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REVIEW ARTICLE

Bioactivities of *Ximenia americana* L. with a spotlight on probing its anti-*Helicobacter pylori* potential: A bioprospection review coupled molecular docking study

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Abstract

The medicinal plant *Ximenia americana* L., often called hog plum or wild olive, is indigenous to Africa and some regions of India and is well -known for its wide range of therapeutic uses. The phytochemical contents and therapeutic potential of *X. americana* are the primary focus of this review, which focuses on the antibacterial (anti-*Helicobacter pylori*) and antioxidant potentials. People have long used this plant to cure various illnesses, including fever, gastrointestinal issues and skin infections. Recent research has emphasized its substantial antioxidant potential, with leaf extracts demonstrating potent free radical scavenging properties. Studies on antimicrobials have validated their historical use in folk medicine, demonstrating notable efficacy against various harmful bacteria and fungi. However, researchers are still investigating their potential to combat *H. pylori*. Phytochemical analyses from multiple studies found a wealth of bioactive substances, including flavonoids, tannins and saponins, adding to its therapeutic advantages. This review highlights the value of *X. americana* as a natural resource and the need for more study to thoroughly understand its mechanism of action and possible uses in contemporary medicine, especially for illnesses linked to oxidative stress and infections caused by *Helicobacter pylori*. The anti-*H. pylori* action of epicatechin, rutin, cumaroyl-o-galloyl-glucose, quinic acid and procyanidin derived from *X. americana* via molecular docking is therefore highlighted in this review along with the bioprospection. Our results, however, call for more research on the bioactive extrolites of *X. americana* and their unique interaction with PPX/GppA in complex with GNP proteins to better acknowledge their potential as a treatment for *H. pylori*.

Keywords: antioxidant activity; antimicrobial activity; bioactive extrolites; *Helicobacter pylori*; molecular docking; *Ximenia americana*

Introduction

The investigation of medicinal plants as possible antimicrobials and antioxidants has attracted much attention because of their capacity to lessen tissue damage from free radicals, which are linked to several illnesses (1, 2). Since these natural compounds might be safer than synthetic antioxidants, applying plant-based antioxidants in food, cosmetics and pharmaceuticals is incredibly alluring. Numerous plants have been found to have significant antioxidant qualities due to phenolic chemicals, particularly flavonoids (3). Flavonoids are a broad class of polyphenolic substances, including flavonols, flavones, flavanones, catechins, anthocyanidins and chalcones. These compounds provide several health benefits for people and fulfil vital plant roles. Due to their ability to modulate immunological and antioxidant responses and function as "biological response modifiers", much research has been done on their antibacterial, antiviral and antioxidant properties (4). However, relatively little has been established about their effects on *H.pylori* infections. These substances also serve as scavengers of free radicals, lowering oxidative stress and preventing the action of hydrolytic enzymes (5). One therapeutic plant is *X.americana*, which has many bioactive components with potent antioxidant and antibacterial qualities. Known as "wild olive" or "plum," this medicinal plant grows all over Africa and parts of India. It has traditionally been used in Northern Nigeria to treat various ailments, including skin infections, ulcers, fever and malaria (6). Different parts of the plant are used for multiple medical applications and it has been claimed to have trypanocidal, antitrypanosomal and anti-inflammatory activities (7-9).

Saponins, cyanogenic glycosides, flavonoids and tannins are among the phytochemical components of *Ximenia americana* that support its medicinal properties (6). The ability of *Ximenia americana* to combat oxidative stress, a primary cause of diseases like cancer, atherosclerosis and malaria, has drawn attention as interest in natural antioxidants has grown (10). Its antirheumatic, moluscicide and antioxidant qualities have been emphasized in recent

research, highlighting its applicability in both conventional and alternative medicine. This review emphasizes the importance of *X. americana* as a natural resource and the need for additional research to fully comprehend its mechanisms of action and potential applications in modern medicine, particularly for conditions associated with oxidative stress and microbial infections. Therefore, this study highlights the anti-*H. pylori* action of procyanidin, quinic acid, epicatechin, rutin and cumaroyl-o-galloyl-glucose that were produced from *X. americana* using molecular docking, coupled with the ioprospection. To fully recognize their potential as a therapy for *H. pylori*, our findings, however, necessitate more investigation into the bioactive extrolites of *X. americana* and their distinct interaction with PPX/GppA in complex with GNP proteins.

Botanical description of *Ximenia americana*

Ximenia americana is a semi-scandent shrub or small tree that belongs to the *Olacaceae* family. It is often referred to by several popular names, including "hog plum," "wild plum," and "false sandalwood" in English, "inkoy" and "kol" in Amharic and "Mlehtta" and "Mullo" in Tigrigna. The average height of this plant is between 2-7 meters and its trunk diameter hardly ever goes above 10 cm. The bark exhibits dark brown to pale grey colours and can be smooth or scaly. Waxy blooms accompany the purple-red branchlets and stiff, slender spines that ornament its branches. Interestingly, *X. americana* may have semiparasitic traits, developing haustoria on its roots to take nutrients from nearby plants (11). The lanceolate to elliptic leaves are alternately arranged and range in size from 3 to 8 cm in length and 1.5 to 4 cm in width. The leaf tips can have a semi-succulent to thin texture and be either obtuse or emarginate. The petioles are short and thin, up to 6 mm long and each leaf has three to seven pairs of veins. Because of cyanogenic glycosides, the plant's young, crushed leaves have a distinctively bitter almond-like odour (12). Pedunculate axillary racemes or umbels that are 3 to 7 mm long support the branched inflorescences of *X. americana*, which have flowers that are white, yellow-green and occasionally pink (Fig. 1). The plant produces globose to ellipsoidal drupes that are about 2.5 cm in diameter and 3 cm long. When ripe, they change from their original greenish colour to yellow or, sometimes, orange-red. A solitary seed with a fleshy pulp surrounds each fruit, with a fatty kernel inside a light yellow, woody, brittle shell. The seeds can grow up to 1.5 cm in length and 1.2 cm in thickness (13).

Flowering and fruiting can occur at any time of the year and are not severely controlled by climate. Its fruits are dispersed mainly by animals, which helps it expand throughout many ecosystems. Because of its extreme adaptability, *X. americana* may flourish in various habitats, including savannas, riverbanks, dry woods and rural areas. It can withstand temperatures ranging from 14 to 30 °C and grow 900 to 2000 m above sea level. Dry, nutrient-poor soils, such as sandy clay, clay loam, loamy sand and sandy clay loam, are ideal for the plant, which needs 300 to 1250 mm of rainfall annually (11, 13). Because of its ecological and therapeutic value and its ability to adapt to harsh environmental circumstances, *X. americana* is a species that warrants more study and conservation.

Traditional uses of *Ximenia americana*

In many civilizations, especially in Africa and India, *X. americana* has been used in traditional medicine. The herb treats various illnesses, demonstrating its significance in regional therapeutic customs. The leaves are frequently used to treat cuts, wounds and other skin ailments. One of the most significant qualities is its ability to prevent infections due to its effective antibacterial properties. *X. americana* has long been used to treat the symptoms of fever and malaria. Its bioactive compounds might help diminish how severe these illnesses are.

Additionally, the plant is well-known for its ability to treat gastrointestinal conditions like dysentery and diarrhoea. *X. americana* is used topically to reduce pain and inflammation, highlighting its value in conventional treatments. The plant has long been used to treat rheumatism and arthritis symptoms. Because of its alleged antioxidant properties, *X. americana* is used by indigenous people to promote general health and well-being (10-13).

Bioactive components and their potential therapeutic applications

Antimicrobial activity of *Ximenia americana*

Studies on *Ximenia americana* have demonstrated its significant antimicrobial potential. Leaf and stem bark extracts exhibit activity against bacterial strains like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (6, 7). Methanolic extracts often show more substantial antibacterial effects, especially against *S. aureus*, while ethanol and aqueous extracts also display inhibitory activity, though their effectiveness varies depending on the

Fig. 1. Leaves and fruits of *Ximenia americana.*

microorganism (14). Phytochemical analysis across studies consistently identifies bioactive compounds, including flavonoids, tannins, saponins and alkaloids, supporting the plant's traditional use in treating infections (6, 7). Some studies report enhanced effects when combined with antibiotics or nanoparticles, suggesting broader therapeutic applications (15). The antibacterial and antidiarrheal properties of phenol acid-rich fractions made from roots of *X. americana* demonstrated strong antibacterial efficacy against bacterial strains resistant to antibiotics, consistent with the traditional uses of *X. americana* in treating infectious disorders (14). The phenol acid-rich fractions in animal models also showed antidiarrheal properties, successfully preventing enteropooling and castor oil-induced diarrhoea. Although the study backs up the traditional use of roots of *X. americana*, more investigation is necessary to pinpoint the precise phytochemical components causing these medicinal benefits.

Antioxidant and anticancer activity of *Ximenia americana*

Significant antioxidant and anticancer activities of *X. americana* have been discovered through research. Because of the presence of bioactive substances such as flavonoids, phenolic compounds, saponins and tannins, the plant's stem bark, leaves, seeds and fruits all have significant levels of antioxidant activity (16-25). Strong free radical scavenging effects have been shown in studies employing various extraction techniques (methanol, ethanol and aqueous), with epicatechin and quercetin playing essential roles (26). The anticancer potential has been associated with proteins resembling the plant's ribosome-inactivating proteins (RIPs). The plant may be used in food and medication to prevent cancer and other disorders linked to oxidative stress. However, comprehensive research is needed to evaluate safety and efficacy and identify the plant chemicals in *X. americana* that are responsible for the documented therapeutic effects. Furthermore, specific investigations have revealed anticancer potential, with extracts from *X. americana* exhibiting antiproliferative on different cell lines (27, 28). These results demonstrate the potential for creating novel anticancer treatments and lend credence to its use in traditional medicine to treat cancer.

Anti-diabetic activity

The pathogenesis of diabetes is exacerbated by persistent hyperglycemia, which causes inflammation and oxidative stress (OS). Extracts from the leaves and roots of *X. americana* have been shown in numerous investigations to have anti-diabetic properties. The chloroform extract of *X. americana* leaves included important phytochemicals such as oleic acid and n-hexadecanoic acid. The aqueous extract of 9,12-octadecadienoic acid showed the most anti-diabetic effect *in vitro* (29). Similarly, the methanolic extract of *X. americana* leaves showed dose-dependent impact, with the peak activity at 600 mg/kg body weight and dramatically lowered blood glucose levels in rats with alloxan-induced diabetes (30). The *in silico* research demonstrated that the stigmasterol and 4,4-Dimethylcyclohex-2-en-1-ol in *X. americana* has anti-diabetic effects by inhibiting enzymes associated with inflammation and OS (31). In streptozotocin-induced diabetic rats, the tannin-rich root extract of *X. americana* also established hepatoprotective and antioxidant properties, lowering serum lipid peroxides and blood glucose levels while raising insulin levels (32). All of these results point to the possibility of *X. americana* extracts as a treatment option for diabetes and other illnesses associated with oxidative stress.

Antitrypanosomal, gastroprotective and antidepressant activities of *X. americana*

Numerous promising medicinal properties are displayed by *X. americana*, especially in the areas of antitrypanosomal, gastroprotective and depressive actions. With flavonoids identified as active components, extracts from the stem bark and roots have demonstrated potential against *Trypanosoma congolense* and *Trypanosoma brucei* in terms of antitrypanosomal activity (33, 34). Studies showed a notable decrease in gastrointestinal lesions brought on by different irritants, ascribed to substances like procyanidins and catechins, provided evidence for gastroprotective qualities of *X. americana* (35). Furthermore, new studies indicated that the dehydrocostus lactone of root bark may block monoamine oxidase-A and have antidepressant-like effects (36). As mentioned above these results highlight the medicinal potential of *X. americana* and call for more research into its pharmacological uses and active ingredients.

Analgesic and anti-inflammatory activities of *Ximenia americana*

Traditional medicine has acknowledged analgesic and anti-inflammatory activities of *X. americana* showed that total polysaccharides (TPL-Xa) derived from *X. americana* bark inhibited nociception without affecting systemic toxicity, demonstrating that the bark contains polysaccharides that exhibit significant antinociceptive effects in various animal models (37). Additionally, the methanol extracts contain bioactive compounds such as flavonoids and tannins that support their analgesic effects (38). Other research supported the traditional use of stem extracts and certain flavonoids, such as catechin of *X. americana*, in pain management by validating their potent antinociceptive and anti-inflammatory qualities (39, 40). Furthermore, aqueous ethanol extracts from the root bark of *X. americana* have been reported to have anti-inflammatory properties, effectively decreasing leukocyte migration and edema in inflammation models (41). The therapeutic potential of *X. americana* in treating inflammatory diseases was emphasized through the regulation of cannabinoid receptors (42). These results point to *X.americana* as a viable option for creating natural anti-inflammatory and analgesic therapies, which calls for more investigation into its active ingredients (30, 42, 43).

Antipyretic activities of *Ximenia americana*

Numerous investigations have shown that *X. americana* has strong antipyretic properties, suggesting that it could be used as a herbal treatment for fevers. Extracts from leaves and stem bark of *X. americana* successfully lowered the rectal temperatures of rats by 0.45 % to 2.13 %, similar to aspirin's antipyretic effects (44). The phytochemical study detected bioactive substances such as alkaloids, flavonoids, saponins and terpenoids, which are frequently linked to antipyretic impacts. Additionally, the antipyretic effectiveness of aqueous extracts of *X. americana* in a yeast-induced hyperthermia model using Wagner's fractionation method was also investigated (45). The findings also suggested that

saponins function as prostaglandin inhibitors since specific fractions had better and longer-lasting antipyretic effects when compared to the lysine acetylsalicylate, a standard medication. The methanolic dichloromethane extracts further supported these results and showed a substantial decrease in fever in a pyrexia model (46). The data above backs up the historical use of *X. americana* as a potent antipyretic and emphasizes its potential as a natural source for creating herbal fever remedies.

Antiulcer effect of *Ximenia americana*

Numerous researchers have assessed the antiulcer properties of *X. americana*, demonstrating notable gastric cytoprotective effects. In models of ulcers caused by hydrochloric acid and hypothermic stress, aqueous ethanolic extracts of *X. americana*, at a concentration of 10 mg/kg body weight, produced a significant decrease in the ulceration index by more than 65 % (47). Interestingly, the same concentration promoted full ulcer recovery. With an IC50 for DPPH radical scavenging of less than 5 µg/mL and a total ferric reducing antioxidant capacity of more than 77 mg EQAA/100 mg (Ascorbic acid equivalents per 100 mg of sample), the extracts demonstrated high antioxidant activity. Although more research is needed to clarify the underlying mechanisms, the high total polyphenolic concentration of 53.75 ± 1.39 mg EGA/g (milligrams of gallic acid equivalent (GAE) per gram) further supports the potential of *X. americana* for promoting ulcer healing and stomach cytoprotection. The phytochemical components of *X. americana* stem bark and their potential gastroprotective effects were investigated. Through preliminary phytochemical screening, the study found a range of bioactive substances, such as terpenoids, alkaloids, flavonoids and saponins. The stem bark extract dramatically decreased mean ulcer spots in a dose-dependent manner, according to tests of the antiulcer activity conducted on Wistar rats that had ulcer models brought on by ethanol and indomethacin. The observed effects were similar to those of common drugs like misoprostol and cimetidine. Crucially, the extract successfully reduced the development of severe ulcer patches even at larger dosages (500 and 1000 mg/kg), confirming the traditional usage of *X. americana* stem bark in ulcer treatment (47). Overall, strong scientific evidence supports the antiulcer action of *X. americana*, suggesting that it may be used as a treatment for gastric ulcers. Thus, *X. americana* is a viable candidate for more pharmacological research aimed at comprehending its mechanisms of action and clinical applications because of various phytochemicals and the demonstrated antioxidant and antimicrobial properties.

A comprehensive literature search was conducted utilizing several widely used scientific search engines, including Google Scholar, PubMed, Scopus, Wiley, Science Direct and others. The search phrase "phytochemicals and *X. americana*" and bioactivities or anti-*Helicobacter pylori* were used to pick keywords primarily relevant to the bioactivities of natural compounds generated from *X. americana*. As mentioned above, various biologically active compounds and their activities were chosen and tabulated (Tables 1-3). The entire body of research and the search results were thoroughly examined. After carefully reviewing the research, natural substances derived from this plant's strong microbial activity were chosen for computational docking studies.

Table 1. Summary of studies conducted to assess the antimicrobial activity of *Ximenia americana* along with significant findings

Study	Plant Part/ Extract	Microbial Strains Tested	Main Findings	Phytochemicals Identified
(6)	Leaf extract	*Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli*	Highest activity against *P. aeruginosa*, comparable to penicillin (2 µg).	Flavonoids, tannins, cyanogenic glycosides, saponins
(51)	Bark, leaves, stem, root (methanol, water, chloroform)	*Staphylococcus aureus*, *Candida albicans*	Methanol extract is most effective, especially against *S. aureus*; aqueous extract is also effective.	-
(52)	Stem bark, leaves, roots (ethanol and water extracts)	*Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*	Ethanol extract is effective against *S. aureus*; water extract is effective against *E. coli* and *S. aureus*.	Saponins, tannins, volatile oils, phenols, flavonoids, alkaloids, glycosides
(7)	Stem bark (methanol and water extracts)	*E. coli*, *P. aeruginosa*, *S. aureus*, *P. vulgaris*, *B. subtilis*, *C. albicans*	Significant activity against *E. coli*, *S. aureus* and *P. aeruginosa*.	Alkaloids, saponins, flavonoids, cardiac glycosides, terpenoids, tannins
(53)	Leaves (methanol and water extracts)	Bacterial isolates from post-surgical wounds	No activity against test bacteria.	Flavonoids, steroids, tannins, reducing sugars, alkaloids, saponins
(54)	Stem bark extract	*Staphylococcus aureus*	Synergistic effect with norfloxacin, increased efficacy. Anti-kinetoplastida activity.	Quercitrin, caffeic acid
(55)	Roots, stem, bark, leaves (methanol and water extracts)	*S. aureus*, *Klebsiella pneumoniae*, *Shigella flexneri*	Inhibited *S. aureus* and *K. pneumoniae*. Methanol and aqueous extracts were effective against *S. flexneri*.	Cardiac glycosides, saponins, tannins, flavonoids, carbohydrates
(56)	Whole plant extracts	Various bacterial species	Significant antimicrobial activity against several bacterial strains.	Saponins, alkaloids, tannins, flavonoids, terpenes, sterols, coumarins
(57)	Trunk bark (ethanol, hydro ethanol and water extracts)	*S. aureus*, *K. pneumoniae*, *S. typhi*, *E. coli*	Polyphenol-rich extracts showed significant antibacterial and antioxidant activity.	Saponins, catechin tannins, flavonoids, sterols
(15)	Stem bark (ethanol extract combined with ZnO/ AgNPs)	Skin wound healing assay	Enhanced wound healing through collagen deposition and reduced inflammation.	Myricetin, catechin, (-)-epicatechin, rutin

Table 2. Summary of antioxidant and anticancer activity of *Ximenia americana*

Study	Plant Part/ Extract	Bioactive extrolites	Activity	Main Findings
(16)	Stem bark	Flavonoids, saponins	Antioxidant	Significant antioxidant activity (DPPH test, Rc50=8)
(17)	Fruits	Yellow flavonoids, anthocyanins, polyphenols	Antioxidant	High antioxidant activity, potential use in food and medicine
(26)	Leaves, stem bark	Epicatechin, quercetin	Antioxidant	Strong antioxidant activity, high phenolic content (DPPH method)
(18)	Leaves	Flavonoids, phenolic compounds	Antioxidant	Aqueous extract showed high antioxidant activity
(19)	Fruits (red, yellow)	Flavonoids, total phenols	Antioxidant	Over 90 % DPPH scavenging, high nutritional and health-promoting potential
(20)	Seeds, pulp	Polyphenols, flavonoids, vitamin C	Antioxidant	Strong antioxidant activity related to polyphenols and vitamin C
(21)	Leaves (var. caffra)	23 phenolic compounds	Antioxidant, anti-aging	*In vitro* and *in vivo* antioxidant activity, biofilm inhibition, anti-aging
(22)	Ethanolic extract	Polyphenols	Antioxidant	Best action on DPPH radical, strong reducing activity
(23)	Seeds	Pentacyclic triterpenes, phenolic compounds	Antioxidant, antibacterial	Antioxidant and weak antibacterial activity, potential for food applications
(24)	Root	Phenolics, flavonoids	Antioxidant	Strong DPPH free radical scavenging activity
(25)	Root	Catalase, glutathione-S-transferase, superoxide dismutase	Antioxidant	Increased activity of antioxidant enzymes, strong antioxidant properties
(27)	Powder (traditional medicine)	Galactose-affinity proteins, RIP proteins	Anticancer	Antineoplastic activity, related to type II RIP family proteins
(28)	Leaves	Flavonoids, tannins, saponins	Anticancer	Significant antiproliferative activity, potential in breast cancer treatment

Table 3. Overview of bioactivities of *X. americana*, along with key findings and mechanisms or compounds associated with respective activity

Study reference	Activity	Extract used	Main findings	Mechanism/key compounds
(29)	Anti-diabetic	Aqueou-s extract	Highest anti-diabetic effectiveness among solvent extracts; significant hypoglycemic activity *in vitro*.	Presence of oleic acid, hexadecanoic acid and more.
(30)	Anti-diabetic	Methan-olic extract	Reduced blood glucose levels in diabetic rats after 7 days of administration (200, 400, 600 mg/kg).	Dose-dependent hypoglycemic effect compared to glibenclamide.
(31)	Anti-diabetic	In-silico analysis	Stigmasterol and 4,4-Dimethylcyclohex-2-en-1-ol showed potential antihyperglycemic effects, such as inhibition of oxidative stress and inflammation processes.	Stigmasterol, 4,4-Dimethylcyclohex-2-en-1-ol.
(32)	Anti-diabetic	Tannin-rich root extract	Increased insulin levels in STZ-diabetic rats and reduced blood glucose and serum lipid peroxide levels.	Tannins and catechin derivatives.
(33)	Anti-trypanosomal	Methanolic stem bark extract	Flavonoid fraction reduced motility of *Trypanosoma congolense*; effective *in vitro* and *in vivo* at 25 mg/mL.	Flavonoids.
(34)	Anti-trypanosomal	Various solvent extracts	Methanol extract showed highest activity against *Trypanosoma brucei;* TLC-MS and LC-MS analysis identified several phytochemicals.	Gallic acid, quercetin and others.
(43)	Anti-trypanosomal	Aqueous extract	Antitrypanosomal potential with 55 % and 90 % immobilization of trypanosomes.	Requires further characterization of active principles.
(35)	Gastroprotective	Aqueous extract	Significant reduction in gastric lesions caused by ethanol, acidified ethanol and indomethacin.	Procyanidins B and C, catechin/ epicatechin.
(36)	Antidepressant	Root bark extract	Dehydrocostus lactone showed antidepressant-like effects; decreased immobility; potential MAO-A inhibition.	Dehydrocostus lactone.
(59)	Antidepressant	Hydroalcoholic extract	Reduced inactivity and malondialdehyde levels in mice; showed total antioxidant capacity; reduced harmful effects of sodium fluoride.	Antioxidant compounds in hydroalcoholic extract.

Molecular docking assisted anti-*Helicobacter pylori* activity of phytochemicals derived from *X. americana*

Computational docking has been used as a powerful strategy for understanding and predicting the molecular interaction of ligands with various biological receptors, such as protein active sites. This interesting protein-ligand interaction can guide the design of molecules and experiments, providing a large set of candidates in medicinal applications. In continuation of the aforementioned comprehensive review that covered recent advances in the domain of the effect of various botanically derived natural products of *X. americana* as an alternative treatment approach against ulcer, pyretic, inflammation, trypanosomiasis, depression, diabetes, cancer, oxidative stress and microbial infections, simultaneously, the docking analyses was performed to reveal the anti-*Helicobacter pylori* activity of procyanidin, quinic acid, epicatechin, rutin and cumaroyl-o-galloyl-glucose derived from *X. americana* as promising inhibitors of active sites of (exopolyphosphatase) PPX/GppA (guanosine pentaphosphate phospho-hydrolase) in complex with GNP (phosphoaminophosphonic acid-guanylate ester) proteins present in *Helicobacter pylori*.

Guanosine pentaphosphate (pppGpp) and tetraphosphate (ppGpp) are cytoplasmic alarmones that control stringent response, an adaptive process that allows bacteria to regulate global gene expression under a variety of stress conditions (48). The ppGpp is derived from guanosine-diphosphate (GDP), while pppGpp is derived from guanosine triphosphate (GTP) by the RelA/SpoT proteins (49). During amino acid starvation, the (p)ppGpp nucleotides are accumulated, reaching levels nearly equal to that of GTP. Accumulation of (p)ppGpp affects bacterial cell growth and general metabolism by the alarmones bound directly to RNA polymerase and the enzymes involved in nucleotide synthesis and uptake (50). In this way, the alarmones alert bacteria to the presence of stress and provide a signal to curtail unnecessary processes to adapt to environmental changes. Consequently, there is a significant focus on developing new drugs that target and modulate the levels of pppGpp as a potential strategy for controlling bacterial infections. Elevated levels of pppGpp are associated with antibiotic persistence, where bacteria enter a dormant state, leading to recurring and recalcitrant infections and antibiotic resistance mutations. Thus, targeting the stringent response is a promising strategy to combat bacterial antibiotic persistence and resistance (50). Taking into consideration those as mentioned above, the *Helicobacter pylori* (exopolyphosphatase) PPX/GppA (guanosine pentaphosphate phospho-hydrolase) complex with GNP (phosphor aminophosphonic acid-guanylate ester) proteins was chosen as a target for molecular docking study.

Computational docking methodology

The 3-D structure of the PPX/GppA in complex with GNP proteins was downloaded from the protein data bank (PDB) with PDB ID: 6CP2. Molecular docking between five selected plant compounds (procyanidin, quinic acid, epicatechin, rutin and cumaroyl-o-galloyl-glucose) and the target protein was performed using AutoDock 4.2.6 software. The two-dimensional structure of five selected plant compounds was downloaded from the PubChem server (Table 4).

Two main criteria, binding affinity and hydrogen bonding, are mainly emphasized for molecular docking. AutoDock was used to do automated molecular docking and all default parameters were utilized apart from the number of runs. The ligand and target molecules were retained in a non-flexible state during the rigid docking process (without changing the bond angle, length, or torsion angle). All the amide bonds were fixed in place, but all ligand bonds were free to move around. The AUTOGRID algorithm allocated each type of atom in the ligand molecule a pre-calculated grid map. The grid's X, Y and Z dimensions were fixed to 72 Å with 0.375 Å distance between grid points. The area of the binding site of PPX/GppA in complex with GNP proteins is found at ASP9, GLU115, ASP136 and GLU143 active sites (48).

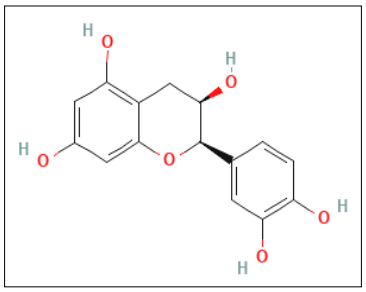
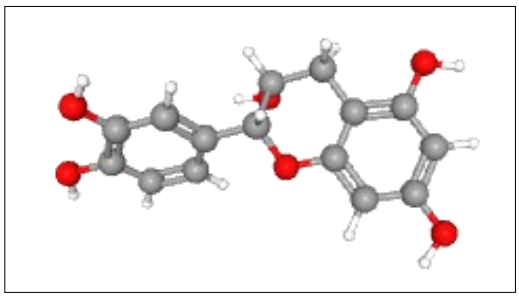
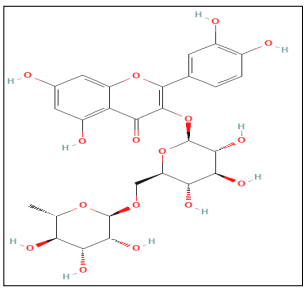
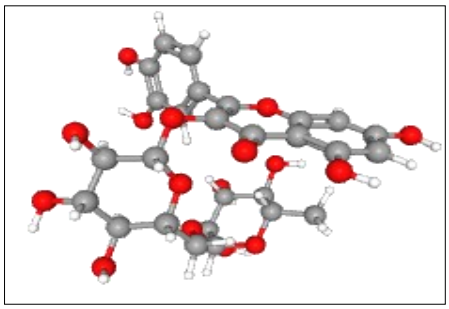
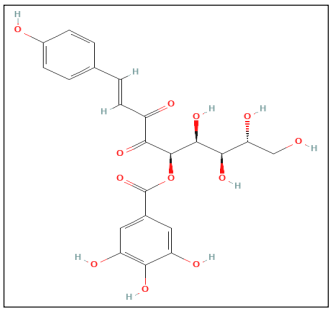
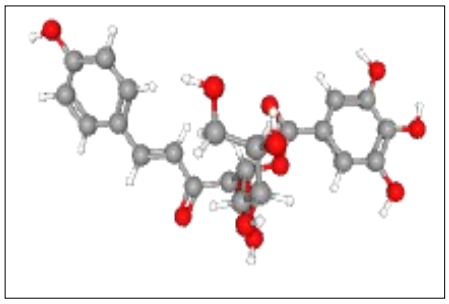
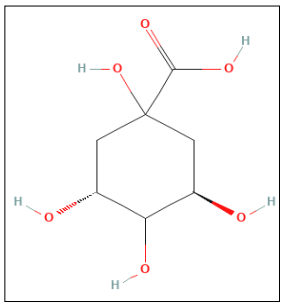
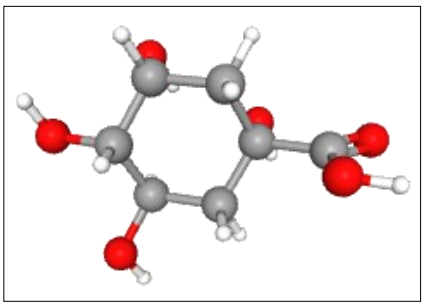
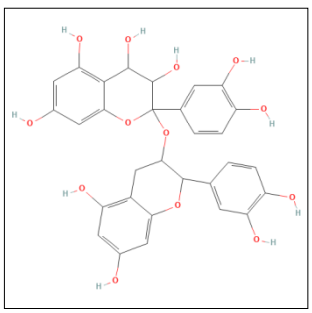
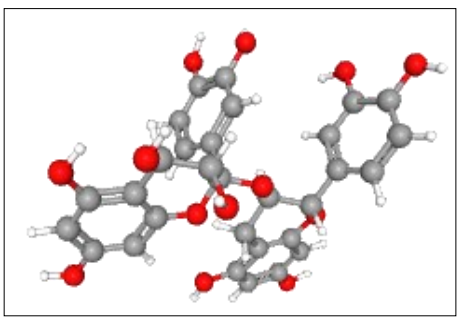
Repercussion of the computational docking method

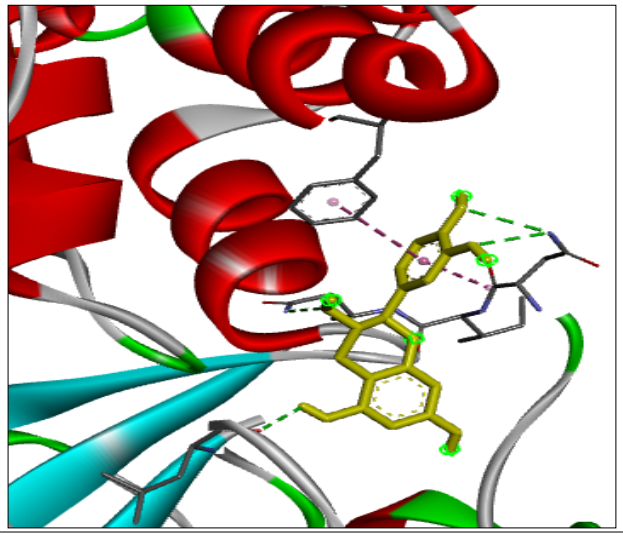
The study revealed that the lower the binding energy, the more hydrogen bonds interact with the amino acid residue, ensuing in more stable and stronger interactions. Fig. 2 (a-e) presents the molecular docking of PPX/GppA (in complex with GNP proteins) with epicatechin, rutin, cumaroyl-o-galloyl-glucose, quinic acid and procyanidin respectively. Only quinic acid showed interaction with ASP9 and GLU143 amino acids and it is reported by active site in the study (48). It was also observed that GLU115 and GLU143 as active sites of PPX/GppA (in complex with GNP proteins) but in our study we did not get this interaction with interactions of epicatechin, rutin, cumaroyl-o-galloyl-glucose and procyanidin. The result indicated that rutin achieved the highest binding affinity and docking score of 9.1 K.cal/mole with PPX/GppA (in complex with GNP proteins) through 08 hydrogen bonds with amino acids ASP216, PHE215, HIS414, TYR217, GLY224, SER388, GLU226, SER416 (Fig. 2b). Quinic acid displayed the second active top scoring of 9.0 K.cal/mole against PPX/GppA (in complex with GNP proteins). It interacted with 06 hydrogen bonds with amino acids SER12, GLY140, THR83, SER141, ASP9, GLU143 (Fig. 2d). Analysis of the binding modes of epicatechin, cumaroyl-o-galloyl-glucose and procyanidin considered as the third, fourth and fifth active compounds due the display of 8.3, 7.7 and 6.8 K.cal/mole binding affinity score with PPX/GppA (in complex with GNP proteins), revealed that three H bonds with ASN306, ASN308, LEU127; ALA124, PHE303, ASN125; and TYR225, ASP227, GLY224 respectively (Fig. 2a, 2c and 2e).

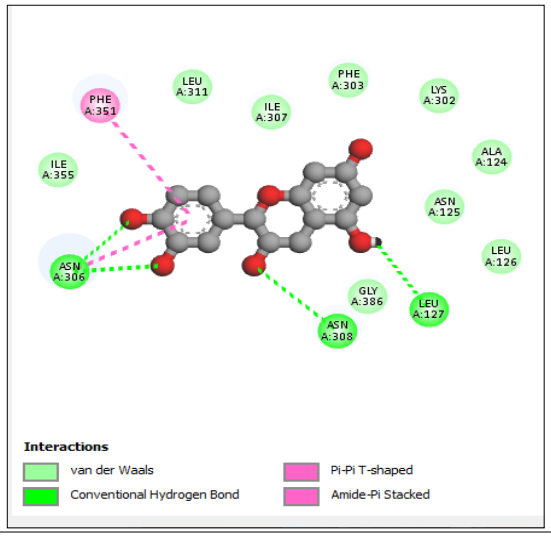
Conclusion

Ximenia americana, a plant with a rich history of traditional medicinal use, has demonstrated significant therapeutic potential through its diverse phytochemical profile. The bioactive compounds identified in this plant exhibit promising pharmacological activities, including antimicrobial, antidiarrheal, antioxidant, anticancer, anti-diabetic, antitrypanosomal, gastro protective, antidepressant, analgesic, antipyretic, anti-inflammatory and antiulcer effects.

Table 4. Ligands (chemical compounds) derived from *X. americana*

Compound Name (ID)	2D structure	3D structure
Epicatechin (72276)		
Rutin (5280805)		
Coumaroyl-o-galloyl-glucose (146170723)		
Quinic acid (6508)		
Procyanidin (107876)		

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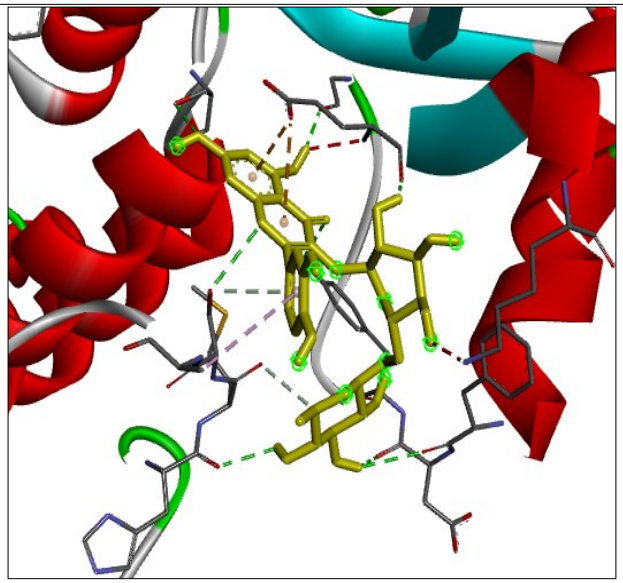
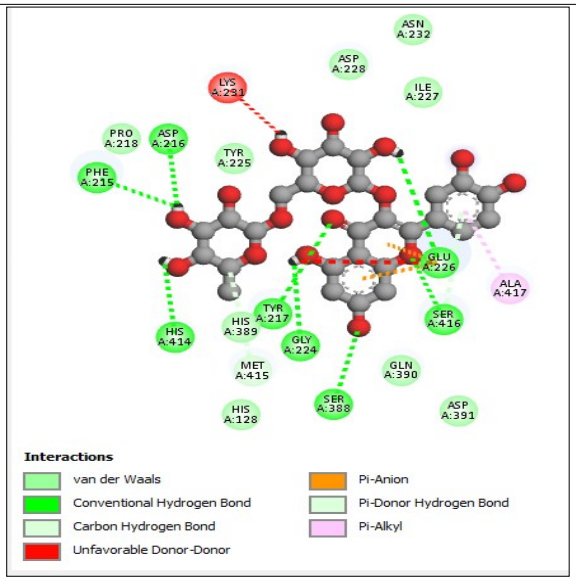
ASN A:306
GLY A:386
LEU A:127
ASN A:308
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Pi T-shaped
Amide-Pi Stacked

a

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ILE A:227
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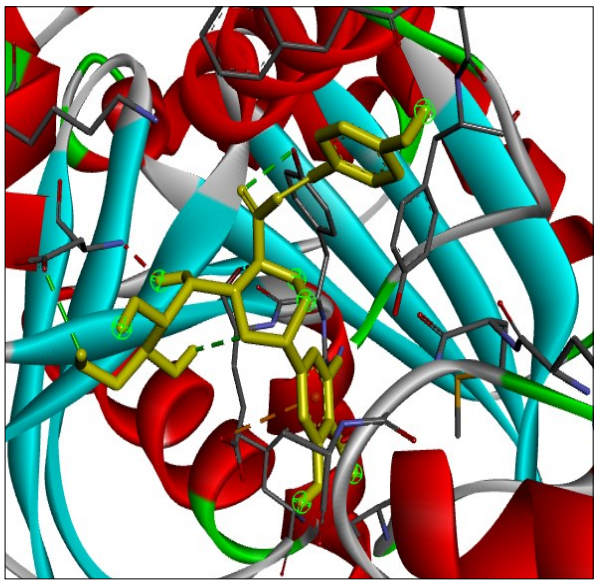
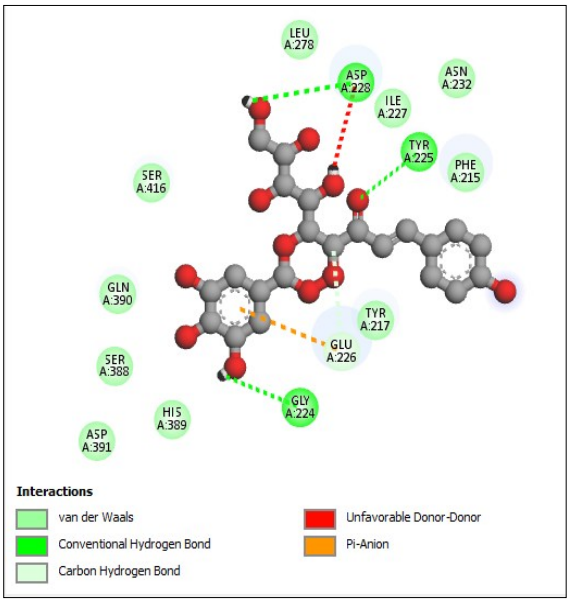
ALA A:417
TYR A:217
HIS A:389
SER A:416
HIS A:414
GLY A:224
MET A:415
GLN A:390
SER A:388
HIS A:128
ASP A:391
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor
Pi-Anion
Pi-Donor Hydrogen Bond
Pi-Alkyl

b

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SER A:388
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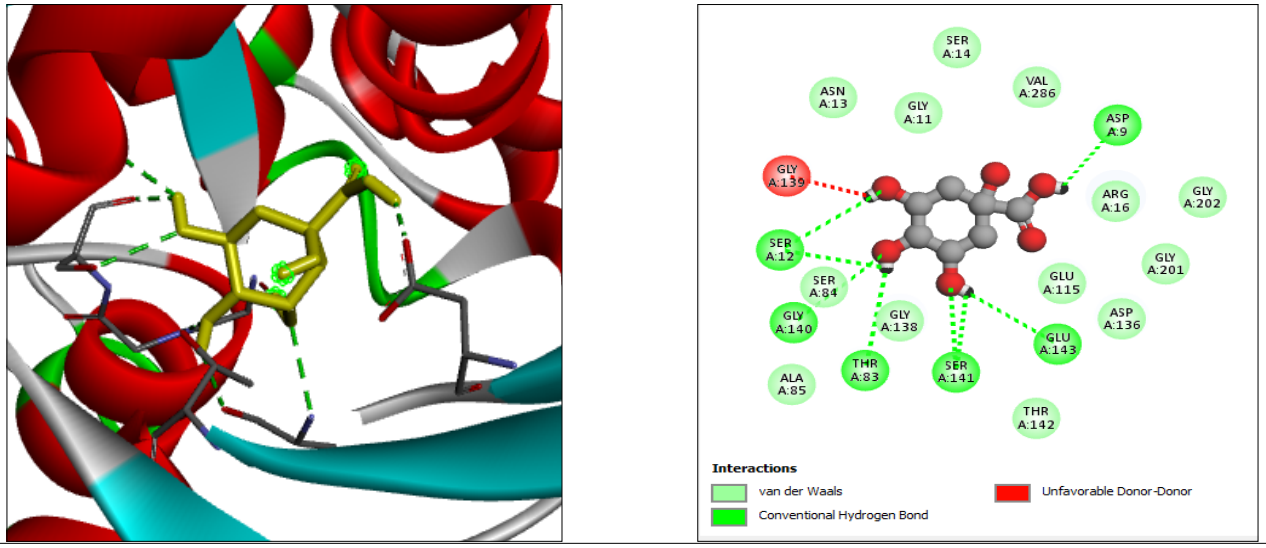
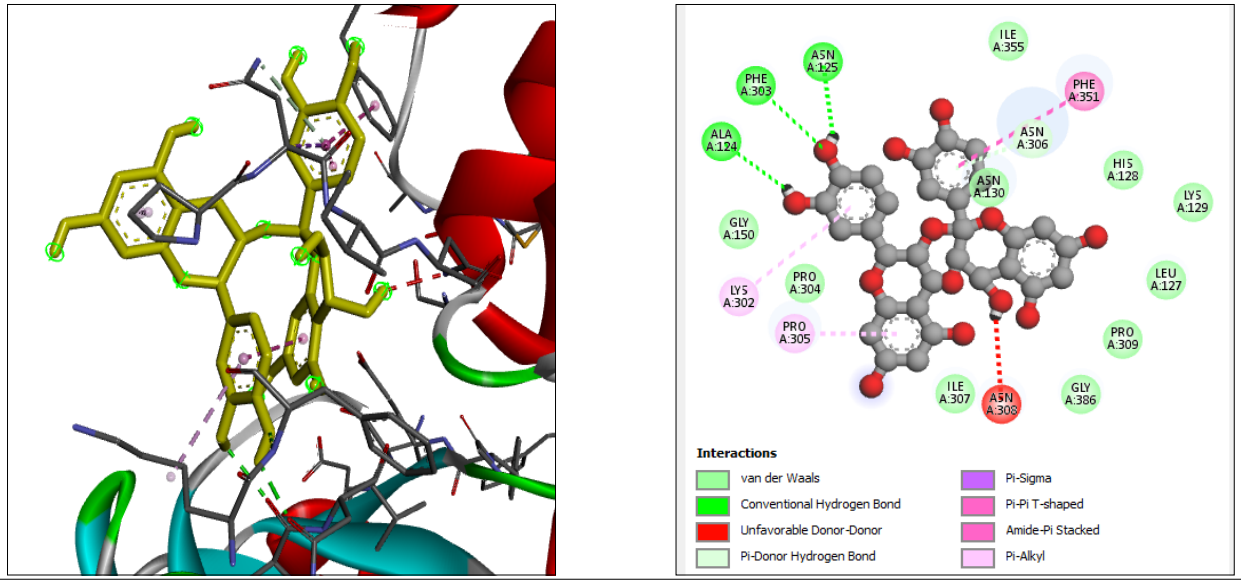
ASP A:391
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor
Pi-Anion

c



d



e

Fig. 2. Molecular docking interaction of Protein (GTP)- Ligand (derived from *X.americana*) complex: a. Epicatechin- PPX/GppA in complex with GNP proteins; b. Rutin-PPX/GppA in complex with GNP proteins; c. Coumaroyl-o-galloyl-glucose- PPX/GppA in complex with GNP proteins; d. Quinic acid- PPX/GppA in complex with GNP proteins; e. Procyanidin- PPX/GppA in complex with GNP proteins

Helicobacter pylori is spreading quickly throughout the healthcare sector, causing stomach pain, ulcers and stomach cancer. Because of these challenges, researchers are concentrating on anti-Helicobacter pylori medicines. Plant-based natural substances are safer, less expensive, easier to obtain and less dangerous. The PPX/GppA and GNP proteins' molecular docking studies demonstrate encouraging in silico anti-Helicobacter pylori characteristics. The exact dosage and efficacy of these substances and the development of complementary therapies require further investigation.

Integrating traditional knowledge with advanced computational approaches, such as molecular docking, offers a robust framework for identifying lead compounds for pharmaceutical applications. However, further *in vivo* studies, clinical trials and safety assessments are essential to translate these findings into practical therapeutic interventions. Overall, *Ximenia americana* represents a valuable natural resource with immense potential to contribute to modern medicine.

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Authors' contributions

TL and US helped in choosing the review article topic and its overall outline. PT, SI and VR provided insights and drafted the manuscript, critical corrections and subsequent revisions. All authors read and approved the final version.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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