Efficacy and Molecular Docking Study of Main Constituents of *Murraya paniculata* Biomass Extract Against *Helicobacter pylori*

Marwa Yousry A. Mohamed [a], Aisha M. H. Al-Rajhi [b,*], Seham M. Hamed,[a] Ibrahim Masmali,[c] HananTaher Hamza [d,e], Manal J. Kiki [f], and Asmaa A. Alharbi [g]

Natural compounds have received extra attention through the current decade to suppress *Helicobacter pylori* growth. This study investigated the phytochemical characterization of *Murraya paniculata* fruit extract (MPFE) and its estimation against different activities of *H. pylori*. Moreover, the molecular docking interactions (MDI) of catechin and kaempferol with *H. pylori* proteins were examined. Several compounds were detected *via* high performance liquid chromatography in MPFE with various concentrations. Of these, catechin, kaempferol, chlorogenic acid, and vanillin were measured as 11,000, 4960, 4610, and 65.8 µg/g, respectively. Excellent inhibition of *H. pylori* was recorded with an inhibition zone 24.3 mm using MPFE compared to the activity of standard antibiotic (16.2 mm). Both minimum inhibitory concentration and minimal bactericidal concentration (MBC) of MPFE were 60.5 µg/mL, whereas it was 15.6 µg/mL using standard antibiotic. The biofilm of *H. pylori* was inhibited by 25, 50, and 75% of MPFE MBC to a level of 68.2, 84.1, and 90.4%, respectively. Hemolysis caused by MPFE was prevented to a level of 21.2, 6.8, and, 3.3% at 25, 50, and 75% of MIC, respectively. The authors implemented the MDI using Molecular Operating Environment (MOE) software. The screened compounds interacted well with the *H. pylori* protein (PDB ID: 3K1H).

DOI: 10.15376/biores.20.2.3299-3314

Keywords: Murraya paniculata; Helicobacter pylori; Phenols; Flavonoids; Inhibition

Contact information: a: Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), P. O. Box: 90950, Riyadh 11623, Saudi Arabia, b: Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; c: Jazan University Hospital, Jazan University, P.O. Box 114, Jazan 45124, Saudi Arabia; d: Biology Department, College of Science, Jouf University, P.O. Box: 2014, Sakaka, Saudi Arabia; e: Zoology Department, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt; f: Department of Biological Sciences, Jeddah University, Jeddah 23218, Saudi Arabia; g: Department of Biology, College of Science, Jazan University, Jazan, Kingdom of Saudi Arabia;

** Corresponding author: amoalrajhi@pnu.edu.sa*

INTRODUCTION

Over 150 genera belong to the Rutaceae family that are spread in several regions of the world. *Murraya* represent one of the Rutaceae genera that includes 17 species growing in Australia and Asia as well as in the Pacific region. A varied range of uses are recognized

for *Murraya* in ethnobotanical and traditional medicine, such as the management of pain, fever, and dysentery. Additinally, antihyperlipidemic, anti-inflammatory, anti-diabetic, antimalarial, antimicrobial, and antidiarrheal activities of *Murraya* were investigated (Zaatout *et al.* 2022; Yohanes *et al.* 2023). As reported previously, its oil possesses anti-protozoa activity. *M. paniculata* has been applied to treat headache, gastralgia bruises, stomachaches, skin irritation, rheumatism, and snake bite (Joshi and Gohil 2023). Based on previous reports, *M. paniculata* leaves have been prepared in powder form as an additive to some foods in numerous Malay and Indian plates because of their strong smell (Ng *et al.* 2012). Several active compounds were identified in species of *Murraya*. For instance, naringin, isoquercitrin, rutin, hesperidin, and naringenin-7-O-β-D-glucopyranoside were found in *M. tetramera* stem and leaves. *M. paniculata* can be regarded as a highly valuable species of *Murraya* due to its medicinal features. It is cultivated in either tropical or subtropical climates with white flowers, oblong fruits, and rigid wood (Narkhede *et al.* 2012). A review article about the pharmacological applications of *M. paniculata* and *M. exotica* indicates that managements of cancer, diabetics, Alzheimer dissease, microbial pathogens, analgesic, and inflammation are associated with these plants (Qi *et al*. 2024).

Human illness arising from *Helicobacter pylori* represents a big problem worldwide (Yahya *et al.* 2022; Qanash *et al.* 2023a). Colonization by *H. pylori* in the human stomach is an effective danger agent for gastric adenocarcinoma in half of the world's individuals. The latest research has studied changes in immune responses and infection by *H. pylori*, which might contribute to hemolytic factors (Kumar *et al.* 2021). Hemolysis and peptic ulcers are two different but clinically relevant disorders that have historically been studied in the fields of hematology and gastroenterology, respectively (Obeagu and Obeagu 2024). Kumar *et al.* (2021) reported the connection among *H. pylori* infection and levels of immune responses. This association may lead to hemolytic conditions, while the precise mechanisms persist unknown. An *in vivo* experiment by Lu *et al.* (2021) documented that stomach lesions of rats were improved and that the levels of cytokine, which is linked to inflammation, were reduced. The virulence factors of *H pylori* with their capacity to develop biofilms make it more resistant to traditional antibiotics, which increase the need for novel compounds and approaches to treat *H. pylori* infection (Spósito *et al*. 2024). Plant extracts, such as *Aloe vera* (Yahya *et al.* 2022), laurel (Al-Rajhi *et al.* 2023a), *Acacia nilotica* (Al-Rajhi *et al.* 2023b), berberine, curcumin (Fonseca *et al.* 2024), and rosemary (Bakri *et al.* 2024) have been investigated against *H. pylori* as safe alternatives to synthetic drugs. *In vitro* studies were carried out on some species of *Murraya* to evaluate its antioxidant activity. According to published papers, *M. koenigii* (Aju *et al.* 2017), and *M. paniculata* (Beltagy *et al.* 2024) leaf extracts exhibited excellent antioxidant activity, which was attributed to flavonoids and phenolics content.

Biologists have become interested in molecular docking studies in the past 10 years as a means of determining a natural or synthetic molecule's affinity for a certain biological target. Additionally, it allows for a reduction in the time and expense needed to carry out the same biological activity processes (Qanash *et al.* 2022; Alsalamah *et al.* 2023; Al-Rajhi *et al*. 2024). As stated in the present introduction, there has been no investigation on the influence of *M. paniculata* fruit extract (MPFE) on *H. pylori*, but other plants have been applied for the management of *H. pylori*. Therefore, the current paper aims to estimate the chemical profile of MPFE and its influence on growth and biofilm of *H. pylori*, in addition to hemolysis inhibition in the presence of *H. pylori*. Also, the molecular docking

investigation of the main constituents of MPFE against *H. pylori* was the goal of present study.

EXPERIMENTAL

Plant Fruits and Extraction

Fruits of *Murraya paniculata* were collected from an agricultural field planted with ornamental plants of the corresponding author (Aisha M. H. Al-Rajhi) during the autumn season (April 2024) in Saudi Arabia. The identification of plant was documented by a botanist at the biology department, Faculty of Science, Jazan University, Saudi Arabia. The collected fruits were dried in air for 20 days under shadow conditions with stirring each day. Then, the dried fruits were ground, followed by extraction with methanol (20 g/100 mL). Methanol was chosen because it has been used for extraction of most active compounds (Selim *et al*. 2024; Binsaleh *et al.* 2025). The yield of extract was concentrated and dissolved in dimethyl sulfoxide (10%) (DMSO) for further experiments.

Phenolics and Flavonoids Detection in the MPFE by HPLC

The compounds of flavonoids and phenols in MPFE were determined using high-performance liquid chromatography (HPLC) (GLSciences Inc., Tokyo, Japan). The constituents of the solvents as well as the gradient elution were applied as described earlier (Kubola and Siriamornpun 2011). Briefly, the MPFE was dissolved as one mg/mL of DMSO (10%). One % of acetic acid in water and acetonitrile were employed as a gradient of solvents A and B, respectively, proceeded at a flow level of 0.8 mL/min. The elution gradient was carried out as next: 5% to 9%, 9% to 11%, 11% to 18%, 18% to 23%, 23% to 90%, 90% to 80%, and 80% of solvent B from 0 to 5 min, 5 to 15 min, 15 to 22 min, 22 to 38 min, 38 to 43min, 43 to 44 min, 44 to 45 min, and 45 to 55 min, respectively. The spectra were measured at a range of 200 to 600 nm. The detected compounds of phenols and flavonoids were recognized by matching its retention time (RT) and UV spectra with standard compounds.

Measuring the Anti-*H. pylori* Potential of MPFE

To conduct this experiment, *H. pylori* was applied in this test using a well agar diffusion procedure. *H. pylori* was obtained from University Hospital of Ain Shams at Cairo, Egypt. Briefly, 100 µL suspensions at level of 1.0×10^8 colony forming units (CFUs)/mL of *H. pylori* was inoculated in Mueller Hinton agar supplemented with 10% blood, then poured in petri dishes. A 6-mm diameter hole was punched aseptically with a sterile cork borer from the agar layer. Then, 100 µL of the MPFE was poured into the well. The well containing DMSO was served as the control; whereas the loaded well with 0.05 mg/mL of clarithromycin was served as the standard control. The petri dishes containing *H. pylori* were incubated at under microaerophilic with humidity at 37 °C for 72 h. Then, the radii of the appeared zones of inhibition were recorded (French 2006).

Measuring the Minimal Inhibitory Concentration (MIC) of MPFE

The MPFE underwent testing to determine its MIC *versus H. pylori* using a broth of micro-dilution, which was made from Mueller–Hinton broth that had been broken down

to include lysed horse blood. Sequences of dilutions (0.98 to 1000 µg/mL) of MPFE were created. For each dilution that was suitable, 200 µL of MPFE was poured in each well of a microtitrate plate. The culture of *H. pylori* was grown in sterile saline solution (0.85% NaCl) to match the stock of 1.0 McFarland turbidity. A sum of 2 µL of *H. pylori* culture was introduced into each well to achieve a concentration of 3.0×10^6 CFUs/mL. The plates were then placed in an environment with certain conditions (15% CO_2/35 °C/3 days. Next, the MIC was judged visually, indicating the extent of inhibition of *H. pylori*. *H. pylori* culture without MPFE served as a positive control, whereas MPFE deprived of *H. pylori* culture was operated as a negative control in every microplate (Andrews 2001). Three replicates for each treatment were performed.

Measuring the Minimal Bactericidal Concentration (MBC) of MPFE

The MBC test was carried out by transferring 100 mL culture of the *H. pylori* from each well showing strong growth inhibition to the Mueller–Hinton agar plates (5% horse blood) through sub-culturing. These plates were then placed in an environment with low oxygen levels (15% CO_2) at 35 °C/3 days. Following this, the MBC was visually assessed to determine the lowest concentration of MPFE that completely stopped *H. pylori* growth. To identify whether the MPFE was bactericidal or bacteriostatic, the MBC/MIC proportion was designed. If the MBC/MIC ratio was less than 4 intervals that of the MIC, it indicated that the MPFE had bactericidal properties (Andrews 2001).

Measuring the Antibiofilm Activity of MPFE

The impact of MPFE on the biofilm development of *H. pylori* was evaluated using plates of 96-well polystyrene flatbottom (Stepanović 2007). Each plate was filled with fresh trypticase soy yeast broth that had been injected with *H. pylori* (250 µL, each containing 10^6 CFU/mL) and then evenly distributed with varying doses of MBC (25, 50, and 75%), as observed in the study. The plates were then left to incubate for 48 h at 37 °C. After this time, the liquid above the cells was carefully removed. Next, sterile distilled water was used to wash away any cells that were floating freely. The plates were then left to air-dry for 30 min. The resulting *H. pylori* biofilm was then colored with 0.1% of crystal violet (aqueous) (15 min /25 °C), after which the excess crystal violet was washed off with sterile distilled water. To fully eliminate the crystal violet dye attached to the *H. pylori* cells, 250 µL of ethanol (95%) was introduced into each well to act as a dye-solubilizing agent. Following this, all wells were left to incubate for 15 min, and the absorbance was recorded at 570 nm employing a reader of microplate.

$$\text{Biofilm development inhibition (\%)} = 1 - \frac{\text{MPFE Absorbance} - \text{Blank Absorbance}}{\text{Control Absorbance} - \text{Blank Absorbance}} \times 100 \quad (1)$$

The media absorbance is considered as the Blank, whereas *H. pylori* treated is the absorbance of MPFE, and *H. pylori* deprived of any management is considered control absorbance.

Measuring the Antioxidant Potential of MPFE

Using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging technique, the free radical scavenging capacity of the MPFE was determined as follows: 0.1 mM DPPH

solution in methanol was made. Three millilitres of methanol was added to 1 mL of the solution at varying doses (1.95 to 1000 µg/mL). After offering the mixture a suitable shaking, it was left at room temperature for 30 min. At 517 nm, the absorbance was then recorded using a ultraviolet visible (UV-VIS) Shimadzu spectrophotometer. The utilised reference was ascorbic acid, ranging from 1000 to 1.95 µg/mL. The quantity of MPFE necessary to stop the DPPH free radical at level (50%) is known as the IC_{50} value. The log dosage inhibition curve was employed to decide the sample's IC_{50} quantity. The subsequent calculation was employed to compute the percent DPPH scavenging influence:

$$\text{DPPH scavenging effect (\%)} = 100 \times \frac{\text{Absorbance in presence of the MPFE}}{\text{Absorbance of the control reaction}} \quad (2)$$

Molecular Docking Interaction of Catechin and Kaempferol with *H. pylori* Receptor Proteins

The computational study was performed on an Intel Core i7 processor through the Windows 10 operating system (OS). The molecular docking was accomplished manipulating Molecular Operating Environment (MOE) version 2019.0102 software. The MOE model tool was used to estimate the binding interactions between catechin/kaempferol and *H. pylori* receptor proteins. The structure of catechin and kaempferol were retrieved as SDF (standard data file) files from the database PubChem (PubChem ID: CID_73160, 5280863) for MOE to show. The three-dimensional (3D) protein structure of (3K1H) as an *H. pylori* target was retrieved through an online browser (http://www.rcsb.org/pdb) from protein data bank (PDB). Hydrogen atoms were involved subsequent to removal of water molecules across the protein. Through the MMFF94x force field, the charges and parameters were recorded. After generating alpha-site spheres operating MOE's site finder module, the selected compounds were docked inside the active region utilizing MOE's DOCK module. The dock scoring for the MOE programme was established employing the London dG scoring mode, with location, such as triangle matcher, retention 10, and refinement as the force field. The leading formations of the docked ligands were recognized by respecting the values of root mean square deviations (RMSD) and binding energies, as well as binding manners with the selected residues.

Analysis of Findings

The software SPSS version 15.0 was employed to investigate results (SPSS Inc. Chicago, IL, USA). The average of three results was calculated to provide the ± standard deviation (SD).

RESULTS and DISCUSSION

Some studies were performed on the biological activities of *M. paniculata* leaves but not on fruits, especially anti-*H. pylori*. Figure 1 illustrates the main performed experiments on *M. paniculata* fruits extract (MPFE). Table 1 and HPLC chromatogram (Fig. 2) reflect the investigation of phenols and flavonoids in MPFE. High content of catechin (11.0 µg/g) was associated to MPFE. Moreover, kaempferol, chlorogenic acid, caffeic acid, gallic acid, and rutin were detected in the following concentrations 4960, 4610, 3700, 2660, and 2180 µg/g, respectively. In contrast, a low quantity (65.8 µg/g) of

vanillin followed by querectin 117 µg/g and cinnamic acid 120 µg/g were recognized in MPFE. Other compounds were noticed in moderate doses like syringic acid, coumaric acid, methyl gallate, and naringenin (Table 1). However, rosmarinic acid was loaded in HPLC as standards but it was not discovered in the extract. Phenol and flavonoid compounds in *M. paniculata* leaves extract perform a key role in its the therapeutic roles. These compounds contribute to its antibacterial, anti-inflammatory, and antioxidant features (Ashibuogwu *et al.* 2022; Joshi and Gohil 2023). The acids gallic, chlorogenic, caffeic, ellagic, plus rutin, epicatechin, querectin, and catechin were detected in leaves extract of *M. paniculata* according to previous study (Menezes *et al*. 2015).

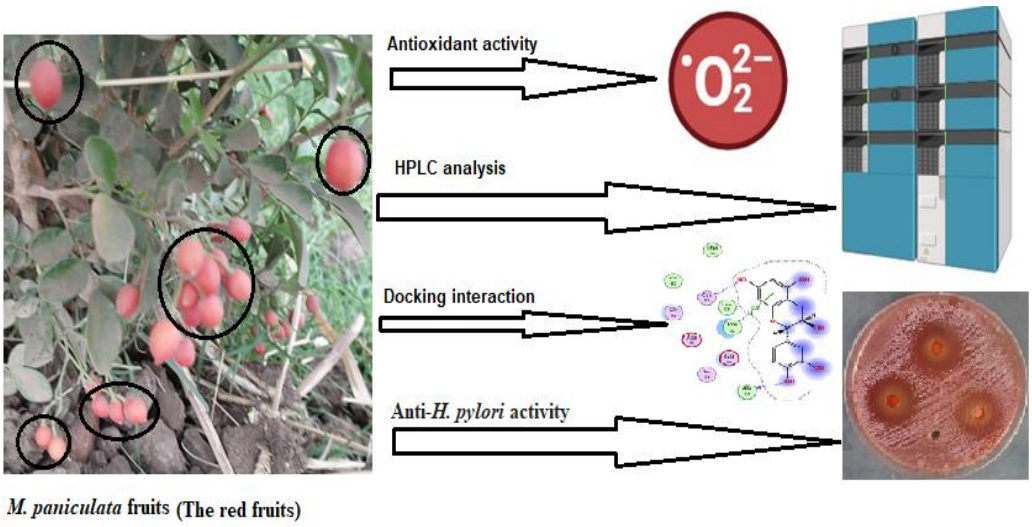

Fig. 1. *M. paniculata* fruits with their performed analysis and biological activities

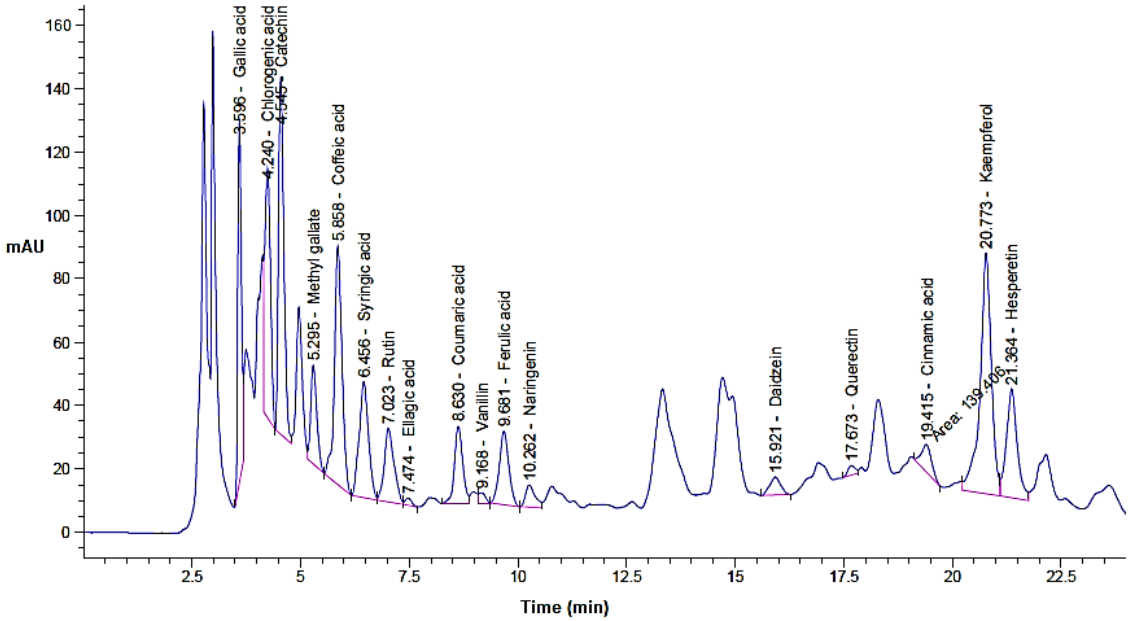

Fig. 2. Different peaks of HPLC chromatogram show the identified phenolic and flavonoid compounds in MPFE

The MPFE exhibited anti-*H. pylori* with a higher clear zone of 24.3 mm than that caused by standard antibiotic (16 mm) (Table 2 and Fig. 3). Moreover the value of MIC and MBC of MPFE were encouraging (62.5 µg/mL), while the values employing standard antibiotic was 15.6 (Table 2). MPFE was characterized as a bactericidal agent, this feature was documented through the calculation of MBC/MIC index. Some detected compounds in the MPFE exhibited antimicrobial activities but from other plants as described in published papers. For instance, growth inhibition was attributed to rutin *versus H. pylori* (Qanash *et al.* 2023a), gallic acid and hesperetin *versus Enterococcus faecalis* and *Candida albicans* (Al-Rajhi *et al.* 2023c), ellagic acid and chlorogenic acid *versus Candida albicans* and *Geotrichum candidum*. The extracted oil from *M. paniculata* L. leaves reflected antimicrobial activities against various bacteria of Gram positive and Gram negative as well as *Candida albicans* (Zaatout *et al.* 2022). Few studies have reported on the fruits extract of other species of *Murraya*, such as *M. koenigii*, which reflected therapeutic potential for ulcer treatment caused by *H. pylori* (Waghmare 2015).

The ability of *H. pylori* to create biofilm was documented in numerous investigations, which is associated with virulence of this species. The tested MPFE succeeds in preventing the *H. pylori* biofilm with different levels depending on the applied dose. Antibiofilm activity % of MPFE was 68.2, 84.1, and 90.4% at 25, 50, and 75% of MBC (Fig. 4). Standard antibiotic clarithromycin reflected 100% antibiofilm (this data not tabulated). The obtained findings indicate that the extract is an efficient agent for the prevention of biofilm formation. Urease is a virulent agent in stomach ulcers caused by *H. pylori*. Ashibuogwu *et al.* (2022) recorded the inhibition of urease by *M. paniculata* leaves extract. Several factors are associated with the process of hemolysis. For example, toxins from some microorganisms are considered one of the most influencing factors causing hemolysis. Therefore, the prevention of hemolysis has attracted the attention of pharmacologists.

Table 1. Phenols and Flavonoids Analysis in MPFE *via* HPLC

Detected Constituent	RT	Area (mAU*s)	Concentration (µg/g)
Gallic acid	3.596	661.31	2660
Chlorogenic acid	4.240	722.03	4610
Catechin	4.545	963.91	11.0
Methyl gallate	5.295	300.25	770
Caffeic acid	5.858	907.95	3700
Syringic acid	6.456	544.08	1620
Rutin	7.023	359.03	2180
Ellagic acid	7.474	23.06	142
Coumaric acid	8.630	320.46	530
Vanillin	9.168	35.70	65.8
Ferulic acid	9.681	351.50	980
Naringenin	10.262	105.97	480
Rosmarinic acid	11.922	0.00	0.00
Daidzein	15.921	98.69	310
Querectin	17.673	35.06	117
Cinnamic acid	19.415	139.41	120
Kaempferol	20.773	1384.77	4960
Hesperetin	21.364	593.18	1420

According to von Recklinghausen *et al.* (1998), sheep blood was lysed by *H. pylori*-free culture with a 57% hemolysis occurrence. The performed experiment expressed the activity of the extract to repress the blood hemolysis. At different doses of the obtained MBC, the hemolysis decreased with increasing the applied dose of MPFE (Fig. 5). The hemolysis inhibition was 21.2 and 3.3% at 25 and 75% of MBC, respectively, compared to complete hemolysis. These outcomes may be attributed to the incidence of some active compounds that minimize the toxicity level of *H. pylori*.

Table 2. Inhibitory Parameters (Clear Zone), MIC, MBC, and MBC/MIC Index (µg/mL) of MPFE

Treatment	Zone of Inhibition (mm)	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC Index
Clarithromycin	16.25 ± 0.50	15.62	15.62	1
MPFE	24.33 ± 0.66	62.50	62.50	1

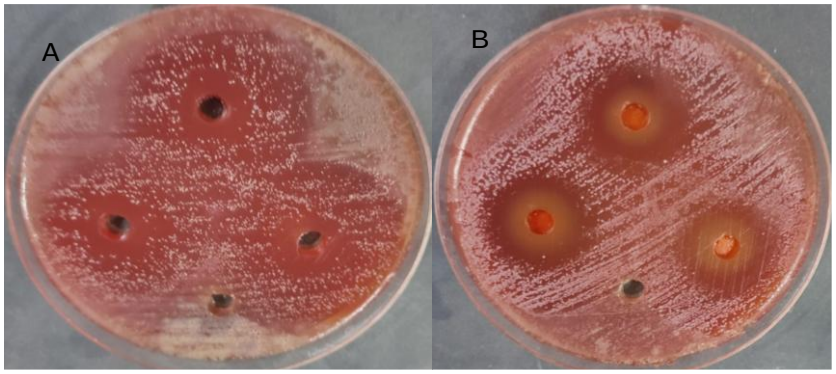

Fig. 3. Inhibition of *H. pylori* by MPFE (A) and standard antibiotic (B). The three wells in each plate showed clear zones containing extract or antibiotic, while the well that was not surrounded by a clear zone contains DMSO

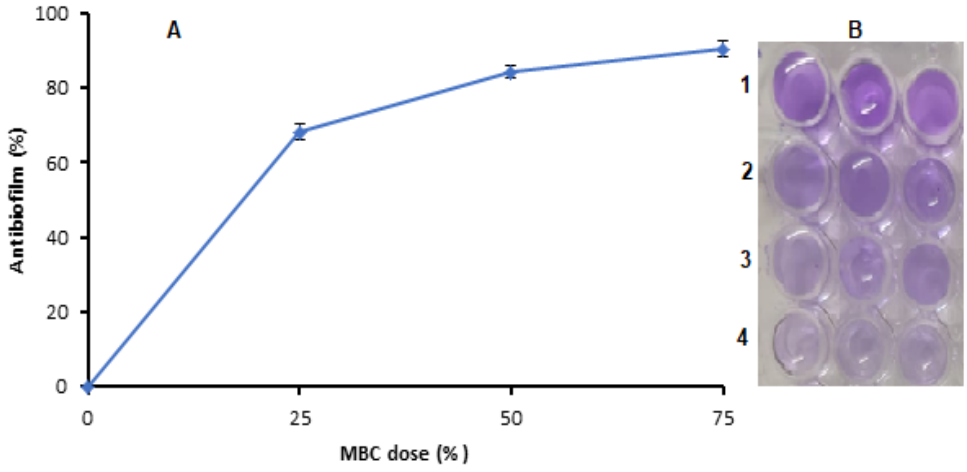

Fig. 4. *H. pylori* biofilm inhibition at different doses of MBC of MPFE (A), Microtiter plate showing the color stain shifts as an indicator for inhibition of biofilm development (B) at different doses involving *H. pylori* without treatment (1), *H. pylori* treated by 25% of MBC (2); *H. pylori* treated by 50% of MBC (3), and *H. pylori* treated by 75% of MBC (4)

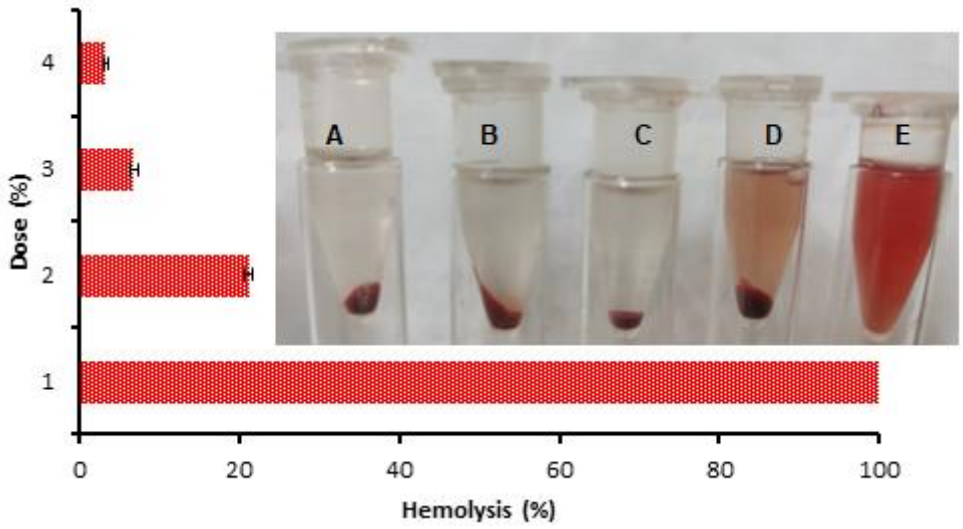

Fig. 5. Hemolysis activity of *H. pylori* exposed to different doses of MBC of MPFE. Doses were as follows: control without treatment (1), treatment by 25% (2), 50% (3), and 75% (4) of MBC. The Eppendorf tubes indicate hemolysis at different doses of MBC of MPFE including positive control (A), 25% MIC (B), 50% MIC (C), 75% MIC (D), and without treatment (E)

The outcomes in Fig. 6 indicate that the MPFE possessed excellent antioxidant activity. This property was documented through the calculated IC_{50} (5.39 µg/mL) with the comparison of ascorbic acid IC_{50} (2.58 µg/mL). Moreover, as the MPFE dose increased, the DPPH scavenging % increased with dose dependent manner as shown also in case of ascorbic acid. For illustration, at 1.95, 15.62, 125, and 1000 µg/mL of the MPFE, DPPH scavenging % was 43.8, 66.7, 90.7, and 97.9 %, respectively.

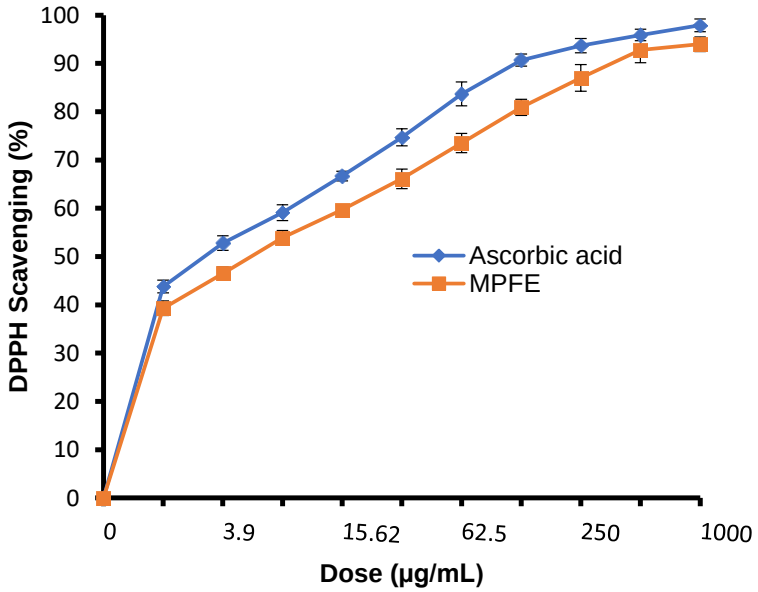

Fig. 6. Antioxidant properties of MPFE and ascorbic acid

The antioxidant potential of *M. paniculata* leaves was recognized but with more IC_{50} (ranging from 87.6 to 259 µg/mL according to used solvent) (Ahmed *et al.* 2019) than that of fruits in the current investigation. Zhu *et al*. (2015) mentioned that extract of *M. paniculata* leaves displayed greater antioxidant activity than trolox (antioxidant drug). The findings of antioxidant potential may associate well with the presence of flavonoids and phenolic in MPFE, as mentioned in other investigations but using another organ of *M. paniculata* (Sonter *et al.* 2021) or other plants (Al-Rajhi *et al.* 2022, 2023c; Alawlaqi *et al.* 2023).

Some studies were performed on the biological activities of *M. paniculata* leaves but not on fruits, especially anti-*H. pylori*. Figure 1 illustrates the main performed experiments on MPFE. Table 1 and HPLC chromatogram (Fig. 2) reflected the investigation of phenols and flavonoids in MPFE. High catechin content (11,000 µg/g) was associated to MPFE. Moreover, kaempferol, chlorogenic acid, caffeic acid, and gallic acid, in addition to rutin were detected in the following concentrations 4960, 4610, 3700, 2660, and 2180 µg/g, respectively. In contrast, a low quantity (65.8 µg/g) of vanillin followed by querectin 117 µg/g and cinnamic acid 119 µg/g were recognized in MPFE.

Molecular docking interaction has been applied in numerous investigations for several reasons, such as to evaluate the compounds activity, design of new therapeutic compounds, and discover bioactive constituents for management of many issues. Molecular docking interaction is achieved *via* targeting of specific proteins and bioactive compounds (Alghonaim *et al.* 2023; Al-Rajhi and Abdelghany 2023a,b; Qanash *et al.* 2023b). Molecular docking analysis is an *in silico* mode that simulates the binding potential of tiny compounds to a specific protein.

The docking investigation revealed that catechin and kaempferol (catechin and kaempferol represent the main detected compounds in the MPFE with concentrations 11,000 and 4960 µg/g, respectively; therefore, it was selected for docking interaction study) attach perfectly to the active site of the targeted protein *H. pylori* (PDB: 3K1H) by virtue of their similar molecular structures and binding orientation (Figs. 7 and 8). The docking results, which were collected in Table 3, indicate that catechin and kaempferol have low binding free energy of -4.76 and -4.89 kcal mol^{-1} respectively. These designed structural molecules (catechin and kaempferol) were found making key hydrogen bond interactions with 3K1H residues. Catechin interacts with CYS 86, ALA 90, and PRO 92 amino acid residues through O 4, O 6, and 6-ring in ligand. While kaempferol binds to amino acid pocket molecules ASP 95 *via* O 3 atom.

Table 4 shows the interactions between practically all atoms in the ligands and 3K1H amino acid residues. The present results are in agreement with other studies on molecular docking. Several investigators studied the activity of flavonoid and phenols compounds *via* docking interaction against target proteins. For instance, the 4HI0 protein of *H. pylori* was targeted by ferulic acid, chlorogenic acid, and rutin, which reflected low energy scores of − 5.58 kcal/mol (Al-Rajhi *et al.* 2023a), − 6.49 kcal/mol (Yahya *et al.* 2022), and − 7.48 kcal/mol (Qanash *et al.* 2023a), respectively. According to some studies, the compounds that give a high degree of the negative score are more effective than those which give low negative score values (Al-Rajhi and Abdelghany 2023a,b). Figure 9 shows the key for the types of interactions among catechin/kaempferol and protein receptors.

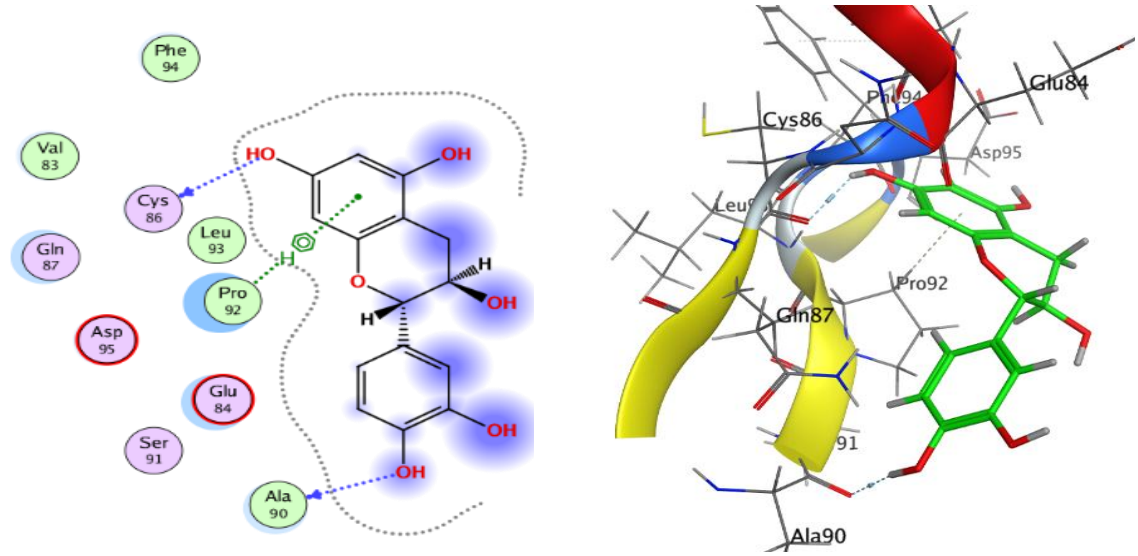

Fig. 7. 2D and 3D illustrations display the interaction among catechin and active sites of *H. pylori* 3K1H protein

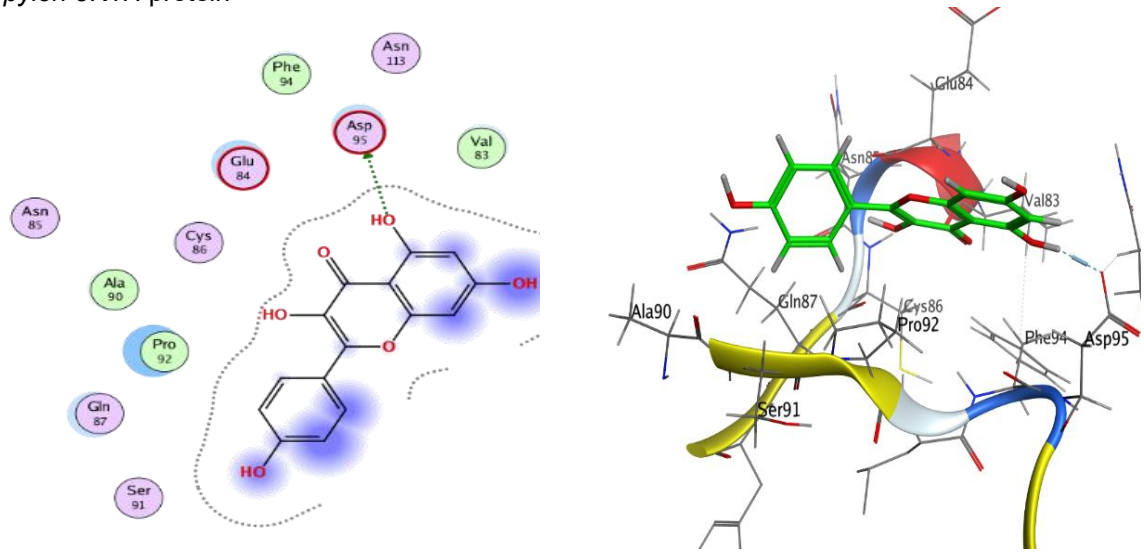

Fig. 8. 2D and 3D illustrations display the interaction among Kaempferol and active sites of *H.pylori* 3K1H protein

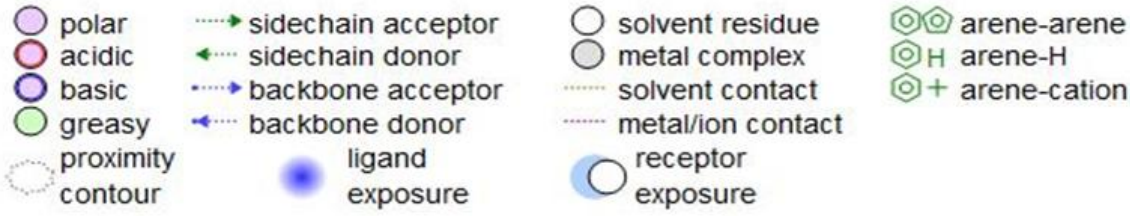

Fig. 9. The illustrative key for the kinds of interaction among catechin/Kaempferol and protein receptors

Table 4. Docking Scores and Energies of Catechin and Kaempferol with *H. pylori* (PDB ID:3K1H) Receptors

Mol	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
Catechin	-4.75896	2.96747	-16.1254	-47.1809	-9.91079	-22.0836	-4.75896
Catechin	-4.74825	1.132875	-14.321	-38.7649	-9.67824	-22.2848	-4.74825
Catechin	-4.68648	1.511018	-16.9585	-47.3669	-9.77006	-21.0769	-4.68648
Catechin	-4.67424	1.440543	-16.5329	-51.1967	-10.1376	-21.6961	-4.67424
Catechin	-4.65091	2.031837	-15.9015	-53.9201	-9.73125	-21.669	-4.65091
Kaempferol	-4.8859	1.014926	3.055002	-64.7749	-9.75998	-24.6066	-4.88590
Kaempferol	-4.67286	1.744309	3.854871	-58.5196	-10.2336	-21.8348	-4.67286
Kaempferol	-4.56481	1.61925	3.155271	-48.3284	-9.2691	-20.3878	-4.56481
Kaempferol	-4.52378	2.005444	1.137617	-60.1243	-9.3297	-21.0934	-4.52378
Kaempferol	-4.51962	1.876773	3.051854	-37.7939	-9.29751	-20.1781	-4.51962

Table 5. Interaction of Catechin and Kaempferol with *H. pylori* (PDB ID: 3K1H) Receptors

Mol	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
Catechin	O 4	O CYS 86 (A)	H-donor	2.99	-2.0
	O 6	O ALA 90 (A)	H-donor	3.06	-1.0
	6-Ring	CB PRO 92 (A)	pi-H	3.63	-0.5
Kaempferol	O 3	OD2 ASP 95 (A)	H-donor	2.78	-4.0

CONCLUSIONS

1. The *Murraya paniculata* fruit extract (MPFE) was found to possess excellent anti-*H. pylori* activity (24.33 ± 0.66 mm inhibition zone) *via* the well diffusion assay, minimal inhibitory concentration (MIC) (62.5 µg/mL), minimal bactericidal concentration (MBC) (62.5 µg/mL), and its activity against biofilm formation of *Helicobacter pylori* with biofilm inhibition 68.2, 84.1, and 90.4% at 25, 50, and 75% of MBC, respectively.
2. Blood hemolysis caused by *H. pylori* was inhibited by the different doses of MPFE MBC.
3. The free radical scavenging was an excellent pointer for the antioxidant activity of MPFE.
4. The high performance liquid chromatography (HPLC) analysis showed the presence of numerous phenols and flavonoids in MPFE. The main detected compounds (catechin and kaempferol) were docked with *H. pylori* protein.
5. Based on the docking analysis, catechin and kaempferol were discovered as possible *H. pylori* specific inhibitors.

ACKNOWLEDGMENTS

To Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R217), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia

Funding

This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R217), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia

REFERENCES CITED

Ahmed, W. S., Abdel-Lateef, E. E. S., El-Wakil, E. A., and Abdel-Hameed, E. S. S. (2019). "*In vitro* antioxidant and antimicrobial properties of *Murraya paniculata* L. extracts as well as identification of their active secondary metabolites by HPLC-ESI-MS," *Der Pharma Chemica* 11(3), 1-8.

Aju, B. Y., Rajalakshmi, R., and Mini, S. (2017). "Evaluation of antioxidant activity of *Murraya koenigii* (L.) Spreng using different *in vitro* methods," *Journal of Pharmacognosy and Phytochemistry* 6(4), 939-942.

Alawlaqi, M. M., Aisha, M. H., Tarek M. A., Ganash, M., and Moawad, H. (2023). "Evaluation of biomedical applications for linseed extract: Antimicrobial, antioxidant, anti-diabetic, and anti-inflammatory activities *in vitro*," *Journal of Functional Biomaterials* 14(6), article 300. DOI: 10.3390/jfb14060300

Alghonaim, M. I., Alsalamah, S. A., Alsolami, A., and Abdelghany, T. M. (2023). "Characterization and efficiency of *Ganoderma lucidum* biomass as an antimicrobial and anticancer agent," *BioResources* 18(4), 8037-8061. DOI: 10.15376/biores.18.4.8037-8061

Al-Rajhi, A. M. H., and Abdelghany T. M. (2023a). "*In vitro* repress of breast cancer by bio-product of edible *Pleurotus ostreatus* loaded with chitosan nanoparticles," *Applied Biology & Chemistry* 66, article 33. DOI: 10.1186/s13765-023-00788-0

Al-Rajhi, A. M. H., and Abdelghany, T. M. (2023b). "Nanoemulsions of some edible oils and their antimicrobial, antioxidant, and anti-hemolytic activities," *BioResources* 18(1), 1465-1481. DOI: 10.15376/biores.18.1.1465-1481

Al-Rajhi, A. M. H., Bakri, M. M., Qanash, H., Alzahrani, H. Y., Halawani, H., Algaydi, M. A., and Abdelghany T. M. (2023c). "Antimicrobial, antidiabetic, antioxidant, and anticoagulant activities of *Cupressus sempervirens in vitro* and *in silico*," *Molecules* 28(21), article 7402 DOI: 10.3390/molecules28217402

Al-Rajhi, A. M. H., Ganash, M., Alshammari, A. N., Alsalamah, S. A., and Abdelghany, T. (2024). "*In vitro* and molecular docking evaluation of target proteins of lipase and protease for the degradation of aflatoxins," *BioResources* 19(2), 2701-2713. DOI: 10.15376/biores.19.2.2701-2713

Al-Rajhi, A. M. H., Qanash, H., Almashjary, M. N., Hazzazi, M. S., Felemban, H. R., and Abdelghany, T. M. (2023a). "Anti-*Helicobacter pylori*, antioxidant, antidiabetic, and anti-alzheimer's activities of laurel leaf extract treated by moist heat and molecular

docking of its flavonoid constituent, naringenin, against acetylcholinesterase and butyrylcholinesterase," *Life* 13(7), article 1512. DOI: 10.3390/life13071512

Al-Rajhi, A. M. H., Qanash, H., Bazaid, A. S., Binsaleh, N. K., and Abdelghany, T. M. (2023b). "Pharmacological evaluation of *Acacia nilotica* flower extract against *Helicobacter pylori* and human hepatocellular carcinoma *in vitro and in silico*," *Journal of Functional Biomaterials* 14(4), article 237. DOI: 10.3390/jfb14040237

Al-Rajhi, A. M., Yahya, R., Abdelghany, T. M., Fareid, M. A., Mohamed, A. M., Amin, B. H., and Masrahi, A. S. (2022). "Anticancer, anticoagulant, antioxidant and antimicrobial activities of *Thevetia peruviana* latex with molecular docking of antimicrobial and anticancer activities," *Molecules* 27(10), article 3165. DOI: 10.3390/molecules27103165

Alsalamah, S. A., Alghonaim, M. I., Jusstaniah, M., and Abdelghany, T. M. (2023). "Anti-yeasts, antioxidant and healing properties of henna pre-treated by moist heat and molecular docking of its major constituents, chlorogenic and ellagic acids, with *Candida albicans* and *Geotrichum candidum* proteins," *Life* 13(9), article 1839. DOI: 10.3390/life13091839

Andrews, J. M. (2001). "Determination of minimum inhibitory concentrations," *Journal of Antimicrobial Chemotherapy* 48(Suppl_1), 5-16. DOI: 10.1093/jac/48.suppl_1.5

Ashibuogwu, A. I., Afieroho, O. E., Suleiman, M., and Abo, K. A. (2022). "Anti-urease and antioxidant activities of the leaf extracts from *Murraya paniculata* (L.) jack (Rutaceae)," *GSC Advanced Research and Reviews* 11(1), 156-164. DOI: 10.30574/gscarr.2022.11.1.0099

Bakri, M. M., Alghonaim, M. I., Alsalamah, S. A., Yahya, R. O., Ismail, K. S., and Abdelghany, T. M. (2024). "Impact of moist heat on phytochemical constituents, anti-*Helicobacter pylori*, antioxidant, anti-diabetic, hemolytic and healing properties of rosemary plant extract *in vitro*," *Waste and Biomass Valorization* 15, 4965-4979. DOI: 10.1007/s12649-024-02490-8

Beltagy, A. M., Omran, G. A., and Zaatout, H. H. (2024). "*In-vitro* promising anticancer activity of *Murraya paniculata* L. leaves extract against three cell lines and isolation of two new major compounds from the extract," *Egyptian Journal of Chemistry* 68(1), 91-97. DOI: 10.21608/ejchem.2024.266841.9268

Binsaleh, N. K., Bazaid, A.S., Barnawi, H. Bandar Alharbi, Ahmed Alsolami, Albatool Y. Babsail, Samy Selim, Tarek M. Abdelghany, Reham M. Elbaz, and Qanash, H. (2025). "Chemical characterization, anticancer, antioxidant and anti-obesity activities with molecular docking studies of *Pleurotus ostreatus* biomass exposed to moist heat," *Food Measure*. DOI: 10.1007/s11694-025-03125-9

Fonseca, D. R., Chitas, R., Parreira, P., and Martins, M. C. L. (2024). "How to manage *Helicobacter pylori* infection beyond antibiotics: The bioengineering quest," *Applied Materials Today* 37, article ID 102123. DOI: 10.1016/j.apmt.2024.102123

French, G. L. (2006). "Bactericidal agents in the treatment of MRSA infections – the potential role of daptomycin," *Journal of Antimicrobial Chemotherapy* 58(6), 1107-1117. DOI: 10.1093/jac/dkl393

Joshi, D., and Gohil, K. J. (2023). "A brief review on *Murraya paniculata* (orange jasmine): Pharmacognosy, phytochemistry and ethanomedicinal uses," *Journal of Pharmacopuncture* 26(1), 10-17. DOI: 10.3831%2FKPI.2023.26.1.10

Kubola, J., and Siriamornpun, S. (2011). "Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng)," *Food Chemistry* 127, 1138-1145.

Kumar, S., Patel, G. K., Ghoshal, U. C. (2021). "*Helicobacter pylori*-induced inflammation: Possible factors modulating the risk of gastric cancer," *Pathogens* 10(9), article 1099. DOI: 10.3390/pathogens10091099

Lu, M., Du, Z., Yuan, S., Ma, Q., Han, Z., Tu, P., and Jiang, Y. (2021). "Comparison of the preventive effects of *Murraya exotica* and *Murraya paniculata* on alcohol-induced gastric lesions by pharmacodynamics and metabolomics," *Journal of Ethnopharmacology* 281, article ID 114567. DOI: 10.1016/j.jep.2021.114567

Menezes, I. R., Santana, T. I., Varela, V. J., Saraiva, R. A., Matias, E. F., Boligon, A. A., and Rocha, J. B. (2015). "Chemical composition and evaluation of acute toxicological, antimicrobial and modulatory resistance of the extract of *Murraya paniculata*," *Pharmaceutical Biology* 53(2), 185-191. DOI: 10.3109/13880209.2014.913068

Narkhede, M., Ajmire, P., and Wagh, A. (2012). "Evaluation of antinociceptive and anti-inflammatory activity of ethanol extract of *Murraya paniculata* leaves in experimental rodents," *International Journal of Pharmacy and Pharmaceutical Sciences* 4(1), 247-250.

Ng, M. K., Abdulhadi-Noaman, Y., Cheah, Y. K., Yeap, S. K., and Alitheen, N. B. (2012). "Bioactivity studies and chemical constituents of *Murraya paniculata* (Linn) Jack," *International Food Research Journal* 19(4), 1307-1312.

Obeagu, E. I., and Obeagu, G. U. (2024). "Exploring the intersection: Peptic ulcers and hemolysis-Unraveling the complex relationship," *Medicine (Baltimore)* 103(11), article e37565. DOI: 10.1097/MD.0000000000037565

Qanash, H., Al-Rajhi, A. M. H., Almashjary, M. N., Basabrain, A. A., Hazzazi, M. S., and Abdelghany, T. M. (2023a). "Inhibitory potential of rutin and rutin nano-crystals against *Helicobacter pylori*, colon cancer, hemolysis and Butyrylcholinesterase *in vitro* and *in silico*," *Applied Biology and Chemistry* 66, article 79. DOI: 10.1186/s13765-023-00832-z

Qanash, H., Bazaid, A. S., Aldarhami, A., Alharbi, B., Almashjary, M. N., Hazzazi, M. S., Felemban, H. R., and Abdelghany, T. M. (2023b). "Phytochemical characterization and efficacy of *Artemisia judaica* extract loaded chitosan nanoparticles as inhibitors of cancer proliferation and microbial growth," *Polymers* 15(2), article 391. DOI: 10.3390/polym15020391

Qanash, H., Yahya, R., Bakri, M. M., Bazaid, A. S., Qanash, S., Shater, A. F., and Abdelghany, T. M.. (2022). " Anticancer, antioxidant, antiviral and antimicrobial activities of Kei Apple (*Dovyalis caffra*) fruit," *Scientific Reports* 12(1), article 5914. . DOI: 10.1038/s41598-022-09993-1

Qi, Y., Wang, L., Wang, N., Wang, S., Zhu, X., Zhao, T., and Jiang, Q. (2024). "A comprehensive review of the botany, phytochemistry, pharmacology, and toxicology of *Murrayae folium* et Cacumen," *Frontiers in Pharmacology* 15, article ID 1337161. DOI: 10.3389/fphar.2024.1337161

Selim, S., Alruwaili, Y., Ejaz, H., Abdalla, A. E., Almuhayawi, M. S., Nagshabandi, M. K., and Abdelghany, T. M. (2024). "Estimation and action mechanisms of cinnamon bark via oxidative enzymes and ultrastructures as antimicrobial, anti-biofilm,

antioxidant, anti-diabetic, and anticancer agents," *BioResources* 19(4), 7019-7041. DOI: 10.15376/biores.19.4.7019-7041

Sonter, S., Mishra, S., Dwivedi, M. K., and Singh, P. K. (2021). "Chemical profiling, *in vitro* antioxidant, membrane stabilizing and antimicrobial properties of wild growing *Murraya paniculata* from Amarkantak (MP)," *Scientific Reports* 11(1), article 9691. DOI: 10.1038/s41598-021-87404-7

Spósito, L., Fonseca, D., Carvalho, S. G., Sábio, R. M., Marena, G. D., Bauab, T. M., and Chorilli, M. (2024). "Engineering resveratrol-loaded chitosan nanoparticles for potential use against *Helicobacter pylori* infection," *European Journal of Pharmaceutics and Biopharmaceutics* 199, article ID 114280. DOI: 10.1016/j.ejpb.2024.114280

Stepanović, S., Vuković, D., Hola, V., Di Bonaventura, G., Djukić, S., Cirković, I., and Ruzicka, F. (2007). "Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by Staphylococci," *Apmis* 115(8), 891-899. DOI: 10.1111/j.1600-0463.2007.apm_630.x

von Recklinghausen, U., von Recklinghausen, G., and Ansorg, R. (1998). "Haemolytic activity of *Helicobacter pylori* against human and animal erythrocytes," *Zentralblatt für Bakteriologie* 288(2), 207-215. DOI: 10.1016/S0934-8840(98)80041-8

Waghmare, A. N. (2015). "Evaluation of ethanolic fruit extract of *Murraya koenigii* for its anti-ulcer activity against ethanol and pylorus ligation induced gastric ulcer model," *International Journal of Advances in Pharmaceutical Research* 6(05), 118-123.

Yahya, R., Al-Rajhi, A. M. H., Alzaid, S. Z., Al Abboud, M. A., Almuhayawi, M. S., Al Jaouni, S. K., and Abdelghany, T. M. (2022). "Molecular docking and efficacy of *Aloe vera* gel based on chitosan nanoparticles against *Helicobacter pylori* and its antioxidant and anti-inflammatory activities," *Polymers* 14, article 2994. DOI: 10.3390/POLYM14152994

Yohanes, R., Harneti, D., Supratman, U., Fajriah, S., Rudiana, T. (2023). "Phytochemistry and biological activities of *Murraya* species," *Molecules* 28(15), article 5901. DOI: 10.3390/molecules28155901

Zaatout, H. H., Al-Koriety, H. A., Omran, G. A., and Beltagy, A. M. (2022). "Profiling of the essential oil of *Murraya paniculata* cultivated in Egypt over four different seasons using gas chromatography-mass spectrometry and screening for antimicrobial and anticancer activities," *Egyptian Journal of Chemistry* 65(12), 751-759 DOI: 10.21608/ejchem.2022.148077.6408

Zhu, C. H., Lei, Z. L, and Luo, Y. P. (2015). "Studies on antioxidative activities of methanol extract from *Murraya paniculata*," *Food Science and Human Wellness* 4(3), 108-114. DOI: 10.1016/j.fshw.2015.07.001

Article submitted: January 26, 2025; Peer review competed: March 1, 2025; Revised version received: March 4, 2025 and accepted; Published: March 12, 2025.
DOI: 10.15376/biores.20.2.3299-3314