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Spécialité : Biologie moléculaire et cellulaire

THÈME

In Silico Molecular Docking study of anti- Inflammatory and antioxidant effect of phenolic compounds.

Présenté par :

– **Abdi Oussama Elias**

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Devant le jury composé de :

Mme MATMOURA A.	**MAA**	**USDB1**	**Présidente**
Mme ZERROUTI K.	**MAA**	**USDB1**	**Examinatrice**
Mme KADRI F.	**MCA**	**USDB1**	**Promotrice**
Mme ELBASYOUNI	**Assistance**	**USDB1**	**Co-promotrice**

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Abstract

Phenols are natural compounds with pharmacological properties and possible drug like molecules. In this research a docking study of selected phenolic compounds with key elements of inflammation and oxidative stress (Ikappa kinase beta (Ikkβ), cyclooxygenase-2(COX-2(and Xanthine oxidase) was performed to predict their possible anti-oxidative and anti-inflammatory effect, alongside ADMET (Absorption ,Distribution , Metabolism , Excretion, Toxicity) profiling for more accurate results on their effectiveness.

Molecular modeling with BIOVIA Discovery Studio Visualizer and virtual screening with Pyrx application allowed to find that phenolic acids (4-Hydroxybenzoic, Ferulic and caffeic acids), Anthocyanins (Apigeninidin, Delphinidin, Pelargonidin and Luteolinidin) and Flavonols (Fisetin and Taxifolin) are probable Ikkβ inhibitory sources. all of the tested phenolic compounds interacted with Cox-2 and Cox-1active sites. The phenolic acids (Caffeic and Ferulic acids), flavonoids ,Pelargonidin, Delphinidin , Gallocatechin, Fisetin and Butein showed the best non-specific binding conformation towards Cox-2 ,while Genistein only interacted with Cox-2. From all the tested phenolic compounds only 4-Hydroxybenzoic acid, Luteolinidin and Apigeninidin were found to be possible XO inhibiters.

As to the pharmacological profile, all the tested phenolic were accepted in Lipinski's rule of five and showed good absorption, distribution, metabolism and excretion results. As to the toxicity profile, Epicatechin, Luteolin, Butein, Fisetin, Taxifolin, Luteolinidin and Pelargonidin were suggested be mutagenic, only Caffeic acid was registered to be hepatotoxic and Ferulic acid, Genistein, Naringenin, Butein, Luteolinidin and Apigeninidin were suggested to possibly be carcinogenic.

Key word: natural compounds, Ikappa kinase beta, Cyclooxygenase-2, Xanthine Oxidase, Phenolic compounds,Docking, Inhibiter, in silico, Autodock Vina, BIOVIA Discovery Studio Visualizer.

Résumé

Les phénols sont des composés bioactifs dotés de propriétés pharmacologiques et de possibles molécules ressemblant à des médicaments. Dans cette recherche, une étude d'amarrage de composés phénoliques sélectionnés avec des éléments clés de l'inflammation et du stress (Ikappa kinase beta (Ikkβ), cyclooxygenase-2(COX-2) et Xanthine oxidase) a été réalisée pour prédire leur possible effet antioxydant et anti-inflammatoire, ainsi que le profilage ADMET (Absorption ,Distribution , Metabolism , Excretion, Toxicity) pour des résultats plus précis. sur leur efficacité.

la modélisation moléculaire avec BIOVIA Discovery Studio Visualizer et le criblage virtuel avec l'application Pyrx ont permis de découvrir que les acides phénoliques (acides 4-hydroxybenzoïque, férulique et caféique), les anthocyanes (Apigéninidine, Delphinidine, Pelargonidine et Lutéolinidine) et les Flavonols (Fisetine et Taxifoline) sont probables Ikkβ sources inhibitrices. tous les composés phénoliques testés interagissaient avec les sites actifs Cox-2 et Cox-1. Les acides phénoliques (acides caféique et férulique), les flavonoïdes, la pélargonidine, la delphinidine, la gallocatéchine, la fisétine et la butéine ont montré la meilleure conformation de liaison non spécifique envers la Cox-2, tandis que la génistéine n'interagissait qu'avec la Cox-2. tous les composés phénoliques testés, seuls l'acide 4-hydroxybenzoïque, la lutéolinidine et l'apigéninidine se sont avérés être des inhibiteurs de XO possibles.

En ce qui concerne le profil pharmacologique, tous les composés phénoliques testés ont été acceptés dans la règle de cinq de Lipinski et ont montré de bons résultats d'absorption, de distribution, de métabolisme et d'excrétion. En ce qui concerne le profil de toxicité, l'épicatéchine, la lutéoline, la butéine, la fisétine, la taxifoline, la lutéolinidine et la pélargonidine ont été suggérées comme mutagènes, seul l'acide caféique a été enregistré comme étant hépatotoxique et l'acide férulique, la génistéine, la naringénine, la butéine, la lutéolinidine et l'apigéninidine ont été suggérés comme pouvant être cancérigène..

Mot clé : natural compounds, Ikappa kinase beta, Cyclooxygenase-2, Xanthine Oxidase, Phenolic compounds,Docking, Inhibiter, in silico, Autodock Vina, BIOVIA Discovery Studio Visualizer.

Résumé

الملخص

البوليفينول عبارة عن مركبات نشطة بيولوجيًا لها خصائص دوائية محتملة. في هذا البحث ، تم إجراء دراسة
DOCKING لمجموعة من البوليفينول مع العناصر الرئيسية للالتهاب والإجهاد التأكسدي, (ikkβ) ikappa kinas beta)
cycloxigenase -2 (COX-2 , Xanthin Oxidas), للتنبؤ بالتأثير المضاد للأكسدة والمضاد للالتهاب المحتمل للمركبات
الفينولية ، إلى جانب اختبار ADMET (Toxicity, Excretion , Metabolism , Distribution, Absorption)
للحصول على نتائج أكثر دقة حول فعالية المركبات الفينولية.

سمحت النمذجة الجزيئية باستخدام BIOVIA Discovery Studio Visualizer والفحص الافتراضي باستخدام
تطبيق Pyrx باستنتاج أن الأحماض الفينولية (Apigeninidin, ,(4-Hydroxybenzoic, Ferulic and caffeic acids)
Delphinidin, Pelargonidin and Luteolinidin) والفلافونولز (Fisetin and Taxifolin) مثبطة لـIkkβ. بالنظر إلى
Cox-2 ، تفاعلت جميع المركبات الفينولية المختبرة مع الموقع الفعال لـ Cox-1 و Cox-2 . أظهرت الأحماض الفينولية
الفينولية (Caffeic and Ferulic acids) ، والفلافونيدات Pelargonidin, Delphinidin , Gallocatechin, Fisetin and
Butein أفضل ارتباط غير محدد تجاه Cox-2، مع تفاعل Genistein فقط مع Cox-2. من بين جميع المركبات الفينولية
المختبرة ، تم استنتاج Hydroxybenzoic acid, Luteolinidin and Apigeninidin4 كمثبطات محتملة لـXO .

فيما يتعلق بالجانب الصيدلاني، تم قبول جميع الفينولات المختبرة في قاعدة ليبينسكي وأظهرت نتائج جيدة في
الامتصاص والتوزيع والتمثيل الغذائي والإفراز. فيما يتعلق بملف السمية ، تم اكتشلف مواد مطفرة مثل Epicatechin,
Luteolin, Butein, Fisetin, Taxifolin, Luteolinidin and Pelargonidin، وتم تسجيل caffeic acid كمادة سامة
للكبد Ferulic acid, Genistein, Naringenin, Butein, Luteolinidin and Apigeninidin كمواد مسرطنة.

summary

Figure list

Figure list

Table list

Introduction

Introduction

Inflammation plays a major role in chronic diseases such as Chronic liver disease, gallbladder disease , chronic respiratory diseases, heart disorders, cancer, obesity, and diabetes (Bengmark 2004; Netea et al. 2017; Pahwa et al. 2021).

oxidative stress is thought to be involved in many neurodegenerative diseases , chronic kidney disease, cancer and several others (Liguori et al. 2018).

It has been proven throughout the years that oxidative stress interconnects with inflammation by activating certain transcriptional factors such as NF-κB (Reuter et al. 2010).

Numerous reports have identified therapeutic roles of plants and their extracts because they contain phytochemicals which are secondary metabolite with anti-inflammatory and anti-oxidative effects (Choi et al 2010; Mueller et al. 2010).

Phenolic compounds express anti-inflammatory activity by modulating the inflammatory responses through inhibition of inflammatory pathways and down-regulating the expression of IL-1β, IL-6 ,TNF-α, INF-γ and COX-2 (Bisht et al2010; H. Zhang and Tsao 2016). It was found that phenolic compounds regulate the NFkB pathway mediated inflammation by suppressing its DNA-binding ability and preventing the phosphorylation and degradation of IkBα (Singh and Aggarwal 1995; Chiu et al. 2015) . Same as antioxidants, some polyphenolic compounds play a role in the suppression of ROS formation by either inhibiting the enzymes involved in their production, scavenging of ROS, or by upregulation and protection of antioxidant defenses(Hussain et al. 2016).

Virtual screening is an in silico method that is rapidly dominating the field of hit recognition and drug discovery due to its increasing accuracy and low cost utilization (Pinzi and Rastelli 2019). Molecular docking is the most commonly used virtual screening method, it is performed between a small molecule (ligand) and a target macromolecule (protein) and gives us as a result the binding affinity and the structure of the protein–ligand complex which we can use for structure–activity studies and lead optimization (Morris and Lim-Wilby 2008; Wang and Zhu 2016). In this study we aim to identify anti-inflammatory and anti-oxidative properties of a selected set of phenolic compounds by conducting a docking analysis to find possible inhibitory activity towards Ikkβ , Cox2 and xanthine oxidase , along sides pharmacological and toxicity analysis .

Bibliography

I.   Inflammation :

Inflammation is the immune system's response to pathogens, damaged cells, toxic substances, or irradiation (Medzhitov 2010) and it works by eliminating the source of the response and commencing the healing process (Ferrero-Miliani et al. 2007)Thus, inflammation is a necessary defense mechanism for health (Furman et al. 2019; Lordan, Tsoupras, and Zabetakis 2019).

At the tissue level, inflammation manifests as redness, swelling, heat, discomfort, and loss of tissue function, all of which are the result of local immunological, vascular, and inflammatory cell responses to infection or injury (Takeuchi and Akira 2010). During the inflammatory process, significant microcirculatory events occur, including changes in vascular permeability, leukocyte recruitment and accumulation, and inflammatory mediator release (Chertov et al. 2000)

I.1 Types of Inflammation

a)   Acute inflammation

It can be induced by Tissue damage ,microbial invasion or chemical compounds ;it lasts for a few hours or days (Table 1), marked by secretion of fluid and plasma and leukocyte emigration (mainly neutrophils), and when the immune system is able to eliminate those pathogenic elements, the reaction disappears.(Ambriz-Pérez et al., 2016 ; Pahwa et al., 2021)

b)   Chronic inflammation

Due to a Failure in eliminating the pathogen , An autoimmune disorder ,A defect in the cells responsible for mediating inflammation the host can enter a long and persistent chronic inflammation phase (table 3), associated with the presence of lymphocytes and macrophages, vascular proliferation, fibrosis, and tissue destruction (Ambriz-Pérez et al., 2016; Pahwa et al., 2021).

It has been proven throughout several studies that a person entering the chronic inflammation leads to an overproduction of pro-inflammatory mediators (TNF-α, TGFb, IL-6) and it plays a major role in chronic diseases such as Chronic liver disease and gallbladder disease , chronic respiratory diseases, heart disorders, cancer, obesity, and diabetes (Bengmark, 2004; Netea et al., 2017; Pahwa et al., 2021).

Table 1 : Acute inflammation Vs chronic inflammation

	Acute inflammation	**chronic inflammation**
Trigger	PAMPs (infection), DAMPs (cellular stress, trauma)	DAMPs ('exposome', metabolic dysfunction, tissue damage)
Duration	Short-term	Persistent, non-resolving
Magnitude	High-grade	Low-grade
Outcome(s)	Healing, trigger removal, tissue repair	Collateral damage
Age-related	No	Yes
Biomarkers	IL-6, TNF-α, IL-1β, CRP	Silent—no canonical standard biomarkers

I.2 **Inflammatory response**

I.2.1 Pattern recognition receptor activation

The inflammatory response is set into action once the host cells recognizes pathogen associated molecular patterns (PAMPs) or danger associated molecular patterns (DAMPs) using pattern recognition receptors (PRRs) (Netea et al., 2017). In mammals, these recognition receptors are known as toll like receptors (TLR) , and are able to recognize various PAMPs like:

- lipopolysaccharide (LPS) (detected by TLR4),

- bacterial lipoproteins and lipoteichoic acids (detected by TLR2),

- Flagellin (detected by TLR5).

- The unmethylated CpG DNA of bacteria and viruses are detected by TLR9.

- The double-stranded RNA are detected by TLR3

- Single-stranded viral RNA (by TLR7) (Iwasaki and Medzhitov 2004).

The combined activity of PAMPs and TLRs lead to the activation of several signaling pathways such as nuclear factor-κB (NF-κB) , Nod-like receptor pyrin domain containing 3 (NLRP3) ,the mitogen-activated protein kinase and JAK–STAT signaling pathways that incites the secretion of different pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α,interleukin IL-1 (Afonina et al. 2017) (Figure 1).
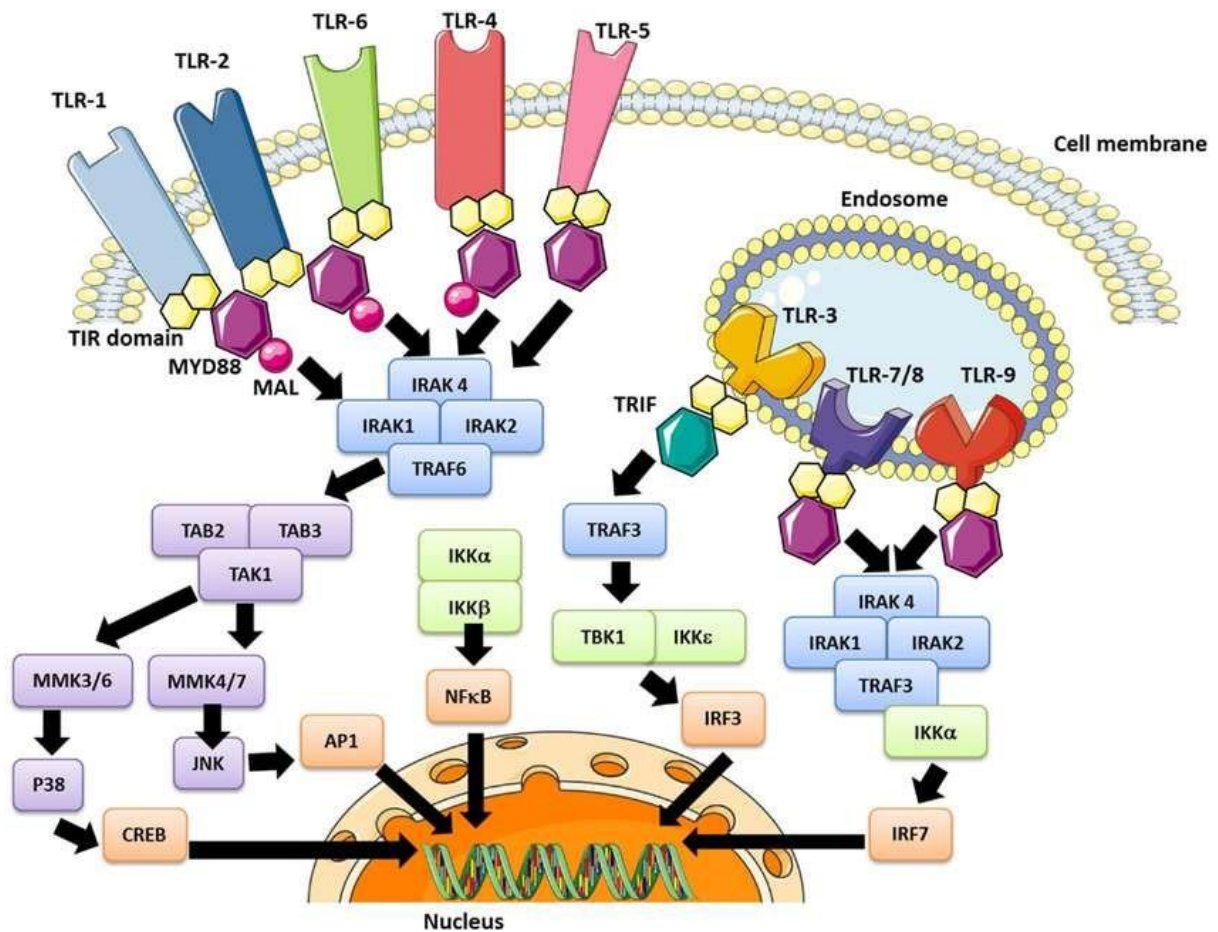
Figure 1: TLR signaling (Cognasse et al. 2015)

I.2.2 Activation of inflammatory pathways

1. NF-κB pathway

The NF-κB transcription factor is involved in numerous activities including inflammation, immunological response, survival, and apoptosis ((Girard et al. 2009; Peng et al. 2020). The NF-κB family of transcription factors is comprised of five members: P50, p52, RelA (p65), RelB, and c-Rel(figure 2) (Moynagh 2015; Hoffmann, Natoli, and Ghosh 2006) . NF-κB exist in the cytoplasm in an inactive state in the form of homo/heterodimers under the inhibitory effect of IkB family (Figure 2) (Bonizzi and Karin 2004)
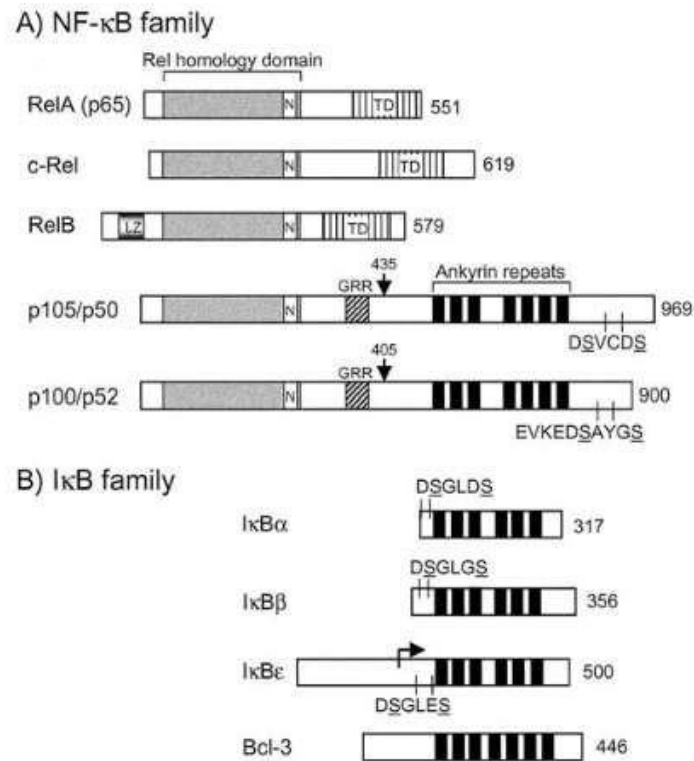
Figure 2: NF-KB and IKB family members (Nishikori 2005)

NF-κB signaling pathways (canonical and non-canonical) are prompted by a various insinuators such as proinflammatory cytokines (TNF-α and IL-1), T and B cell mitogens, bacterial lipopolysaccharide(LPS), viruses, viral proteins, double-stranded RNA, and physical and chemical stress (Karin and Ben-Neriah 2000) (Figure 2).

- **Canonical pathway**

Upon proper activation of the canonical NF-κB pathway commonly represented by TNFα/TNFR stimulus , IkB are ubiquitinated due to phosphorylation of Ser32 and Ser36 residues under the effect of IkB kinase IKK , a complex composed of three subunits , IKKα and IKKβ , the catalytic subunits , and NEMO or IKKγ the regulatory subunit . IkB degradation leads to the exposure of the NLS (nuclear localization signal) of the NF-κB dimers allowing them to pass through the nuclear import pathways and express their gene transcription activity (Lin et al. 2010; Ghosh and Karin 2002). (Figure 3)

- **Non-canonical pathway**

The non-canonical pathway is mainly activated by non-death receptor members of the TNF receptor family like CD40, lymphotoxin beta (LTβ), and B-cell-activating factor (BAF) and viral proteins such as LMP-1 from Epstein-Barr virus (EBV). This pathway is dependent on NF-κB-inducing kinase (NIK)-mediated activation of IKKα subunit of the IKK complex, which leads to the cleavage of p100 to create p52. Then p52 forms a complex with RelB and translocate to the nucleus to enhance gene expression (Ghosh and Karin 2002;Lin et al. 2010).(Figure 3)
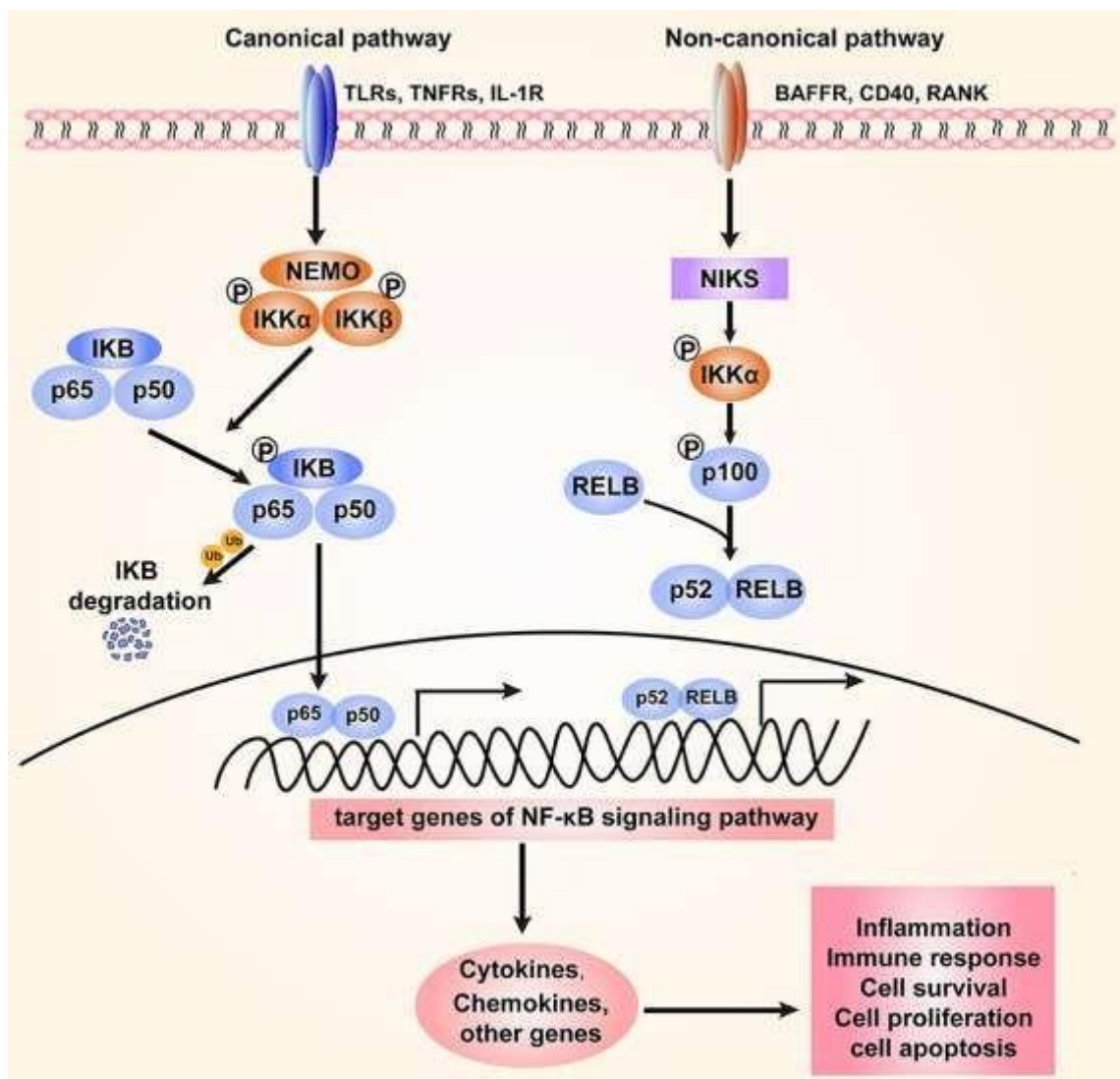
Figure 3 : The canonical and non-canonical NF-κB signaling pathway (Peng et al. 2020)

Without any major surprise research shows that NF-κB signaling plays a major role in many inflammatory diseases as well as cancer (Zhang et al., 2017; Ben-Neriah and Karin, 2011). Making the steps of activating the NFkB pathway (IKK activation, IkB degradation and NF-κB nuclear translocation and DNA binding) interesting targets for therapeutic inhibition.(Lin et al., 2010).

a.1 IKK kinase

The IKK kinase complex is an important component in the NF-κB signaling pathway. It is essentially made of two kinases (IKKα and IKKβ) and a regulatory subunit, NEMO/IKKγ (Israël 2010). The structure of hIKKβ consists of an N-terminal kinase domain, KD (1–309), the central ubiquitin-like domain, ULD (310–404), and the C-terminal dimerization domain, SDD (408–664) (Polley et al. 2013) (figure 4).
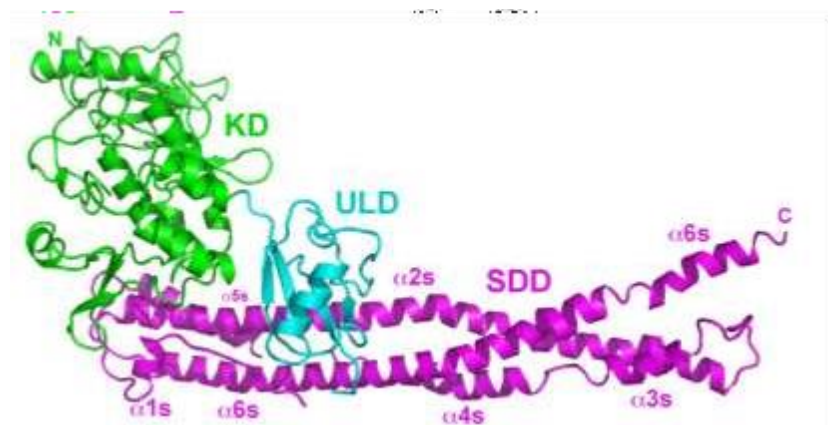
Figure 4 : crystal structure of hIKKβ (Polley et al., 2013)

In determining inhibitory specificity residue Met96 plays a major role as the "gatekeeper" residue, which controls the access of the inhibitor to the hydrophobic pocket ,while Glu97, Tyr98 and Cys99 form the hinge region of the KD of IKKβ. The backbone groups of Glu97 and Cys99 are able to provide hydrogen-bonding interactions with the inhibitor. In addition, the ATP binding site of IKKβ is partly covered by an activation loop comprised of serine, threonine and tyrosine residues in the unphosphorylated state, While the N-terminalside of the activation loop contains the Asp166, Leu167 and Gly168 triad which is involved in catalytic transfer of the γ-phosphate group in most kinase ATP binding sites (Hotchkiss et al. 2021).

2. Cyclooxygenase (COX)

Cyclooxygenase (COX) is responsible of synthetizing prostaglandin. It is an enzyme that catalysis the oxidation of arachidonic acid, the first steps in the synthesis of prostanoids such as prostaglandin, prostacyclin, and thromboxane, a large family of arachidonic acid metabolites that are the inflammation mediators (Minghetti, 2004).

Cyclooxygenase exist in two isoforms, COX-1 a constitutive isoform that exists almost in all cell types and is thought to mediate physiological activities through the synthesis of certain prostaglandins that regulate renal hemodynamics and water/electrolyte balance, protect the gastrointestinal mucosal lining, and limit gastric acid secretion and thromboxane A2 (TXA2). Arachidonate metabolite formed by COX-1 stimulates platelet aggregation and thus maintains normal hemostasis (Figure 5).

COX-2 is an inducible isoform expressed in several cell types in response to growth factors, cytokines, and pro-inflammatory molecules ; It's been noticed that prostaglandins produced via COX-2 (prostaglandin E2, A1, A2, D2…) are intimately involved in the induction of inflammation by enhancing vascular permeability, mediating vasodilation. Iin addition, PGE2 is considered as a chemoattractant for leukocytes (Simon, 1999; Minghetti 2004; Lim et al., 2001). Cox-2 is further more linked to inflammation because of the nonspecific inhibition of its enzymatic activity by the non-steroidal anti-inflammatory drugs (NSAIDs)(Simon, 1999),That is why it is very crucial to develop drugs that have limited side effects and specific to COX-2.
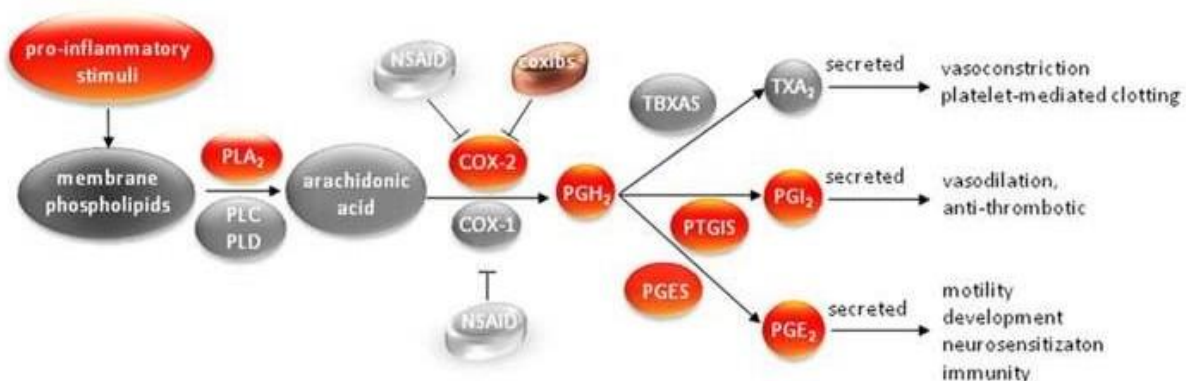
Figure 5: COX pathway (Stasinopoulos et al. 2013)

Considering their structure, when the sequences of Cox-1 and Cox-2 are compared they show a similarity of 61%, and a 87% similarity when only the active site sequence is compared

The Cox active site is divided into three distinctive areas, a hydrophobic pocket defined by the residues Tyr385, Trp387,Phe518, Ala201,Tyr248 and Leu352. The mouth of the active site, with three hydrophilic residues guarding its entrance: Arg120,Glu524,Tyr355 and a side pocket that includes several conserved residues like His90 and non-conserved residues His/Arg513and Ile/Val523 , also interacting with Ser530 and Phe518 is an indication to a possible inhibitory activity (Llorens et al. 2002). (Figure 6).
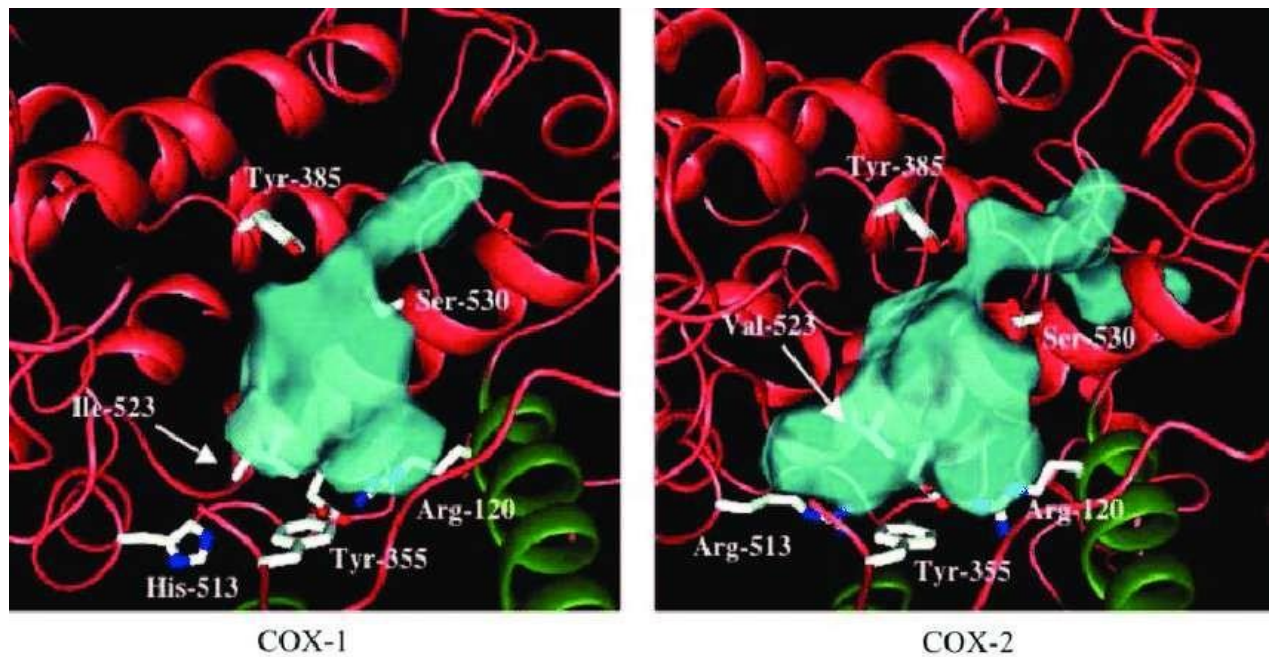
Figure 6 : Comparison of the cyclooxygenase active sites of COX-1and COX-2 .(Kudalkar, Rouzer, and Marnett2015)

II.   Oxydative stress

Oxidative stress is seen as an imbalance between the concentration of reactive oxygen species (ROS) and antioxidants in a cell, due to the overexpression of ROSs or the incapacity of antioxidants (Betteridge 2000).

II.1   Reactive oxygen species (ROS)

Reactive oxygen species or free radicals are every atom or molecule that have one or more unpaired electrons in the outer orbit. This feature gives these molecules or atoms a higher chemical reactivity (Halliwell, 1994).

ROS can be created from external sources, like being exposed to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals. They can be generated by using oxygen O_2 as a first substrate in endogenous enzymatic reaction in different cell compartments such as cytoplasm, cell membrane, endoplasmic reticulum (ER), mitochondria(aerobic respiration), and peroxisome. Various enzymes like NADPH oxidase, xanthine oxidase, D-amino acid oxidase and dihydrolipoamide dehydrogenase are implicated in ROS generation (Forrester et al. 2018; Li et al. 2016; Kalam et al. 2015).

Some of the most famous ROSs are superoxide anion ($O_2–•$). It is formed by adding an electron to oxygen O_2. Another is hydrogen pyroxidas (H_2O_2) made by adding another electron with 2 protons. Hydroxyl radical ($OH•$) is the most reactive free radical and it is formed by the reaction of $O_2–•$ with H_2O_2 in the presence of Fe^{2+} or Cu^+ (the Fenton reaction) (Kalam et al. 2015; Li et al. 2016;Forrester et al. 2018).

II.2   Negative actions of ROS

Free radicals can cause the activation of redox-sensitive transcription factors such as AP-1, p53 and NF-κB. This activation leads to an increased levels of proinflammatory enzymes like COX-2, interleukin 1ß and TNF-α and ROS-induced activation of protein kinases (MAPK). This generally promotes cell survival and proliferation, which can create a perfect environmentfor cancer development. ROS can also cause the loss of intracellular Ca^{2+} homeostasis which activates diverse Ca^{2+} sensitive signaling pathways, alongside with causing lipid, protein,

DNA and mtDNA damage (Burton and Jauniaux 2011). These actions carried out by free radicals made Oxidative stress implicated in many diseases and disorders including cancer, neural disorders, cardiovascular disease, Alzheimer's disease, alcohol induced liver disease, and ageing (Mishra et al. 2013).

II.3   Xanthine oxidase

Xanthine oxidase is widely distributed throughout the liver, gut, kidney, heart, capillary endothellial cells, brain, lung and plasma; it is made up of two identical subunits of approximately 145Kda each. Each catalytically independent subunit contains two non-identical Fe2S2 iron-sulfur centers located in the N-terminal (20 kDa) domain, flavin adenine dinucleotide (FAD) cofactor in the intermediate (40 kDa) domain, and molybdopterin cofactor in the C-terminal (85 kDa) domain (figure12) (Šmelcerović et al. 2017) (Figure 7)

Asn768, Glu802, Leu873, Arg880, Phe914,Phe649 ,Phe1009, Thr1010, Leu1014 and Glu1261 are key residues in the process of oxidative hydroxylation of hypoxanthine and xanthine to uric acid (Šmelcerović et al. 2017).
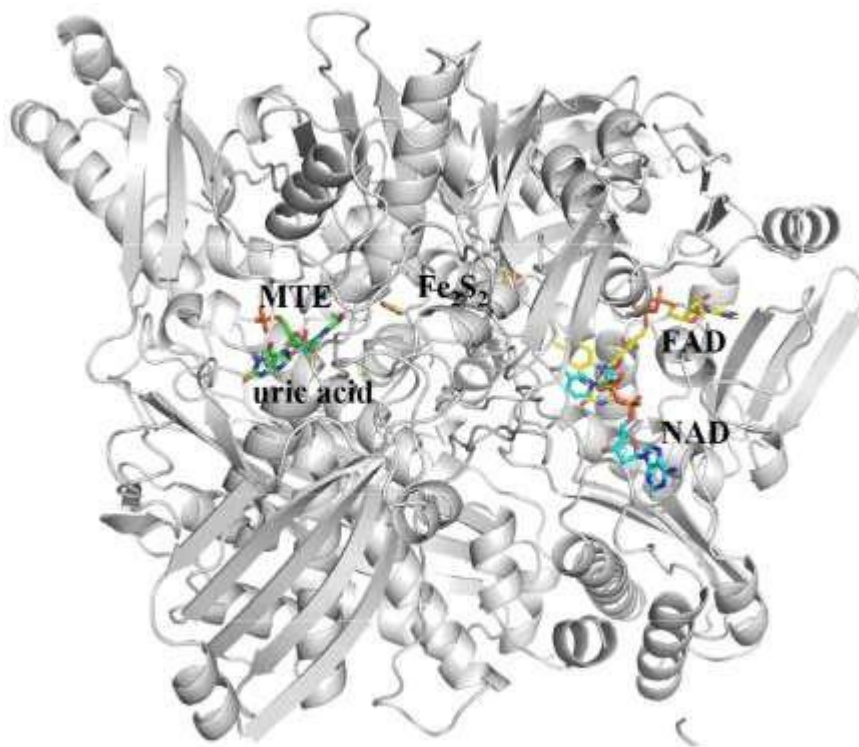
Figure 7: Crystal structure of bovine xanthine oxidas (Šmelcerović et al. 2017)

III.   Phenolic compounds

Phenolic compounds are widely found in fruits, vegetables, cereals and beverages with more than 8,000 compounds.(Table 2) , These molecules are considered as secondary metabolites of plants and are involved in the defense against ultraviolet radiation and/or aggression by pathogens (Pandey and Rizvi 2009; Belščak-Cvitanović et al. 2018).

Table 2: food sources of phenolic compounds.(M. B. Hussain et al. 2019)

Phenolic compound	Source
Phenolic acids (gallic acid)	Red wine
Anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin)	Blackberry, blueberry, black grape, cherry, strawberry, red wine, plum
Condensed tannins (procyanidin)	Red wine, chocolate, cranberry juice and apples
Flavan-3-ols (catechin)	Fruits, vegetables, chocolate, lentil, green and black tea, wine, grapes and ginkgo
Flavanones (hespertin, naringenin)	Orange, grapefruit and lemon juices
Flavones (apigenin, luteolin)	Parsley, celery, capsicum pepper and grape
Flavonols (quercetin, kaempferol)	Fruits, vegetables, and beverages such as tea and red wine
Isoflavones (genistein)	Soy
Stilbenes (resveratrol)	Legumes, grapes, red wine, soy, peanuts and peanut products

III.1. Classification of Phenolic compounds

phytochemicals can be classified according to the number of phenol rings that they contain and the structural elements that bind these rings to each other into four main classes: phenolic acids, flavonoids, stilbenes and lignans (Manach et al. 2004) (Figure 8).
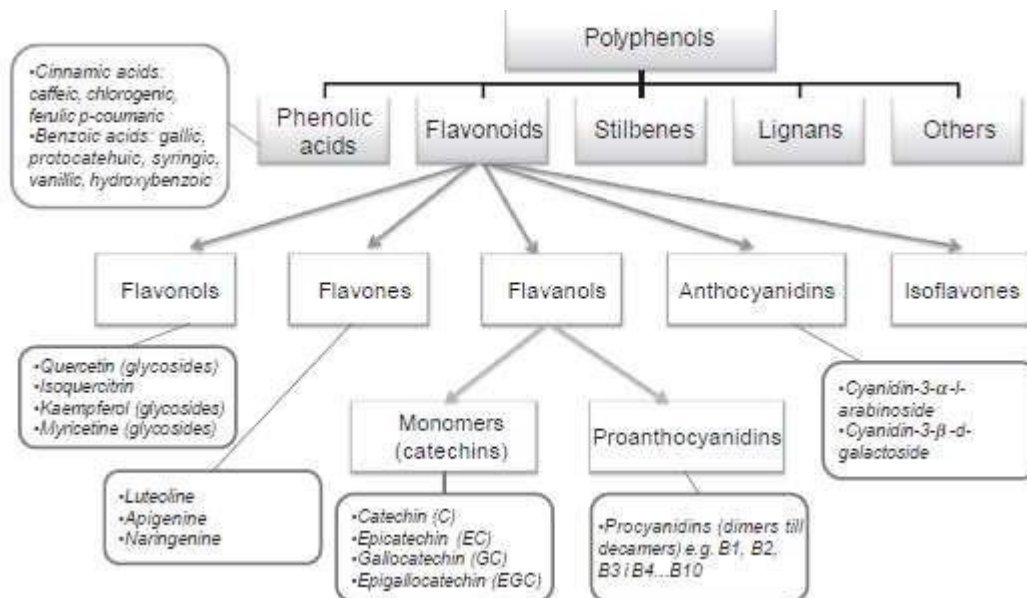
Figure 8: Classification of phenolic compounds (Panche et al., 2016)

1.**Phenolic acids**:

They Are phenolic molecules that can be Found in coffee, tea, cinnamon, blueberries, kiwis, plums, apples, and cherries (Kumar Ganesan and Baojun Xu 2017), with great deal of health benefits such as anti-inflammatory and ant-oxidative activities (Cheng et al. 2007;Ambriz-Pérez et al. 2016). We can distinguish between Two classes of phenolic acids hydroxybenzoic acids and hydroxycinnamic acids .
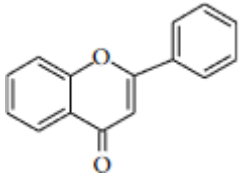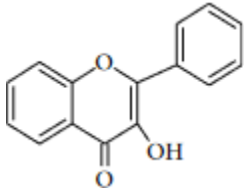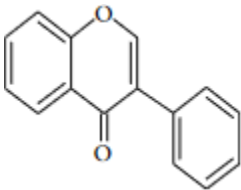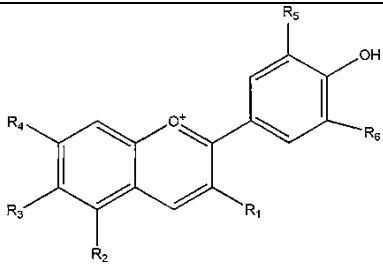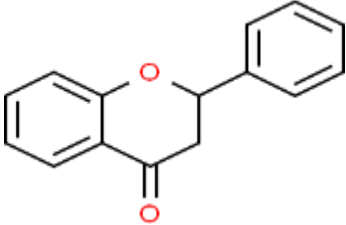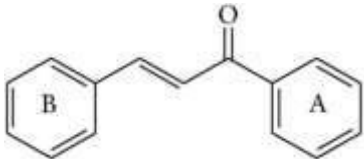
Hydroxybenzoic acids include gallic,p-hydroxybenzoic, vanillic, syringic, and protocatechuic acids. hydroxycinnamic acids are commonly found in foods and beverages like p-coumaric acid, caffeic acid, ferulic acid, sinapic acid and cinnamic acids (Chandrasekara 2019).

2.**Flavonoids**

Flavonoids make two third of the polyphenol population ,they are found in onions , tea , oranges , soy , dry beans, red wine , chocolate and many others. They are the most abundantly found compounds, with Quercetin being the main flavonol in our day to day dietary intake alongside Genistein and Daidzein. (Scalbert and Williamson 2000; Bravo 2009).

The chemical structure of flavonoids is made up of two benzene rings A and B, linked via a heterocyclic pyran ring C. Flavonoids can be divided into several classes according to the properties exhibited by the C ring (flavones ,Flavonols ,Isoflavones ,Anthocyanins ,Flavanones, Chalcones …) (Table 3) (Kumar and Pandey 2013; Panche, Diwan, and Chandra 2016).

Table 3: structure of some flavonoids

Class (Compounds)	Structure
flavones (Luteolin, apigenin and tangeritin)	
Flavonols (Quercitin , myrcitin , rutin)	
isoflavones (genistein and daidzein)	
Anthocyanins (Cyanidin, , pelargonidin, peonidin, malvidin)	
Flavanones (Naringin, naringenin, taxifolin,and hesperidin)	
Chalcones (phloridzin, arbutin, phloretin and chalconaringenin)	

a) Flavonols

Flavonols are flavonoids that include a double bond between the carbon atoms C2 and C3, as well as a hydroxyl group at the carbon atom C3 and a carboxyl group at the carbon atom C4. This structure adds up to three functional groups that are accessible to react with other substances. These flavonoids are present in a wide variety of edible and medicinal plants and are currently undergoing extensive research due to their numerous bioactivities. The most well-known chemicals in this category are kaempferol, myricetin, and quercetin (Kumar and Pandey 2013; Panche et al., 2016).

b) Flavones

Flavones have a double bond between carbon atoms C2 and C3, and a structure similar to Flavonols, but without a hydroxyl group at carbon atom C3. Luteolin and Apigenin are the most prevalent flavones. Their antioxidant action is due to the presence of free hydroxyl groups in their rings A and B (Panche, Diwan, and Chandra 2016)

c) Flavanones

There are about 160 different forms of Flavanones, which are found in 36 different plant families and serve as precursors to a large number of additional flavonoids (Cristina et al. 2017; Durazzo et al. 2019). Chemically, these compounds are defined by the presence of a carboxyl group at position 4 and the absence of a double bond between C2 and C3. Citrus fruits are the primary source of flavanones (Table 3). The most extensively studied chemicals in this class are naringenin and hesperidin (Cristina et al. 2017; Durazzo et al. 2019)

d) Flavanols

Flavanols, sometimes called flavan-3-ols, are the most abundant subclass of flavonoids. The presence of a functional hydroxyl group at position 3 is all that distinguishes this class. Catechin and Epicatechin are the two major members of this class. Flavan-3-ols are present in a variety of fruits and plants (Table 3). Among these sources, green tea (Camellia sinensis L.) stands out for its high concentration of these compounds, and consumption of this tea has been related with a reduced incidence of chromic cardiovascular disease due to the bioactivities of flavan-3-ols (Cristina et al. 2017; Durazzo et al. 2019)

e) Isoflavonoids

Isoflavonoids are the only flavonoids having a benzenoid substituent at position 3, which gives them a structure similar to endogenous estrogens and enables them to interact with estrogen receptors in both an agonistic and antagonistic manner. Over 2000 isoflavonoids have been found, with isoflavones being the most well-known (Panche, Diwan, and Chandra 2016)

f) Anthocyanins

Anthocyanins are are responsible for the various red, blue and purple color of various fruits, vegetables, and flowers , they considered as flavonoids although they have a positive charge at the oxygen atom of the C-ring of basic flavonoid structure (Khoo et al. 2017).

g) Lignans

Lignans are secondary plant metabolites with a variety of chemical configurations; nonetheless, their basic structure is composed of phenylpropanoid dimers (C6-C3) connected by the central carbons of the side chains. Lignans are formed of two phenylpropane units, they can be found in cereals (triticale and wheat), fruit (pears, prunes) and certain vegetables (garlic, asparagus, carrots) with sesame and flax seeds being the most concentrated lignans sources. the most famous lignans are Secoisolariciresinol and Matairesinol , among others such enterodiol, enterolactone, sesamin, syringaresinol, medioresinols have anti-estrogenic, antioxidant and anti-carcinogenic activities (Manach et al., 2004; Rodríguez-García et al., 2019).

III.2. Biological effect of phenolic compounds

Nowadays, phenolic compounds (PC) are one of the most studied groups of bioactive molecules by the scientific community. These molecules have numerous documented health benefits, consuming a diet high in these compounds on a regular basis is critical for overall well-being (Domínguez-Avila et al., 2017).

Additional beneficial bioactivities for health maintenance have been associated with these compounds, including anti-inflammatory, antimicrobial, and anti-proliferative activities (Soto, Falqué, and Domínguez 2015; Cristina et al. 2017; Durazzo et al. 2019). The characteristics of polyphenols are summarized in Figure 5.These biological activities have sparked interest in the use of these molecules in the formulation of nutraceutical products (Cristina et al. 2017; Durazzo et al. 2019) (Figure 9).
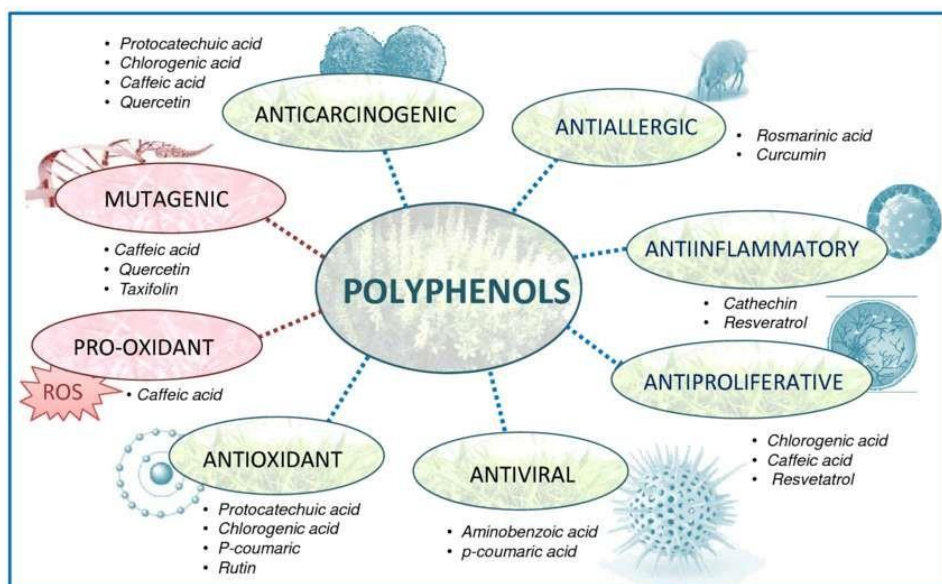

Figure 9: polyphenol properties (Zitka et al. 2011)

III.3. Anti-inflammatory effect

Phenolic compounds express anti-inflammatory activity by modulating the inflammatory responses through various mechanisms, such as:

A. Reduction of cytokine pathways (Zhang and Tsao, 2016),

B. Down-regulating the expression of IL-1β, IL-6 ,TNF-α, INF-γ and COX-2 (Bisht et al., 2010),

C. Inhibiting of NO production (Taofiq et al., 2015) and TNF-α cytotoxicity (Kassim et al., 2010),

D. Nuclear factor-kappa B (NF-κB) DNA-binding ability suppression (Chiu et al., 2015),

E. Inhibition of NF-kappaB activation in a TNF-α induced signaling by preventing the phosphorylation and degradation of IkBα (Singh and Aggarwal, 1995),

F. Inhibition of mitogen-activated protein kinase (MAPK) pathway (Seelinger et al., 2008).

III.4. Anti-Oxidative effect

Polyphenols are regarded antioxidants because they donate a hydrogen atom and/or an electron to free radicals, so interrupting the chain reaction of oxidation. As a result, the antioxidant effect is dependent on the amount and position of the hydroxyl groups (Cristina et al. 2017)

In the study of Sevgi, Tepe, and Sarikurkcu (2015), the antioxidant activity of 10 phenolic acids was reported, with the ferulic acid showing the highest antioxidant activity compared to caffeic,chlorogenic, cinnamic, gallic, p-hydroxybenzoic, protocatechuic, rosmarinic, syringic, p-coumaric,and vanillic acids. In vivo, ferulic acid significantly blocked the free radicals,therefore preventing the oxidative stress correlated with alcohol and polyunsaturated fatty acids

induced toxicity (Rukkumani et al. 2004)

Luteolin-6-C-neohesperidoside is a flavone that have been found to have ant oxidative effects in rats that were subjected to intense physical activity (forced swimming) by reducing lipid peroxidation and the activation of Nrf2/ARE, there for inducing the expression of antioxidant enzymes(Duan et al. 2017) .

in another study a 1% quercetin diet was applied on mice which increased the expression of antioxidant enzymes in the liver and in the epididymal adipose tissues by activating the nuclear factor Nrf2(Kobori et al. 2015)

in a study of Teucrium poliumL. (Lamiaceae) aerial part extracts ,Rutin and Apigenin was found to be active antioxidants according to DDBH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay results (Sharififar, Dehghn-Nudeh, and Mirtajaldini 2009) .In a study The flavonoid Naringenin was administrated orally to rats and was found to reduce elevated anti-oxidative enzymes activity (Wojnar, Zych, and Kaczmarczyk-Sedlak 2018)

IV.   Molecular docking

IV.1. In silico drug design

Modern medicinal chemistry methodologies, including molecular modeling, have become more important in the analysis of structure-activity correlations (SAR) (Hughes et al. 2011). Along with pharmacodynamics data (e.g., potency, affinity, effectiveness, and selectivity), these approaches have been used to investigate pharmacokinetic features (ADMET: absorption, distribution, metabolism, excretion, and toxicity) (Lipinski et al. 2012) The area has advanced in lockstep with advancements in bio- molecular spectroscopic techniques such as X-ray crystallography and nuclear magnetic resonance (NMR), which have enabled dramatic improvements in molecular and structural biology.

These approaches have enabled the resolution of over 100,000 three-dimensional protein structures, thereby revealing critical structural information about important macromolecular drug targets (Berman et al. 2000)Efforts to store, organize, and explore such data have resulted in an increase in the demand for strong and advanced computational tools. From this vantage point, the precise integration of in silico and experimental methodologies has resulted in a comprehensive understanding of the delicate elements of intermolecular recognition (Weigelt 2010)

IV.2. Molecular docking

The molecular docking approach is used to mimic the atomic level interaction between a small molecule and a macromolecule (protein), allowing us to define the interaction pattern and the binding region of target proteins (McConkey, Sobolev, and Edelman 2002).

Docking is a two-step method that begins with the prediction of the ligand structure, as well as its position and orientation inside these sites (often referred to as pose), and ends with the determination of the binding affinity. These two phase sare associated with sampling methods and scoring schemes (Drwal and Griffith 2013)(Figure 10).
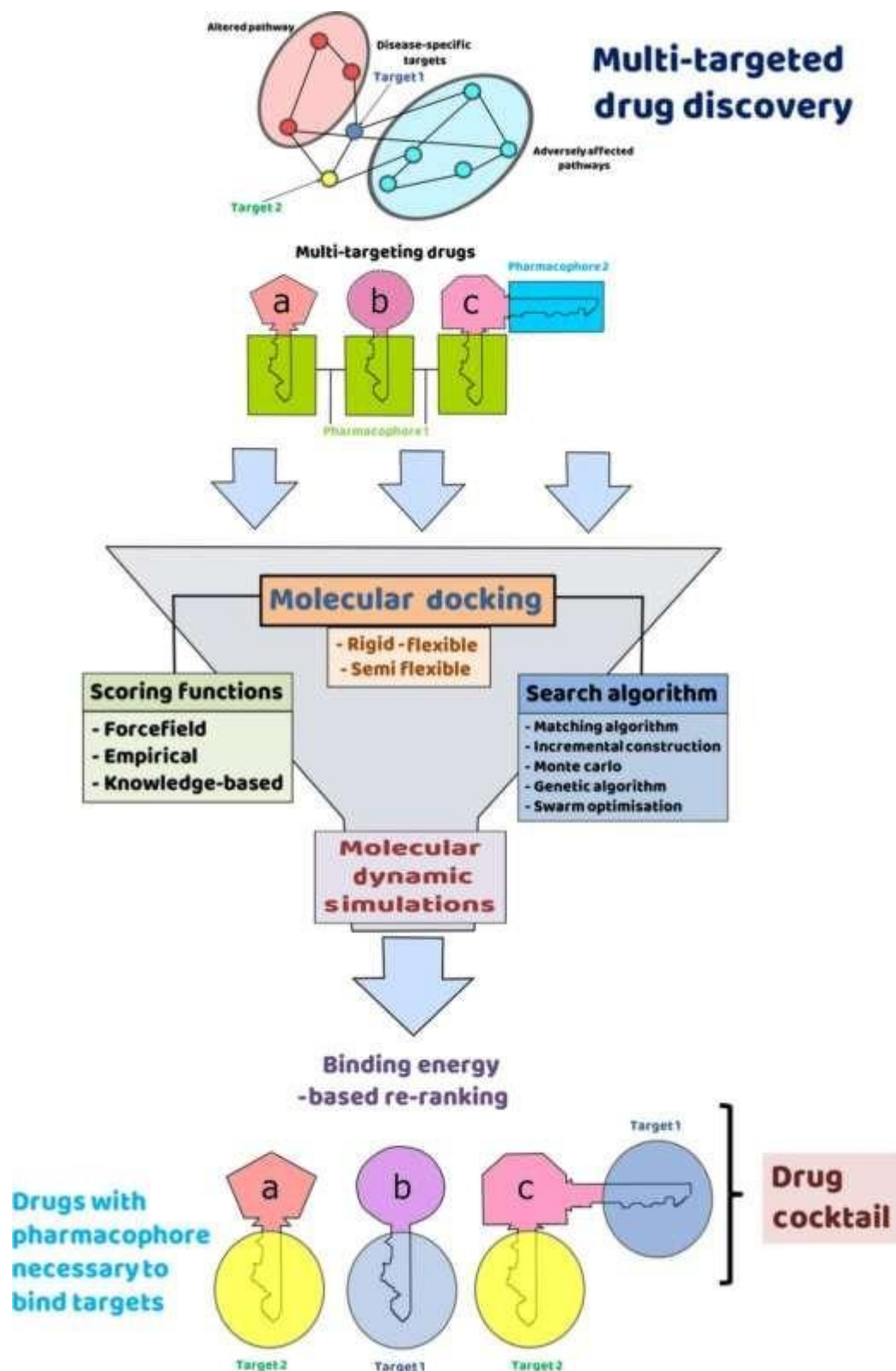
Figure 10: Utility and implementation of molecular docking in drug discovery and drugdesign (Sivakumar et al. 2020)

Before the docking simulation it is better to know the location of the binding site because it considerably improves docking accuracy. (Kalyaanamoorthy and Chen 2011) Additionally, information about the sites can be obtained by

Comparing the target protein to a family of proteins with comparable functions or to proteins co-crystallized with other ligands. Without knowledge of the binding locations, cavity detection tools and online servers like as GRID, POCKET, SurfNet, PASS, and MMC can be used to find probable active sites within proteins (Glaser et al. 2006).

V. In silico pharmacology

Absorption, distribution, metabolism, excretion along sides toxicity are considered to be very important data in discovering and developing new drugs, it helps researchers find drug like molecules that possesses physicochemical properties that might enable them to become a therapeutic drugs (Zhong, 2017; Chandrasekaran et al., 2018)

a) Absorption

Absorption is considered to be the ability of a molecule to penetrate the gastrique cell membrane; there are two types of trans-membrane activity: (Chandrasekaran et al., 2018; Zhong, 2017).

➢ Passive membrane transport: compounds are transported by the effect of a concentration gradient; here we take into consideration the lipophilicity and size of the molecule to predict the possibility of absorption.

➢ Active membrane transport: this type of trans-membrane activity requires the mediation of carrier proteins that are selective and energy dependent, like P-glycoprotein (MDR1), in this case the interaction between the molecule and the carrier protein is taken into consideration.

b) Distribution

Drug distribution means the movement of a drug from the circulatory systems (blood or lymphatic) to the tissue; the prediction of drug distribution is made possible by mainly examining the blood–brain barrier (BBB) permeability, the volume of distribution (VD), and the plasma protein binding (PPB) (Zhong, 2017; Chandrasekaran et al., 2018)

c) Metabolism

Drug metabolism is the biotransformation (oxidation, reduction, hydrolysis, and carboxylation.) of viable drugs into metabolites; most of this reactions occur in the liver and are carried out by cytochrome P450 (P450 or CYP) with about 100 CYPs isoforms found in humans ,The most active CYPs for drug metabolism are CYP2C, CYP2D, and CYP3A subfamilies. (Zhong, 2017).

d) Excretion

Excretion is the elimination of a drug from the body achieved by either the kidney and/or the liver where drugs are eliminated in the form of urine or bile; drug excretion prediction help build the required drug concentration to maintain the therapeutics effects.(Zhong 2017; Chandrasekaran et al., 2018).

e) Toxicity Profile

Prediction of Toxicity Profiles considered as a critical to Developers; toxicity was tested by using laboratory animals but now in silico toxicology is applied for toxicity optimization and to minimize the risks of animal toxicity testing by predicting the toxicity of compounds towards certain organ(liver , kidney …).(Chandrasekaran et al. 2018) (Nathan and Aihao 2010)

Materials and Methods

I. Materials

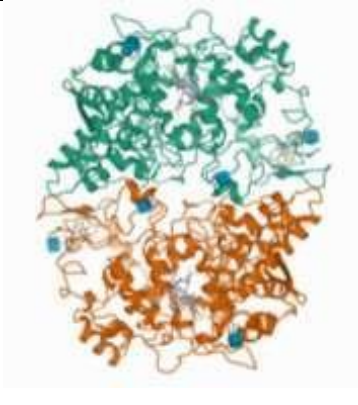**I.1. Data bases**
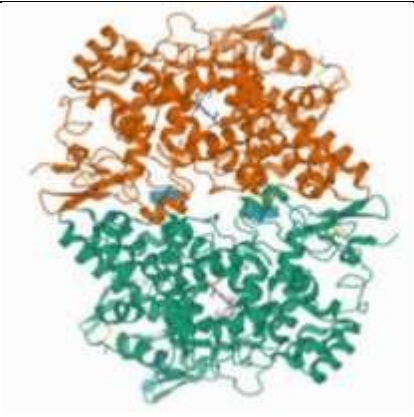
a) PDB

The Protein Data Bank (PDB) is the first open access digital data resource in biology and medicine. It provides free access to 3D structure data for large biological molecules (proteins, DNA, and RNA) (www.rcsb.org). PDB was explored to download the targeted proteins 3D structures in PDB format. In our study, we retrieved 3D structure of IKB, COX-1 and 2, and Xanthine-oxydase according to their IDs (Table 4).

Table 4: 3D structures and IDs of target proteins downloaded from PDB

Targeted protein (macromolecule)	RCSB ID	structure
Human IkB kinase beta	4KIK	
Bovine Xanthine Oxidase in Complex with Quercetin	3NVY	

Cyclooxygenase-2 (prostaglandin synthase-2) with a selective inhibitor, sc-558 (cox-2)	1CX2	
Ovine Cyclooxygenase-1 Complex with Meloxicam	4O1Z	

b) PubChem

PubChem is an open chemistry database. Since its launch in 2004, it became a key chemical information resource for scientists, students, and the public. Mostly, PubChem contains small molecules, but also larger molecules such as nucleotides, carbohydrates, lipids, peptides, and chemically modified macromolecules. It collects information on chemical structures, identifiers, chemical and physical properties, biological activities, patents, health, safety, toxicity data, and many others. (pubchem.ncbi.nlm.nih.gov).

We used PubChem to download 2D structures of phenolic compounds from different classes according to their IDs (Table 5).

<p align="center">**Table 5:** PubChem IDs of targeted polyphenol compounds.</p>

Phenolic compound	PubChem ID	Phenolic compound	PubChem ID
4-Hydroxybenzoic acid	135	Fisetin	5281614
Apigenin	5280443	Gallocatechin	9882981
Apigeninidin	441647	Genistein	5280961
Butein	5281222	Luteolin	5280445
Caffeic acid	689043	Luteolinidin	441701
Delphinidin	128853	Naringenin	439246
Epicatechin	72276	Pelargonidin	440832
Ferulic acid	445858	Taxifolin	439533

I.2. Software

    a) BIOVIA Discovery Studio Visualiser

It's considered as a free molecular modeling application for viewing and analyzing proteins and small molecules data, with an easy interactive environment for viewing and editing molecular structures.('ADMETlab 2.0' n.d.)

    b) Pyrx

Pyrx is considered as virtual screening software for structure-based drug design that can be used to screen compounds against potential drug targets; Pyrx enables Virtual Screening from data preparation to job submission and analysis of the results with an easy-to-use user interface, which makes it a valuable tool for Computer-Aided Drug Design. (Pyrx,) .it's used in this study due to its free availability and easy work interface.

c) OpenBabel

Conversion of chemical structures between multiple formats is a recurrent issue in computational modeling. While standard interchange formats (for example, Chemical Markup Language) and de facto standards (for example, the SMILES format) exist, the need to interconvert formats continues to be a problem due to the variety of different applications for chemistry data and the differences in the data stored by different formats (0D versus 3D, for example).

With the release of Open Babel 2.3, Open Babel supports 111 chemical file formats in total. It can read 82 formats and write 85 formats. These encompass:

- Common formats used in cheminformatics (SMILES, InChI, MOL, MOL2),
- Input and output files from a variety of computational chemistry packages (GAMESS, Gaussian, MOPAC),
- Crystallographic file formats (CIF, ShelX),
- Reaction formats (MDL RXN),
- File formats used by molecular dynamics and docking packages (AutoDock, Amber),
- Formats used by 2D drawing packages (ChemDraw),
- 3D viewers (Chem3D, Molden) and,
- Chemical kinetics and thermodynamics (ChemKin, Thermo).

I.3. Webserver

a) **ADMETlab 2.0:**

Is an enhanced version of the widely used ADMETlab for systematical evaluation of Pharmacological properties, with significant updates to functional modules, predictive models, explanations, and the user interface ('ADMETlab 2.0')

II.   Methods

II.1. macromolecules preparation

The selected macromolecules were purified using BIOVIA Discovery Studio Visualizer, heteroatoms (water, ions, etc.) were deleted, polar hydrogen was added, and the final macromolecules saved in PDB format.

II.2. Ligands preparation

The SDF files for the phenolic compounds were obtained from the PubChem database,

II.3. Molecular docking process

Virtual molecular screening is a technique that involves docking small-molecule libraries to a macromolecule in order to identify lead compounds with desirable biological properties. This in silico method is widely used in computer-aided drug design. We describe how to conduct virtual screening of small molecules using PyRx. Additionally, the specific methods for using PyRx are outlined, as well as considerations for data preparation, docking, and analysis as explained below :

- After opening our downloaded target molecule using Discovery studio visualizer we delete the heatatoms and add polar hydrogens and finally save the modified molecule in PDB format. (Figure 11 and Figure 12).
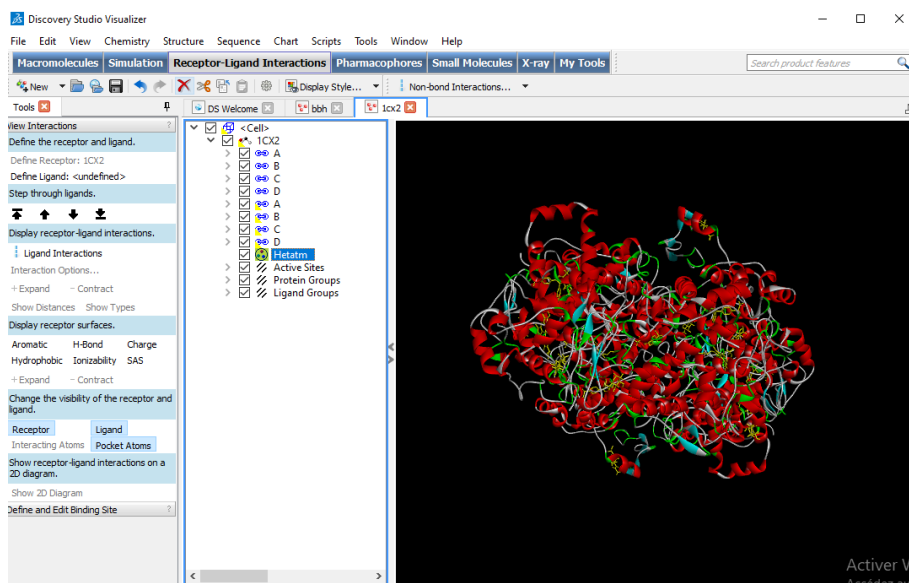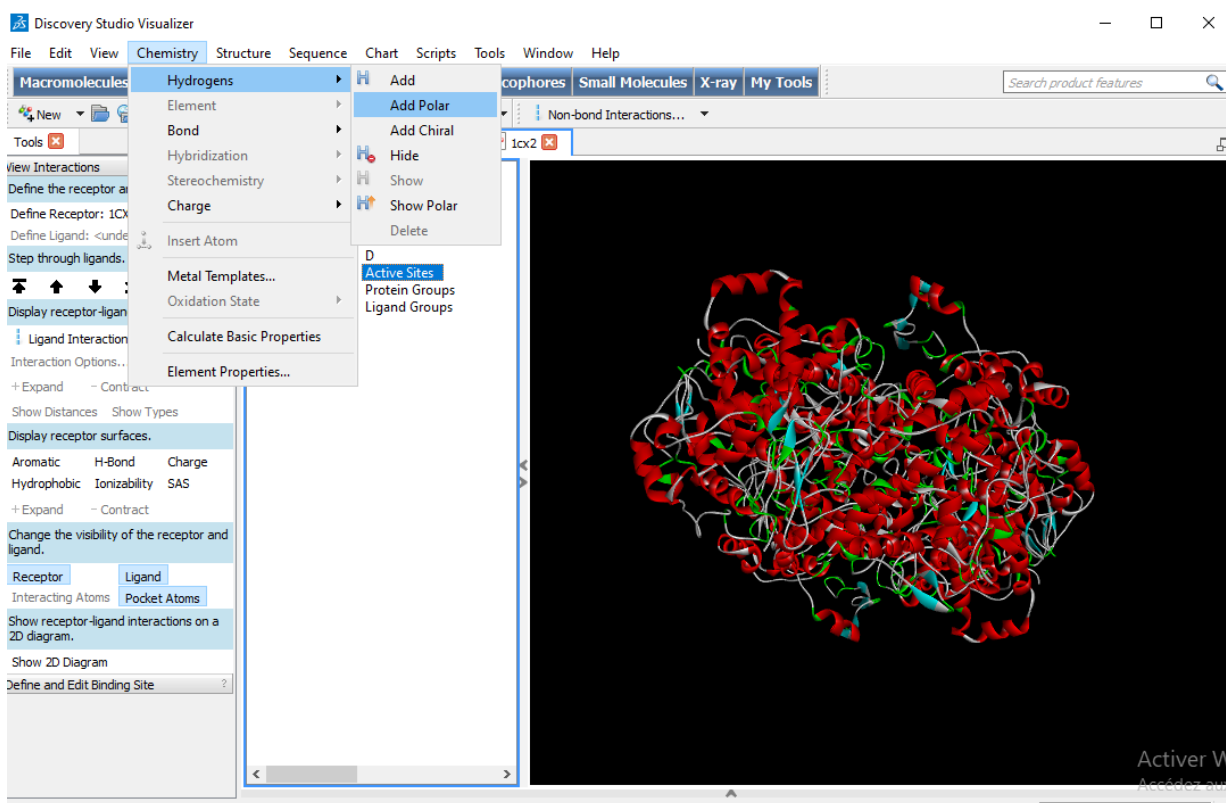

Figure 11: deleting heatatoms.

Figure 12 : adding polar hydrogen.

- after opening Pyrex we right click using the mouse on the white space and click on load molecule and choose our PDB format molecule (Figure13).
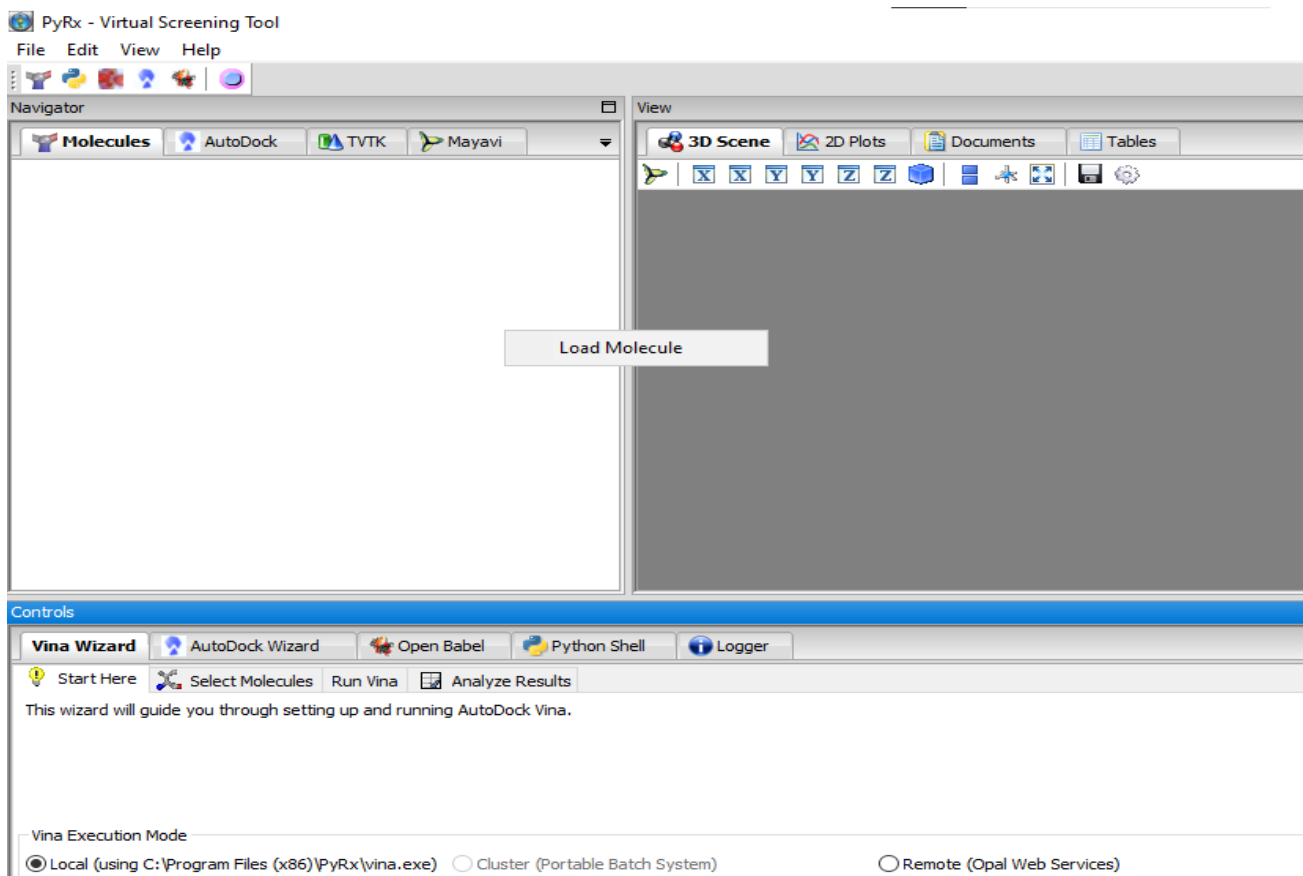

Figure13: loading macromolecules.

- 2 after our macromolecule have been loaded into Pyrex we right click on it and choose Autodock then click on "make macromolecule." (figure 14)
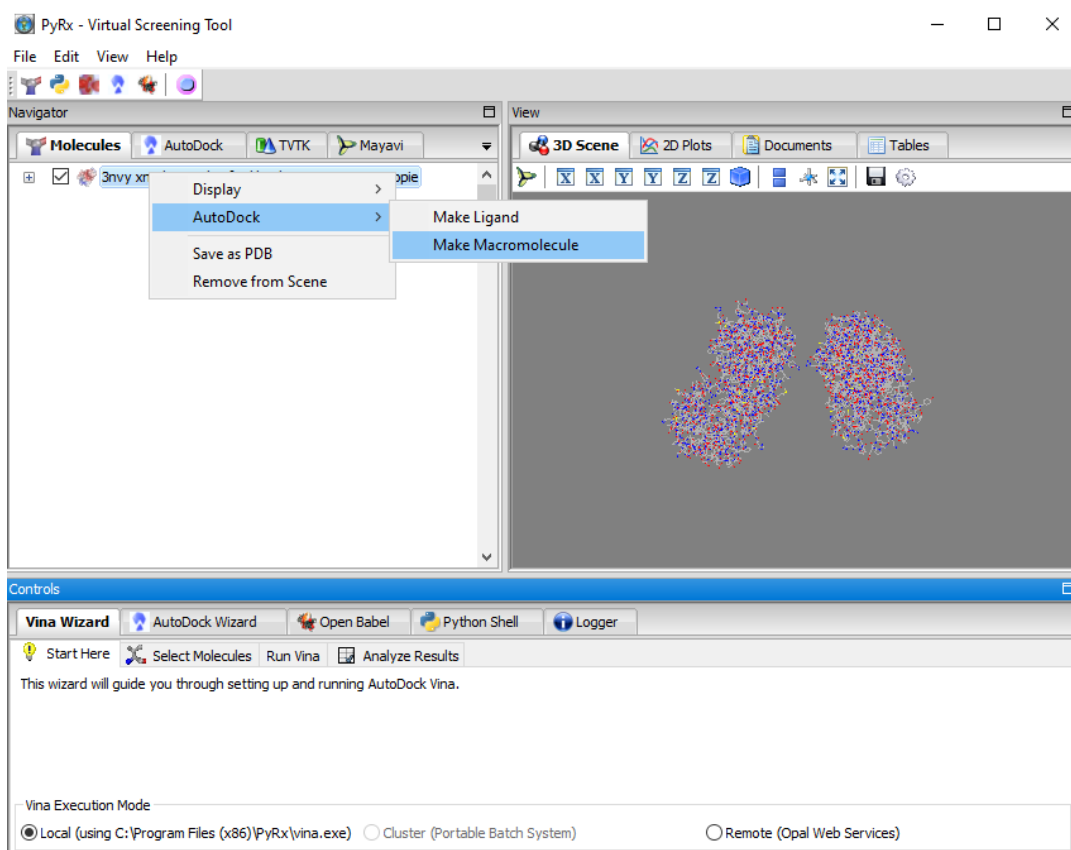

Figure 14 : transforming the macromolecule into Autodock macromolecule.

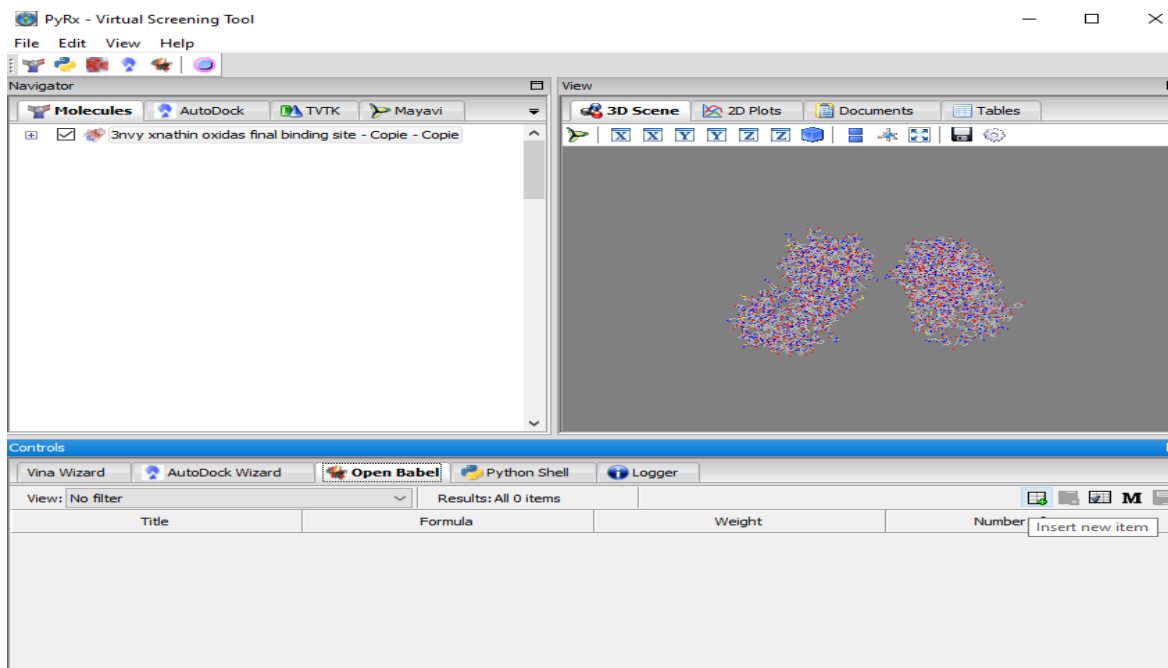- we click on Open babel then click on the Insert new item icon and choose our ligands (Figure15)

Figure 15 : loading the ligands.

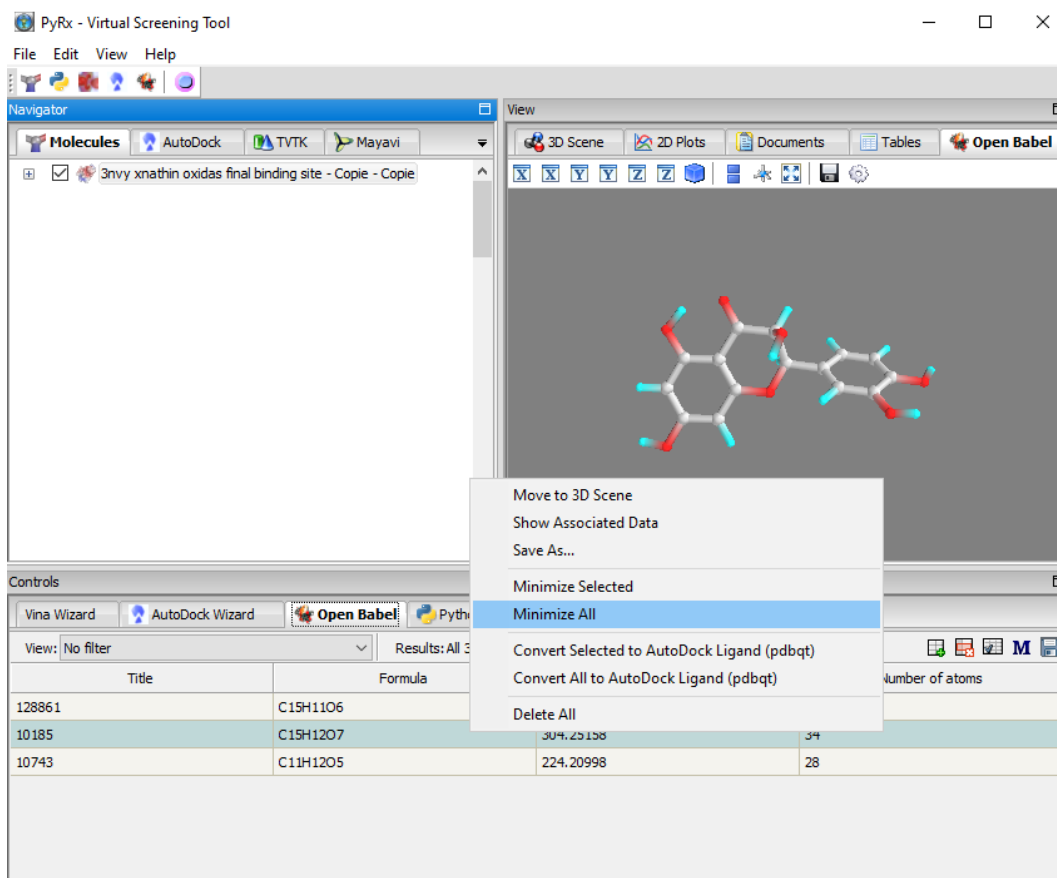- After choosing the ligands we right click on it and choose minimize all (Figure 16).

Figure 16: minimizing ligands

- After the minimization is completed we right click and choose convert all to Autodock ligand (Figure 17)

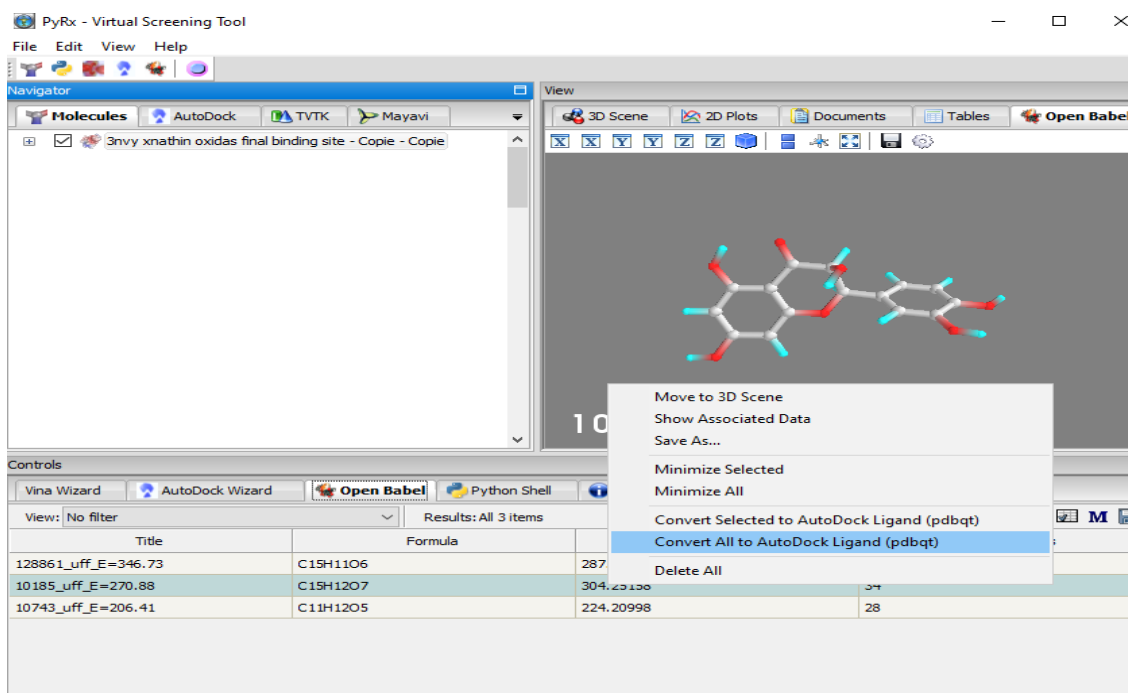**Figure 17 :** converting ligands to Autodock ligands.

- By clicking on the Autodock window we can see our loaded and converted ligands and macromolecules (Figure 18)
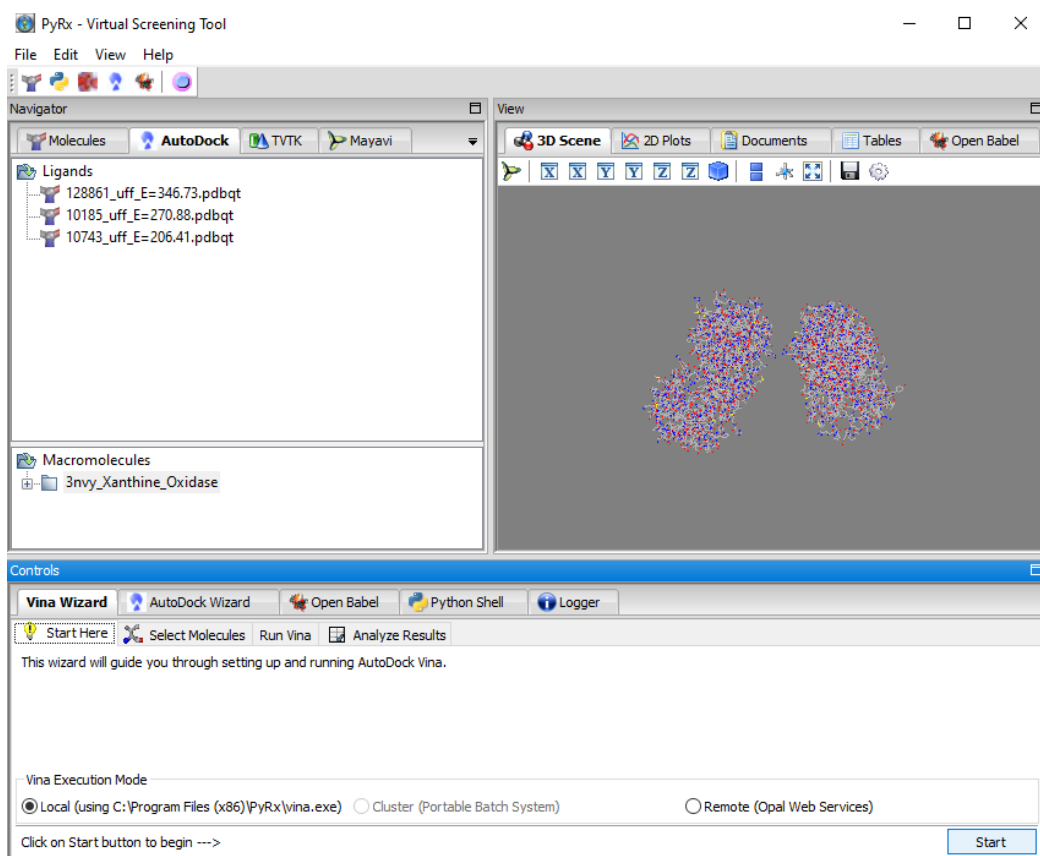

Figure 18 : Autodock window view.

- After clicking on the start button and choosing our Targeted ligands and macromolecule we click on "forward"(Figure 19)
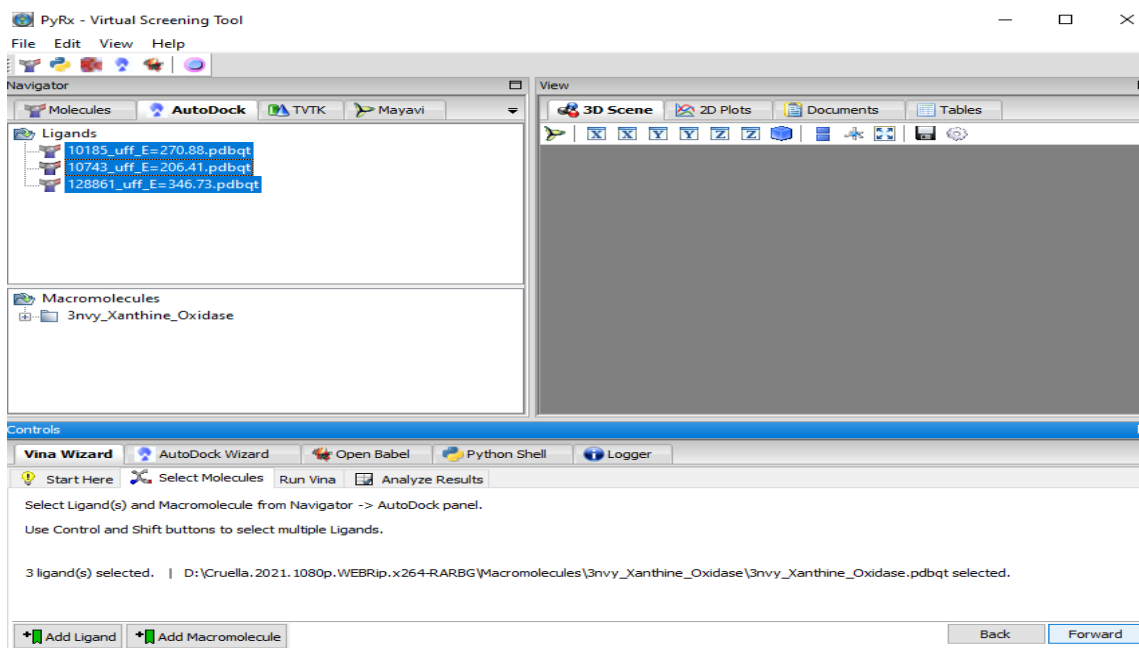
Figure 19 : selecting targeted ligands and macromolecule.

- We maximize our Vina search space for optimal results and click on the forward button again to start our docking process (Figure 20).

Figure 20 : starting the docking process.

- Once the calculations are done, results will be show the Binding Affinity (kcal/mol) values. More negative the binding affinity better the orientation of the ligand in the binding site.

- Exporting Vina Results: Results can be exported to Biovina discovery studio visualizer software for analysis,

II.4. Pharmacological properties

They were predicted using Lipinski's rule of five and ADMET' features.

II.4.1 Lipinski rule of Five

In drug research setting the rule of five predicts that poor absorption and permeation is less likely when there are:
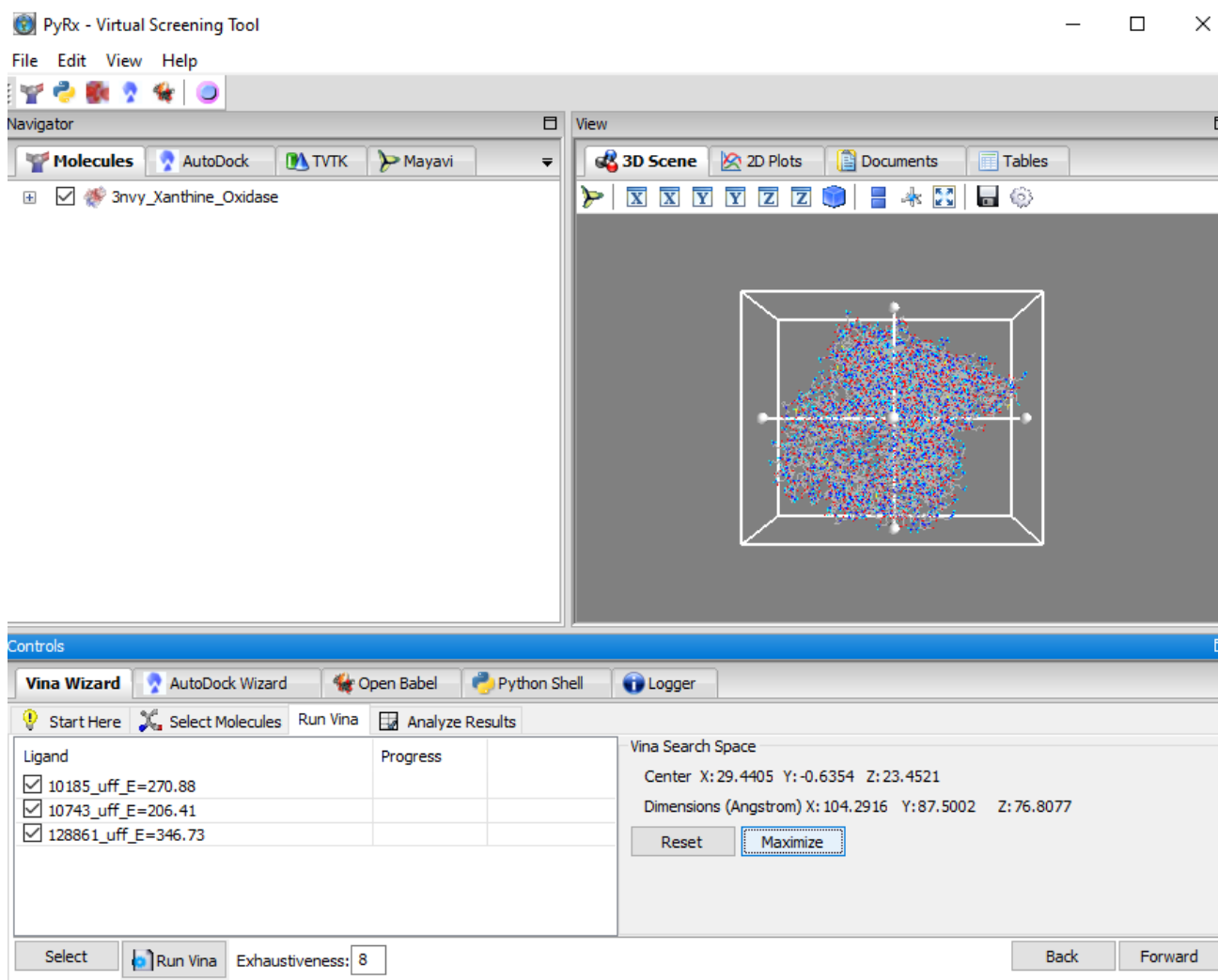
- less 5 hydrogen bonds donors (the total number of nitrogen– hydrogen and oxygen–hydrogen bonds);

- less than 10 hydrogen bonds acceptors (all nitrogen or oxygen atoms);

- the molecular weight is less than 500 Daltons;

- the calculated octanol-water partition coefficient (log P) is less than 5 (Lipinski et al., 2001).

II.4.2. ADMET Features

Chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) ,these features were predicted using ADMETlAB2.0 server (table 6)

Table 6: Utility of pharmacology features used in this study

Pharmacology features	Parameters	Significance
Absorption	**Caco-2 permeability**	A compound is considered to have a proper Caco-2 permeability and is easy to absorb if the predicted Caco-2 permeability value is >-5.15 log cm/s.
	HIA	Compounds with absorbance of more than 30% are considered to be greatly absorbed, while compounds with absorbance less than 30% are considered poorly absorbed.
	Pgp-substrate and Pgp-inhibitor	A compound is considered to have a low absorption if it was an inhibitor or a substrat for P-glycoprotein.
Distribution	**Plasma protein binding (PPB)**	A compound is considered to have a proper PPB if it has a predicted value < 90%; drugs with high protein-bound may have a low therapeutic index.
	Volume Distribution (VD)	A Compound is considered to have a proper VD if it has a predicted value in the range of 0.04-20 L/kg,
	Fraction unbound in plasma (FU)	The fraction unbound in plasma. Most drugs in plasma will exist either in a bound or an unbound state to serum proteins, the more that is bound the less efficiently the drug molecule activity can be. $\geq$ 5%: excellent ;< 5% : poor.
	The blood–brain barrier (BBB)	the ability of a drug to cross into the brain: BBB+ is considered good blood–brain barrier permeability while BBB- is a low blood–brain barrier.

a) **Metabolism:** Metabolism was predicted based on the CYP models for substrate and inhibition of CYP2D6, CYP3A4, CYP1A2, CYP2C19 and CYP2C9.

b) **Excretion :** Excretion was predicted based on the total clearance:

- o High clearance : >15 ml/min/kg
- o Moderate clearance: 5-15 ml/min/kg:
- o Low clearance : <5 ml/min/kg

c) **Toxicity:** The toxicity prediction of drugs was based on AMES toxicity, hERG inhibition, hepatotoxicity and Carcinogenicity.
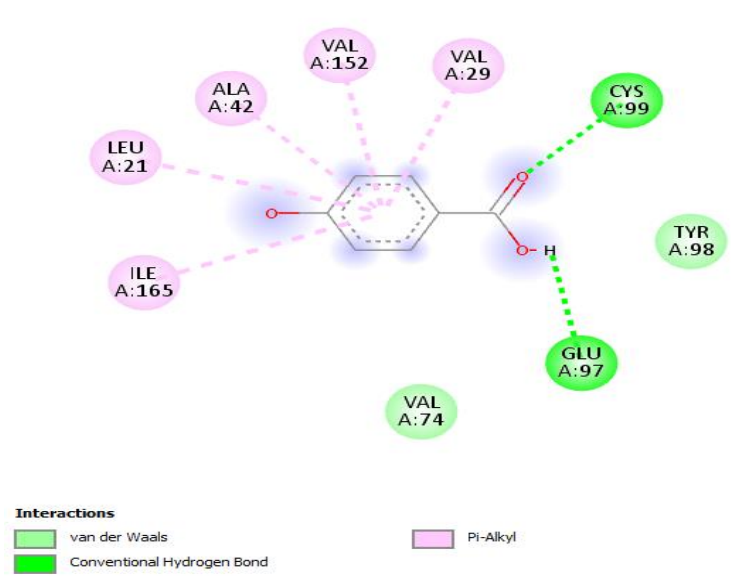
Results and discussion

I.   Ikkβ

II.1   Ikkβ interaction profile

In our study we found that polyphenols could bind to the Met 96 gatekeeper with Vander Waals interaction (Ferulic acid, Caffeic acid, Gallocatechin, Fisetin and Narnigenin), Pi- Sulfur bound (Pelargonidin, Genistein, Delphinidin, Apigeninidin and Luteolin) or Pi-Alkyl with and Luteolinidin. (Figure 21)

In addition, we found that the hinge region of the KD of IKKβ successfully interacted with 4-Hydroxybenzoic acid, Ferulic acid, Caffeic acid, Gallocatechin, Fisetin, Taxifolin, Pelargonidin, Delphinidin, Apigeninidin, Narnigenin, Luteolinidin and Luteolin. Epicatechin and Butein do not interact with kinase domain of IKKβ residues. (Figure 21)

Regarding the interaction with the activation loop, we found that almost all the phenolic compound display an interaction bound with Asp166 while Epicatechin displayed an interaction with Gly168. 4-Hydroxybenzoic acid, Caffeic acid, Butein and Fisetin do not interact with none of the triad residues. Leu167 do not interact with any of the phenolic compounds.(Figure 21)
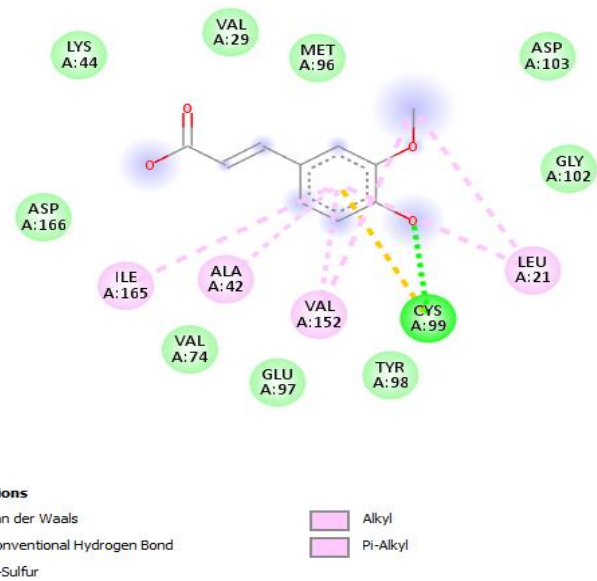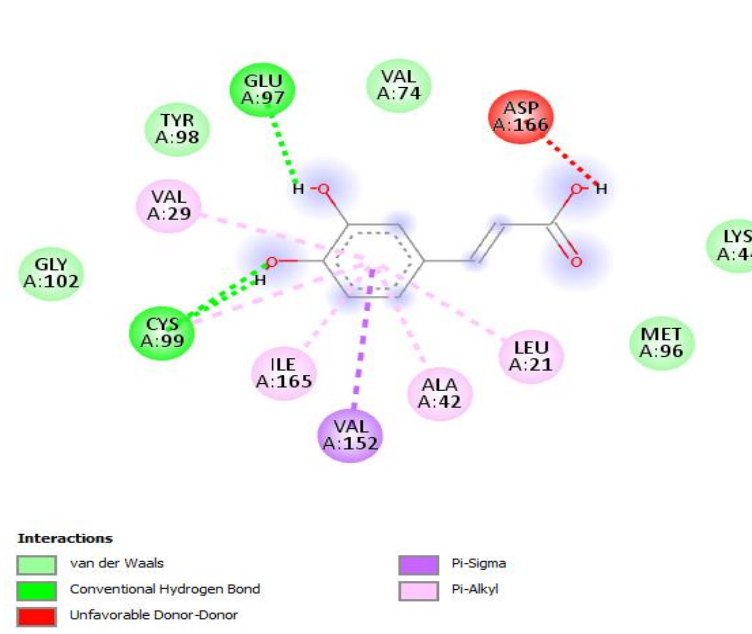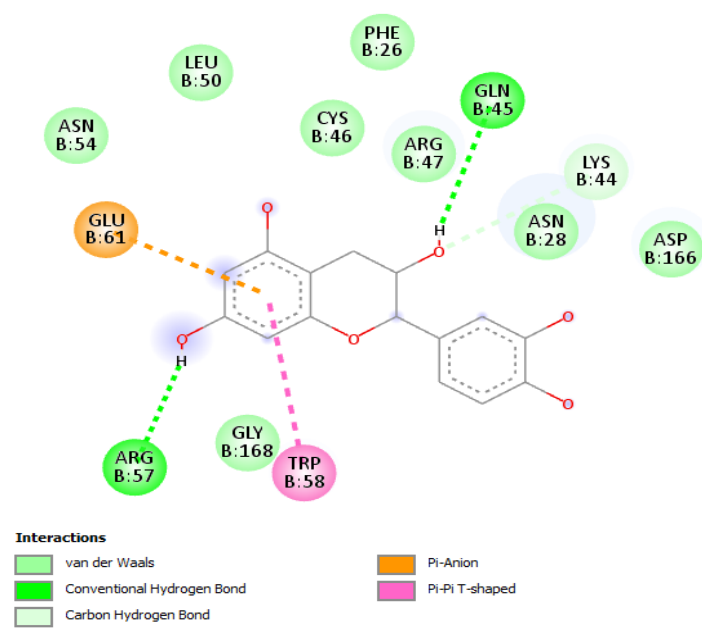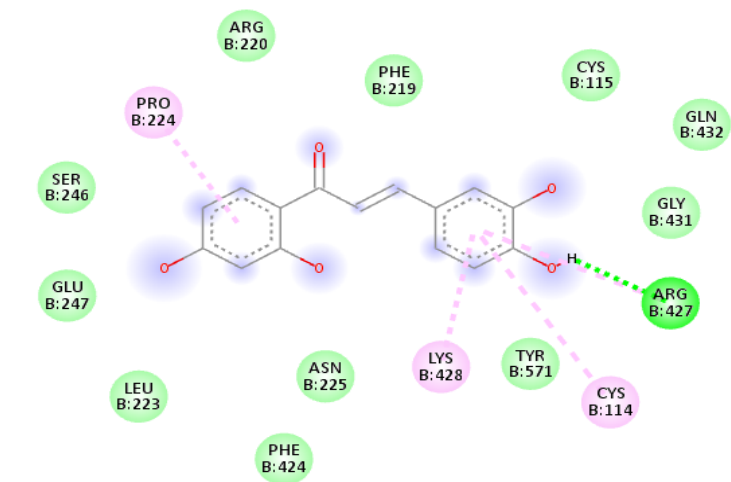
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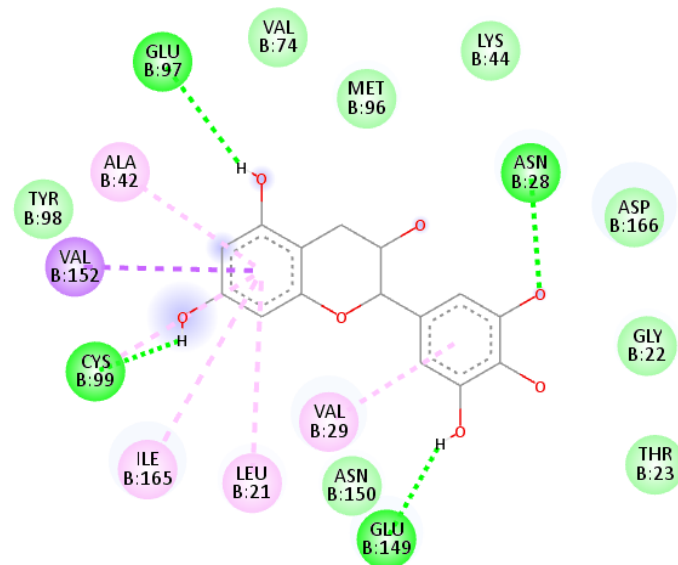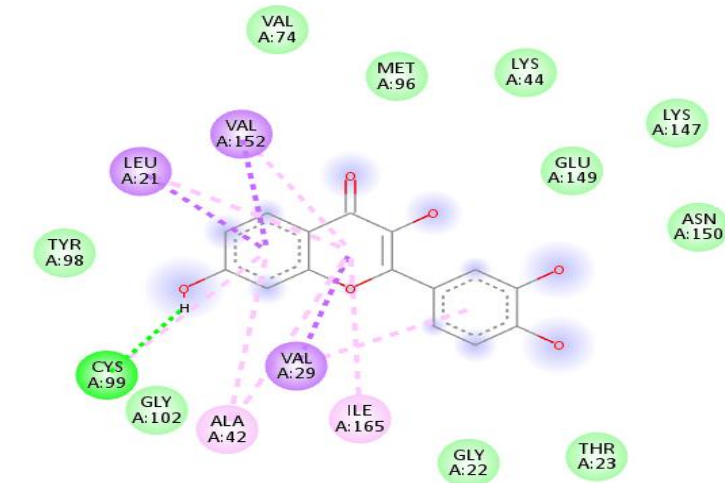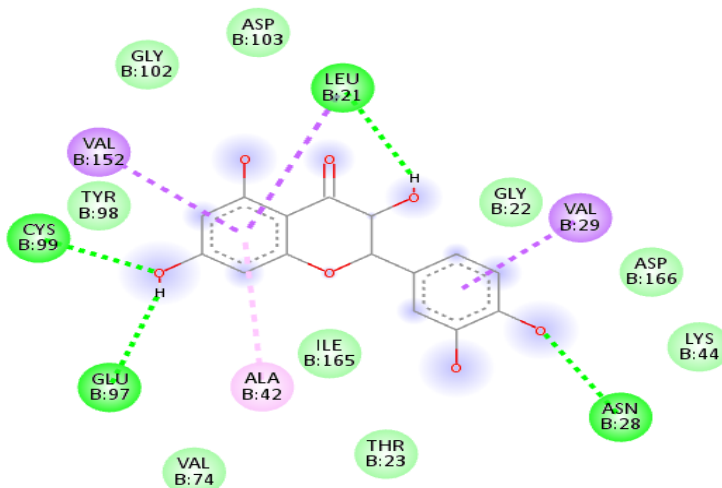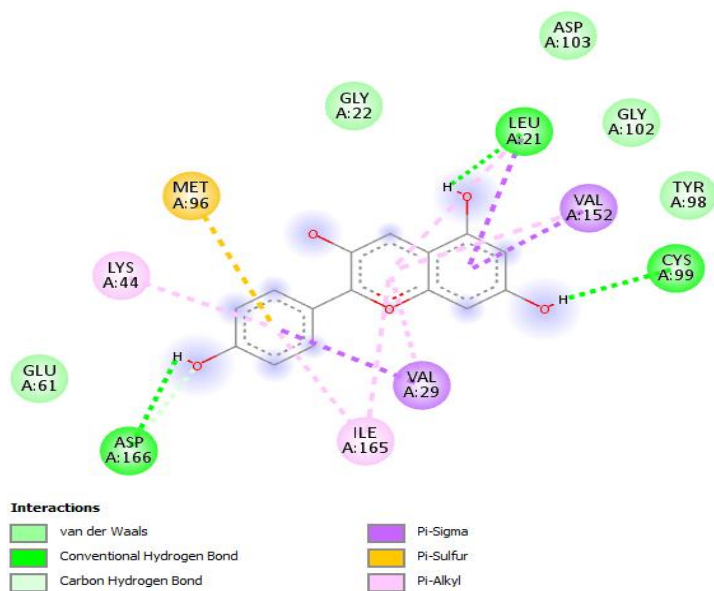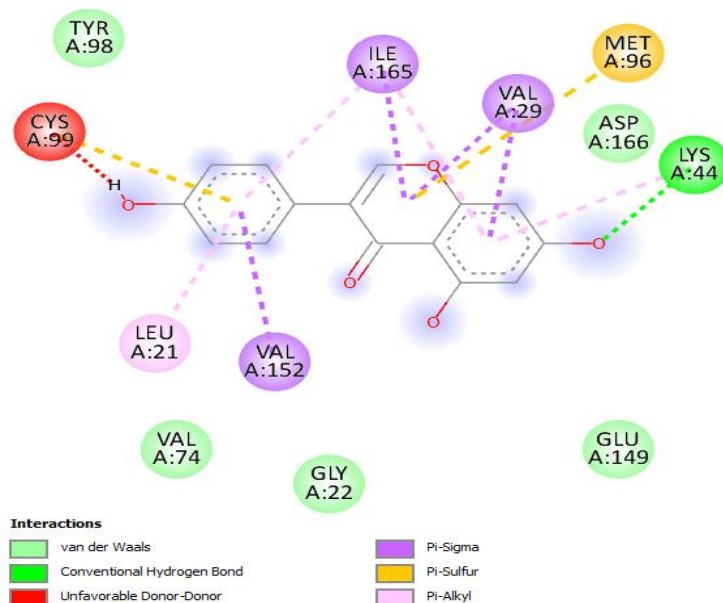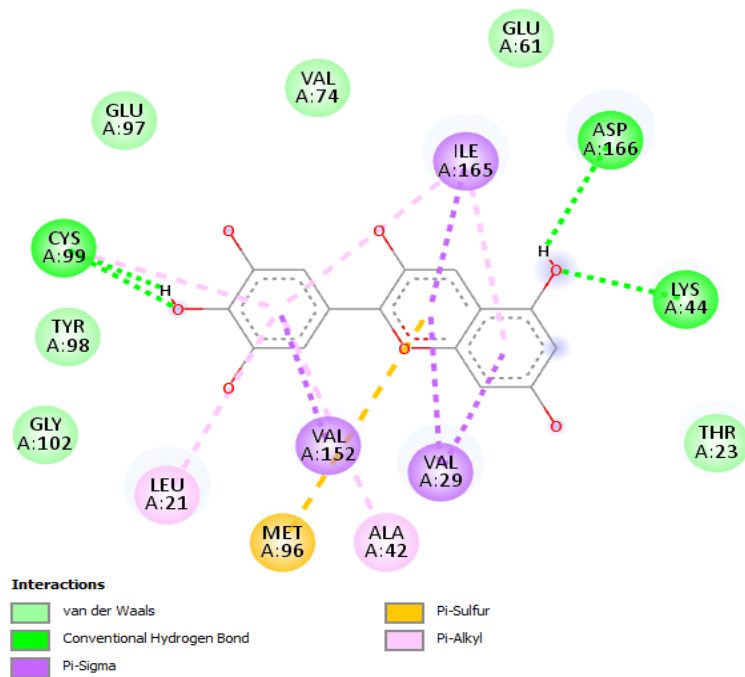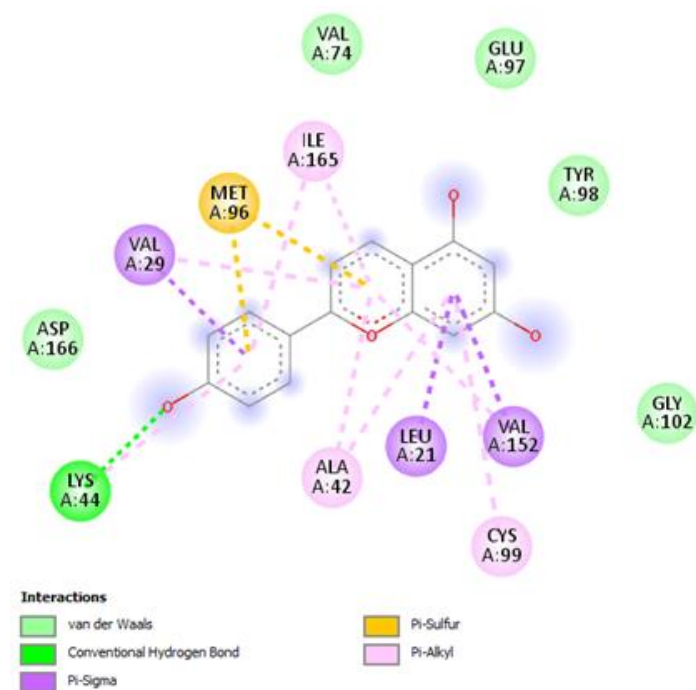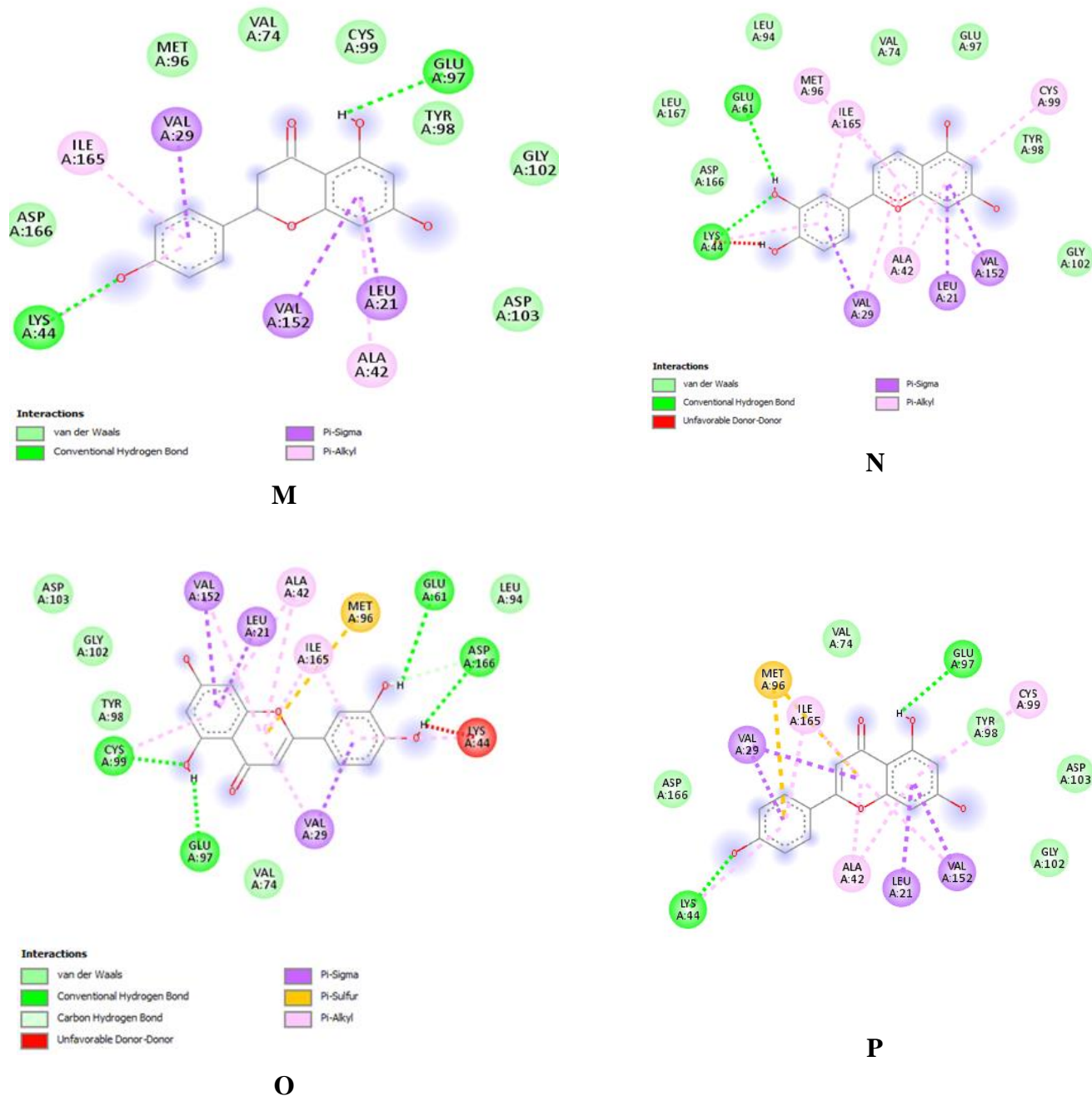
Figure 21: interaction profile of phenolic compounds with Ikkβ. **A** : 4-Hydroxybenzoic acid,**B :** Ferulic acid , **C:** Caffeic acid,**D**: Epicatechin ,**E** Butein ,**F**: Gallocatechin,**G**: Fisetin ,**H:** Taxifolin, **I :** Pelargonidin ,**J :** Genistein ,**K :** Delphinidin ; **L :** Apigeninidin,**M :** Narnigenin, **N :** Luteolinidin,**O**: Luteolin, **P**: Apiginin

I.2 Ikkβ Binding energy results :

All the phenolic compounds exhibited a high binding affinity with IKKβ as shown in table 7.

Table 7: The binding energies displayed by phenolic compounds-IKKβ complexes

Phenolic compounds	Binding energy (kcal/mol)
4-Hydroxybenzoic acid	-5.8
Ferulic acid	-6.8
Caffeic acid	-6.9
Epicatechin	-7.1
Butein	-7.9
Gallocatechin	-8.5
Fisetin	-8.5
Taxifolin	-9
Pelargonidin	-9.1
Genistein	-9.1
Delphinidin	-9.3
Apigeninidin	-9.5
Naringenin	-9.6
Apigenin	-9.6
Luteolinidin	-9.9
Luteolin	-10

The docking results of this research are similar to the study of the molecular interaction between celastrol and its 36 analogues with IKKβ done by (Veerappan et al. 2016); were they found that celastrol and 25 of its analogues inhibit IKKβ, Celastrol formed two hydrogen bonds with Glu97 and one with Met96, gatekeeper residue, which controls the access of inhibitor to binding pocket ,An additional hydrogen bond with Asn150 and multiple hydrophobic interactions with glycine loop (residues 20-30) and activation loop (residues 166- 194) greatly stabilizes the celastrol IKKβ interaction with the binding score of −10.56 kcal/mol.

In another study Hammoudi et al. (2020) docked thirty 2-amino-3-cyano-4-alkyl-6-(2- hydrox-yphenyl) pyridine derivatives with IKKβ , the results showed docking score valuesranging from -5.710 to -8.441 kcal/mol with a binding mode similar to k252-A a known inhibiter ofIKKβ, and interacting with residues such as Leu21, Glu97, Cys99, Asp166, Glu149,Tyr98, Cys99,Asp103.

II.   Cox-1 and Cox-2 :
I.   Cox-2 interaction profile :

During this research we found that all the tested phenolic compounds interact with residues of the hydrophobic pocket Tyr385, Tyr387, Phe518 and Leu352 mostly by Van Der Waals and Pi-Alkylbonds (Figure 22)

Considering the mouth of the active site Apigenin, Luteolin, Naringenin, Ferulic acid and Caffeicacid formed Van Der Waals interaction with Tyr355 residue. Apigeninidin and Pelargonidin formed Pi-Cation interaction with residue Arg120 (Figure 22).

Regarding the interaction with the side pocket , Caffeic acid formed Hydrogen bonds with both His90 and Val523 and Van Der Waals interaction with Arg513 and Ser530 , while Ferulic acid formed Pi-Alkyl bonds with Val523 and Van Der Waals bonds with Ser530,Arg513 and His90.(Figure 22)

The flavonoids, Delphinidin ,Luteolin , Apigenin ,Genistein and Naringenin all formed Van Der Waals bond with residue Val523 , while Fisetin, Apigeninidin and Pelargonidin formed Pi-Alkyl bond with Val523 , the two Anthocyanins also formed Van der Waals bond with Ser530 (Figure 22).

A

B

C

A

E

B

G

C

I

D

K

E

M

F

O

G

Figure 22: Phenolic compounds interaction with Cox-2. **A** : 4-Hydroxybenzoic acid,**B** : Ferulic acid, **C**: Caffeic acid, **D**: Epicatechin, **E** : Butein, **F** : Gallocatechin , **G** : Fisetin, **H** : Taxifolin, **J** : Genistein, **K** : Delphinidin, **L**: Apigeninidin, **M** : Apigenin, **N** : Luteolinidin , **O** : Luteolin, **P** : Naringenin.

II.   Cox-2 Binding energy results:

This study found binding energy of Cox-2 to be between -6.1 and -8.5 kcal/mol .(Table 8).

Table 8: Cox-2 Binding energy results

Phenolic compounds	Binding energy (kcal/mol
4-Hydroxybenzoic acid	-6.1
Caffeic acid	-6.8
Ferulic acid	-6.9
Epicatechin	-7.5
Gallocatechin	-7.7
Butein	-7.9
Taxifolin	-7.9
Delphinidin	-7.9
Fisetin	-8.1
Luteolinidin	-8.1
Naringenin	-8.2
Genistein	-8.3
Luteolin	-8.3
Apigenin	-8.3
Pelargonidin	-8.4
Apigeninidin	-8.5

III.   Cox-1 interaction profile :

In this study we found that all the tested phenolic compounds interact with residues of the hydrophobic pocket .4-Hydroxibenzoic acid, Caffeic acid ,Epicatechin, Gallocatechin, Taxifolin, Luteolin and Naringenin all formed Van Der Waals interaction with Trp387 and Tyr385. Ferulic acid, Fisetin and Delphinidin formed Hydrogen bonds with Tyr385.Pelargonidin and Apigeninidin showed Pi-Alkyl bond with Leu352 and Van Der Waals bond with Trp387. Butein interacted with the hydrophobic pocket via Van Der Waals interactions with Phe518, Tyr385, Trp387 and Leu 352 (Figure 23).

Considering the mouth of the active site the flavonoids, Butein, Pelargonidin, Apigenin, Apigeninidin and Luteolinidin formed Van Der Waals bond with Tyr355, a key residue of the mouth of the active site (Figure 23). Regarding the interaction with the side pocket residues, Pelargonidin and Apigeninidin formed Van Der Waals bond with Ser530 and Ile523.Butein and Luteolinidin formed Pi-Alkyl bond with Ile523 and Van Der Waals bond with Ser530, Apigenin on the other hand formed hydrogen bond with Ser530 and P-Alkyl bond with Ile523. Genistein didn't show any interaction with Cox-1 active site.(Figure 23).
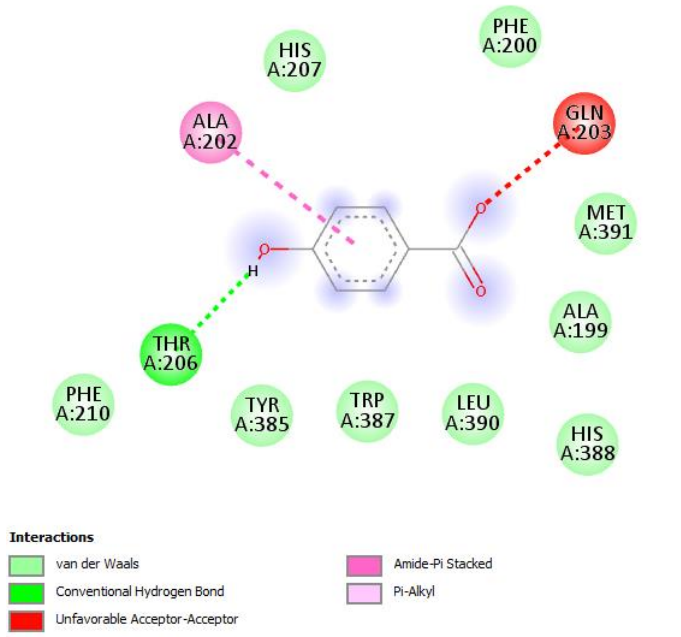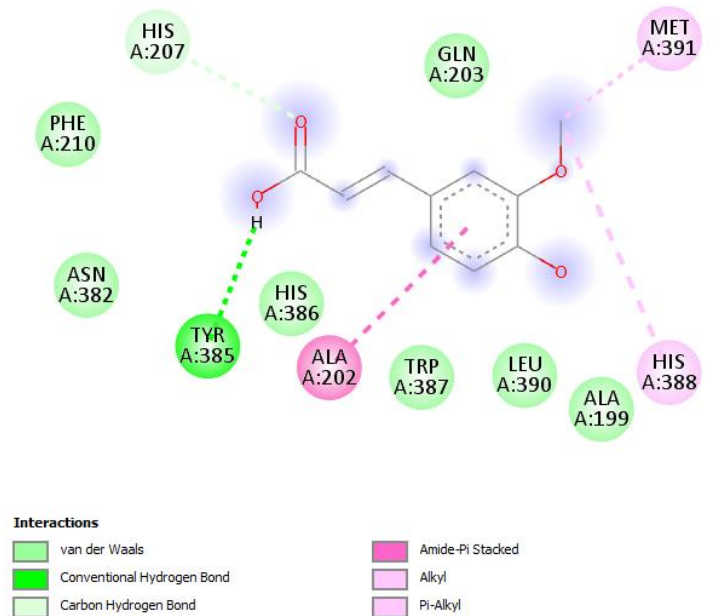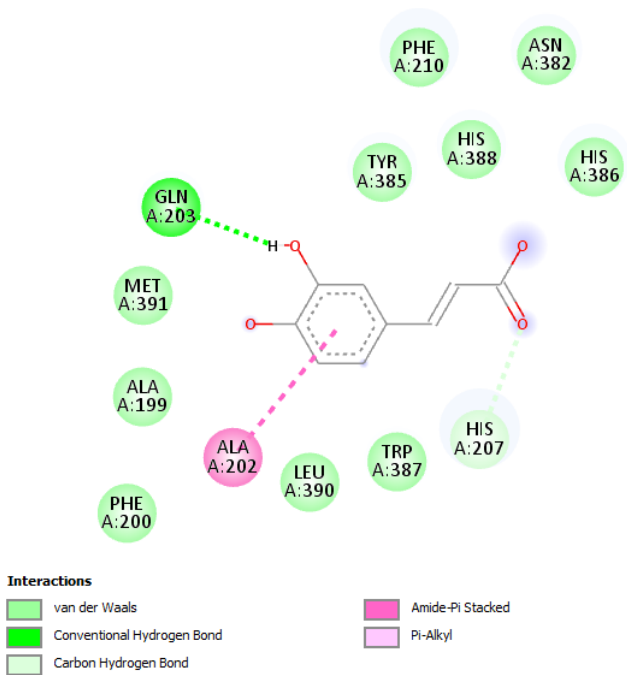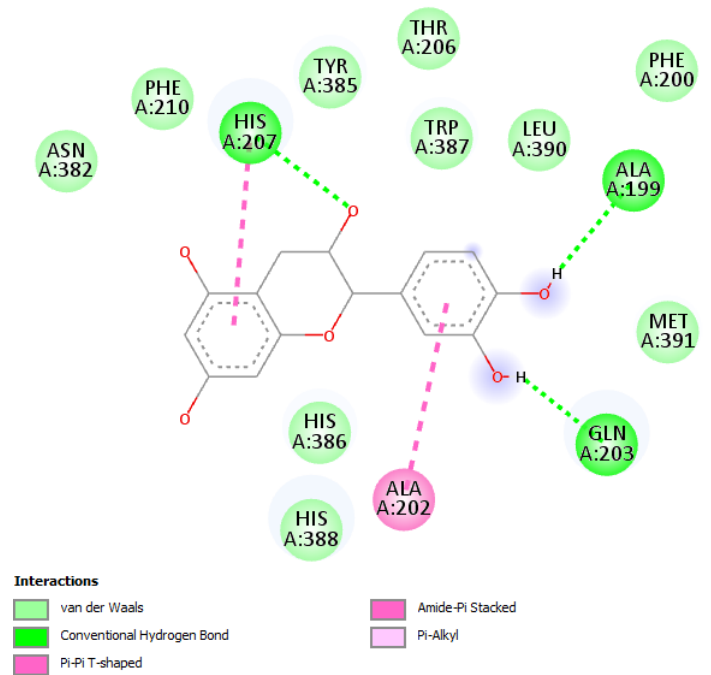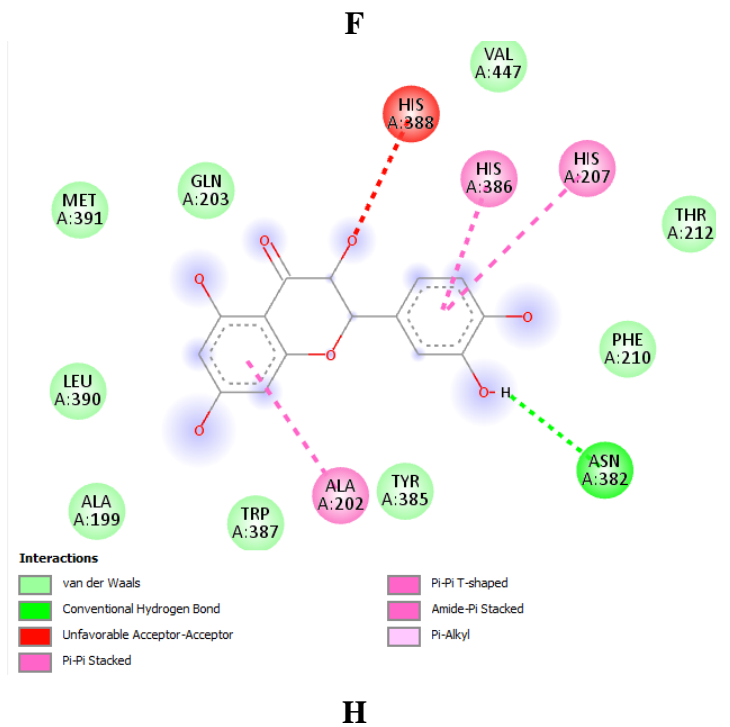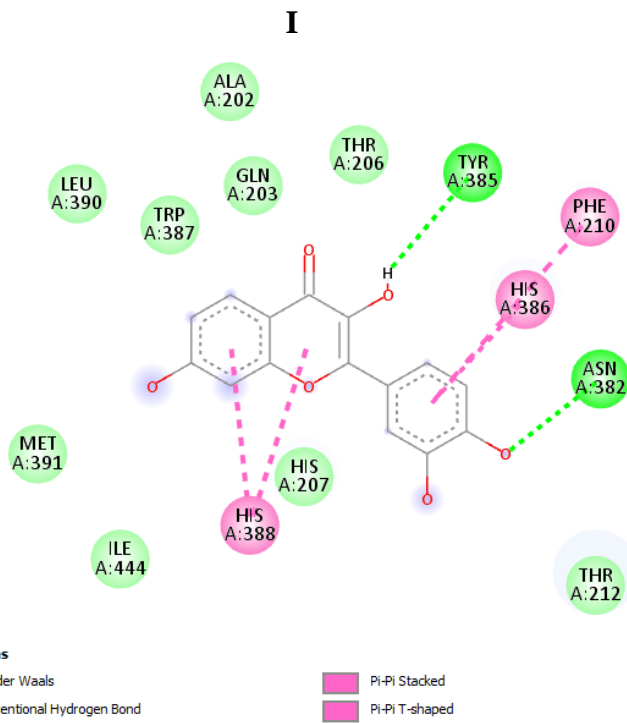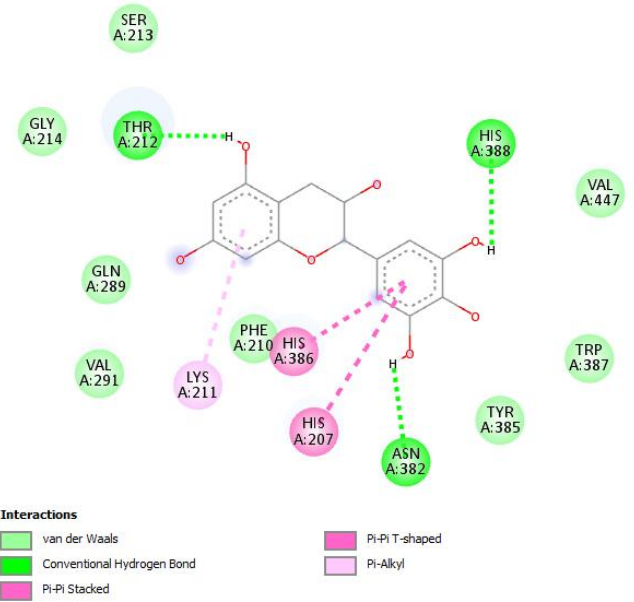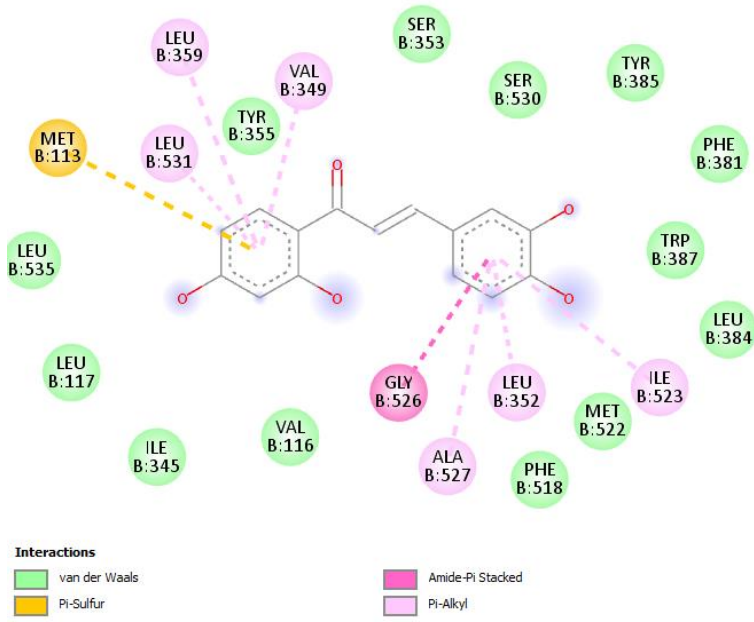
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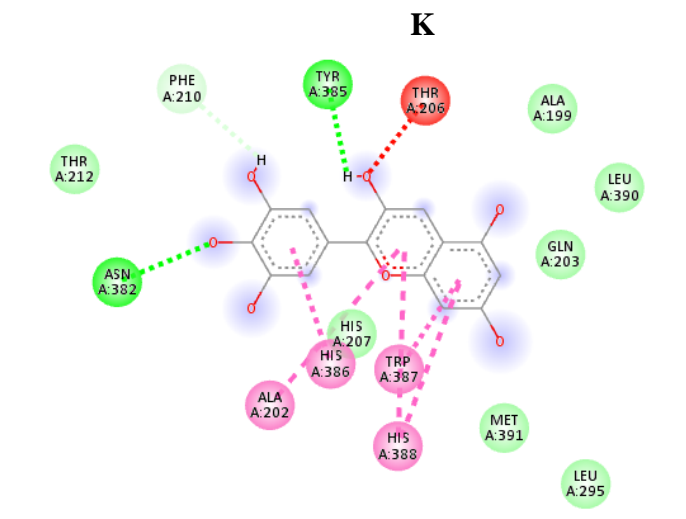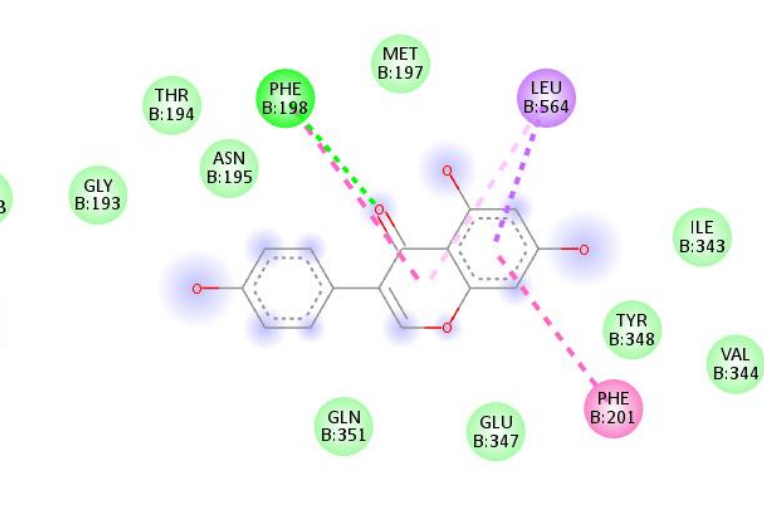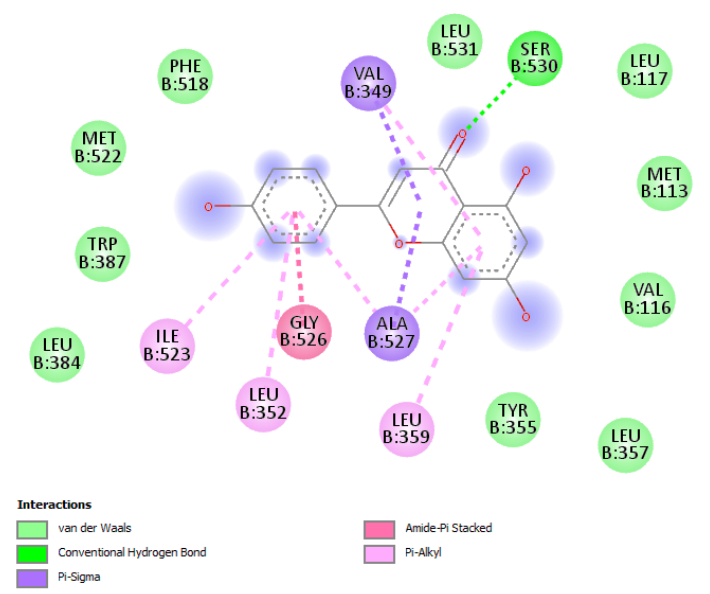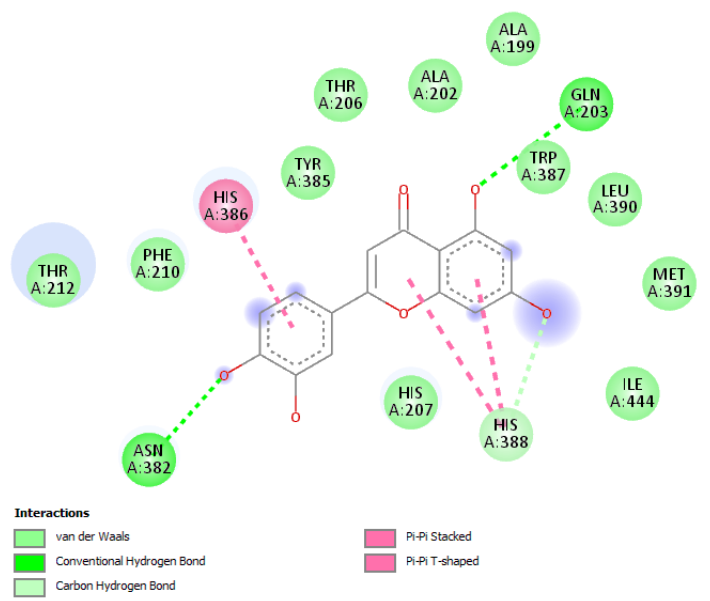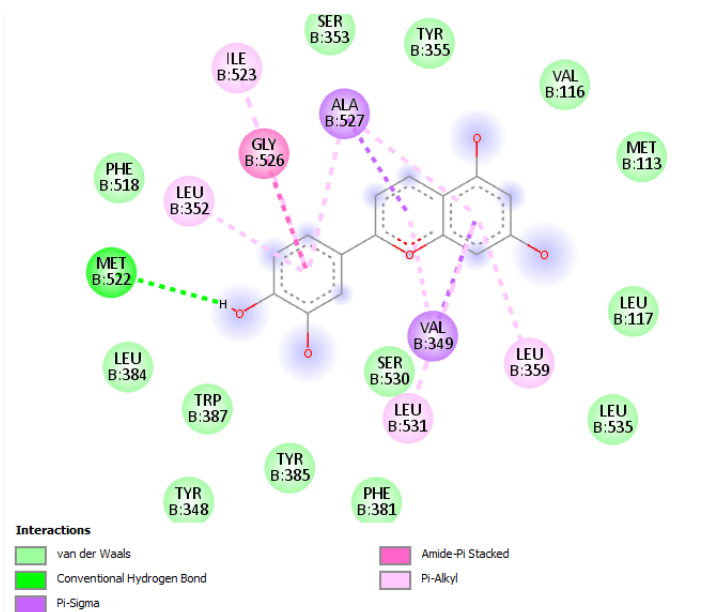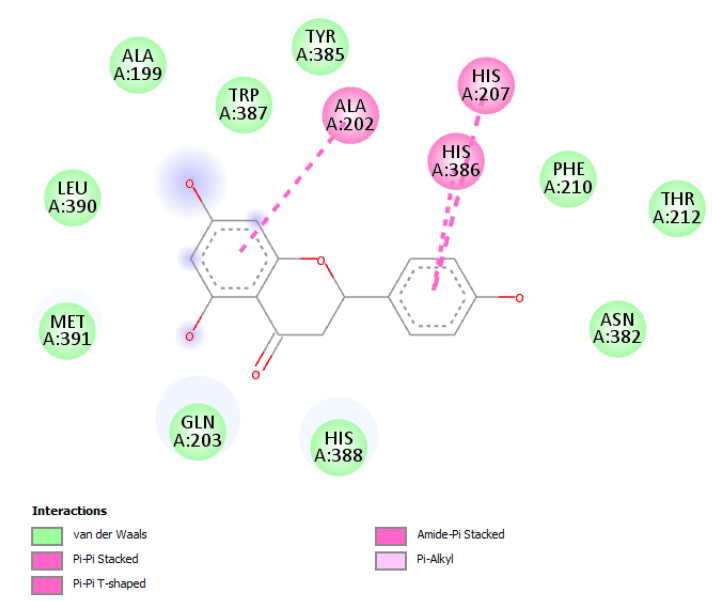
Figure 23 : Phenolic compounds interaction with Cox-1. **A** : 4-Hydroxybenzoic acid, **B** : Ferulic acid, **C**: Caffeic acid, **D**: Epicatechin, **E** : Butein, **F** : Gallocatechin , **G** : Fisetin, **H** : Taxifolin, **J** : Genistein, **K** : Delphinidin, **L**: Apigeninidin, **M** : Apigenin, **N** : Luteolinidin , **O** : Luteolin, **P** : Naringenin.

IV. Cox-1 binding energy results :

Cox-1 binding energy ranged between -6.2 and -9,.5 kcal/mol (Table 9).

Table 9: Cox-1 Binding energy results

Phenolic compounds	Binding energy (kcal/mol
4-Hydroxybenzoic acid	-6.2
Caffeic acid	-6.6
Ferulic acid	-6.7
Gallocatechin	-7.2
Genistein	-7.4
Epicatechin	-7.7
Butein	-8.1
Naringenin	-8.2
Delphinidin	-8.3
Pelargonidin	-8.5
Taxifolin	-8.5
Fisetin	-8.6
Luteolin	-8.6
Apigenin	-8.6
Apigeninidin	-8.6
Luteolinidin	-8.8

the results of Kurumbail et al. (1996) showed that SC-558 , a cyclooxygenase inhibitor that shows inhibitory selectivity for COX-2 over COX-1 formed Alkyl and Pi-Alkyl bonds with residues of the Hydrophobic pocket Trp387, Tyr358 and Leu352, Hydrogen bonds with residues from the mouth of the active site Arg120 and Tyr355. SC-558 also interacted with the side pocket by forming Pi-Sulfur bond with His90 and Pi-Sigma bond with Val523 (Figure 24) (Kurumbail etal. 1996).
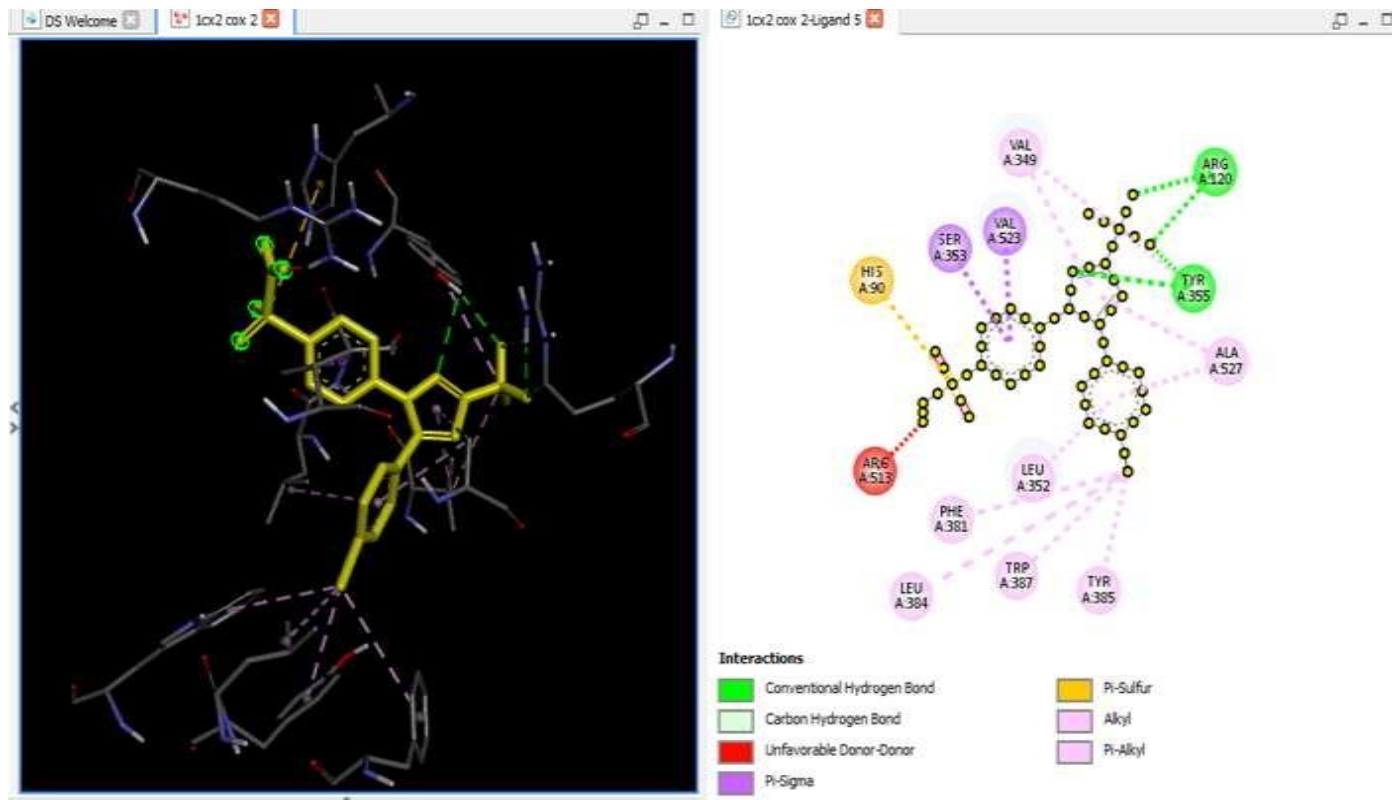
Figure 24 Interactions between selective inhibitor SC-558 and COX-2.

In a Molecular docking study of active phytocompounds from the methanolic leaf extract of vitex negundo against cyclooxygenase-2 , the resulted interactions of the tested compounds were similar to the results of this research, the tested compounds interacted with residues Ser530, Try355, His90 ,Arg120 and Val523 and were labeled as potential inhibiters for Cox-2 (Murugesan, Ponnuswamy, and Gopalan, 2014).

III.   Xanthine oxidase

III.1   Xanthin oxidas interaction profile

Apigeninidin showed the most interesting interaction with XO by forming hydrogen bonds with Thr1010 and Ser876 ,Pi-Pi T shaped and Pi-Pi Stacked interaction with Phe914 and Phe1009 ,Pi-Alkyl bond with residues Leu1014 ,Val1011and Leu873 and Van Der Waals interaction with Glu802 (Figure14).4-Hydroxybenzoic acid formed hydrogen bond with Gln1216 AND Van Der Waals interaction with residue Phe914. Luteolinidin formed hydrogen bonds with Glu1261, and Van Der Waals interaction with Phe914 (Figure 25).

The rest of the tested phenolic compounds didn't show any interaction with the desired residues.

A

B
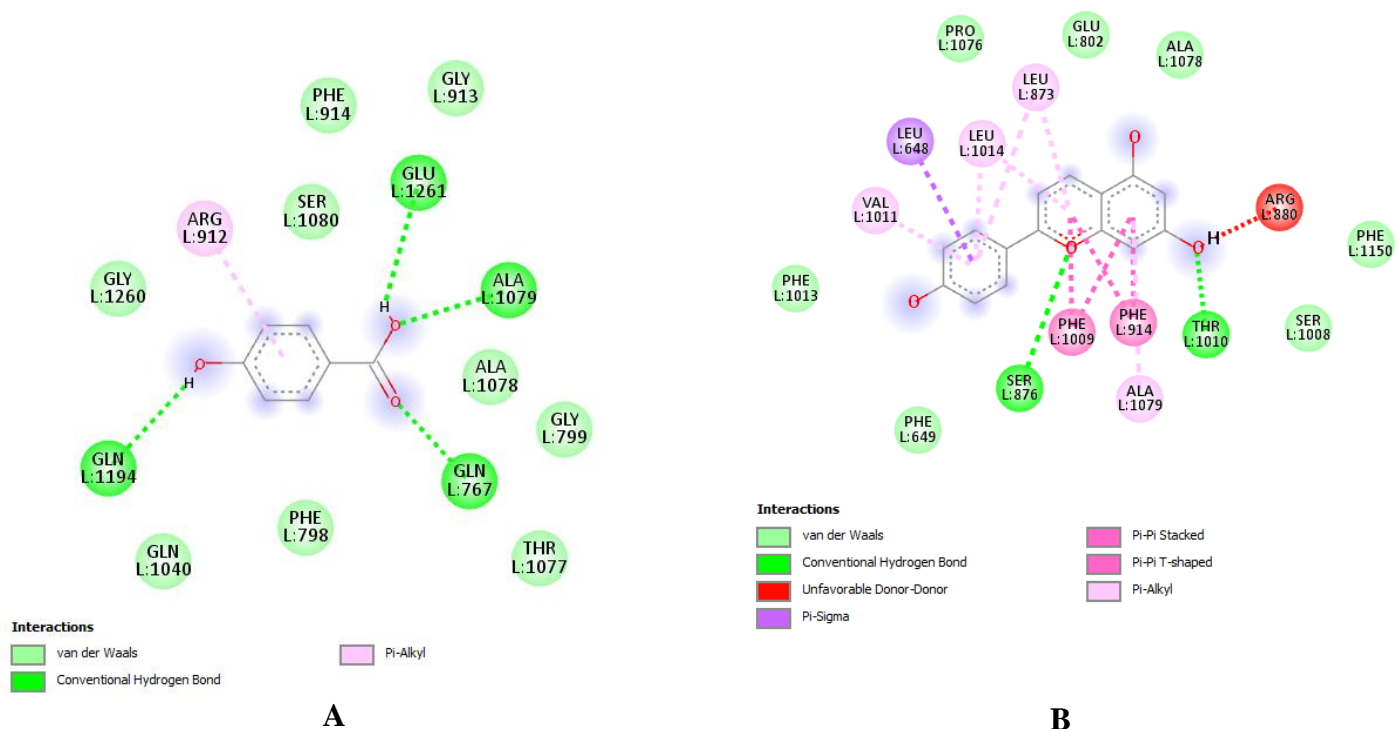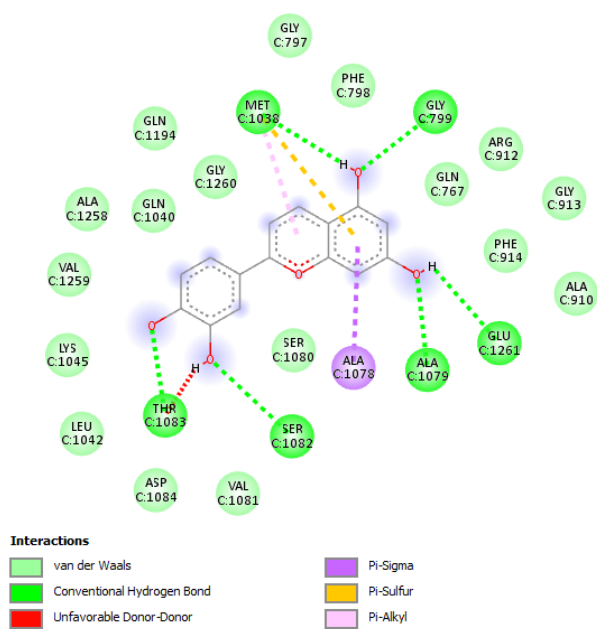
C

Figure 25 phenolic compounds interaction with Xanthine Oxidase .**A:** 4-Hydroxybenzoic acid,

B: Apigeninidin, **C:** Luteolinidin.

III.2 Xanthin oxidas binding energy :

The binding energy results of the 4-Hydroxybenzoic acid, Apigeninidin and Luteolinidin ranged between -6.1 and -9.6 kcal/mol.

Table 10 : binding energy results of xanthin oxidas with 4-Hydroxybenzoic acid,

Apigeninidin and Luteolinidin.

Phenolic compounds	Classification	Binding energy (kcal/mol)
4-Hydroxybenzoic acid	Phenolic acids :Hydroxybenzoic acids	-6.1
Apigeninidin	flavonoids : Anthocyannins	-9.3
Luteolinidin	flavonoids : Anthocyannins	-9.6

The results of this docking study are similar to the results of (Cao, Pauff, and Hille 2014); where they docked the Flavonoid Quercetin with XO and found interactions with catalytically important residues Arg880 and Glu802, as well as residues involved in purine substrate binding Phe 914,Phe1009, and Thr1010, and also residues forming the extended solvent-accessible channel leading to the molybdenum center Leu873, Leu1014,Val 1011 (Figure 26).
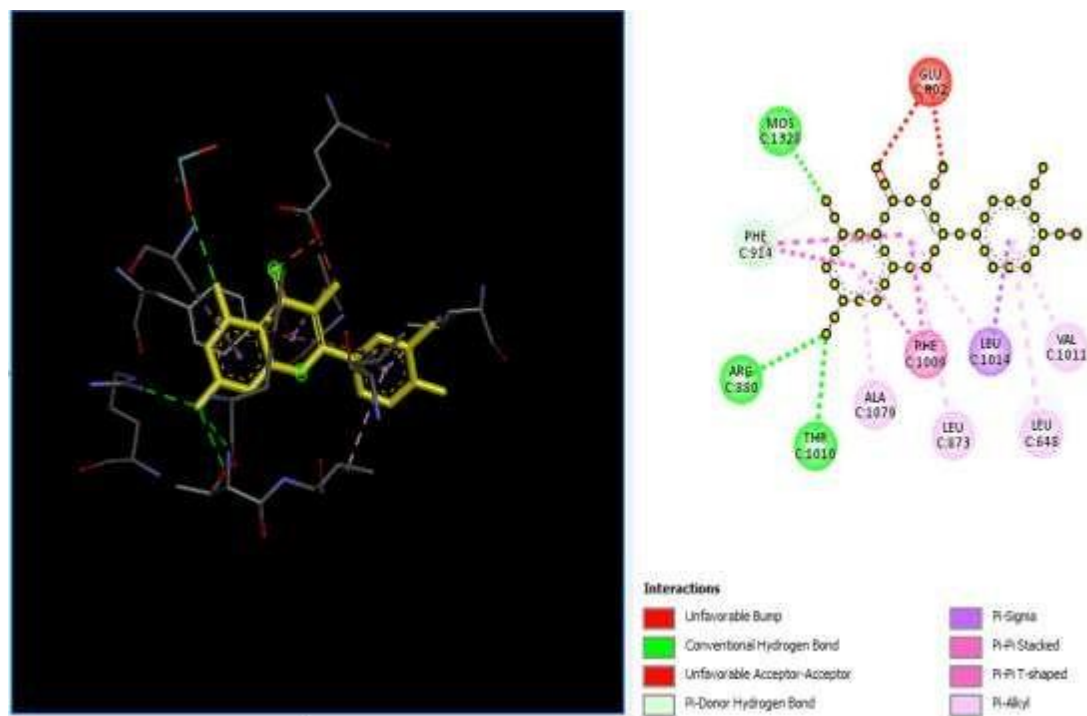
Figure 26 : Interaction of Quercetin with Xanthine Oxidase.

IV. Pharmacological properties

IV.1 Drug Ability

All the tested phenolic compounds were accepted according to Lipinski Rules parameters (Table 11).

Table 11 : Lipinski Rule results

Phenolic compounds	Molecular weight	LogP	Hydrogen donners	Hydrogen accepters	
4-Hydroxybenzoic acid	138.122	1.090	2	2	Accepted
Apigenin	270.24	2.419	3	5	Accepted
Apigeninidin	255.070	3.649	3	4	Accepted
Butein	272.256	2.405	4	5	Accepted
Caffeic acid	180.159	1.196	3	3	Accepted
Ferulic acid	109.060	1.803	2	4	Accepted
Delphinidin	303.050	2.488	6	7	Accepted
Epicatechin	290.271	1.546	5	6	Accepted
Fisetin	286.050	2.248	4	6	Accepted
Gallocatechin	306.27	1.252	6	7	Accepted
Genistein	270.24	2.577	3	5	Accepted
Luteolin	286.239	2.282	4	6	Accepted
Luteolinidin	271.060	3.317	4	5	Accepted
Naringenin	272.256	2.51	3	5	Accepted
Pelargonidin	271.248	3.203	4	4	Accepted
Taxifolin	304.060	0.449	5	7	Accepted

V. ADMET profile

a) Absorption

All the tested phenolic compounds are non-Pgp inhibitors and have a High Human intestinal absorption. Only Apigenin and Genistein were suggested to be possible Pgp-substrates. Furthermore, the results suggested that 4-hydroxybenzoic acid, Caffeic acid, Gallocatechin, Epicatechin, Taxifolin and Delphinidin have a low Caco-2 Permeability as shown in table 12.

Table 12: In silico Caco-2 permeability prediction of phenolic compounds

Phenolic compounds	Caco-2 Permeability (cm/s)	Pgp-inhibitor	Pgp-substrat	HIA
4-hydroxybenzoic acid	-5.270	No	No	High
Caffeic acid	-5.220	No	No	High
Ferulic acid	-4.902	No	No	High
Genistein	-4.764	No	Yes	High
Gallocatechin	-6.306	No	No	High
Epicatechin	-5.971	No	No	High

Luteolin	-5.028	No	No	High
Apigenin	-4.847	No	Yes	High
Naringenin	-4.803	No	No	High
Butein	-4.931	No	No	High
Fisetin	-4.987	No	No	High
Taxifolin	-6.055	No	No	High
Luteolinidin	-4.989	No	No	High
Apigeninidin	-4.843	No	No	High
Pelargonidin	-4.965	No	No	High
Delphinidin	-5.871	No	No	High

b) Distribution

All of the phenolic compounds showed no BBB penetration and had a proper volume distribution as shown in table 13.

Table 13: In silico prediction of distribution features

Phenolic compounds	PPB (%)	VD (L/kg)	Fu (%)
4-hydroxybenzoic acid	38.354	0.291	49.502
Apigenin	97.255	0.510	3.668
Apigeninidin	98.450	0.693	2.384
Butein	99.308	0.466	1.430
Caffeic acid	87.705	0.370	11.072
Delphinidin	92.202	0.649	8.692
Epicatechin	92.065	0.661	8.871
Ferulic acid	89.754	0.339	6.395
Fisetin	97.043	0.477	5.171
Gallocatechin	91.158	0.572	9.800
Genistein	97.558	0.471	2.088
Luteolin	95.436	0.533	5.985
Luteolinidin	97.627	0.610	3.290
Naringenin	93.763	0.502	5.654
Pelargonidin	97.777	0.652	3.287
Taxifolin	85.443	0.681	15.910

Only 4-hydroxybenzoic, Caffeic and Ferulic acid and Taxifolin showed a proper Plasma protein binding (<90%). Other polyphenols showed a low fraction unbound in plasma and may have a low therapeutic index. It is well established that phenolic chemicals have a low oral

bioavailability and undergo significant biotransformation in enterocytes, the liver, and the gut microbiota (Luca et al. 2020).

c) Metabolism

We found that 4-hydroxybenzoic, Caffeic acid, Ferulic acid, Gallocatechin and Delphinidin are neither substrates nor inhibitors of CYP enzymes. Other phenolic compounds are either inhibitors or substrate to one or more of the cytochrome P450 family as shown in table 14.

Table14: Prediction of metabolism features of the phenolic compounds

Phenolic compounds	Substrate	Inhibitor			
			Luteolin	CYP2C9	CYP1A2, CYP2C9, CYP3A4
4-hydroxybenzoic acid	None	None	**Luteolinidin**	CYP2C9	CYP1A2
Apigenin	CYP2C9	CYP1A2, CYP2C9, CYP3A4	**Naringenin**	CYP2C9	CYP1A2, CYP2C9, CYP3A4
Apigeninidin	CYP2C9	CYP1A2, CYP2C9, CYP3A4	**Pelargonidin**	CYP2C9	CYP1A2
Butein	CYP2C9	CYP1A2, CYP2C9, CYP3A4	**Taxifolin**	CYP2C9	CYP1A2, CYP2C9, CYP3A4
Caffeic acid	None	None	**Gallocatechin**	None	None
Delphinidin	None	None	**Genistein**	CYP2C9	CYP1A, CYP3A4
Epicatechin	CYP2C9	None			
Ferulic acid	None	None			
Fisetin	CYP2C9	CYP1A2, CYP3A4, CYP2C9, CYP3A4			

d) Excretion

The results in this research suggest that all the tested phenolic compounds have a good excretion with clearance values ranging between 3.840 and 17.388 ml/min/kg (Table 15).

Table 15: clearance values of Phenolic compounds

Phenolic compounds	Clearance (ml/min/kg)	Phenolic compounds	Clearance (ml/min/kg)
4-hydroxybenzoic acid	7.575	Gallocatechin	17.081
Apigenin	7.022	Genistein	7.844
Apigeninidin	11.101	Hesperetin	15.680
Astilbin	3.840	Luteolin	8.146
Butein	16.320	Luteolinidin	12.924
Caffeic acid	10.973	Naringenin	17.388
Delphinidin	14.752	Pelargonidin	13.405
Epicatechin	17.911	Quercetin	8.284
Ferulic acid	7.480	Taxifolin	9.517
Fisetin	8.273		

e) Toxicity

The toxicity results showed that Epicatechin, Luteolin, Butein, Fisetin, Taxifolin, Luteolinidin and Pelargonidin might be mutagenic. All the tested phenolic compounds were suggested to be non hERG Blockers. Only Caffeic acid was registered to be hepatotoxic. Considering carcinogenicity Ferulic acid, Genistein, Naringenin, Butein, Luteolinidin and Apigeninidin were suggested to possibly be carcinogenic as shown in table 16.

Table 16: Toxicity prediction of the tested Phenolic compounds

Phenolic compound	AMES Toxicity	hERG Blockers	H-HT	Carcinogenicity
4-hydroxybenzoic acid	AMES -	Non-blocker	H-HT-	Non-carcinogenic
Caffeic acid	AMES -	Non-blocker	H-HT+	Non-carcinogenic
Ferulic acid	AMES -	Non-blocker	H-HT-	possibly carcinogenic
Genistein	AMES -	Non-blocker	H-HT-	possibly carcinogenic
Gallocatechin	AMES -	Non-blocker	H-HT-	Non-carcinogenic
Epicatechin	AMES+	Non-blocker	H-HT-	Non-carcinogenic
Luteolin	AMES +	Non-blocker	H-HT-	Non-carcinogenic
Apigenin	AMES -	Non-blocker	H-HT-	Non-carcinogenic
Naringenin	AMES -	Non-blocker	H-HT-	possibly carcinogenic
Butein	AMES +	Non-blocker	H-HT-	possibly carcinogenic
Fisetin	AMES+	Non-blocker	H-HT-	Non-carcinogenic
Taxifolin	AMES+	Non-blocker	H-HT-	Non-carcinogenic
Luteolinidin	AMES+	Non-blocker	H-HT-	possibly carcinogenic
Apigeninidin	AMES -	Non-blocker	H-HT-	possibly carcinogenic
Pelargonidin	AMES+	Non-blocker	H-HT-	Non-carcinogenic
Delphinidin	AMES -	Non-blocker	H-HT-	Non-carcinogenic

Results and discussion

Despite the many health benefits of flavonoids , they show a low bioavailability due to the sulfation, methylation and glucuronidation in the small intestine and the liver(Thilakarathna and Rupasinghe 2013),

Konishi, Zhao, and Shimizu (2006) measured plasma concentration of phenolic acids dosed in a rat stomach and reported an increasing order of the gastric absorption efficiency, Gallic acid=chlorogenic acid<caffeic acid<p-coumaric acid= ferulic acid.

Zhao, Egashira, and Sanada (2003) who studied the absorption sites of ferulic acid in rats showed aquasitotal absorption of ferulic acid in the upper part of the gut and found that FA has a very high bioavailability, which was evaluated on the basis of the high urinary recovery of FA and the high total FA plasma concentration.

The final results of Wang et al. (2014) showed that Caffeic acid was shown to have low oral bioavailability in rats, low intestinal absorption, and poor permeability across Caco-2 cells.

Considering flavonoids researcg bsyggests that they have low intestinal bioavailability and rapid urinary and biliary excretion. The bioavailability of them varies between different kinds of flavonoids(Akhlaghi and Foshati 2017)

In a study done by Franke, Lai, and Halm (2014) they found that Isoflavonoids have a biphasic absorption pattern After soy intake, first in the small intestine and then in the large intestine , with Daidzein (DE) and Genistein started to be absorbed minutes after intake

In an interesting study of Bioavailability of green tea flavan-3-ols in humans by (Del Rio et al. 2010; Stalmach et al. 2009) it was found that green tea flavan-3-ols are highly absorbed and rapidly excreted via the kidneys .

in the study of Azzini et al. (2010) after researching the bioavailability of phytochemicals in fresh and stored strawberry it was found that Pelargonidin glucuronide, pelargonidin glucoside and pelargonidin aglycone peaked in urine within 2 h of strawberry consumption.

Conclusion

Conclusion

In conclusion, this study showed that phenolic acids (4-Hydroxybenzoic acid, Ferulic acid and caffeic acid) , Anthocyanins (Apigeninidin ,Delphinidin and Pelargonidin and Luteolinidin) and Flavonols Fisetin and Taxifolin) are probable Ikkβ inhibitory sources due to their interactions with Met96 ,a gate keeper residue or Glu97, Tyr98 and Cys99 , the residue of the hinge region of the KD and Asp166, Leu167 and Gly168 residues the N-terminal side of the activation loop.

Considering Cox-2, All of the tested phenolic compounds interacted with Cox-2 and Cox-1active site ,showing interactions with the hydrophobic pocket residues Tyr385, Trp387, Phe518, Ala201,Tyr248 and Leu352, the mouth of the active site hydrophilic residues Arg120,Tyr355 and the conserved and non-conserved residues of the side pocket His90, Val523, Arg513 and Ile523.Caffeic acid, Ferulic acid, Pelargonidin, Delphinidin, Gallocatechin, Fisetin and Butein showed the best non-specific binding conformation towards Cox-2 with Genistein only interacted with Cox-2.

From all the tested phenolic compounds only 4-Hydroxybenzoic acid, Luteolinidin and Apigeninidin were found to be possible Xanthine Oxidase inhibiters due to their interaction with catalytically important residue Glu802 as well as residues involved in purine substrate binding, Phe 914,Phe1009, and Thr1010, and also residues forming the extended solvent-accessible channel leading to the molybdenum center, Leu873, Leu1014,Val 1011.

All of the tested phenolic compounds had promising absorption, distribution, metabolism and excretion results. Considering toxicity Epicatechin, Luteolin, Butein, Fisetin, Taxifolin, Luteolinidin and Pelargonidin might be mutagenic, only Caffeic acid was registered to be hepatotoxic and Ferulic acid, Genistein, Naringenin, Butein, Luteolinidin and Apigeninidin were suggested to possibly be carcinogenic.

All in all phenolic compound or plant based compounds in general show a great deal of interesting anti-inflammatory and anti-oxidative properties and should be intensively studies to provide a more safer and effective medicinal drugs.

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