Bioactivity of *Piper crocatum* Extract: Apoptosis Induction and Anticancer Activity in A549 Cell Line Supported by Multi-Target Molecular Docking

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Bogor, July 2026

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SUMMARY

RAMZY ALOTHMANY. Bioactivity of Piper crocatum Extract: Apoptosis Induction and Anticancer Activity in A549 Cell Line Supported by Multi-Target Molecular Docking Supervised by MEGA SAFITHRI and RIKA INDRI ASTUTI. Lung cancer is one of the leading causes of cancer-related mortality worldwide, creating an urgent need for safer and more effective therapeutic agents.

Piper crocatum Ruiz & Pav. (red betel) is a medicinal plant known for its antioxidant and anticancer properties. However, its apoptosis-inducing activity associated with mitochondrial dysfunction and its antiproliferative effects against A549 human lung cancer cells remain insufficiently explored. This study aimed to evaluate the phytochemical composition, antioxidant activity, apoptosis-inducing potential, antiproliferative activity, and molecular interactions of *P. crocatum* extract and fractions. Leaves of *P. crocatum* were extracted with 70% ethanol and fractionated into n-hexane, ethyl acetate, and aqueous fractions. Phytochemical constituents, including phenolics, flavonoids, and alkaloids, were determined, and antioxidant activity was evaluated using the DPPH assay. Apoptosis-like activity was assessed in *Saccharomyces cerevisiae* through petite-frequency and rhodamine B fluorescence assays, while antiproliferative activity against A549 lung cancer cells was determined using the MTT assay. In addition, LC-MS-identified phytochemicals were subjected to multi-target molecular docking against EGFR, BCL-2, and KRAS G12C to investigate their potential molecular interactions.The ethyl acetate fraction exhibited the highest phytochemical contents and strong antioxidant activity. In yeast, the crude extract at 10 ppm induced the highest petite frequency and the greatest reduction in rhodamine B fluorescence, indicating pronounced mitochondrial dysfunction and apoptosis-like induction. The ethyl acetate fraction also showed the strongest antiproliferative activity against A549 cells, with an IC_{50} of approximately 111 ppm. Molecular docking demonstrated that several compounds, particularly Vitexin 2"-O-β-L-rhamnoside, Kaempferitrin, Vitexin, Flemiphilippinin, and Fusarin C, exhibited favorable binding affinities toward EGFR, BCL-2, and KRAS G12C, suggesting their potential to regulate multiple pathways involved in cancer progression and apoptosis.Overall, the findings demonstrate that *P. crocatum* possesses significant antioxidant, apoptosis-inducing, and antiproliferative activities. The integration of in vitro experiments with in silico multi-target molecular docking provides mechanistic insight into its anticancer potential and supports the development of *P. crocatum* as a promising natural source of multitarget anticancer agents.

Keywords: Red betel (*Piper crocatum*); antiproliferative activity; mitochondrial dysfunction; lung cancer; molecular docking

RINGKASAN

RAMZY ALOTHMANY. *Bioactivity of Piper crocatum Extract: Apoptosis Induction and Anticancer Activity in A549 Cell Line Supported by Multi-Target Molecular Docking.* Dibimbing oleh MEGA SAFITHRI dan RIKA INDRI ASTUTI.

Kanker paru merupakan salah satu penyebab utama kematian akibat kanker di dunia sehingga diperlukan pengembangan agen terapi yang lebih efektif dan aman. *Piper crocatum* Ruiz & Pav. (sirih merah) merupakan tanaman obat yang diketahui memiliki aktivitas antioksidan dan antikanker. Namun, aktivitasnya dalam menginduksi apoptosis yang berkaitan dengan disfungsi mitokondria serta efek antiproliferatifnya terhadap sel kanker paru manusia A549 masih belum banyak dieksplorasi. Penelitian ini bertujuan untuk mengevaluasi kandungan fitokimia, aktivitas antioksidan, kemampuan menginduksi apoptosis, aktivitas antiproliferatif, serta interaksi molekuler ekstrak dan fraksi *P. crocatum*. Daun *P. crocatum* diekstraksi menggunakan etanol 70% dan difraksinasi menjadi fraksi *n*-heksana, etil asetat, dan air. Kandungan fenolik, flavonoid, dan alkaloid dianalisis, sedangkan aktivitas antioksidan diuji menggunakan metode DPPH. Aktivitas apoptosis dievaluasi pada *Saccharomyces cerevisiae* melalui uji *petite frequency* dan pewarnaan Rhodamine B, sedangkan aktivitas antiproliferatif terhadap sel A549 ditentukan menggunakan metode MTT. Sebanyak 30 senyawa fitokimia dari *Piper crocatum* dianalisis secara *in silico* menggunakan *multi-target molecular docking* terhadap protein EGFR, BCL-2, dan KRAS G12C. Hasil penelitian menunjukkan bahwa fraksi etil asetat memiliki kandungan fitokimia tertinggi dan aktivitas antioksidan yang kuat. Pada model khamir, ekstrak kasar konsentrasi 10 ppm menghasilkan frekuensi *petite* tertinggi dan penurunan fluoresensi Rhodamine B terbesar, yang mengindikasikan disfungsi mitokondria dan induksi apoptosis. Fraksi etil asetat juga menunjukkan aktivitas antiproliferatif tertinggi terhadap sel A549 dengan nilai IC_{50} sekitar 111 ppm. Analisis *molecular docking* menunjukkan bahwa Vitexin 2"-O-β-L-rhamnoside, Kaempferitrin, Vitexin, Flemiphilippinin, dan Fusarin C memiliki afinitas ikatan yang baik terhadap EGFR, BCL-2, dan KRAS G12C, yang menunjukkan potensinya dalam memodulasi jalur molekuler yang berperan pada progresi kanker dan regulasi apoptosis. Secara keseluruhan, penelitian ini menunjukkan bahwa *P. crocatum* memiliki aktivitas antioksidan, penginduksi apoptosis, dan antiproliferatif yang signifikan. Integrasi pendekatan *in vitro* dan *in silico* memberikan pemahaman mengenai mekanisme antikanker *P. crocatum* serta mendukung potensinya sebagai sumber senyawa alami untuk pengembangan agen antikanker multitarget.

Kata kunci: Sirih merah; aktivitas antiproliferatif; disfungsi mitokondria; kanker paru; *molecular docking*.

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FOREWORD

The author would like to express his praise and gratitude to Allah Subhanahu wa Ta'ala for all His gifts so that this scientific work was successfully completed. The theme chosen in the research conducted from the month of August 2025 to the month of July 2026 is Natural Products and Cancer, with the title "Bioactivity of Piper crocatum Extract: Apoptosis Induction and Anticancer Activity in A549 Cell Line Supported by Multi-Target Molecular Docking."

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The author hopes that this scientific work will contribute to the advancement of scientific knowledge and serve as a valuable reference for future research in the fields of natural products, pharmacology, biotechnology, and cancer biology.

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Table of Content

TABLE LIST

LIST OF FIGURES

LIST OF ATTACHMENTS