Valorization of *Pimenta racemosa* Essential Oils and Extracts: GC-MS and LC-MS Phytochemical Profiling and Evaluation of *Helicobacter pylori* Inhibitory Activity

Iriny M. Ayoub 1,* , Marwa M. Abdel-Aziz 2, Sameh S. Elhady 3, Alaa A. Bagalagel 4, Rania T. Malatani 4,* and Wafaa M. Elkady 5,*

1 Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt
2 Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo 11651, Egypt
3 Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia
4 Department of Pharmacy Practice, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia
5 Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Future University in Egypt, Cairo 11835, Egypt

*Correspondence: irinyayoub@pharma.asu.edu.eg (I.M.A.); welkady@fue.edu.eg (W.M.E.)

**Abstract:** *Pimenta racemosa* is a commonly known spice used in traditional medicine to treat several ailments. In this study, comprehensive phytochemical profiling of the essential oils and methanol extracts of *P. racemosa* leaves and stems was performed, alongside assessing their potential *Helicobacter pylori* inhibitory activity in vitro and in silico. The essential oils were chemically profiled via GC-MS. Moreover, the methanol extracts were profiled using HPLC-PDA-ESI-MS/MS. The antibacterial activity of the essential oils and methanol extracts against *H. pylori* was determined by adopting the micro-well dilution method. GC-MS analysis unveiled the presence of 21 constituents, where eugenol represented the major component (57.84%) and (59.76%) in both leaves and stems of essential oils, respectively. A total of 61 compounds were annotated in both leaves and stems of *P. racemosa* methanolic extracts displaying richness in phenolic compounds identified as (epi)catechin and (epi)gallocatechin monomers and proanthocyanidins, hydrolyzable tannin derivatives (gallotannins), flavonoids, and phenolic acids. The stem essential oil showed the most promising inhibitory effects on *H. pylori*, exhibiting an MIC value of 3.9 µg/mL, comparable to clarithromycin with an MIC value of 1.95 µg/mL. Additionally, in silico molecular modeling studies revealed that decanal, eugenol, terpineol, delta-cadinene, and amyl vinyl showed potential inhibitory activity on *H. pylori* urease as demonstrated by high-fitting scores indicating good binding to the active sites. These findings indicate that *P. racemosa* comprises valuable phytochemical constituents with promising therapeutic effects, particularly the stem, an economic agro-industrial waste.

**Keywords:** *Pimenta racemosa*; LC-MS; GC-MS; anti-*Helicobacter pylori*; molecular modeling; drug discovery; health care

1. Introduction

*Pimenta racemosa* (Mills) J.W. Moore, a member of the family Myrtaceae, is one of the most commonly known spices. It is also called the Bay or Lemon Bay Rum Spice tree [1]. It is up to 25 ft high. This species is native to the Caribbean region but can be adopted in different warm climates [2]. Traditionally in Egypt and other countries as Cuba, Bahamas and Jamaica, *P. racemosa* leaves have been prepared as tea and used to treat flatulence, colds, or fever [3]. This activity could be assigned to the richness of essential oils and phenolic constituents [4]. The anti-inflammatory and antinociceptive activities of the aqueous leaves extract were also reported [3]. Furthermore, *P. racemosa* exhibited
antimicrobial, antioxidant, analgesic, and anti-inflammatory properties [5]. Polyphenolics have been generally recognized for their beneficial effects on several health problems, including diabetes, cardiovascular diseases, neurodegenerative diseases, cancers, and osteoporosis [6]. This is usually due to their free radical scavenging activity, reducing the risk of different ailments [7]. In addition, essential oils have long been known for their therapeutic activities and their use in the food and cosmetics industries [5].

*Helicobacter pylori* is a gram-negative bacteria with a spiral shape responsible for several illnesses, including asymptomatic gastritis, chronic inflammation, and gastric cancer [8]. Despite the improvements in antimicrobial treatments worldwide, there is still no model therapy for *H. pylori* infection due to drug resistance and numerous side effects of the antibiotics [9]. Plants have usually been considered alternative or complementary medicine for many ailments. Research has been conducted to find a suitable cure for *H. pylori* from natural sources. The research literature revealed that *P. racemosa* leaves and fruits exhibited promising anti-inflammatory and antimicrobial activities [3,5,10]. Moreover, *P. racemosa* bark also showed strong anti-schistosomal activity [11].

Leaves and fruits are the most widely used parts of *P. racemosa* [1]. The stem could be considered a natural and economic agro-industrial waste. However, it could play a role in phytotherapy and the pharmaceutical industry. Hence, the present study aimed to compare the phytochemical profile of the methanol extracts and essential oils of both leaves and stems. Moreover, the potential anti-*H. pylori* inhibitory activity was assessed in vitro, alongside the evaluation of the in silico inhibitory activity of the identified phytoconstituents.

2. Results and Discussion

2.1. GC/MS Analysis of the Essential Oils

Hydrodistillation of fresh *P. racemosa* leaves and stems yielded 0.61% and 0.06% (w/w) essential oil, respectively. Essential oils were pale yellow with a distinctive strong aromatic clove-like odor. GC/MS analysis of the essential oils identified 21 volatile components in the leaves, accounting for 99.57% of the oil composition. Meanwhile, 19 compounds were identified in *P. racemosa* stem essential oil, representing 98.82% of the total oil composition (Table 1, Figures 1 and 2).

### Table 1. Phytochemical profile of the essential oils of *P. racemosa* leaf (PRL-EO) and stem (PRS-EO).

| No. | Rt (min) | Compounds a | Molecular Formula | \( R_{E} \) b | \( R_{I} \) c | Content % PRL-EO | PRS-EO |
|-----|----------|--------------|------------------|---------------|---------------|-----------------|--------|
| 1   | 7.42     | \( \alpha \)-Thujene | C_{10}H_{16}     | 917           | 917           | 0.04            | -      |
| 2   | 7.61     | \( \alpha \)-Pinene | C_{10}H_{16}     | 938           | 938           | 0.89            | 0.48   |
| 3   | 8.92     | \( \beta \)-Pinene | C_{10}H_{16}     | 973           | 973           | 0.06            | -      |
| 4   | 9.05     | 1-Octen-3-ol (Amyl vinyl carbinol) | C_{8}H_{16}O | 976           | 976           | 1.51            | 0.45   |
| 5   | 9.39     | \( \beta \)-Myrcene | C_{10}H_{16}     | 990           | 990           | 16.30           | 17.43  |
| 6   | 9.77     | \( \alpha \)-Phellandrene | C_{10}H_{16}     | 1004          | 1004          | 1.02            | 0.58   |
| 7   | 10.16    | 2-Carene | C_{10}H_{16}     | 1015          | 1014          | 0.11            | 0.07   |
| 8   | 10.42    | \( \rho \)-Cymene | C_{10}H_{14}     | 1023          | 1023          | 0.43            | 0.71   |
| 9   | 10.55    | Limonene | C_{10}H_{16}     | 1029          | 1029          | 7.20            | 7.70   |
| 10  | 10.63    | Eucalyptol | C_{10}H_{18}O   | 1033          | 1033          | 7.31            | 5.45   |
| 11  | 11.16    | \( \beta \)-Ocimene | C_{10}H_{16}     | 1037          | 1037          | 0.36            | 0.92   |
| 12  | 11.50    | \( \gamma \)-Terpinene | C_{10}H_{16}     | 1060          | 1060          | 0.12            | 0.08   |
| 13  | 12.43    | Terpinolene | C_{10}H_{16}     | 1090          | 1090          | 0.07            | 0.07   |
| 14  | 15.26    | Terpinen-4-ol | C_{10}H_{18}O   | 1179          | 1179          | 0.34            | 0.43   |
| 15  | 15.66    | \( \alpha \)-Terpineol | C_{10}H_{18}O   | 1193          | 1193          | 1.68            | 1.49   |
| 16  | 16.05    | \( n \)-Decanal(Capralddehyde) | C_{10}H_{20}O   | 1204          | 1204          | 0.13            | 0.24   |
| 17  | 17.61    | Chavicol(p-Allylphenol) | C_{9}H_{18}O    | 1259          | 1259          | 4.18            | 2.60   |
| 18  | 20.65    | Eugenol | C_{10}H_{12}O_{2} | 1359         | 1359          | 57.84           | 59.76  |
| 19  | 22.27    | Caryophyllene | C_{15}H_{24}    | 1424          | 1424          | 0.05            | 0.16   |
| 20  | 24.96    | \( \delta \)-Cadinene | C_{15}H_{24}    | 1529          | 1529          | 0.05            | 0.10   |
Table 1. Cont.

| No. | Rt (min) | Compounds a | Molecular Formula | R_I<sup>b</sup> | R_I<sup>c</sup> | Content % |
|-----|---------|-------------|------------------|---------------|---------------|-----------|
|     |         |             |                  | PRL-EO        | PRS-EO        |           |
| 21  | 26.56   | Viridiflorol | C<sub>15</sub>H<sub>24</sub> | 1591          | 1591          | 26.48     |
|     |         |             |                  |               |               | 28.04     |

Monoterpenes hydrocarbons
Oxygenated monoterpenes
Sesquiterpenes hydrocarbons
Phenyl propanoids
Others
Total identified %

\(^a\) Compounds are listed based on their elution on an RTX-5MS column. \(^b\) R_I<sup>b</sup>, retention indices were determined experimentally on RTX-5MS column relative to a standard hydrocarbon mixture (C8–C28).\(^c\) R_I<sup>c</sup>, published retention indices. Identification of all the compounds was carried out based on a comparison of their mass spectral data (MS) and retention indices (RI) with those of the NIST Mass Spectral Library (2011), Wiley Registry of Mass Spectral Data 8th edition, and the literature.

Figure 1. GC-MS chromatograms of *P. racemosa* leaf (A) and stem (B) essential oils on the Rtx-5MS column.
Phenyl propanoids represented the most abundant class identified herein. Eugenol was the main compound recognized in both leaf and stem essential oils representing 57.84% and 59.76% of the total oil composition, respectively. Chavicol, a key marker for *P. racemosa* essential oil [1], was detected in both leaves (4.18%) and stems’ (2.6%) essential oils. Oxygenated monoterpenic hydrocarbons could be detected in both leaves (9.33%) and stems (7.37%), represented by eucalyptol, α-terpineol, and terpinen-4-ol. Monoterpenes were also found in a considerable amount, representing 26.48% in leaves and 28.04% in stems, rich in β-Myrcene and D-limonene. However, sesquiterpene hydrocarbons were present in trace amounts. The major components identified are represented in Figure 2.

Our results complied with earlier reports on the chemical composition of *P. racemosa* growing in different regions. Earlier reports showed the effect of the geographical source on essential oil composition, where *P. racemosa* leaves from two different locations in Benin yielded a range from 0.9 to 2.4% (w/w) [12], whereas that from North India yielded 0.02% (w/w) [13]. Besides the essential oils from *P. racemosa* leaves grown in Venezuela, different locations in Benin, India, and Egypt showed richness in eugenol content with variable percentages [1,12–14].

2.2. HPLC-PDA-ESI-MS/MS Analysis

Metabolic profiling of both leaves and stems of *P. racemosa* methanolic extracts was achieved using HPLC-PDA-ESI-MS/MS. Sixty-one compounds were annotated. Most of them belonged to the class of polyphenolics, including (epi)catechin and (epi)gallocatechin monomers and proanthocyanidins (the oligomeric polyflavan-3-ol). Moreover, hydrolyzable tannin derivatives (gallotannins), phenolic acids & flavonoids were also annotated (Table 2 and Figure 3). Compounds were tentatively identified based on the mass of the molecular ion peaks, their tandem mass data, considering fragmentation patterns, neutral mass losses, UV spectra, and comparison with bibliographic references [15–19]. Chemical structures of representative compounds identified in *P. racemosa* leaf and stem methanol extracts are displayed in Figure 4.

2.2.1. Proanthocyanidins

Several classes of proanthocyanidins (PAs) could be detected herein. Proanthocyanidins constituted of (epi)catechin units are known as procyanidins, while proanthocyanidins composed of (epi)gallocatechin units were designated as prodelphinidins. HPLC-PDA-ESI-
MS/MS analysis showed a series of derivatives of polyflavan-3-ol. The pseudomolecular ion [M−H]− at m/z 289 was tentatively identified as (epi)catechin, while [M−H]− ion at m/z 305 was annotated as (epi)gallocatechin. Proanthocyanidins, including dimers, trimers, tetramers, and pentamers, alongside different galloylated procyanidins and prodelphinidins, were annotated in *P. racemosa* leaves and stem extracts.

![Figure 3](image-url)

**Figure 3.** (A) UPLC-ESI-MS base peak chromatogram of *P. racemosa* leaves methanol extract (PRL-Me) in the negative ion mode. (B) UPLC-ESI-MS base peak chromatogram of *P. racemosa* stem methanol extract (PRS-ME) in the negative ion mode. Peaks are numbered relative to compounds listed in Table 2.
Figure 3. (A) UPLC-ESI-MS base peak chromatogram of P. racemosa leaves methanol extract (PRM-ME) in the negative ion mode. Peaks are numbered relative to compounds listed in Table 2.

2.2.2. Organic Acids, Phenolic Acids and Their Derivatives

Herein, several organic acids and phenolic acids were annotated. Among them, compound 3 showed a pseudomolecular ion \([M−H]−\) at \(m/z\) 191 corresponding to a deprotonated quinic acid \([\text{quinic acid−H}]−\) and a product ion at \(m/z\) 173 representing \([\text{quinic acid−H−H}_2\text{O}]−\) ion was observed [20]. Compound 8 showed a parent ion at \(m/z\) 169, typical for gallic acid compared to its mass spectra in literature [10]. Compounds 51 and 56 were annotated as gallic acid dihexoside isomers exhibiting a base peak of the deprotonated molecule at \(m/z\) 493 and a product ion at \(m/z\) 169 of deprotonated gallic acid resulting from the loss of the two hexose units [20].

Moreover, six gallotannins were identified; tri-O-galloyl-hexoside (33 and 38) showed pseudomolecular ion at \(m/z\) 635; and fragment ions at \(m/z\) 483 and 465, corresponding to the loss of a galloyl (digalloylglucose product ion) and a gallate unit respectively. The same principle was applied to recognize pseudomolecular ions \(m/z\) 787, 939 corresponding to tetra-O-galloyl-hexoside (42 and 44) and penta-O-galloyl-hexoside (46 and 50), respectively.

2.2.3. Flavonoids

Quercetin glycosides were the main identified flavonol glycosides. Compound 45 was identified as quercetin-O-hexoside displaying a pseudomolecular ion at \(m/z\) 463. Compound 54 was annotated as quercetin-O-pentoside displaying a pseudomolecular ion at \(m/z\) 433 and quercetin-O-deoxyhexoside (55) at \(m/z\) 447. All revealed quercetin...
aglycone displaying a characteristic product ion at \( m/z \) 301. The sugar moieties could be annotated by calculating the losses of the sugar part, that is, 162 amu (hexose), 152 amu (pentose), and 146 amu (deoxyhexose) [21]. Additionally, compound (57) exhibited a pseudomolecular ion peak at \( m/z \) 625 and a base peak at \( m/z \) 463, inferring the loss of one hexose unit, and a product ion at \( m/z \) 301, implying the loss of a second hexose and denoting quercetin aglycone \([M-H-162-162]^-\). Thus, compound 57 was tentatively identified as quercetin-di-O-hexoside [22].

2.3. Evaluation of Anti-H. pylori Activity

*P. racemosa* leaves and stems essential oils displayed higher *H. pylori* inhibitory activity than the corresponding methanol extracts (Table 3). Interestingly, the essential oil isolated from the stems elicited the highest activity exhibiting a MIC of 3.9 \( \mu \)g/mL comparable to clarithromycin (MIC 1.95 \( \mu \)g/mL).

The promising inhibitory activity of essential oils perceived herein could be ascribed to the high content of phenyl propanoids, monoterpenes, and oxygenated monoterpenes. Eugenol, the main identified compound in both essential oils, could be the reason for such a great activity. It can decrease the viability of *H. pylori*, regardless of the strain [34]. Furthermore, eugenol can generate morphological alterations in some enzymes in the cell wall because of hydrogen bond formation between the phenolic hydroxyl group and the enzyme [14].

Reports have stated that terpenes have a bactericidal effect; this could be due to their nature [35]. Their solubility in water is weak to moderate, but they are readily dissolved in the lipid layer of the biological membranes. This could affect the cell wall permeability, disrupting the lipid structure and inhibiting microbial metabolism. Additionally, monoterpenes have anti-ulcerogenic and healing effects [35]. The anti-*H. pylori* activity of the essential oils is usually correlated to certain terpenoid components such as \( \alpha \)-pinene, \( \beta \)-pinene, and myrcene [8]. Myrcene is present in a considerable amount in both essential oils isolated from leaves and stems, 16.30 and 17.43%, respectively.

Moreover, polyphenolic compounds and tannin content in the methanol extracts of both leaves and stems could be accountable for the observed anti-*H. pylori* activity [9].

2.4. In Silico Evaluation of Anti-H. pylori Activity

*P. racemosa* essential oils exhibited notable *H. pylori* inhibitory activity; thus, an in-silico study was conducted to validate the obtained results. *H. pylori* urease crystal structure was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/ accessed on 20 June 2022) provided with HAE (PDB ID 1E9Y; 3.00 Å). The acetohydroxamic acid (HAE), a co-crystallized ligand, was utilized to identify the amino acid residues constituting the urease active binding site. The 1E9Y protein was used in the docking research. The amino acid Arg338 was involved in creating bonds with the chemicals investigated. The computed free binding energies of phytoconstituents identified in *P. racemosa* essential oils ranged from −29.76 to −13.31 kcal/mol employing both pH-based and rule-based ionization modes (Table 4). The pH-based ionization mode mimics the physiological pH [36]. Meanwhile, the ionization of functional groups as well as amino acid moieties at the active site is explained well by the rule-based ionization method [37]. These values indicated that the identified phytochemicals bind well to the urease active site. Decanal, eugenol, terpineol, \( \delta \)-cadinene, and amyl vinyl had the best affinity and orientation (Figure 5). These compounds were found in both essential oils displaying a higher percentage in the stem essential oil, representing 0.24, 59.76, 1.49, 0.10, and 0.45%, respectively, of the total oil composition.

The occurrence of alcoholic, phenolic, and ketonic groups in the identified volatile oil components allows the formation of hydrogen and ionic bonds with various amino acids. These interactions can efficiently engage with the proteins’ binding sites, causing their 3D shape (conformation) to be disrupted, resulting in activity inhibition [38]. Consequently, it is possible to conclude that the phytoconstituents discovered herein can act as potential inhibitors of *H. pylori* urease.
Table 2. Identified metabolites in *P. racemosa* leaf methanol extract (PRL-ME) and stem methanol extract (PRS-ME) using HPLC-PDA-ESI-MS/MS in negative ion mode.

| Compound Description | Rt (min) | UV λ (nm) | [M – H]− (PRL-ME) | (PRS-ME) | Fragment Ions (MS/MS) | Class | Ref. |
|----------------------|----------|-----------|-------------------|----------|-----------------------|-------|------|
| 1. B-type proanthocyanidin pentamer | 1.37 | 279 | 1425 | - | + | 1257, 1187, 1155 | Proanthocyanidin | [23] |
| 2. Caffeoylglucaric acid | 1.42 | 236, 270 | 371 | + | - | 325, 191 | Phenolic acid | |
| 3. Quinic acid | 1.47 | 236, 265 | 191 | + | - | 173 | Organic acid | [20] |
| 4. B-type proanthocyanidin trimer (EC→EG→EG) | 1.52 | 267 | 897 | + | - | 879, 711, 693, 543, 407, 289 | Proanthocyanidin | |
| 5. B-type proanthocyanidin dimer (EC→EG) | 1.61 | 275 | 593 | + | - | 575, 467, 441, 305, 289 | Proanthocyanidin | |
| 6. Galloylated prodolphinidin dimer (EG→EG)2 g | 4.17 | 273 | 914 | + | - | 727, 559, 423, 305 | Proanthocyanidin | [23] |
| 7. (Epigallocatechin | 4.28 | 273 | 305 | + | - | 287, 261, 221, 19, 179, 165, 125 | Flavonoid | [23,24] |
| 8. Gallic acid | 4.68 | 270 | 169 | + | - | 125 | Phenolic acid | [25] |
| 9. (Epigallocatechin | 5.46 | 273 | 305 | + | - | 287, 261, 221, 19, 179, 165, 125 | Flavonoid | |
| 10. B-type Procyanidin dimer (EC→EC) | 5.71 | 274 | 577 | + | + | 559, 451, 425, 407, 299, 289, 287 | Proanthocyanidin | [23,26] |
| 11. B-type Prodelphinidin dimer (EG→EG) | 5.96 | 274 | 609 | + | - | 591, 483, 441, 423, 305 | Proanthocyanidin | [23] |
| 12. Galloylated procyanidin dimer (EC→EC)2 g | 6.22 | 276 | 881 | + | - | 729, 711 | Proanthocyanidin | [27] |
| 13. B-type proanthocyanidin trimer EG→EG→EC | 6.63 | 277 | 897 | + | + | 879, 771, 729, 711, 593, 407, 289 | Proanthocyanidin | [27,28] |
| 14. B-type procyanidin trimer (EC→EC→EC) | 6.83 | 278 | 865 | + | + | 847, 695, 577, 449, 407, 287 | Proanthocyanidin | [29] |
| 15. Prodelphinidin trimer (EG→EG→EG) | 7.04 | 277 | 913 | + | - | 895, 787, 745, 727, 609, 559, 483, 305 | Proanthocyanidin | [23] |
| 16. B-type procyanidin trimer (EC→EC→EC) | 7.09 | 277 | 865 | + | - | 847, 695, 577, 407, 287 | Proanthocyanidin | |
| 17. A-type procyanidin trimer EC→EC→EC | 7.11 | 277 | 863 | - | + | 737, 711, 693, 591, 575, 289 | Proanthocyanidin | [29] |
| 18. B-type Procyanidin dimer (EC→EC) | 7.14 | 277 | 577 | + | - | 559, 451, 425, 407, 299, 289, 287 | Proanthocyanidin | [27] |
| 19. Proanthocyanidin dimer EC→EG | 7.76 | 278 | 593 | + | + | 575, 467, 425, 407, 305, 289, 245 | Proanthocyanidin | [27] |
| 20. (Epigallocatechin | 8.2 | 278 | 305 | + | - | 287, 261, 221, 19, 179 | Flavonoid | [23,24] |
| 21. B-type proanthocyanidin trimer EG→EG→EC | 8.3 | 277 | 897 | + | - | 879, 771, 729, 711, 593, 577, 305, 289 | Proanthocyanidin | |
| 22. B-type Procyanidin dimer (EC→EC) | 8.55 | 277 | 577 | + | - | 559, 451, 425, 407, 299, 289, 287 | Proanthocyanidin | |
| 23. B-type proanthocyanidin dimer (EC→EC) | 8.79 | 278 | 593 | + | - | 575, 467, 441, 407, 305, 289 | Proanthocyanidin | |
| 24. B-type proanthocyanidin trimer EC→EC→EG | 9.54 | 278 | 881 | + | - | 755, 729, 711, 695, 593, 425, 407, 289 | Proanthocyanidin | [23] |
| Compound | Rt (min) | UV λ (nm) | [M – H]− (PRL-ME) | (PRS-ME) | Fragment Ions (MS/MS) | Class | Ref. |
|----------|----------|-----------|-------------------|---------|----------------------|-------|------|
| 25. (Ep)catechin | 9.73 | 278 | 289 | + | + | 245, 205, 179 | Flavonoid | [24,29] |
| 26. B-type procyanidin tetramer EC→EC→EC→EC | 9.81 | 278 | 577 | - | + | 559, 451, 425, 407, 299, 289, 287 | Proanthocyanidin | [23] |
| 27. B-type Procyanidin dimer (EC→EC) | 9.9 | 278 | 577 | + | - | 559, 451, 425, 407, 299, 289, 287 | Proanthocyanidin | [23] |
| 28. B-type procyanidin tetramer EC→EC→EC→EC | 10.21 | 278 | 1153 | + | + | 983, 863, 695, 575 | Proanthocyanidin | [23] |
| 29. B-type proanthocyanidin dimer (EC→EC) | 10.26 | 278 | 593 | + | + | 575, 467, 441, 407, 305, 289 | Proanthocyanidin | [27] |
| 30. (Ep)catechin | 10.82 | 278 | 289 | + | + | 245, 205, 179, 151 | Flavonoid | [24,30] |
| 31. B-type procyanidin trimer EC→EC→EC→2 g | 10.86 | 278 | 865 | + | + | 847, 695, 577, 575, 407, 289 | Proanthocyanidin | [23] |
| 32. Tri-O-galloyl-hexoside | 11.00 | 278 | 729 | + | + | 711, 603, 577, 559, 425, 407, 289 | Proanthocyanidin | [26] |
| 33. Tri-O-galloyl-hexoside isomer | 11.15 | 278 | 1169 | + | - | 1042, 890, 864, 703, 633, 443, 424 | Proanthocyanidin | [23] |
| 34. B-type proanthocyanidin trimer EA→EC | 11.31 | 278 | 559 | + | - | 541, 453, 407, 321, 289 | Proanthocyanidin | [23] |
| 35. B-type proanthocyanidin pentamer EC→EC→EC→EC→EC | 11.49 | 278 | 1441 | - | + | 1421, 1315, 1271, 1153, 1151, 1027, 865, 863, 739, 575 | Proanthocyanidin | [23] |
| 36. B-type procyanidin trimer (EC→EC) | 11.75 | 278 | 1017 | + | - | 999, 891, 865, 739, 729, 575, 425, 407 | Proanthocyanidin | [23] |
| 37. Tri-O-galloyl-hexoside isomer | 12.38 | 278 | 635 | + | - | 483, 465 | Gallotannin | [20] |
| 39. B-type Procyanidin dimer (EC→EC) | 12.43 | 278 | 577 | + | - | 559, 451, 425, 407, 299, 289, 287 | Proanthocyanidin | [23] |
| 40. Galloylated procyanidin dimer (EC→EC)g | 12.66 | 278 | 729 | + | + | 711, 603, 577, 559, 425, 407, 289 | Proanthocyanidin | [23] |
| 41. Quercetin-O-galloyl hexoside | 12.79 | 279 | 577 | + | + | 559, 451, 425, 407, 299, 289, 287 | Proanthocyanidin | [23] |
| 42. Tetra-O-galloyl hexoside | 13.12 | 277 | 787 | + | - | 635, 617, 465, 331, 313 | Gallotannin | [20] |
| 43. Galloylated procyanidin trimer (EC→EC→EC)g | 13.82 | 278 | 1017 | + | + | 999, 891, 865, 847, 739, 729, 695, 677, 575 | Proanthocyanidin | [23] |
| 44. Tetra-O-galloyl hexoside isomer | 14.57 | 274 | 787 | + | - | 635, 617, 465, 331, 313 | Gallotannin | [20] |
| 45. Quercetin-O-galloyl hexoside | 14.59 | 274, 349 | 463 | + | - | 301, 179, 151 | Flavonoid | [20] |
| 46. Pentahydroxyflavone-C-hexoside | 14.80 | 274 | 939 | + | - | 921, 787, 769, 635, 617, 555, 465, 447, 313, 295 | Gallotannin | [20] |
| 47. Quercetin-O-galloyl hexoside | 15.15 | 272, 351 | 615 | + | - | 463, 301, 300, 179 | Flavonoid | [31] |
| 48. Pentahydroxyflavone-C-hexoside | 15.70 | 266, 353 | 463 | + | - | 445, 373, 343, 301, 179, 151, 133 | Flavonoid | [31] |
| Compound                              | Rt (min) | UV \(\lambda\) (nm) | [M – H]⁻ | (PRL-ME) | (PRS-ME) | Fragment Ions (MS/MS) | Class          | Ref.   |
|---------------------------------------|----------|----------------------|----------|----------|----------|------------------------|----------------|--------|
| 49. Pentahydroxyflavone-C-pentoside   | 15.83    | 271, 352             | 433      | +        | -        | 415, 373, 343, 301, 300, 287, 251, 193, 179, 151, 125 | Flavonoid      |        |
| 50. Penta-O-galloyl hexoside          | 15.96    | 268, 353             | 939      | +        | -        | 921, 787, 769, 635, 617, 555, 465, 447, 313 | Gallotannin    | [20]   |
| 51. Gallic acid dihexoside            | 16.11    | 266                  | 493      | +        | +        | 341, 313, 179, 169    | Phenolic acid  | [20]   |
| 52. Quercetin-O-deoxyhexoside         | 16.55    | 255, 353             | 447      | +        | +        | 301, 255, 179, 151   | Phenolic acid  | [10,20]|
| 53. Ellagic acid-O-pentoside          | 16.88    | 266, 351             | 433      | +        | -        | 301, 191, 169        | Phenolic acid  |        |
| 54. Quercetin-O-pentoside             | 16.92    | 268, 349             | 433      | +        | +        | 415, 301, 300, 179, 151 | Phenolic acid |        |
| 55. Quercetin-O-deoxyhexoside         | 17.05    | 264, 348             | 447      | +        | -        | 301, 255, 179, 151   | Phenolic acid  |        |
| 56. Gallic acid dihexoside isomer     | 17.21    | 273                  | 493      |          |          | 341, 313, 179, 169, 151 | Phenolic acid  | [20]   |
| 57. Quercetin-di-O-hexoside           | 18.25    | 271, 350             | 625      | +        | -        | 463, 301, 179        | Flavonoid      | [22]   |
| 58. Gallic acid derivative            | 19.23    | 268, 348             | 477      | +        | -        | 313, 301, 223, 169   | Phenolic acid  | [31–33]|
| 59. Gallic acid derivative            | 19.38    | 268, 336             | 447      | +        | -        | 313, 301, 269, 169, 125 | Phenolic acid |        |
| 60. Quercetin O-acetyl-deoxyhexoside  | 20.25    | 275, 350             | 489      | +        | -        | 471, 447, 301, 300, 179, 151 | Phenolic acid |        |
| 61. Unidentified                      | 27.03    | 275                  | 313      | +        | -        | 313, 298, 283, 269, 257, 243, 227, 163, 135, 113 | Flavonoid      |        |
| 62. Unidentified                      | 33.32    | 291, 311             | 289      | +        | -        | 245, 163, 119        |                |        |
| 63. Unidentified                      | 36.67    | 279                  | 325      | +        | +        | 325, 310, 307, 295, 281, 252, 191 |                |        |
| 64. Hydroxypalmitic acid              | 48.09    | 271                  |          | +        | -        | 271, 253, 225        | Fatty acid     |        |
| 65. Unidentified                      | 55.83    | 817                  |          | -        | +        | 796, 711             |                |        |
Table 3. Anti-helicobacter pylori activity of essential oils and methanol extracts of *P. racemosa* leaves and stems.

| Sample Conc. (µg/mL) | Inhibition % |
|---------------------|-------------|
|                     |  PRL-EO     | PRS-EO      | PRL-ME | PRS-ME     | Clarithromycin |
| 125                 | 100 ± 0     | 100 ± 0     | 46.52 ± 0.58 | 100 ± 0     | 100 ± 0        |
| 62.5                | 100 ± 0     | 100 ± 0     | 19.85 ± 1.8 | 100 ± 0     | 100 ± 0        |
| 31.25               | 100 ± 0     | 100 ± 0     | 5.74 ± 2.3  | 100 ± 0     | 100 ± 0        |
| 15.63               | 83.25 ± 3.1 | 100 ± 0     | 0       | 100 ± 0    | 100 ± 0        |
| 7.81                | 64.85 ± 1.2 | 100 ± 0     | 0       | 86.32 ± 1.5 | 100 ± 0        |
| 3.9                 | 39.17 ± 2.5 | 100 ± 0     | 0       | 55.34 ± 2.4 | 100 ± 0        |
| 1.95                | 23.14 ± 1.3 | 92.14 ± 0.95| 0       | 34.38 ± 1.3 | 100 ± 0        |
| 0.98                | 9.32 ± 1.2  | 78.95 ± 1.3 | 0       | 26.34 ± 0.69| 92.45 ± 1.2    |
| 0.48                | 0           | 56.38 ± 1.6 | 0       | 19.3 ± 0.95| 87.65 ± 0.58   |
| 0.24                | 0           | 37.28 ± 2.4 | 0       | 7.2 ± 0.83 | 81.35 ± 1.5    |
| 0                   | 0           | 0           | 0       | 0          | 0              |
| MIC (µg/mL)         | 31.25       | 3.9         | >125    | 15.63      | 1.95           |

All experiments were carried out in triplicate. Results are expressed as mean ± SD.

Table 4. Free binding energies (ΔG) calculated in kcal/mol of the identified phytoconstituents within *H. pylori* urease (1E9Y) active site, adopting pH-based and rule-based ionization techniques using Discovery Studio 4.5.

| Compound          | Free Binding Energy (ΔG) (Kcal/mol) |
|-------------------|-------------------------------------|
|                   | pH Based               | Rule-Based            |
| Co-crystalized ligand (HAE) | −22.51                 | −22.51                  |
| Decanal           | −29.76                 | −29.76                  |
| Eugenol           | −29.44                 | −29.44                  |
| α-Terpineol       | −23.09                 | −23.09                  |
| δ-Cadinene        | −22.68                 | −22.68                  |
| Amyl vinyl        | −22.63                 | −22.63                  |
| Chavicol          | −21.97                 | −21.97                  |
| Ocimene           | −21.91                 | −21.91                  |
| Myrcene           | −21.63                 | −21.63                  |
| Terpinolene       | −20.24                 | −20.24                  |
| Terpinene         | −19.76                 | −19.76                  |
| Phellandrene      | −19.71                 | −19.71                  |
| Caryophyllene     | −18.83                 | −18.83                  |
| Limonene          | −18.71                 | −18.71                  |
| Cymene            | −18.51                 | −18.51                  |
| Pinene            | −15.48                 | −15.48                  |
| Eucalyptol        | −13.31                 | −13.31                  |
Co-crystallized ligand (HAE)

Decanal

Eugenol

Figure 5. Cont.
Figure 5. Two-dimensional and three-dimensional binding modes of identified compounds in *P. racemosa* leaf and stem essential oils within *H. pylori* urease (1E9Y) active site employing C-docker protocol.
3. Materials and Methods

3.1. Plant Material

*P. racemosa* leaves and stems were collected in June 2017 from Orman Botanical Garden in Giza, Egypt, and identified by Mrs. Trease Labib, a Plant Taxonomy Consultant at the Egyptian Ministry of Agriculture and Land Reclamation. A voucher specimen (PHG-P-PR-359) was deposited at the herbarium, Ain Shams University, Department of Pharmacognosy, Faculty of Pharmacy.

3.2. Essential Oils Isolation

Fresh *P. racemosa* leaves and stems were hydrodistilled for 4 h using the Clevenger apparatus to isolate their essential oils PRL-EO and PRS-EO, respectively. The yield (\% w/w) per hundred grams of plant material was determined in triplicate. Both isolated oils PRL-EO and PRS-EO were dried over anhydrous Na$_2$SO$_4$ and preserved in tightly sealed amber glass vials for further analyses.

3.3. Preparation of Plant Extracts

Air-dried leaves (50 g) and stems (20 g) were ground and then extracted using methanol (500 mL $\times$ 3; 25–27 $^\circ$C) for 48 h. Extracts were filtered, then concentrated under vacuum using a rotary evaporator (BUCHI Labortechnik, Flawil, Switzerland) at a temperature of 45 $^\circ$C. Then, the dried extracts were lyophilized employing an Alpha 1-4 LSC Christ freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) to yield 4.8 g of *P. racemosa* leaf extract (PRL-ME) and 1.5 g of the stem extract (PRS-ME). Extraction was performed in triplicate. Extracts were preserved in tightly sealed containers at 4 $^\circ$C until further analysis.

3.4. GC/MS Analysis of Essential Oils

A Shimadzu GCMS-QP 2010 (Kyoto, Japan) equipped with a Rtx-5MS capillary column (30 m length with 0.25 mm I.D. and 0.25 m film thickness; Restek, Bellefonte, PA, USA) was used to analyze essential oil samples PRL-EO and (PRS-EO). The oven temperature was set to 45 $^\circ$C for 2 min, then increased to 300 $^\circ$C at a rate of 5 $^\circ$C/min and maintained at 300 $^\circ$C for another 5 min; the injector temperature was set to 250 $^\circ$C. Helium was used as a carrier gas at a flow rate of 1.41 mL/min. Diluted samples (1% v/v) were injected (1 $\mu$L) at a split ratio of 15:1. Mass spectra were acquired in the range of 35–500 amu, EI mode: 70 eV. The interface and the ion source temperatures were set to 280 $^\circ$C and 200 $^\circ$C, respectively. The essential oils were analyzed independently, and the reported data represented the average of the three readings.

3.5. Identification of Essential Oil Components

Peaks were first deconvoluted using AMDIS software, a freely available software on www.amdis.net (accessed on 20 June 2022). Identification of essential oil phytoconstituents was achieved by comparing their mass spectral profiles with mass spectra within the NIST-17 GC-MS database (NIST, Gaithersburg, MD, USA) and literature [39–46]. Retention indices (RI) were calculated from the retention times of C8-C28 all-even n-alkanes injected under the same conditions.

3.6. HPLC-PDA-ESI-MS/MS Analysis

*P. racemosa* leaves and stems (PRL-ME) and PRS-ME methanol extracts were analyzed by HPLC-PDA-ESI-MS/MS, as described by Elkady et al. [21]. Chromatographic separations, UV, and mass spectral analyses were achieved using a Finnigan Surveyor HPLC system composed of a MS pump plus, autosampler, and PDA detector plus equipped with an EC 150/3 Nucleodur 100-3 C18 column (Macherey-Nagel, Dueren, Germany) coupled to a Finnigan LCQ-Duo ion trap with an ESI source mass spectrometer (Thermo Quest, San Jose, CA, USA). Data acquisitions and analyses were performed using XcaliburTM ver. 2.0.7 platform (Thermo Scientific, Waltham, MA, USA).
3.7. Evaluation of Anti-\textit{H. pylori} Activity

Anti-\textit{H. pylori} activity was determined using the MTT assay to assess the minimum inhibitory concentration (MIC) for bacterial growth: a series of concentrations with a final concentration range from 125 to 0.24 \( \mu \text{g/mL} \) was prepared for the methanol extracts, tested oils, or reference drug clarithromycin in dimethyl sulfoxide (DMSO).

The micro-well dilution method was used to assess the potential \textit{H. pylori} (ATCC 43504) inhibitory activity of \textit{P. racemosa} essential oils and methanol extracts PRL-EO, PRS-EO, PRL-ME, and PRS-ME [8]. A 10 \( \mu \text{L} \) inoculum of \textit{H. pylori} (10^6 CFU/mL) was added to 40 \( \mu \text{L} \) of the Brain Heart Infusion (BHI) growth medium in 10% fetal bovine serum (FBS) in each well. Subsequently, 50 \( \mu \text{L} \) aliquots of two-fold serial dilutions of test samples and Clarithromycin (standard reference) in dimethyl sulfoxide (125–0.24 \( \mu \text{g/mL} \)) were added. DMSO and Clarithromycin were used as negative and positive controls, respectively. The plates were incubated in an 85% N\textsubscript{2}, 10% CO\textsubscript{2}, and 5% O\textsubscript{2} atmosphere at 37 \( ^\circ \text{C} \) for 3 days. Afterward, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) reagent at a freshly prepared 0.5 mg/mL concentration in water and 40 \( \mu \text{L} \) were added to each well, then incubated for 30 min where purple color indicated bacterial growth. Absorbance was recorded at 620 nm using an ELISA microplate reader.

The inhibition percentage was calculated from the equation:

\[
\text{% inhibition} = \left[\frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}\right] \times 100.
\]

The MIC was defined as the lowest concentration, where no color change of MTT (inhibitory percentages 100%) was observed [8].

3.8. In Silico Molecular Docking Study

The molecular binding mode of the major identified compounds in the essential oils of \textit{P. racemosa} leaves and stems to the crystal structure of \textit{H. pylori} urease was assessed for their putative \textit{H. pylori} inhibitory activity using Discovery Studio 4.5 (Accelrys Software, Inc., San Diego, CA, USA). The crystal structure Urease X-ray (PDB ID: 1E9Y; 3.00\AA) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb/ accessed on 20 June 2022). Enzyme preparation was performed through the elimination of all water molecules. Hydrogen atoms were subsequently added, and then the protein structure was refined.

The target binding sites in urease were defined based on the interaction of acetohydroxamic acid (HAE), the co-crystallized inhibitor, and the enzyme. Prior to docking simulations, the co-crystallized ligand was removed. ChemDraw Ultra 8.0.3 was used to construct the 2D structures of the compounds identified in both essential oils. Subsequently, ligands were prepared adopting the ligand preparation protocol in Discovery Studio employing rule-based and pH-based ionization methods. Prepared ligands were docked into the active sites of the energy-minimized protein by applying the C-Docker algorithm and adopting the CHARMm force field. The binding energy was computed to assess the enzyme–ligand interactions. The best 10 ligand binding poses were ordered for each ligand according to their C-Docker energies, and the highest ligand binding poses were selected. The root-mean-square deviations (RMSDs) of C-Docker as a docking technique were calculated by superimposing the initial 3D structure and the docked posture of the co-crystallized inhibitor [8].

4. Conclusions

The current study’s findings shed light on the variation in the phytochemical profile of \textit{P. racemosa} leaves and stems. LC/MS-based metabolic profiling of the leaf and stem methanol extracts was unveiled for the first time. A total of 61 secondary metabolites were annotated. Extracts showed richness in polyphenolics. In addition, 21 components were identified in leaf and stem essential oils, with eugenol being the most abundant component. The essential oil from \textit{P. racemosa} stems, an agro-industrial waste, exhibited the strongest in vitro \textit{H. pylori} inhibitory activity, which was further validated by in silico molecular modeling of the identified phytoconstituents on \textit{H. pylori} urease. Eugenol, the major active
component in leaf and stem essential oils, might be implicated in this action. It could be concluded that *P. racemosa* might offer a natural medicine that exhibits a promising *H. pylori* inhibitory activity and could be accepted by a broad category of customers owing to its natural source. Though, more research is needed to examine additional putative biological actions of *P. racemosa* to discover natural source lead medicines.

**Author Contributions:** I.M.A. and W.M.E., conceptualized the study and performed the methodology, designed the phytochemistry work, prepared the plant extracts and essential oils, analyzed GC-MS and LC-MS data, performed molecular docking, and wrote the manuscript. M.M.A.-A. designed and performed anti-*H. pylori* activity analyzed the data and wrote the manuscript. S.S.E., A.A.B., and R.T.M. revised the manuscript, funding acquisition, supervised the whole work, and validated the results. All authors have read and agreed to the published version of the manuscript.

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