrate-like (active site-directed) inhibitors
- Non-substrate-like (mechanism-based) inhibitors.

- **Substrate-Like or Active Site-Directed Inhibitors**

Active site-directed inhibitors mimic the substrate of urease, bridging the two nickel ions in the enzyme's active site, thus disrupting its function. These inhibitors often contain strongly basic groups that resemble the amide bond in urea, the natural substrate. Examples include thiourea and hydroxyurea, which adopt similar orientations to urea within the active site. Other notable compounds in this category are hydroxamic acid derivatives and phosphazenes **(Amtul *et al*., 2002).**

- **Non-Substrate-Like or Mechanism-Based Inhibitors**

Mechanism-based inhibitors, in contrast, do not structurally resemble the substrate. Instead, they are designed to interfere with the enzyme's catalytic process, leading to its inactivation. These inhibitors are initially unreactive and are transformed by the enzyme into reactive species that irreversibly inactivate it. Examples include phosphorodiamidate and imidazoles. Despite their potential, the clinical application of such inhibitors has been limited due to the complex and sometimes flawed hypothetical chemistry underpinning their design. Challenges include incorrect assumptions about the mechanism of action, unintended chemical pathways upon structural modification, and the need for precise product analysis to refine their design. Mechanism-based inhibitors, being unreactive until they encounter the enzyme, offer specificity and reduced toxicity compared to reactive compounds, which can non-selectively react with other enzymes **(Amtul *et al*., 2002).**

2.2.4 Biological properetes of plants used in Algeria known for their anti-urease activity

Expanding our understanding beyond the initial target of anti-urease activity to encompass the broader spectrum of biological activities exhibited by these plants is indeed crucial. By recognizing and exploring these additional activities, we may uncover valuable insights into their potential applications and mechanisms of action.

- ***Nigella sativa***

Nigella sativa oil and its bioactive compounds, including thymoquinone, have potent antioxidant and anti-inflammatory properties. These properties protect against oxidative stress and inflammation-related diseases. The oil also exhibits broad-spectrum antimicrobial and antiviral activities against bacteria, fungi, and viruses, making it useful for treating infectious diseases. *Nigella sativa* has potential as a chemopreventive agent, modulating the immune system and enhancing immune cell function. It also has cardiovascular protective effects, including hypocholesterolemic, antihypertensive, and antithrombotic effects. It also helps regulate blood glucose levels and protects the liver from diabetes and liver diseases **(Hashem-Dabaghian *et al.*, 2016).**

Figure 10: Picture of *Nigella sativa flower*

Xera Plants. (N.d.). *Nigella sativa*. Retrieved from https://xeraplants.com/plants/nigella-sativa/

Figure 11: Picture og *Nigella sativa* seeds

Fruition Seeds. (n.d.). Organic Black Cumin (Nigella). Retrieved from https://www.fruitionseeds.com/shop/herbs/blackcumin/organic-black-cumin-nigella/

- ***Salvia officinalis***

Salvia officinalis essential oil and extracts have shown antimicrobial, antioxidant, anti-inflammatory, anti-nociceptive, anticancer, antitumor, antidiabetic, insecticidal, and allelopathic properties. The plant's bioactive compounds, including carnosic acid and carnosol, have been found to inhibit cancer cell growth and proliferation. It also has antidiabetic effects, helping regulate blood glucose levels and improve insulin sensitivity. The plant's essential oil has shown insecticidal and allelopathic properties, making it a potential source of natural biopesticides. The study also revealed that Salvia officinalis essential oil has anti-urease activity, making it a potential alternative to Salvia essential oils. Further research is needed to determine active ingredients, toxicity, and structure-activity correlations **(Hassan *et al.*, 2019).**

Figure 12: Picture of salvia officinalis

Gardenia. (n.d.). Salvia officinalis. Retrieved from.https://www.gardenia.net/plant/salvia-officinalis

- ***Eucalyptus globules***

Eucalyptus globulus (EGEO) essential oil has potent antimicrobial and antibiofilm activities, particularly against Candida albicans and other fungal species. It also exhibits significant antioxidant potential, neutralizing ABTS free radicals due to its high monoterpene content. EGEO has effective insecticidal properties, killing 100% of Ocnogyna laverae insects at concentrations as low as 25%. The plant has also shown anti-inflammatory, anticancer, neuroprotective, and hepatoprotective effects. Its extracts significantly block the urease enzyme produced by *Helicobacter pylori*, preventing its survival in the stomach **(Abu-Qatouseh *et al.*, 2013)).**

Figure 13: Picture of *eucaluptus globules*

(the Nantes Naturopathe website the Nantes Naturopathe website)

- ***Zingiber officinale***

Ginger essential oil and extracts have numerous biological activities, including antimicrobial, insecticidal, antioxidant, anti-inflammatory, anticancer, antitumor, antidiabetic, and immunomodulatory properties. Ginger essential oil and extracts have potent antimicrobial and antibiofilm effects against bacteria, fungi, and microbes. It also exhibits insecticidal, repellent, and oviposition-inhibiting properties against various insect pests. Ginger's bioactive compounds, such as gingerol and shogaol, have anticancer and antitumor effects. It also has antidiabetic and hepatoprotective effects, regulating blood glucose levels and protecting the liver. Ginger's phenolic acids, such as gallic and cinnamic acids, contribute to its anti-*H. pylori* action by binding to the urease enzyme, which is

essential for *H. pylor*i's survival. Overall, ginger has numerous potential health benefits **(Nanjundaiah *et al* ., 2011).**

Figure 14: Picture of *zingiber officinale*

https://fr.lakpura.com/pages/zingiber-officinale

- ***Fenugreek (Trigonella foenum-graecum L.)***

Fenugreek extracts have been found to have antibacterial, antimicrobial, and anti-urease properties. They have been studied against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumonia. Ethanol extracts of fenugreek seeds have shown potent antimicrobial effects against Streptococcus pyogenes and Staphylococcus aureus. Fenugreek extract also has anti-urease activity against *Helicobacter pylori*, with significant inhibitory activity. The presence of phenolic compounds in fenugreek extract contributes to its antibacterial properties **(Hasna *et al.*, 2023).**

Figure 15: *Fenugreek* (*trigonella foenum -graecumL*)

https://artofcraft.co.za/product/trigonella-foenum-graecum-fenugreek-seed/

3. Molecular docking :

Molecular docking is a computational technique that predicts the preferred orientation of a small molecule (ligand) when it binds to a larger protein molecule (target) and estimates the strength of the binding interaction. This method is widely used in drug discovery and design to identify potential inhibitors or modulators of protein functions. The process involves several stages, including docking (place generation), scoring (affinity evaluation), force-field-based (calculating interaction energy based on physical forces), empirical (using experimentally derived parameters to estimate binding affinity), and knowledge-based (applying statistical data from known protein-ligand complexes to predict binding) **(Stanzione *et al*,. 2021).**

Molecular docking has various applications in drug discovery, protein-protein interactions, mutational analysis, and virtual screening. It helps identify promising drug candidates by predicting which molecules are likely to bind effectively to target proteins involved in disease. It can also study protein-protein interactions, which is important for understanding cellular processes and signaling pathways. By docking ligands to mutant proteins, researchers can investigate how genetic variations affect drug binding and efficacy **(Stanzione *et al*,. 2021).**

Materiels and Method

1. Computer hardware and analysis programs

In the endeavor to find new ways to treat drug-resistant *Helicobacter pylori*, we use computer analysis to research some natural compounds, which might help to inhibit urease. Urease plays a significant role in the survival of *H. pylori,* hence why it is seen as a desirable point of focus for dealing with gastric illnesses.

The aim of this research is to identify potential inhibitors of *H. pylori* urease from different plants by using molecular docking simulations. The goal is to find potential leads for inhibition from a wide range of plant species.

Our aim is to hasten the search for alternative treatments that can be used in fighting against serious *H. pylori* infections by working with both computer predictions and investigations that are done in the laboratory to establish their validity and at the same time enable appropriate interventions for this bacterium given the antibiotic resistance menace.

1.1 Microcomputers

We used two microcomputers one which has a RAM of 4.00 GB and an 11th Gen Intel(R) Core(TM) i3-1115G4 @ 3.00GHz 3.00 GHz processor, Windows 10 pro and other has a RAM of 4.00 GB and Intel(R) Pentium (R) CPU @ 2.16 GHz processor, Windows 10 pro

1.2 Programs and Data Banks PDB (Protein Data Bank): https://www.rcsb.org/

The Protein Data Bank (PDB) isn't exactly a "site" in the traditional web browsing sense. It's a global archive overseen by the Worldwide Protein Data Bank (wwPDB) consortium. This archive specifically stores information about the three-dimensional structures of large biological molecules, mainly proteins and nucleic acids Essentially, PDB is a massive collection of data that unveils the shapes of these crucial building blocks of life. Scientists can access and analyze this information to understand how these molecules function within organisms (**Berman *et al.*, 2000).**

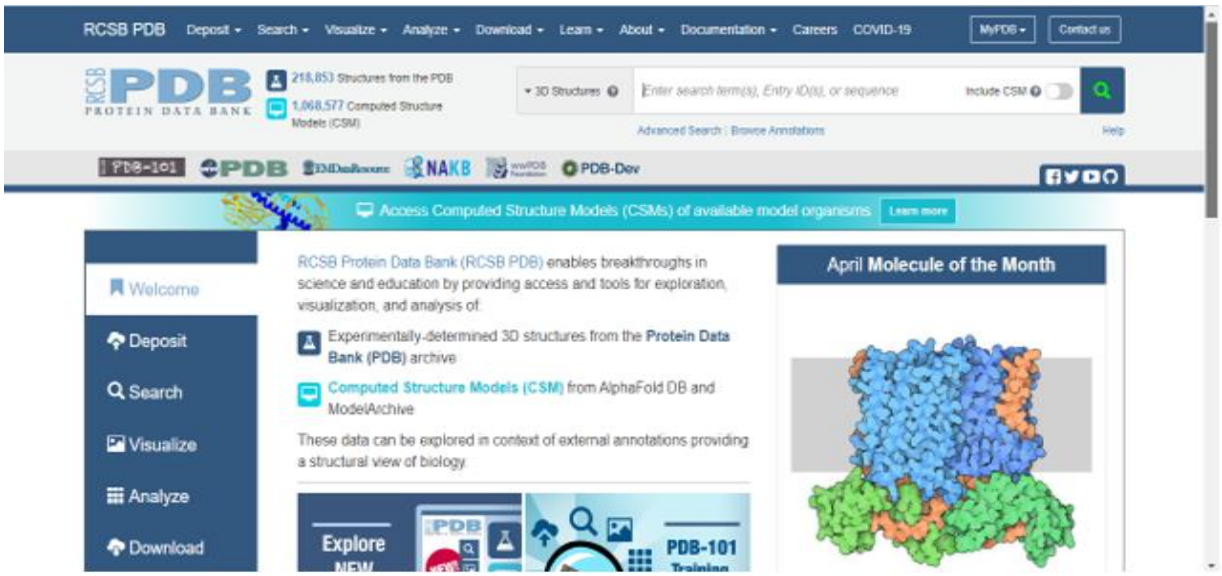


Figure 16: Home page interface of the RCSB PDB

1.3 PubChem: https://pubchem.ncbi.nlm.nih.gov/

PubChem is an open chemistry database managed by the National Institutes of Health (NHI). It contains mostly small molecules, but also larger molecules such as lipids, carbohydrates, nucleotides, peptides and other chemically modified macromolecules. The data in PubChem are organised into three interlinked databases: Substance (as of writing more than 286 million substance descriptions), Compound (over 111 million unique chemical structures) and BioAssay (1.2 million biological assays covering more than 10,000 target protein sequences) **(Stanzione *et al* ,. 2021).**

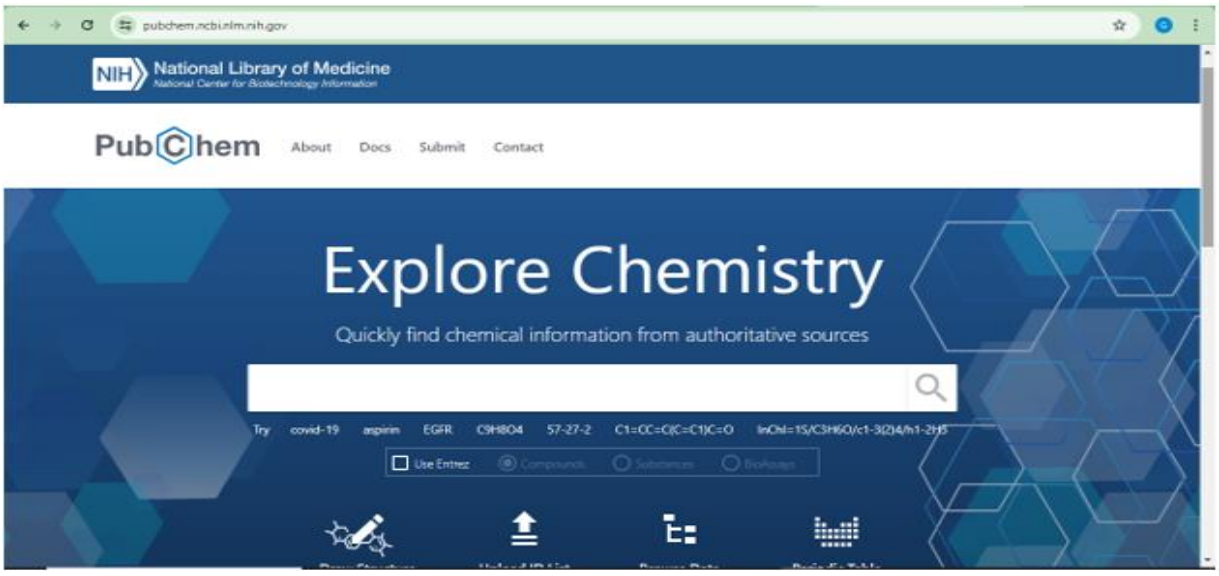


Figure 17: Home page interface of the pub chem.

1.4 AutoDock Tools

AutoDock Tools is a module of the MGL Tools software package specially designed to generate entries (PDBQT files) for AutoDock or Vina and which allows you to:

- Visualize molecules in 3D and prepare them for docking

- Configure the AutoGrid Settings File (GPF) using a visual representation of Grid area and slider-based widgets, launch AutoGrid and AutoDock;

- Read the results of an AutoDock job and display them graphically (**Morris *et al*., 2009).**

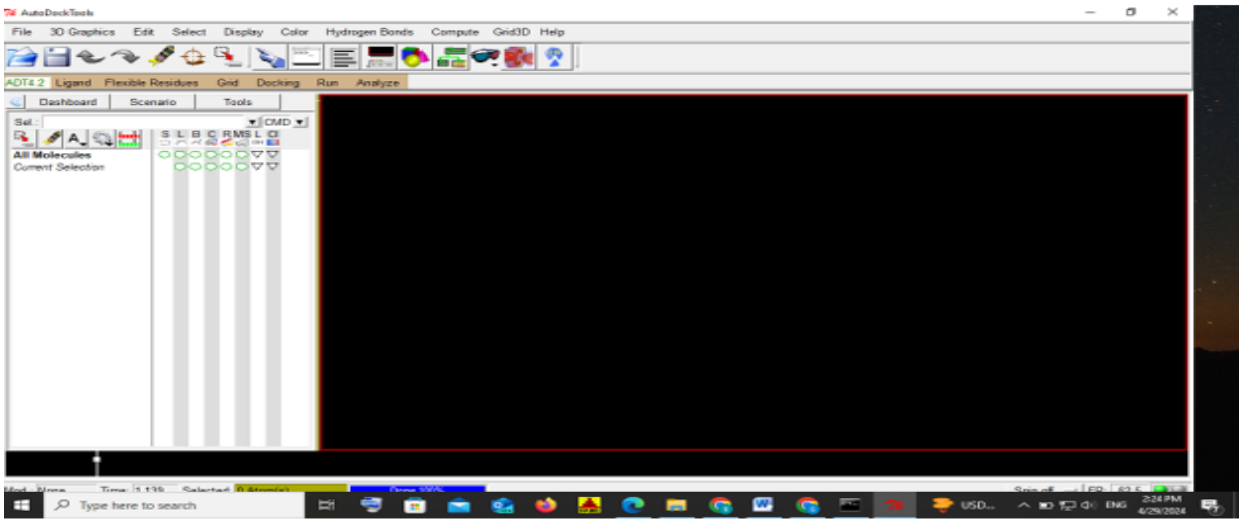


Figure 18: Interface of the home page of the Autodock tools software

1.5 AutoDockVina

AutoDock Vina is a new generation of docking software from MGL (Molecular Graphics Laboratory). It achieves significant improvements in the average accuracy of binding mode predictions, AutoDock Vina does not require choosing atom types and pre-calculating grid maps for them. Instead, it calculates grids internally for the types of atoms needed, and it does so virtually instantly **(Trott et Olson, 2010).**

1.6 BIOVIA Discovery Studio

Biovia Discovery Studio is a visualization tool, which allows a 3D display of a biological molecule structure and the results of the poses obtained by docking software.

It offers several functions such as: the presentation of chemical bonds (hydrogen bond, Van der Waals, pi-sigma, etc.), the measurement of interatomic distances, the annotation of amino acids (name, number), choice of color (according to the atoms, structures…), the ability to hide and then display again the different molecules

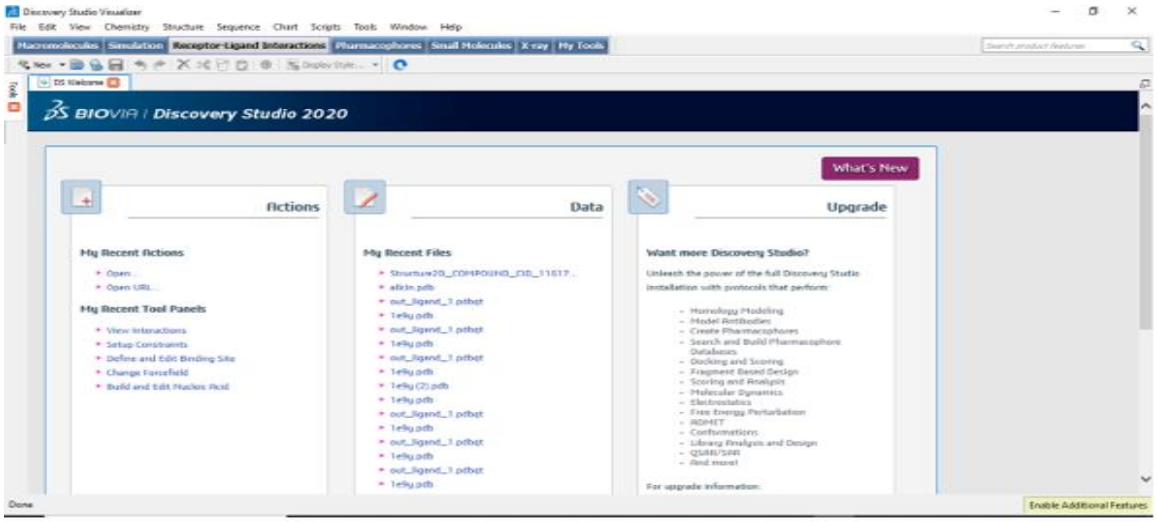


Figure 19: Biovia discovery studio softwareinterface

1.7 ChemDraw

ChemDraw is a comprehensive tool intended for chemists and biologists, integrating a whole range of intelligent tools to facilitate the work of researchers, it was developed in 1985 by David A. Evans and Stewart Rubenstein. It is an essential tool for illustrating chemical and biological concepts, it is simple to use, powerful and allows you to draw intuitively and efficiently in two and three dimensions **(Brown, (2014).**

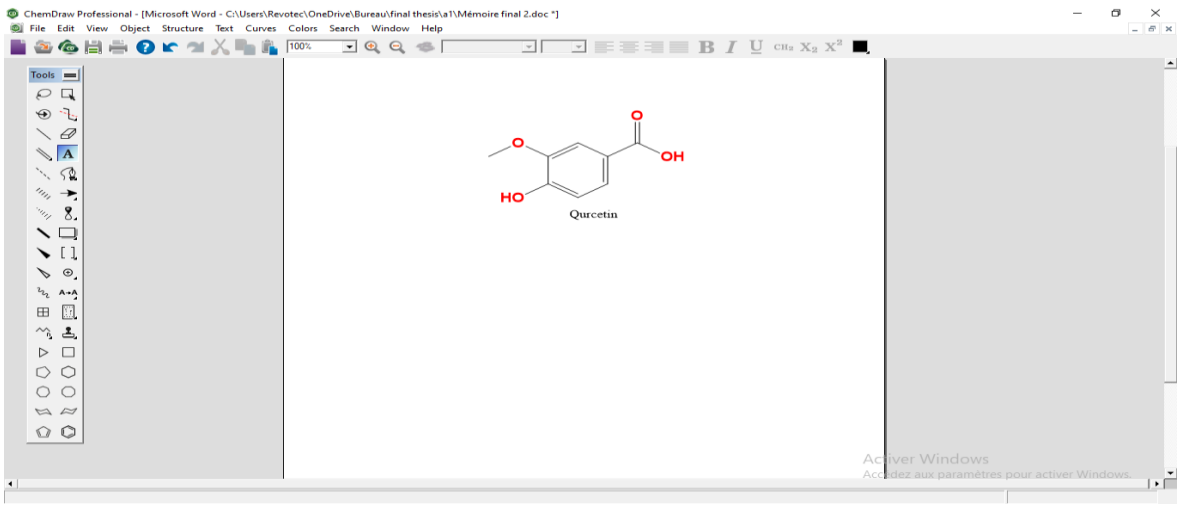


Figure 20 : Chemdeaw softwae software interface

1.8 SwissADME: http://www.swissadme.ch/

A web-based tool created to look at small substance Human Absorption, Distribution, Metabolism, and Excretion problems including pharmacokinetic parameters prediction. Swiss Institute of Bioinformatics made it for computational drug discovery and design as its main goal **(Daina *et al*., (2017).**

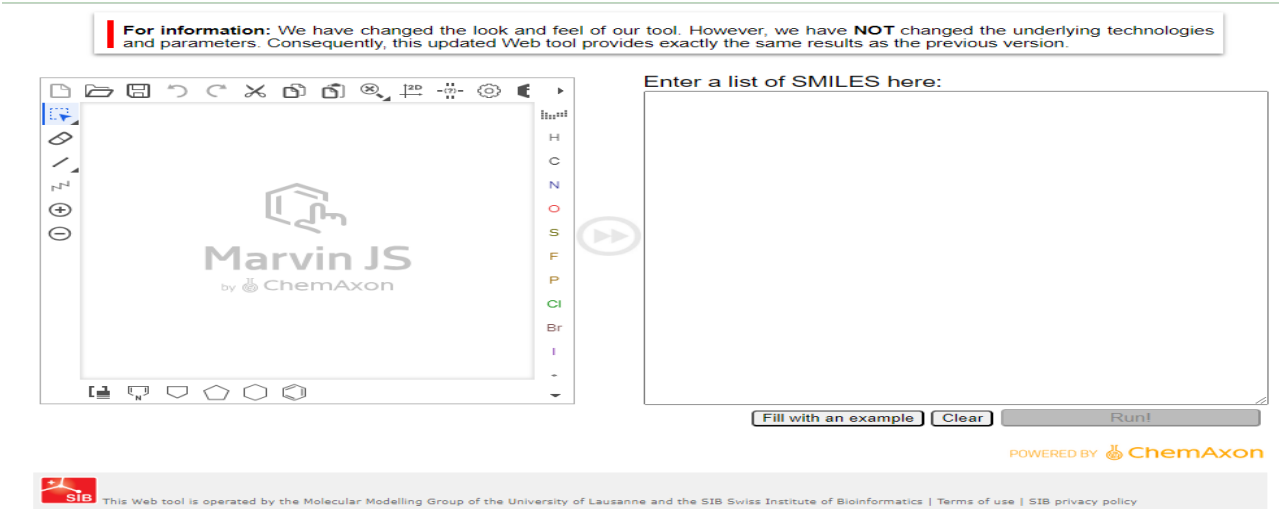


Figure 21: Home page interface of SwissADME

1.9 ADMETlab 2.0 : https://admetmesh.scbdd.com/

Is a web-based tool foretasting the Absorption, Distribution, Metabolism and Excretion (ADME) properties of chemical compounds. It is useful for scientists conducting investigations in the discovery and formulation of drugs by giving an account on the pharmacokinetic properties of possible drug candidates **(Xiong *et al*., 2021).**

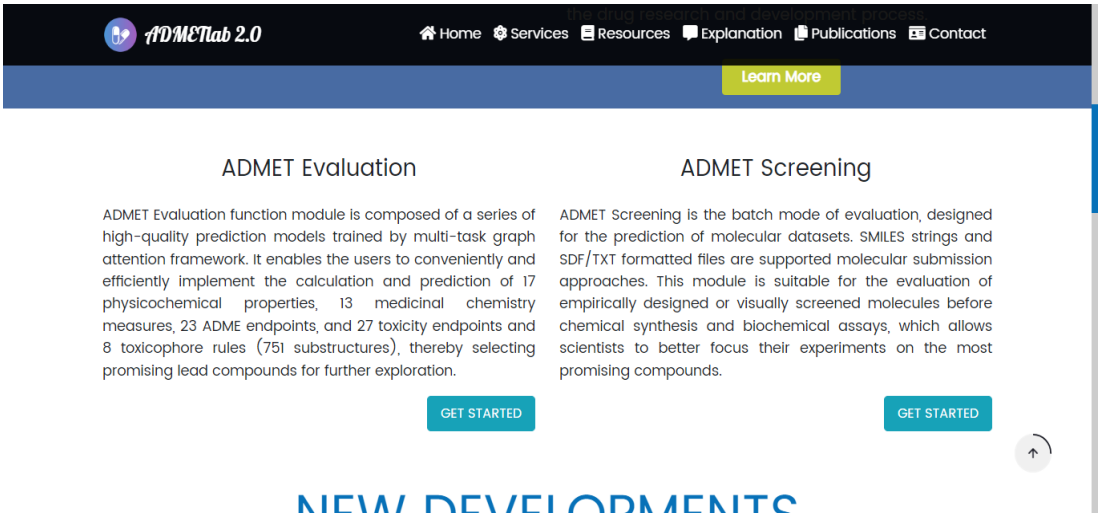


Figure 22: Home page interface of ADMET LAB

1.10 Molsoft : https://www.molsoft.com/

MolSoft is a software company that specializes in computational chemistry and biology tools. Their products and services are widely used in the fields of drug discovery, structural biology, and cheminformatics. The main offerings of MolSoft include various software packages and web-based tools for molecular modeling, visualization, and analysis **(Goswami *et al*., 2024).**

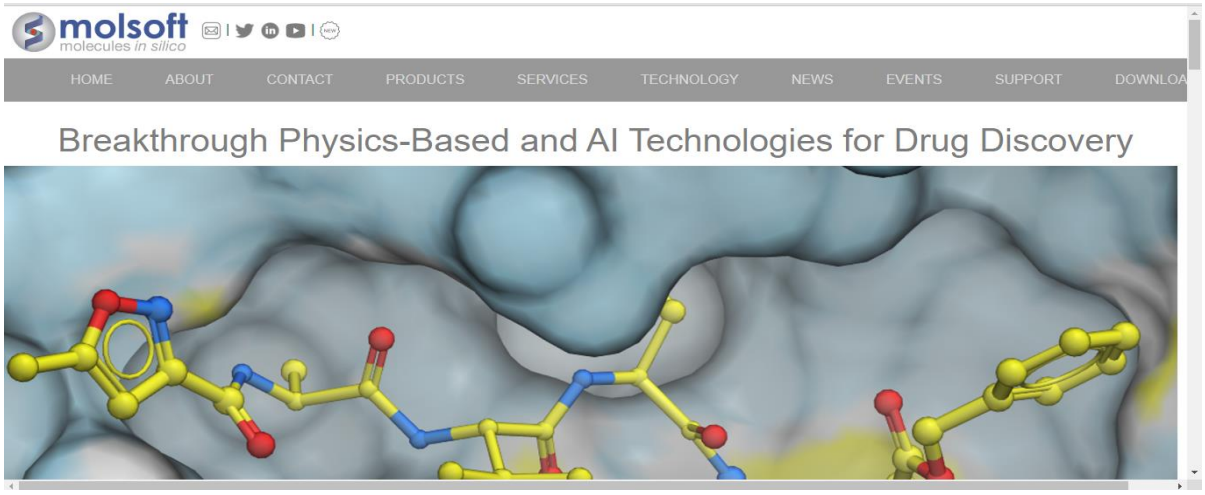


Figure 23: Home pageinterface of the MOLSOFT

2. Methods

2.1 Preparation of molecules for molecular docking

2.1.1 Protein preparation

The enzyme was prepared using the following steps/

- We download the proteins (enzymes) from the PDB site pdb format 1E9Y is in a complex form, bound by co-crystallized ligands.
- Go to the “File” menu.
- Select “Read molecule” (FIG)
- From the "Edit" menu, select "Hydrogens", then choose "Add".
- In the next dialog box, select "Polar Only" and click "OK." (fig)
- Again, under “Edit,” select “Hydrogens,” then “Merge Nonpolar.”
- If any warnings appear during this step, click “Continue” to continue.
- Go to "Edit" and select "Fees."
- Choose "Add Kolman Fee" and click "OK."
- Store the prepared protein:
- Return to the "File" menu.
- Select "Save", then "Write PDB".
- Make sure “Sort Nodes” is checked, then click “OK”.
- If prompted to overwrite, confirm by selecting “YES”

Figure 24: Information about 1E9Y

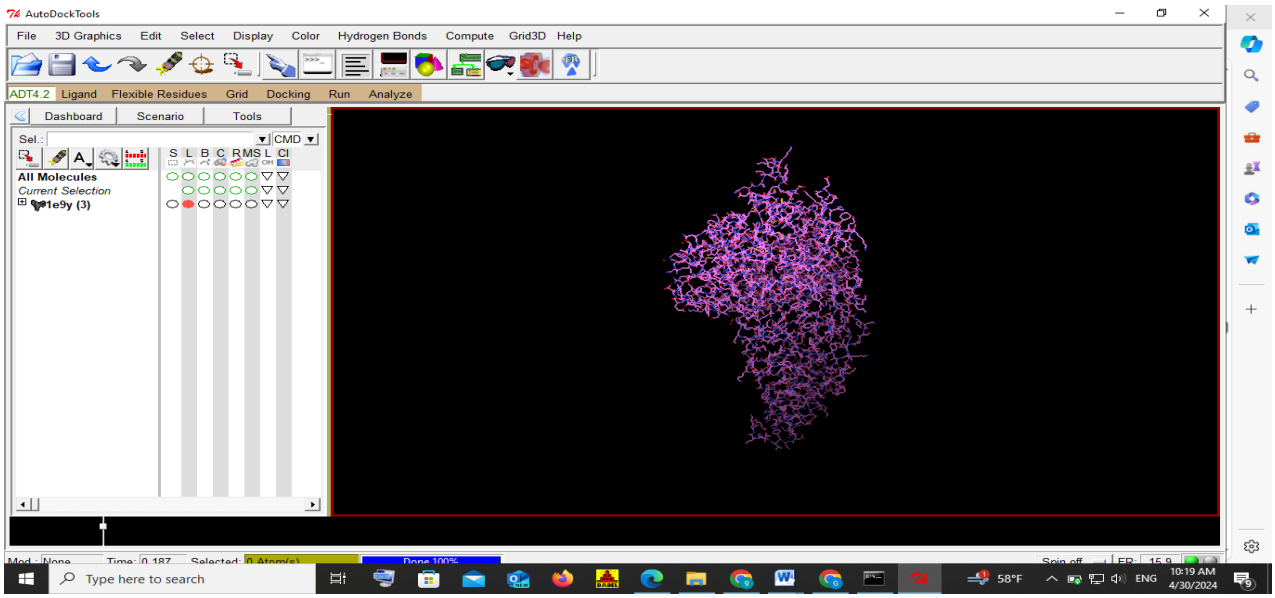


Figure 25: Importing the protein 1E9Y i pdb form on autodock tools

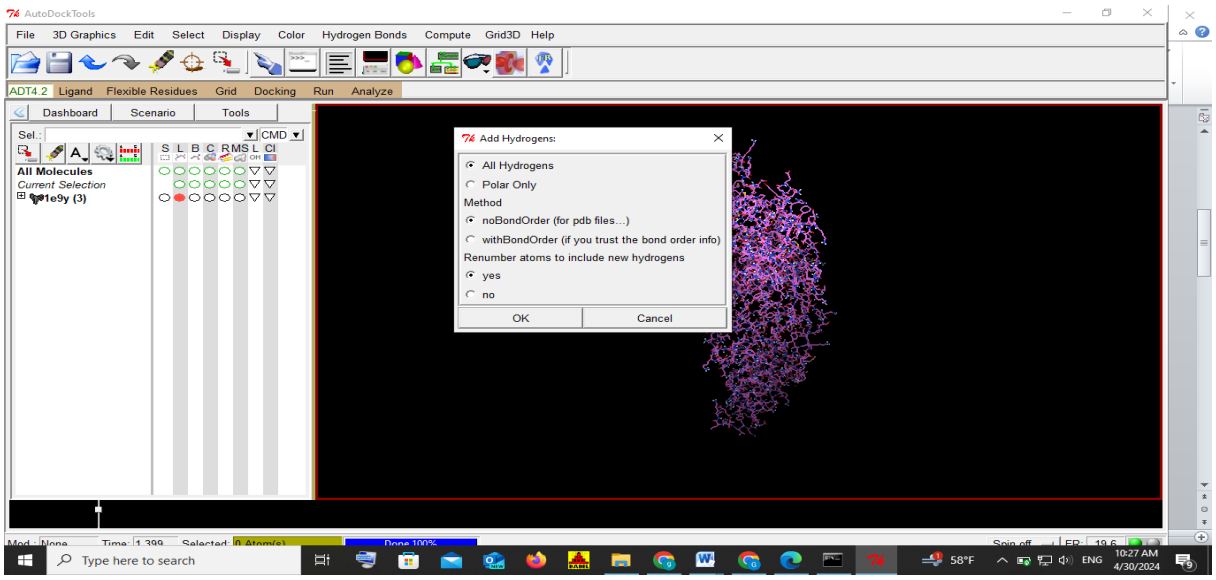


Figure 26: Addition of the polar hydrogens necessary for the diffrent interactions in the structure

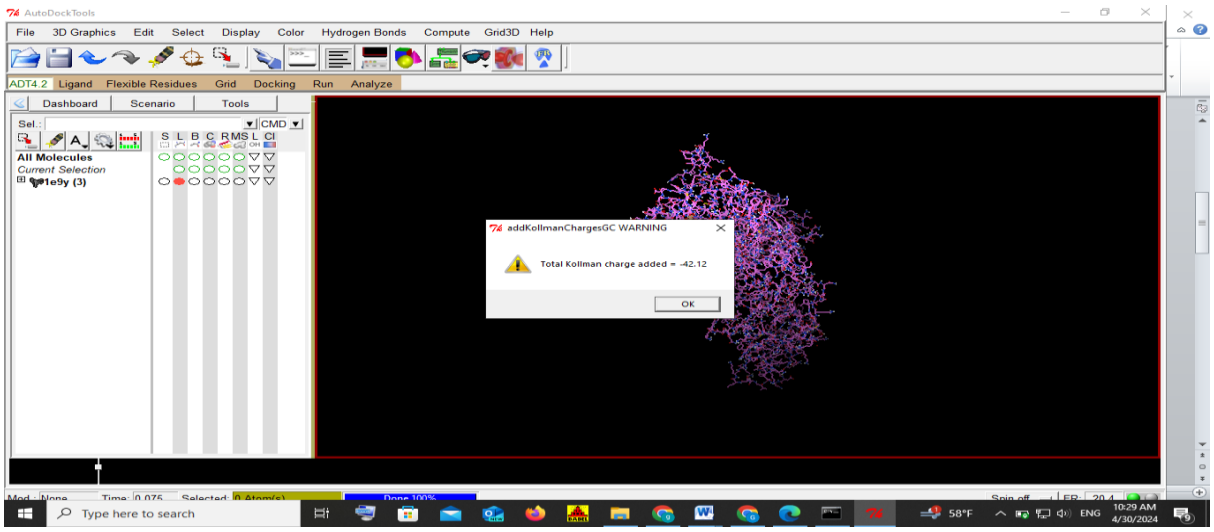


Figure 27: Addition of partial charges (kollman charge)

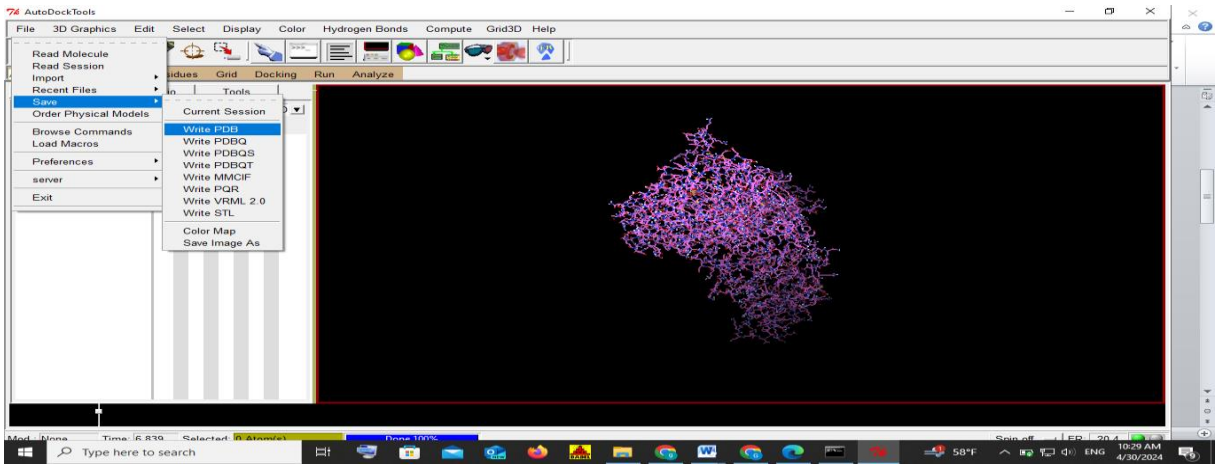


Figure 28: Save the protein in pdbqt form

2.1.2 Ligand preparation

In this study, the ligand ACETOHYDROXAMIC ACID was prepared for molecular docking simulations with the enzyme urease from *H. pylori* using AutoDock Vina, with the aid of Discovery Studio software. The following steps detail the ligand preparation procedure **(Kataria & Khatkar, 2019)**.

- Ligand Structure Retrieval :

The three-dimensional (3D) structure of ACETOHYDROXAMIC ACID was retrieved from the Protein Data Bank (PDB) database using Discovery Studio software. The ligand was selected based on its potential as a urease inhibitor and its relevance to the target enzyme.

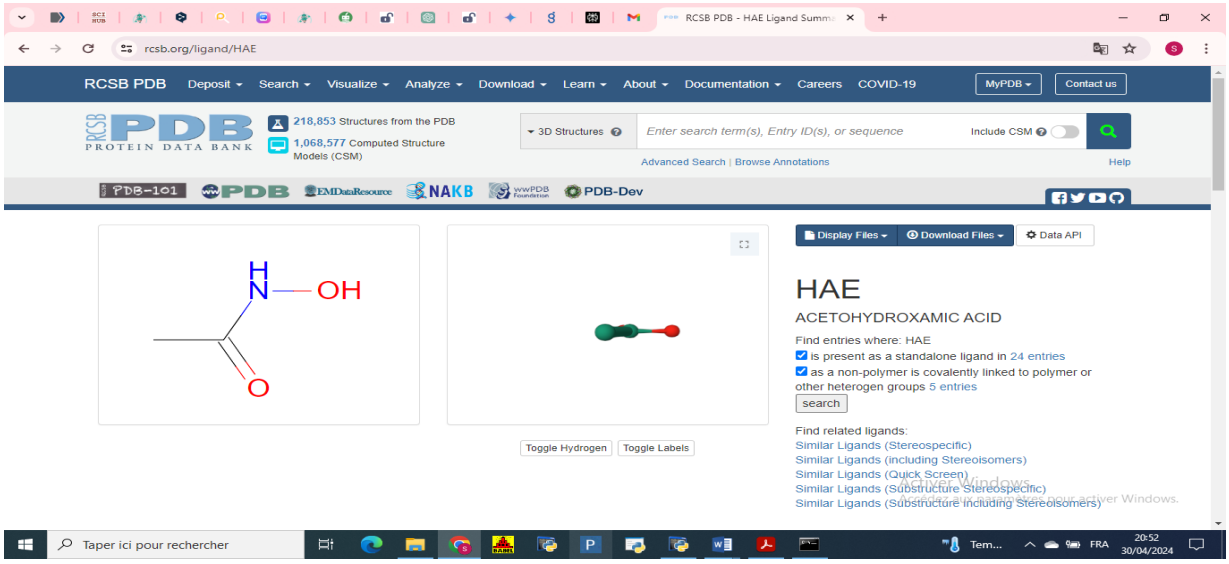


Figure 29: Structure of ACETOHYDROXAMIC ACID from the protein Data Bank (PDB)

➢ <u>Ligand Input and Opening :</u>

In Discovery Studio, the ligand structure was imported by navigating to "Ligand > Input > Open" and selecting the ligand file in ".pdb" format. The file was then opened by clicking "OK".

➢ <u>Ligand Optimization :</u>

The ligand structure was optimized using the built-in tools in Discovery Studio to correct any structural irregularities and minimize steric clashes. Energy minimization techniques, such as the CHARMM force field, were employed to relax the ligand conformation while maintaining its chemical integrity.

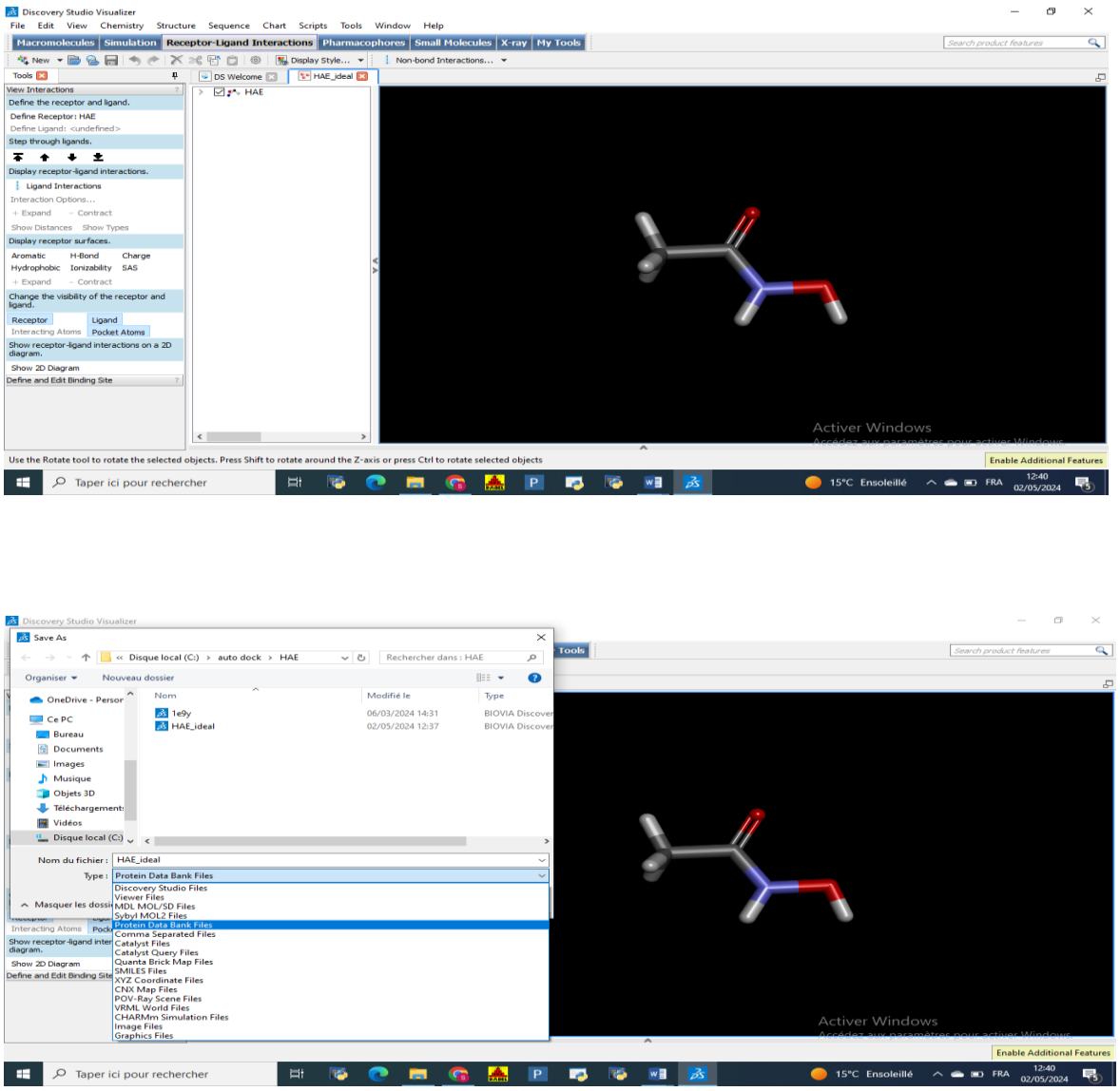


Figure 30: The ligand structure in Discovery studio

➢ <u>Hydrogen Addition and Charge Computation:</u>

Hydrogen atoms were added to the ligand structure using Discovery Studio's hydrogen addition tool. Appropriate protonation states were assigned to the ligand atoms to mimic physiological conditions and ensure proper ligand-receptor interactions during docking simulations. Additionally, charges were computed for the ligand using the Gasteiger method by navigating to "Edit > Charges > Compute Gasteiger".

➢ <u>Ligand Format Conversion:</u>

The prepared ligand structure was converted into the PDBQT format required by AutoDock Vina for docking simulations. This format includes atomic charges, atom types,

and other necessary parameters for docking. The conversion was performed by navigating to "Ligand > Output > Save as PDBQT".

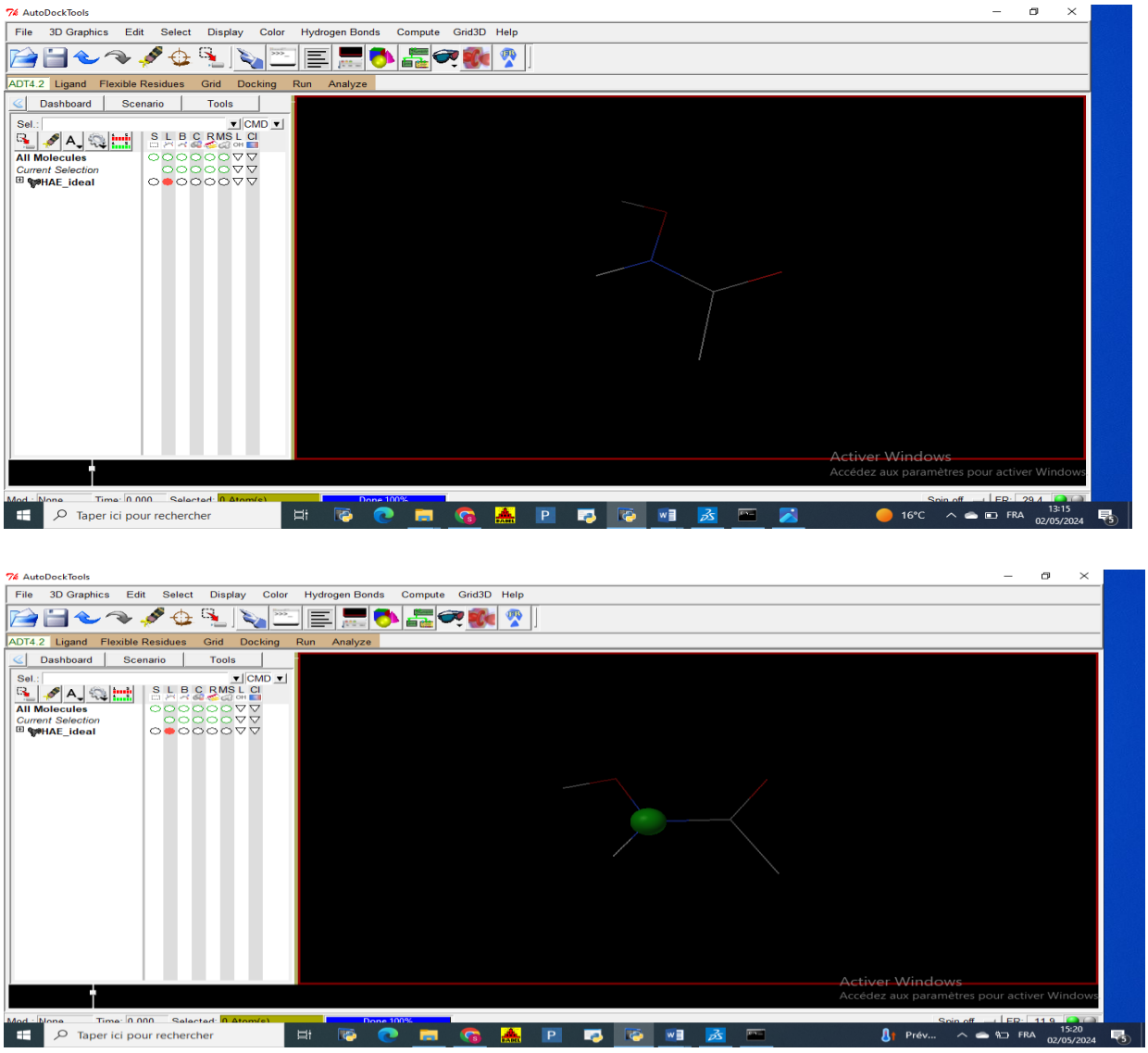


Figure 31 : The ligand preparation in auto dock

➢ <u>Validation of Ligand Preparation</u>

The prepared ligand structure was visually inspected within Discovery Studio to ensure proper atom connectivity, stereochemistry, and protonation states. Additionally, the ligand's properties, such as molecular weight and polar surface area, were verified to be consistent with literature values. Any discrepancies or anomalies were addressed through iterative refinement of the ligand structure.

➢ Grid Box Preparation

A docking grid box was defined around the active site of the enzyme urease *H. pylori* using AutoDock Vina. The dimensions of the grid box were chosen based on the expected ligand-binding region of the protein, ensuring that all key residues involved in ligand recognition were included within the docking grid.

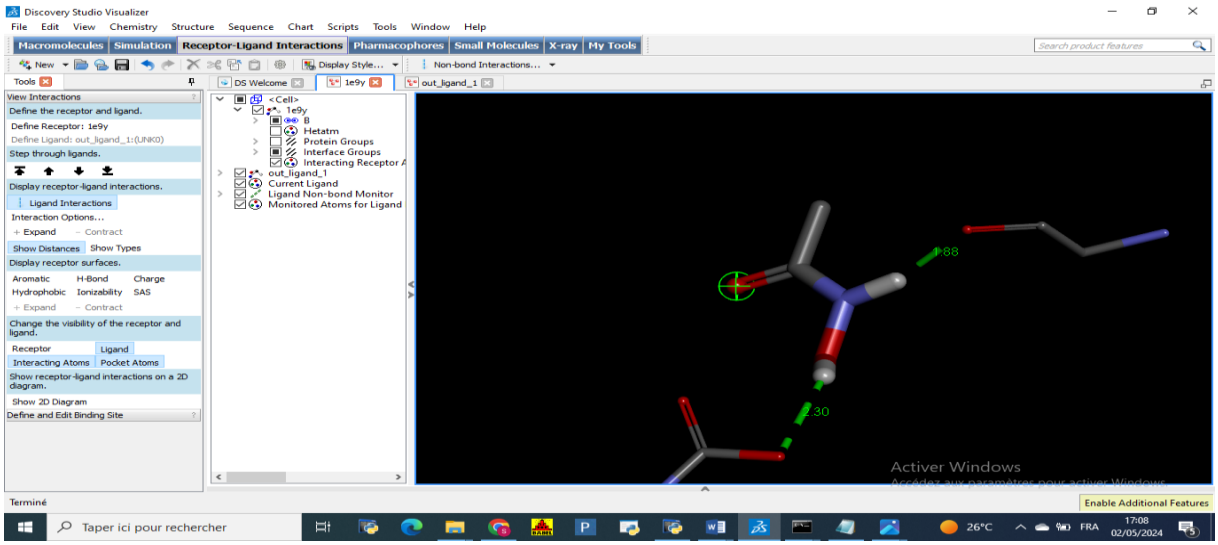


Figure 32: Grid box preparation , the ligand and the enzyme structure confirmations**(**site actives , amino acides ,distanses) using auto dock and discovery studio

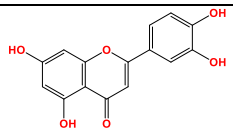
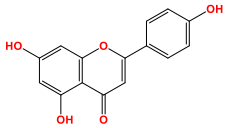
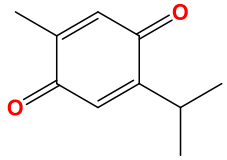
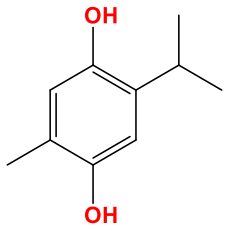
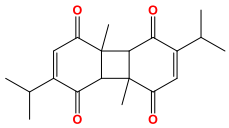
2.1.3 Urease inhibitory molecules preparation

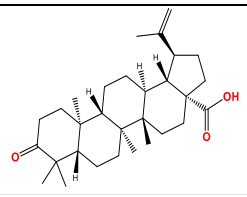
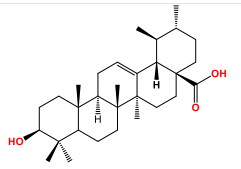
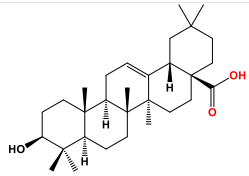
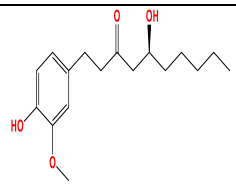
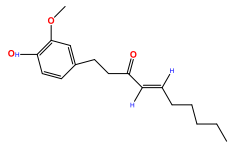
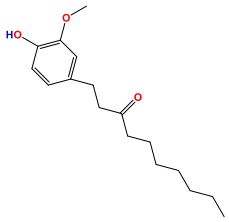
➢ Choosing Inhibitory Molecules for Urease

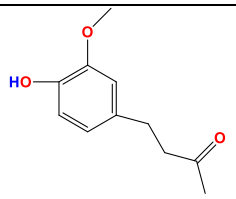
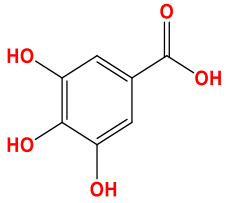
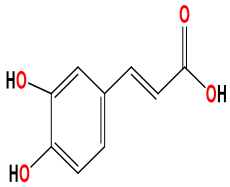
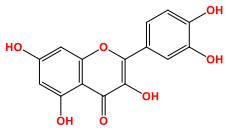
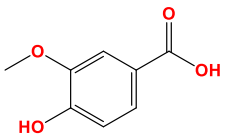
It has long been known that plants are rich sources of bioactive substances, many of which have antibacterial qualities and have been used for millennia in traditional medicine. The abundance of compounds found in plant secondary metabolites has the capacity to suppress urease activity, which in turn prevents *H. pylori* from colonizing and becoming harm **(Mobley et al., 2008).**

Based on their established inhibitory efficacy against *Helicobacter pylori* urease, the urease inhibitory compounds were chosen for the molecular docking investigation. These molecules comprised every compound listed in the bellow table(table 3).

Table 3: presenting inhibitory molecules from medicinal plants known for their anti urease activity

Plants	**molecules that inhibit the urease H pylori**	**family of molecules**	**properties**	**molecular weight g/mol (successes sivly)**	**structure**
Salvia officinalis (Hassan *et al.*, 2019). (Hassan *et al.*, 2019).	• Luteolin • Apigenin	• Flavonoids	• Antioxidant • anti-inflammatory	• 286.24 • 270.24	 • Luteolin  • Apigenin
Nigella sativa (Hashem-Dabaghian et al., 2016).	• Thymoquinone • **Thymohydroquinone** • **Dithymoquinone**	• Quinone • Hydroquinone • Quinone	• Antimicrobial, • anti-inflammatory, • antioxidant. Antimicrobial, • anti-inflammatory, • antioxidant.	• 164.2 g/mol • 166.2 g/mol • 244.3 g/mol	 • Thymoquinone  • Thymohydroquinone  • Dithymoquinone

Eucalyptus globulus **(Abu-Qatouseh et al. (2013)**	• Betulonic acid • Ursolic acid • Oleanolic acid	• terpenoids	• anti-inflammatory, • antioxidant, • anticancer, • hepatoprotective • antimicrobial activities	• 456.71 g/mol • 426.68 g/mol • 456.71 g/mol • 456.71 g/mol	 • Betulonic acid  • Ursolic acid  • Oleanolic acid
Zingiber officinale **(Nanjundaiah, Annaiah, & Dharmesh, 2011)**	• Gingerol • shogaols, • paradols, • zingerone	• phenolic ketones	• antioxidant, • antimicrobial, • anti-inflammatory, • and anticancer properties. antioxidant, • antimicrobial, • anti-inflammatory, and anticancer properties.	• 250.35 g/mol • 276.37 g/mol • 166.19 g/mol • 164.20 g/mol	 • Gingerol  • shogaols,  • paradols,

					 • zingerone
fenugreek (Trigonella foenum-graecum L.) **(Hasna et al., 2023)**	• gallic acid, • caffeic acid, • quercetin, • vanillic acid	• phenolic compounds	• antibacterial • Antioxidant • Anticancer • Anti-inflammatory • Hepatoprotective	• 170.12 g/mol • 180.16 g/mol • 302.24 g/mol • 168.15 g/mol	 • gallic acid,  • caffeic acid,  • quercetin  • vanillic acid

➢ <u>Preparation of urease *H. pylori* inhibitory moleculs for Auto Dock Vina and Discovery Studio</u>

The chosen urease inhibitory compounds' 3D structures were extracted from chemical databases and refined for docking investigations. The molecules were given atom kinds, charges, and torsional degrees of freedom in order to improve their docking software compatibility. Conformational search techniques were utilized to produce and assess multiple conformations of the urease inhibitory compounds.

Similar to ligand preparation, the molecules were protonated, charged appropriately, and optimized for docking simulations in both software platforms.

By following a similar approach to ligand preparation, the urease inhibitory molecules were effectively prepared for molecular docking using AutoDock Vina and Discovery Studio by BIOVIA, ensuring consistency and reliability in the docking studies.

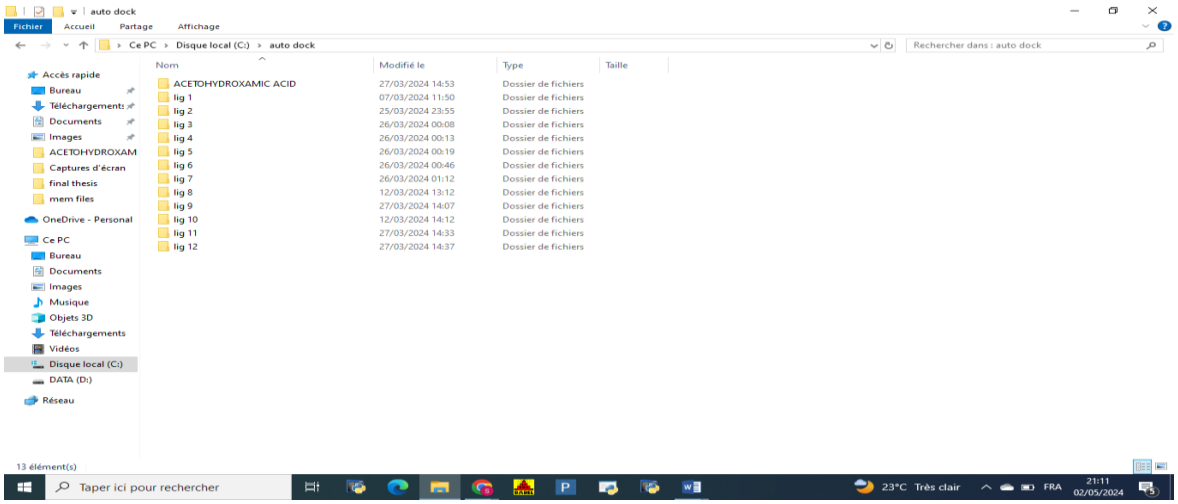


Figure 33 : the ihnibitor molecules preparations

Results and discussion

In this work, we conducted an *in silico* analysis to forecast the mode of action of putative *H. pylori* urease inhibitors. Based on their potential as urease inhibitors and their documented medical benefits, we first chose 17 natural substances. Molecular docking simulations were used for preliminary screening of these drugs. Three chemicals showed the most promising binding affinities and interactions from this first pool: vanillic acid, gallic acid, and quercetin. Subsequently, these three compounds underwent comprehensive docking studies and were juxtaposed with acetohydroxamic acid, a recognized urease inhibitor, to ascertain their binding affinities and interactions within the enzyme's active site

1. Molecular Docking results

We conducted molecular docking simulations with AutoDock Vina and Discovery Studio to investigate the binding interactions of quercetin, vanillic acid, and gallic acid with *H. pylori* urease (PDB ID: 1E9Y). The specific amino acid residues involved in the interactions as well as the binding modalities were revealed in detail by the docking data. A more thorough explanation of the molecular docking procedure and outcomes is given below:

1.1 Preliminary Screening

• **Selection of Natural Compounds**: We started with 17 natural compounds known for their medicinal properties and potential as urease inhibitors.

• **Initial Docking:** Using AutoDock Vina, we performed preliminary docking studies for all 17 compounds to assess their binding affinities to *H. pylori* urease. Compounds with low binding affinities were excluded from further analysis.

• **Comparative Analysis:** The docking results of the 17 compounds were compared to the standard inhibitor, acetohydroxamic acid, focusing on binding affinity and interacting amino acid residues. The comparison aimed to identify compounds with similar binding interactions to the standard.

Table 4: Represente the affinity and amino acid interactions of urease inhibiting compounds against *H pylori*

Ligand	Energy (Kcal/mol)	Amino acids
Acetohydroxamic Acid	**-4.6**	HIS :274 , GLY : 279 ,
Luteolin	-7.5	TYR :39, TYR :9, LYS :2, ILE :4,
Apigenin	-7.6	TYR:9,TYR:39,TYR:13 ,ILE:4, ,LYS:2,ARG:6
Thymoquinone	-5.5	TYR :39,TYR :9
Thymohydroquinone	-5.6	TYR :475, LYS :445, PHE :441
Dithymoquinone	-6.4	PHE :454 ,GLY :91
caffeic acid	-5.8	VAL :473, PHE :441,LYS :445
betulonic acid	-7.6	SER: 567 , LYS: 445
Ursolic acid	-7.9	PHE:569
Oleanolic acid	-7.7	SER: 151

Gingerol	-5.6	LYS: 2 , ILE: 4 ,ARG: 6 ,TYR :13 , TYR: 3
Shogaols	-5.9	ARG :6 , TYR :9 , TYR :39 , ILE :4
Paradols	-5.6	TYR :13 , LYS :2 , ILE :4 , ARG :6 ,TYR : 39, LYS :3
Zingerone	-5.7	LYS :2 , ILE :4 , TYR :39 , TYR : 13 , TYR : 9
gallic acid,	-6.0	ALA :365 , ASN :168 , ASP:223 ,HIS:221 ,ALA:169 , ARG:338
Quercetin	-7.1	ASN:168, ASP:165 , ALA:169 , ALA:365 , HIS:322
vanillic acid	-5.6	HIS :248 , GLU:222 , HIS :221 , HIS : 322 , ALA : 365 ,ALA: 169,CYS:321

• **Selection of Top Inhibitors**:

Selection of Top Inhibitors: Based on the comparison, three Compounds—gallic acid, quercetin, and vanillic acid—were selected for detailed docking studies due to their superior binding affinities and interaction profiles matching those of the standard. Initially, we did not find the same amino acids in the urease catalytic site as the standard. This led us to further investigate the active sites of the enzyme. We discovered that the enzyme possesses three distinct active sites. Among the 17 compounds studied, the three selected compounds.

(gallic acid, quercetin, and vanillic acid) showed binding affinities and interaction profiles at the same site as the standard, while the remaining 13 compounds interacted with other sites (site 2 and site 3).

Table 5: representation of active site of molecules

Molecules	**1st site**	**2nd site**	**3rd site**
HAE	×		
betulonic acid		×	
Ursolic acid		×	
Oleanolic acid		×	
Gingerol			×
Shogaols			×
Paradols			×
Zingerone			×
Gallic acid	×		
Quercetin	×		
vanillic acid	×		
Luteolin		×	
Apigenin		×	
Thymoquinone			×
Thymohydroquinone			×
Dithymoquinone			×
Caffeic acid			×

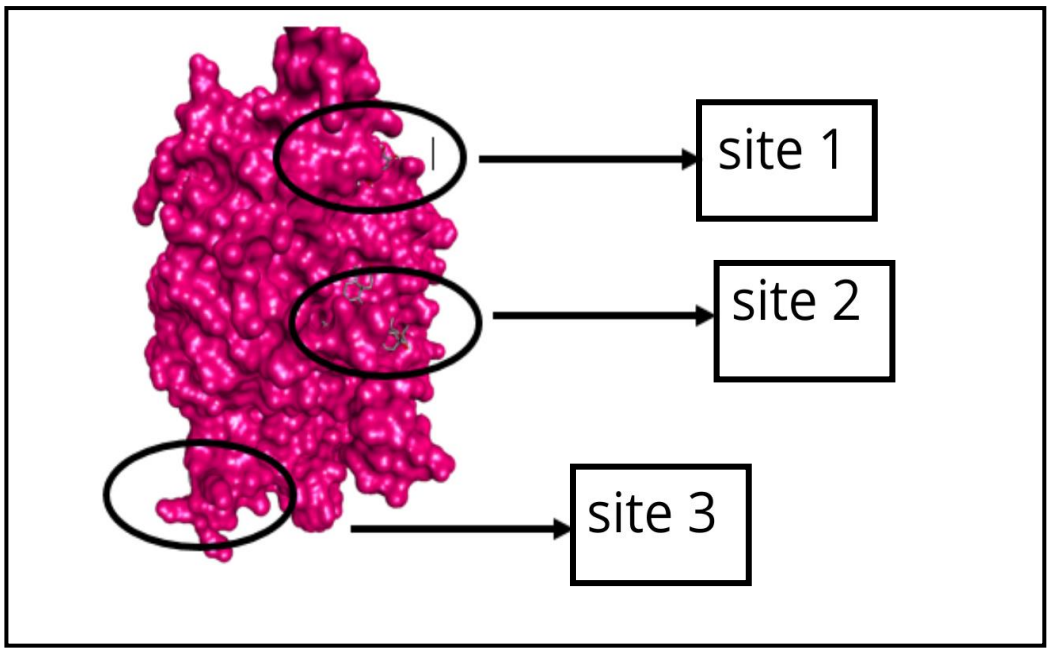


Figure 34: The active sites of molecules with urease enzyme

Detailed Docking Analysis for the detailed docking studies, we utilized both AutoDock Vina and Discovery Studio to ensure the accuracy and reliability of the docking results.

1.2 Molecular interaction between selected moleculesand urease

1.2.1 Quercetin

- **Binding Affinity:** Quercetin exhibited a docking score of-7.1 kcal/mol, indicating a strong binding affinity.
- **Interacting Residues:** The primary interacting residues were ASN 168, ASP 165, ALA 169, ALA 365, HIS322
- **Binding Mode:**

• Hydrogen Bonds: Quercetin formed hydrogen bonds with ASN 168, ASP 165, enhancing the stability of the complex.

• Hydrophobic Interactions: Hydrophobic interactions further contributed to the overall stability and affinity of the complex.

• Comparison with Acetohydroxamic Acid: Quercetin's binding interactions were highly similar to those of acetohydroxamic acid, with additional hydrogen bonding interactions providing a potentially higher inhibitory effect

- **Additional Insights**

• Quercetin demonstrated significant urease inhibitory activity with an IC_{50} of 11.2 ± 0.9 µM against *H. pylori* urease, slightly more potent than acetohydroxamic acid (IC_{50} = 19.4 ± 2.0 µM) **(Xiao *et al.*, 2012).**

• Structure-activity relationship analysis revealed that the 3-hydroxyl group in quercetin is crucial for its activity. Modification or removal of this group significantly reduces potency.

• The kinetic studies indicated that quercetin inhibits *H. pylori* urease through a noncompetitive inhibition mechanism, meaning it binds to a site other than the active site and forms an inactive complex.

• Molecular docking supported this mechanism, showing quercetin binding outside the urea binding pocket, anchoring a helix-turn-helix motif over the active site cavity, and preventing the flap from closing. This was facilitated by multiple hydrogen bonds and hydrophobic interactions. **(Xiao *et al.*, 2012).**

- **Mechanism of Inhibition:**

• Quercetin's noncompetitive inhibition involves binding at a region outside the active site, forming hydrogen bonds that prevent the enzyme's flap region from closing, disrupting catalytic activity indirectly., stabilizing the enzyme in an open conformation, and preventing substrate access. This binding mode involves hydrogen bonding with key residues such as Met366, Gly367, Asp165, and Asn168, further elucidating its inhibitory potency.

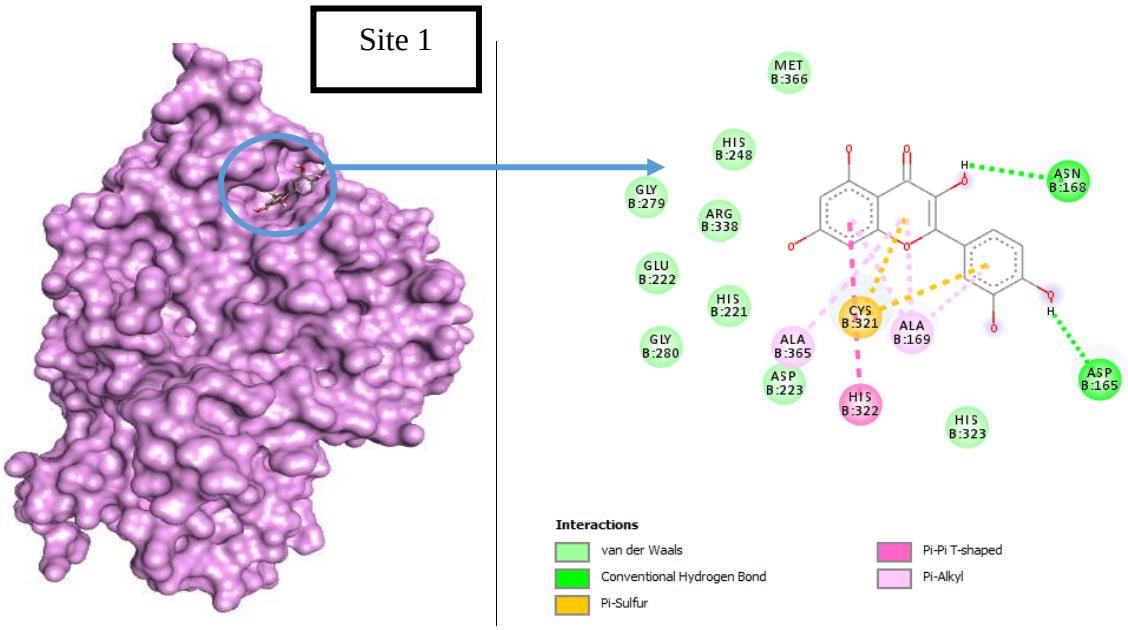


Figure 35: Molecular interactions between quercetin and the active site of the urease enzyme using auto dock

1.2.2 Gallic acid

- **Binding Affinity:** The docking score for gallic acid was -6.0 kcal/mol, indicating a favorable binding interaction with the urease enzyme.
- **Interacting Residues:** The key residues involved in binding included ALA 365 , ASN 168 , ASP 223 ,HIS 221 ,ALA 169 , ARG 338
- **Binding Mode:**

• Hydrogen Bonds: Gallic acid formed hydrogen bonds with ASN 168 , ASP 223 andALA 365 , which significantly contributed to the stability of the complex.

• Hydrophobic Interactions: Hydrophobic interactions helped to anchor the molecule within the active site.

Comparison with Acetohydroxamic Acid: The interaction profile of gallic acid closely matched that of acetohydroxamic acid, particularly in forming hydrogen bonds and hydrophobic interactions

- **Mechanism of Action:** The molecular docking results suggest that gallic acid can inhibit urease by binding to key active site residues, forming stable hydrogen bonds, and participating in hydrophobic interactions that stabilize the binding. This binding prevents the urease enzyme from adopting its active conformation necessary for catalysis.
- **Mechanism of Inhibition**

Binding Details: The docking studies showed that gallic acid interacts with HIS 274 , GLY 279 through hydrogen bonds, which are crucial for its inhibitory action. The presence of these interactions indicates that gallic acid can effectively block the urease activity by occupying the active site and preventing substrate access.

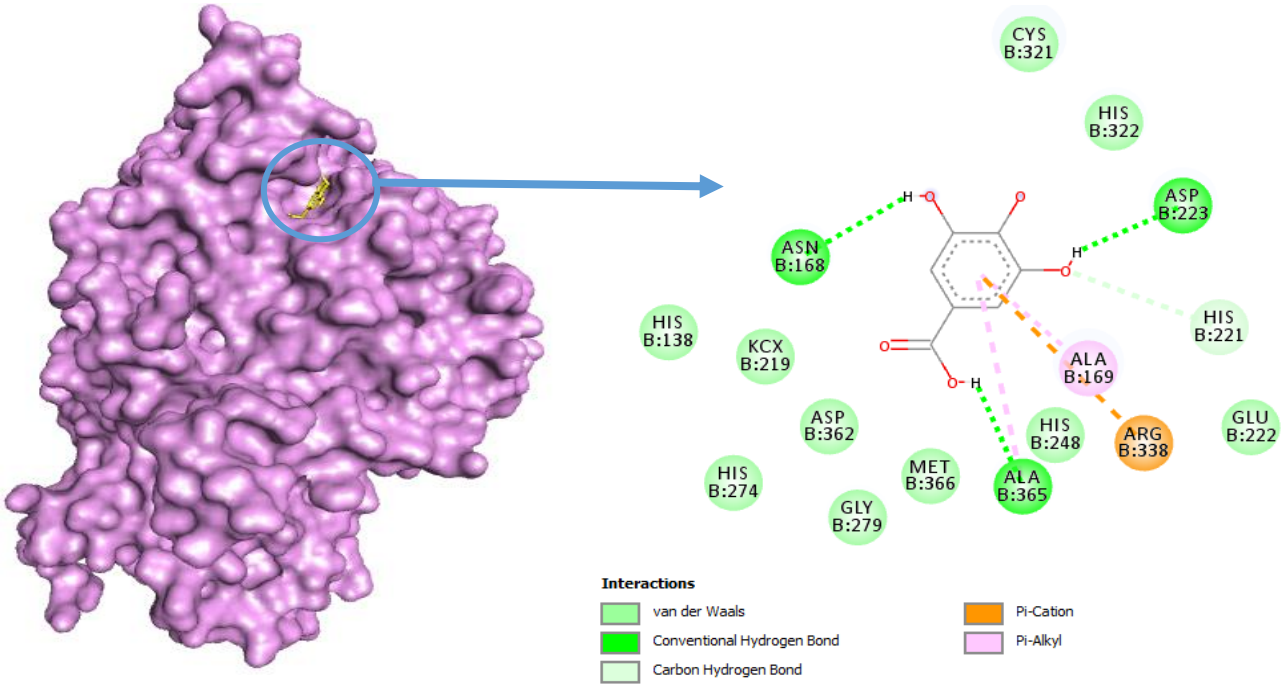


Figure 36 : Molecular interactions between gallic acid and the active site of the urease enzyme using autodock

1.2.3 Vanillic acid

Using the Discovery Studio Visualiser program, it was demonstrated that the complex formed between the urease enzyme and the vanillic acid is stabilized by several types of bonds including a hydrogen bond with the HIS 138, GLY 279, ASP 223 residue, and Pi-cation bond with ARG 338 plus Alkyl and Pi-Alkyl bond with ALA 365, ALA 169, and CYS 321. The ligand is also stabilized by van der Waals bond formed with the amino acids ASP 362, HIS 274, HIS 248, HIS 221 and HIS 322.

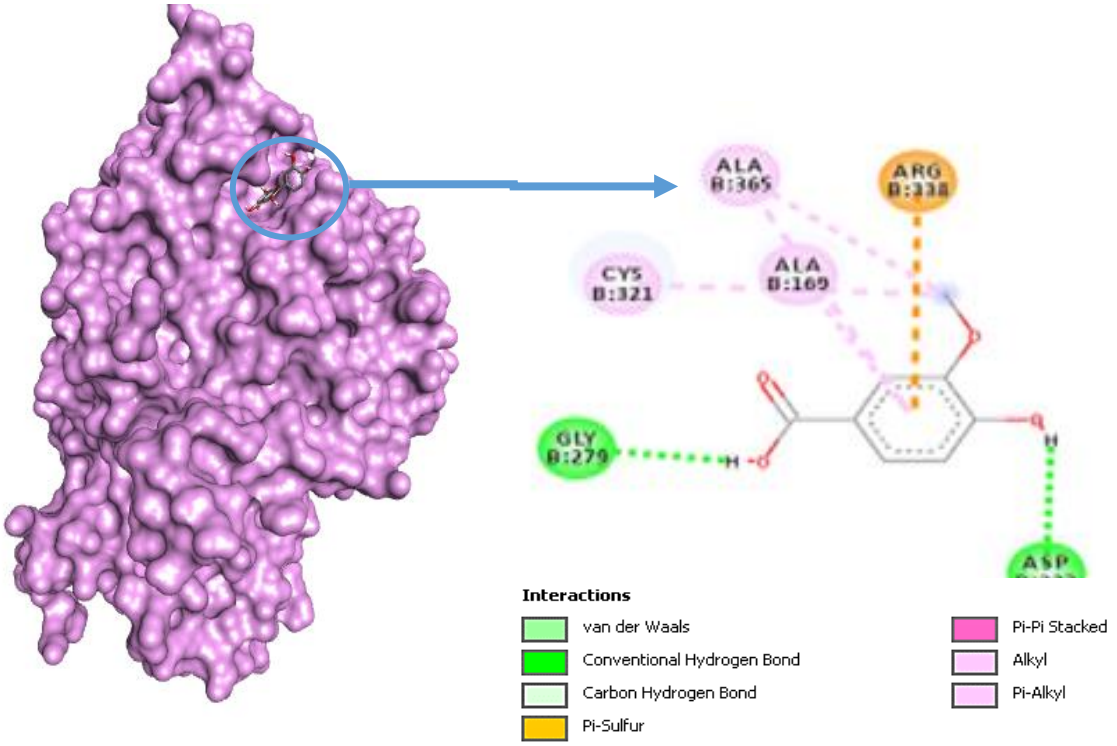


Figure 37: Molecular interactions between vanillic acid and the active siteof the urease enzyme using autodock

We note that urease and vanilic acid ligand have one identical residue: HIS 274 which is van der Waals bond and also By analyzing the binding site of our ligand and the stander(HAE)with the enzyme, we find that they are associated with the same catalytic site (site 1), so that mean vanillic acid is competitive inhibitor.

- **Mechanism of Inhibition**

Vanillic acid inhibits the urease enzyme through competitive inhibition, binding to the active site and competing with urea. Hydrogen bonds with ASP B:223 and GLY B:279, along with hydrophobic and pi interactions, ensure strong binding, preventing urea from accessing the catalytic site. Additionally, vanillic acid induces conformational changes that alter the active site's shape, reducing its affinity for urea. These interactions with ALA, CYS, and ARG residues stabilize the enzyme's altered conformation, enhancing inhibition. By occupying the active site and interacting with key residues, vanillic acid disrupts the enzyme's catalytic activity, potentially blocking essential metal ions like Ni2+.

- **Commonalities**

The analysis highlights that histidine (HIS) and aspartic acid (ASP) residues are consistently involved in the binding interactions of quercetin, gallic acid, and vanillic acid with H. pylori urease, playing a crucial role in stabilizing the enzyme-ligand complex. Quercetin binds to ASP 165 and HIS 322, gallic acid to ASP 223 and HIS 221, and vanillic acid to HIS 138, ASP 223, HIS 221, and HIS 322. Regarding hydrogen bond interactions, quercetin utilizes ASN 168 and ASP 165, gallic acid involves ASN 168, ASP 223, and ALA 365, and vanillic acid interacts with HIS 138, GLY 279, and ASP 223. These findings indicate that while quercetin and gallic acid primarily use hydrogen bonds with ASN and ASP residues, vanillic acid's binding mode is more complex, involving extensive van der Waals and pi interactions. This comparison underscores the significance of HIS and ASP residues in the inhibition of urease activity by these natural compounds.

Molecular docking studies revealed that these compounds exhibit strong binding affinities and stable interactions with the enzyme's active site and nearby regions. Quercetin, in particular, was found to bind non-competitively, interacting with the flap region of the urease, thus preventing the enzyme from adopting its active conformation. Similarly, gallic acid and vanillic acid displayed favorable binding interactions, primarily through hydrogen bonds and hydrophobic interactions with key active site residues. These findings suggest that phenolic compounds could be developed as effective treatments to combat *H. pylori* infections, offering a natural and potent alternative to current urease inhibitors. Further experimental validation and *in vivo* studies are needed to confirm these results and explore their therapeutic potential.

2 Results of pharmacological properties

To comprehensively evaluate the potential of identified urease inhibitors, it is crucial to assess their pharmacokinetic properties alongside their binding affinities. *In silico* absorption, distribution, metabolism, and excretion (ADME) prediction tools, specifically those employing Lipinski's Rule of Five.

Table 6: Lipinski°s rule of five analysis for three ligands

Ligand	MW	Log P	nON	nOHN
Quercetin	302.24 g/mo	1.23	5	7
Galic acid	170.12 g/mol	0.21	4	5
Vanillic acid	168.15 g/mol	1.08	2	4

The molecular weights of quercetin, gallic acid and vanillic acid are less than 500 g/mol, so they easily cross the cell membrane.

We show that the ligands have a hydrogen acceptor number of less than 10 and a hydrogen donor number of less than 5.

The Log P property, which determines solubility, the higher the value of Log P, the lower the solubility. The value of Log P is less than 5 for the molecules. Quercetin, gallic acid and vanillic acid therefore comply with Lipinski's rule.

2.1 Results of pharmacokinetic properties and toxicity

Using the SwissADME prediction engine and ADMETlab to check the pharmacokinetic properties and toxicity of quercetin , gallic acid and vanillic acid. The results are presented in table 07

Table 7: the pharmacokinetic properties and toxicity of the ligands

Properties	**Quercetin**	**Gallic acid**	**Vanillic acid**
GI (Gastro intestinal) absorption	High	High	High
BBB (Blood Brain Barriere)	No	NO	NO
CYP1A2 inhibition	Yes	NO	NO
CYP2C19 inhibition	No	NO	NO
CYP2C9 inhibition	No	NO	NO
CYP2D6 inhibition	Yes	NO	NO
CYP3A4 inhibition	Yes	YES	NO
AMES Toxicity	POSITIVE	NEGATIVE	NEGATIVE
Carcinogenicity	NEGATIVE	NEGATIVE	NEGATIVE
hERG_inhibition	NEGATIVE	NEGATIVE	NEGATIVE

- The SwissADME results for Quercetin, Gallic acid, and Vanillic acid show that they have high gastrointestinal (GI) absorption. This indicates that these compounds can easily pass into the bloodstream when administered orally, suggesting good potential for oral bioavailability.
- The ADMETlab results indicate that none of these compounds cross the blood-brain barrier (BBB). This means they are unlikely to have direct effects on the central nervous system (CNS) or cause CNS-related side effects.
- Quercetin inhibits CYP1A2, CYP2D6, and CYP3A4. This suggests that Quercetin could affect the metabolism of drugs that are substrates for these enzymes, potentially leading to drug interactions.

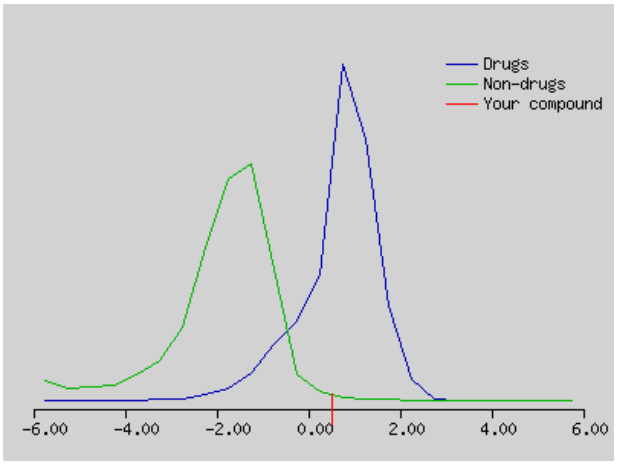


Figure 38: MOLSOFT visualization quercetin _urease docking interaction analysis (score :0.52)

Gallic acid inhibits CYP3A4 but does not inhibit the other tested cytochrome P450 enzymes. Thus, it may still interact with drugs metabolized by CYP3A4, although it has fewer potential interactions compared to Quercetin.

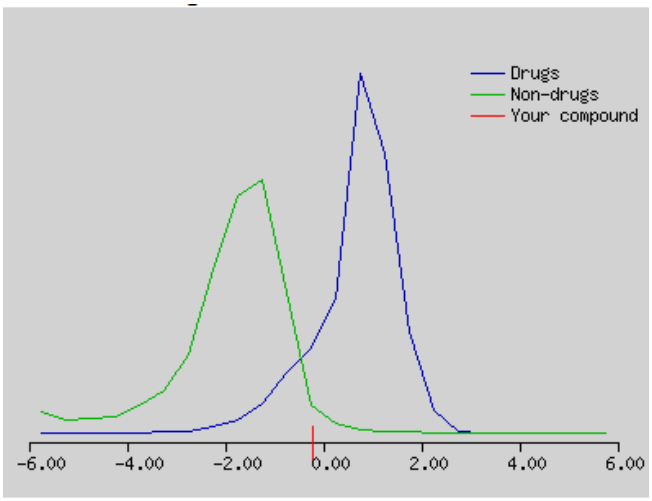


Figure 39: MOLSOFT visualization gallic acid_ urease docking interaction analysis (score:-0.22)

- Vanillic acid does not inhibit any of the tested cytochrome P450 enzymes, suggesting a lower risk for drug-drug interactions related to these enzymes.

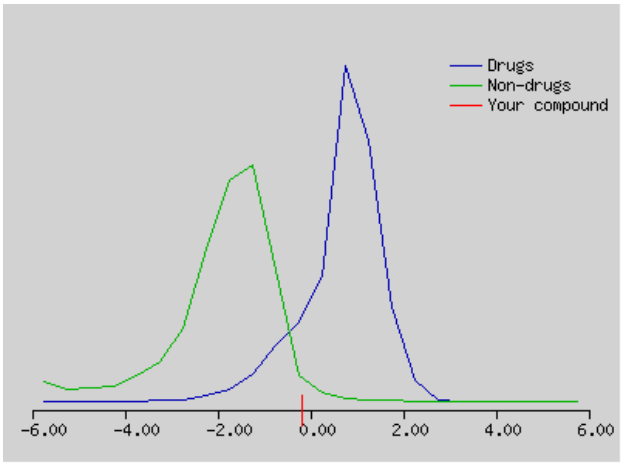


Figure 40 : MOLSOFT visualization vanillic acid -urease docking interaction analysis (score: -0.18)

- Quercetin shows a positive result in the Ames test, indicating potential mutagenicity. In contrast, both Gallic acid and Vanillic acid show negative results, suggesting they are not mutagenic.
- None of the compounds show carcinogenicity, indicating they are unlikely to cause cancer based on the data provided.
- None of the compounds inhibit the hERG channel, suggesting they do not pose a risk for causing cardiac arrhythmias.

2.2 Results of pharmacokinetic properties and toxicity of the rest compounds :

the remaining 13 molecules demonstrated activity through distinct binding sites, suggesting diverse inhibitory mechanisms, potentially including non-competitive pathways. This diversity implies that these molecules may inhibit urease through varied structural interactions and modes of action. Such findings are crucial for developing targeted therapies against urease-related disorders and designing novel inhibitors with tailored mechanisms of action. Further elucidation of these mechanisms could provide valuable insights into their therapeutic potential and applications.

Tableau 8: The pharmacokinetic properties and toxicity of the ramaining 13 molecules

Molecule	Penetration	CYP1A2 Inhibition	CYP2C19 Inhibition	CYP2C9 Inhibition	CYP2D6 Inhibition	CYP3A4 Inhibition	AMES Toxicity	Carcinogenicity	hERG Inhibition
Mol 1	High	No	Yes	No	No	Yes	Negative	Negative	Negative
Mol 2	High	No	Yes	No	No	Yes	Negative	Negative	Negative
Mol 3	High	Yes	No	No	No	No	Negative	Negative	Negative
Mol 4	High	Yes	Yes	No	No	No	Negative	Negative	Negative
Mol 5	High	Yes	No	No	No	No	Negative	Negative	Negative
Mol 6	High	No	No	No	No	No	Negative	Negative	Negative
Mol 7	Low	No	No	No	Yes	No	Negative	Negative	Negative
Mol 8	Low	No	No	No	No	No	Negative	Negative	Negative
Mol 9	Low	No	No	No	No	No	Negative	Negative	Negative
Mol 10	High	Yes	Yes	No	No	Yes	Negative	Negative	Negative
Mol 11	High	Yes	Yes	Yes	No	Yes	Negative	Negative	Negative
Mol 12	High	Yes	No	No	No	No	Negative	Negative	Negative
Mol 13	High	Yes	Yes	No	No	No	Negative	Negative	Negative

Mol 1 : Luteolin ,Mol 2 : Apigenin ,Mol 3 : Thymoquinone, Mol 4 : Thymohydroquinone

Mol 5 : Dithymoquinone, Mol 6 : caffeic acid, Mol 7 : betulonic acid, Mol 8 : Ursolic acid

Mol 9 : Oleanolic acid, Mol 10 : Gingerol, Mol 11 : Shogaols, Mol 12 : Paradols

Conclusion

The use of medicinal plants in herbal medicine and biomedical research is gaining significant recognition, paralleling the importance of traditional chemotherapy. Our research aimed to identify natural inhibitors of the urease enzyme from *Helicobacter pylori*, which is a critical factor in the pathogenesis of various gastrointestinal diseases, including peptic ulcers and gastric cancer. Utilizing an *in silico* approach, we initially screened seventeen natural compounds known for their medicinal properties and potential urease inhibitory activity. From this initial pool, quercetin, gallic acid, and vanillic acid emerged as the most promising candidates.

Molecular docking studies were conducted using AutoDock Vina and Discovery Studio to investigate the binding interactions of these compounds with *H. pylori* urease. The docking studies revealed that quercetin, gallic acid, and vanillic acid exhibit strong binding affinities and stable interactions with the enzyme's active site and adjacent regions. Specifically, quercetin demonstrated a noncompetitive inhibition mechanism, where it binds outside the active site, preventing the enzyme from adopting its active conformation. This binding mode was facilitated by multiple hydrogen bonds and hydrophobic interactions, significantly contributing to the stability and potency of the inhibitory effect. The kinetic studies indicated that quercetin inhibits *H. pylori* urease with an IC50 of 11.2 ± 0.9 μM, which is slightly more potent than the standard inhibitor acetohydroxamic acid (IC50 = 19.4 ± 2.0 μM).

Similarly, gallic acid and vanillic acid showed competitive inhibition by binding directly to the active site of the urease enzyme. Gallic acid formed stable hydrogen bonds and hydrophobic interactions with key residues such as ASN 168, ASP 223, and HIS 221, effectively blocking the enzyme's catalytic activity. Vanillic acid's competitive inhibition was characterized by its binding interactions with residues like HIS 138, GLY 279, and ASP 223, further supported by van der Waals forces and pi-cation interactions. This robust binding prevented urea, the natural substrate, from accessing the catalytic site, thus inhibiting the enzyme's function.

In addition to their binding affinities, we assessed the pharmacokinetic properties of these compounds using *in silico* ADME (Absorption, Distribution, Metabolism, and Excretion) prediction tools. All three compounds exhibited high gastrointestinal absorption, suggesting good potential for oral bioavailability. The compounds also complied with

Lipinski's Rule of Five, indicating favorable drug-like properties. Furthermore, toxicity assessments using SwissADME and ADMETlab showed that quercetin, gallic acid, and vanillic acid do not pose significant mutagenic or carcinogenic risks. While quercetin showed positive results in the Ames test for mutagenicity, gallic acid and vanillic acid were found to be non-mutagenic and non-carcinogenic.

Our comprehensive study underscores the potential of these phenolic compounds as alternative therapeutic agents for *H. pylori* infections. Their comparable, and in some cases superior, binding interactions and pharmacokinetic profiles relative to acetohydroxamic acid highlight their promise in addressing antibiotic resistance and providing natural solutions to combat urease-related pathologies. However, to validate these findings and fully understand their therapeutic potential, further experimental validation through in vitro and *in vivo* studies is necessary. This research not only contributes to the growing body of knowledge on medicinal plants and their bioactive compounds but also emphasizes the importance of continued exploration and integration of phytochemicals in drug discovery and development.

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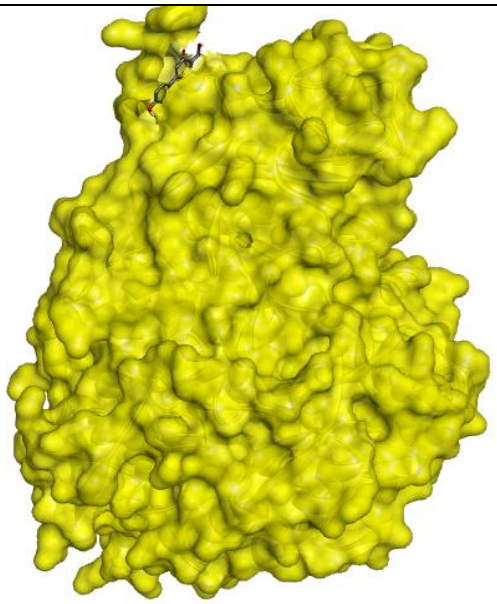
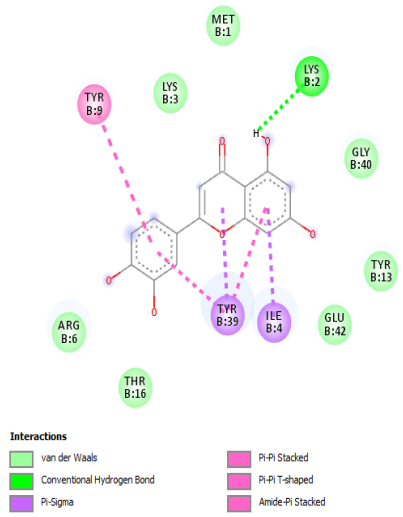
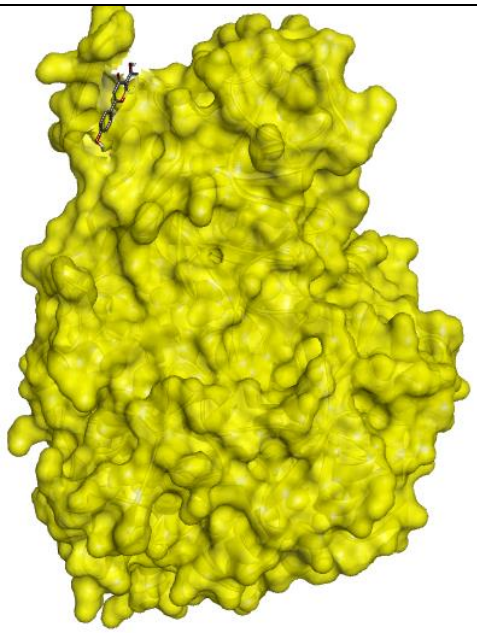
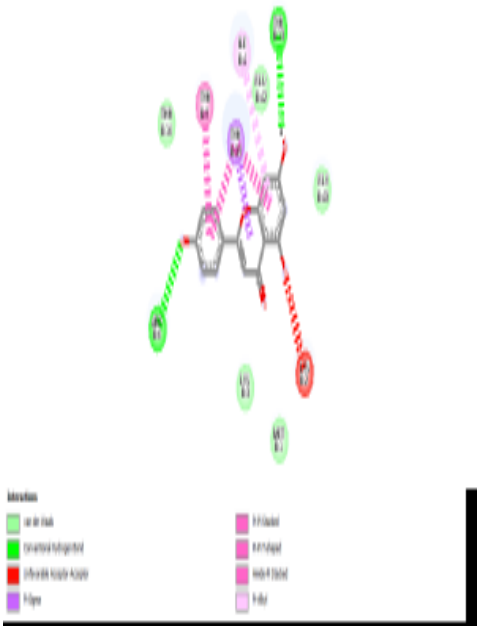
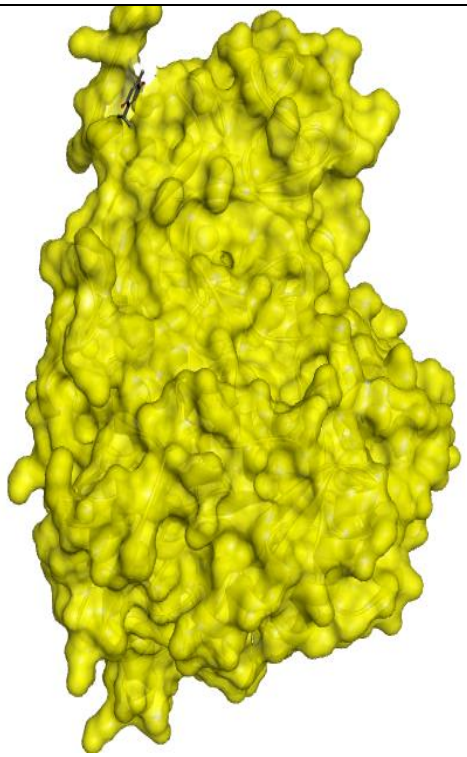
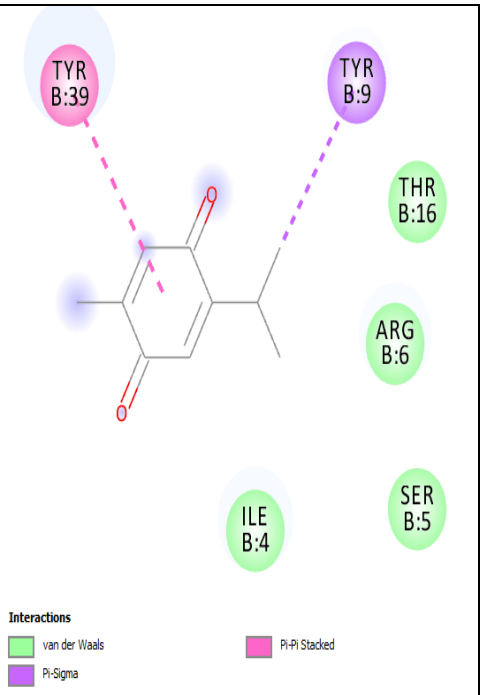
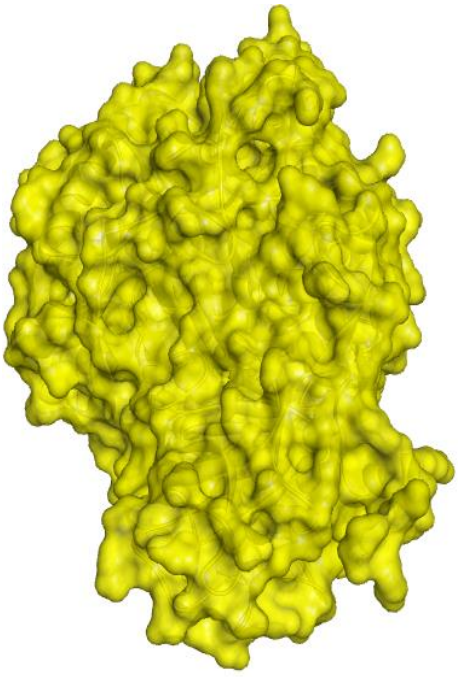
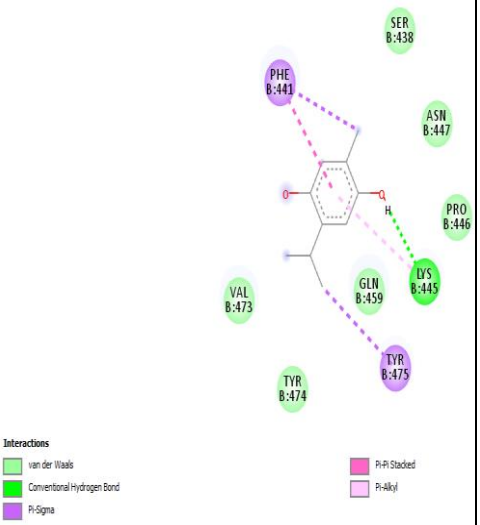
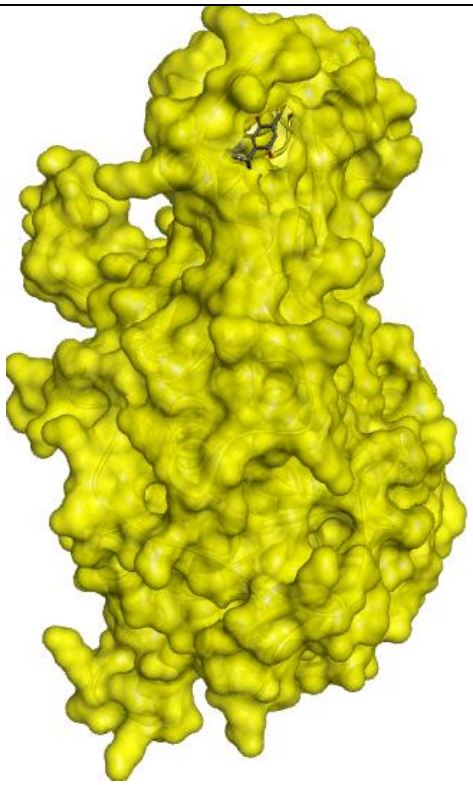
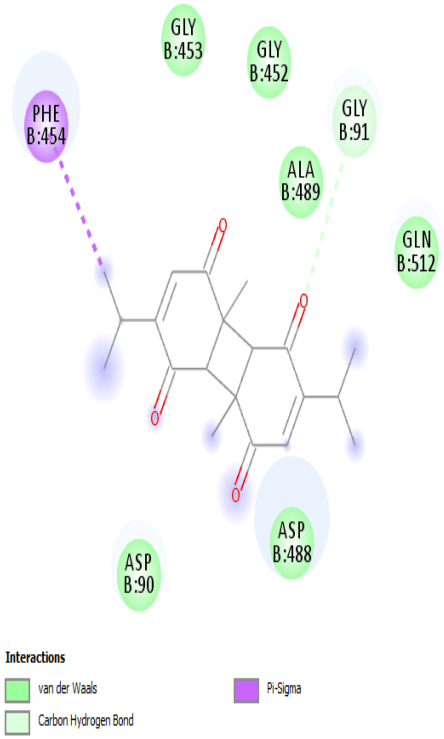
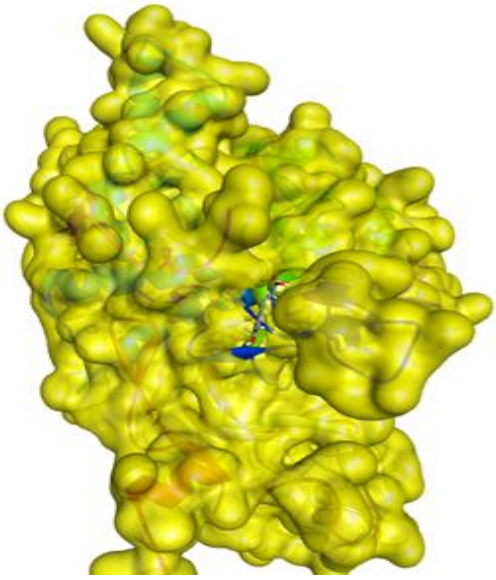
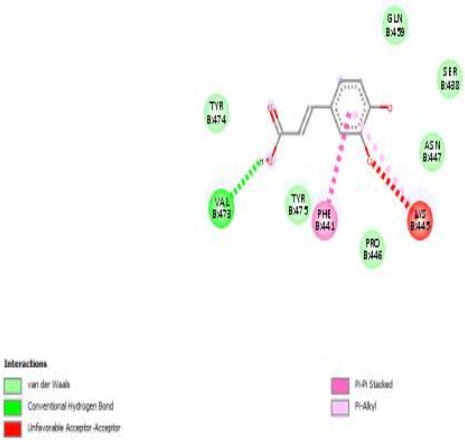
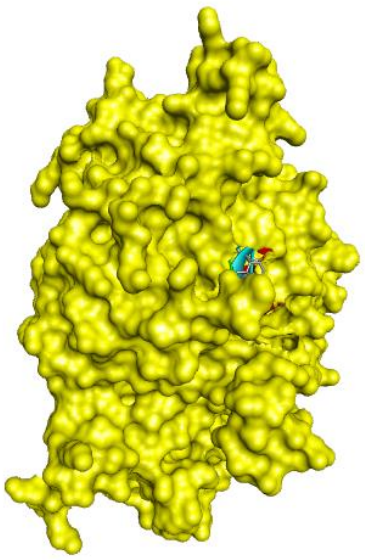
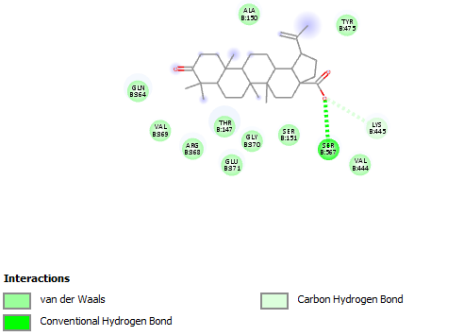
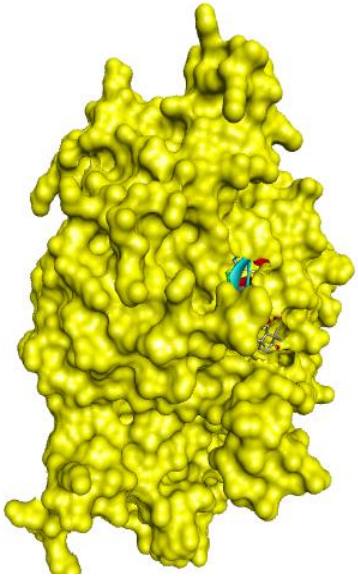
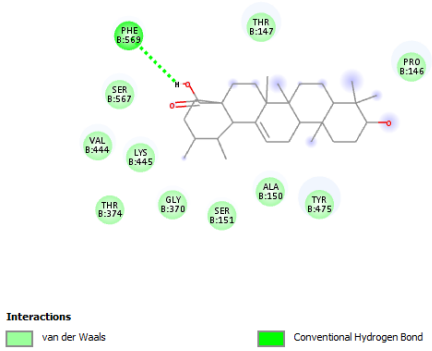
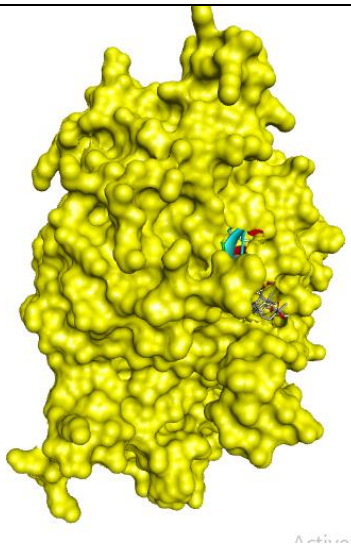
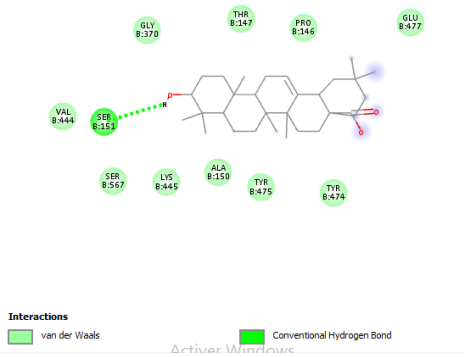
Annexes

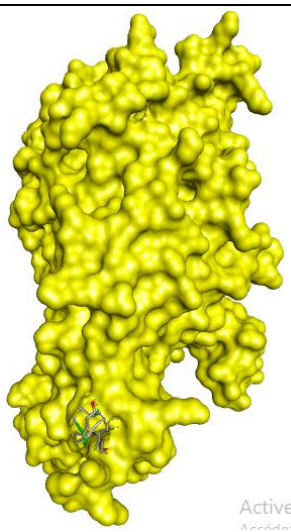
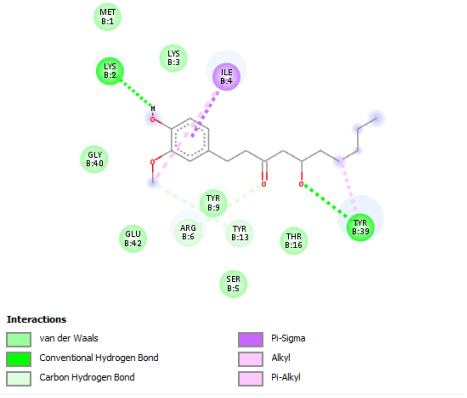
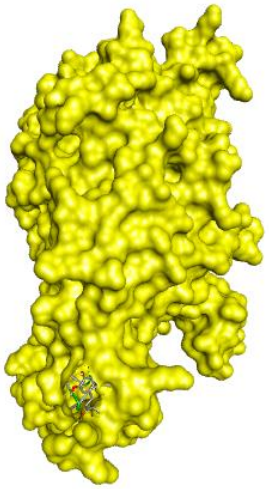
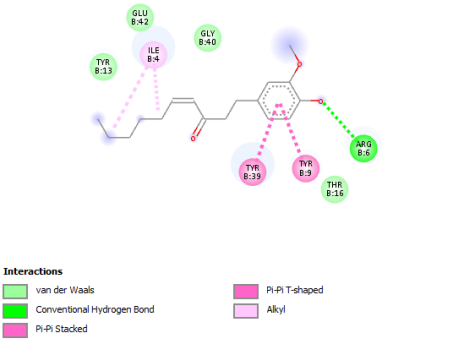
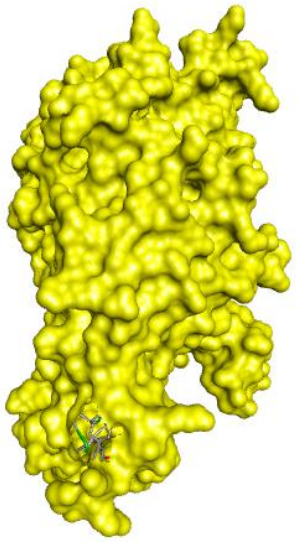
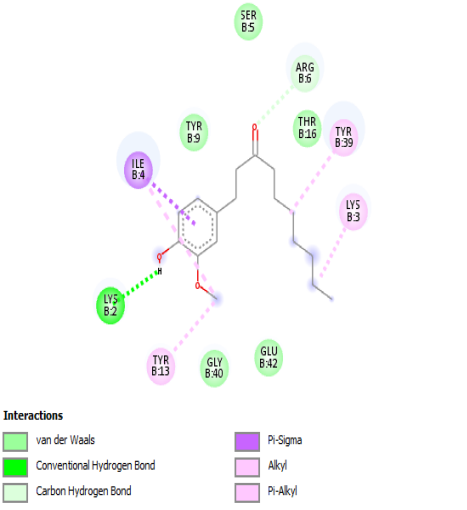
Table 1: Representinf the format of the other molecules

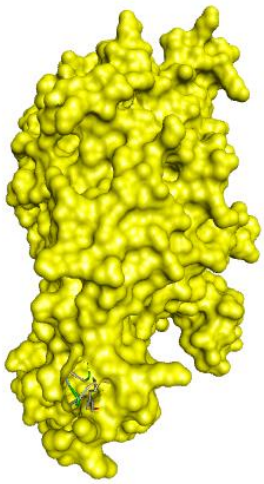
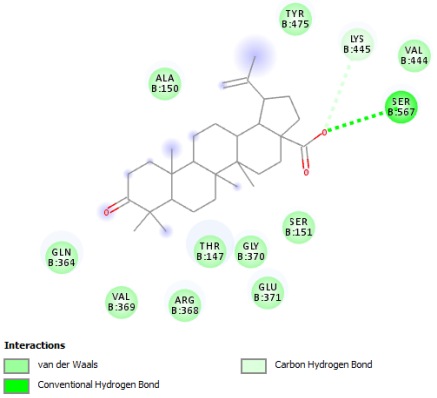
Molecules	**Formats 3D**	**FormatS 2D**
Luteolin		
apigenin		

Thymoquinone		
Thymohydroquinone		

Dithymoquinone		
caffeic acid		

betulonic acid		
Ursolic acid		
Oleanolic acid		

Gingerol		
Shogaols		
Paradols		

Zingerone		 TYR B:475 LYS B:445 VAL B:444 ALA B:150 SER B:567 SER B:151 THR B:147 GLY B:370 GLN B:364 GLU B:371 VAL B:369 ARG B:368 Interactions van der Waals Carbon Hydrogen Bond Conventional Hydrogen Bond