Drug Design, Development and Therapy

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REVIEW

From Phytochemistry to Drug Discovery: A Systematic Review of Multitarget Pharmacology and Lead Compound Prioritization of *Piper crocatum* Ruiz & Pav

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Abstract: *Piper crocatum* Ruiz & Pav. (Piperaceae), commonly known as red betel, is a medicinal plant widely used in Southeast Asian traditional medicine and has attracted increasing interest due to its diverse pharmacological properties. However, the relationship between its phytochemical constituents, biological activities, molecular mechanisms, and drug discovery potential remains insufficiently integrated. This Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-guided review aimed to summarize the phytochemical constituents and pharmacological activities of *P. crocatum* and evaluate their relevance as potential lead structures for drug development. Literature published between 2000 and 2026 was retrieved from Scopus and PubMed. A total of 180 records were identified, and 102 eligible original studies reporting phytochemical and/or biological investigations of *P. crocatum* were included in the final analysis. Forty compounds have been reported from *P. crocatum*, including phenolic compounds, neolignans, flavonoids, terpenoids, and phytosterols. These constituents are associated with antimicrobial, antibiofilm, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, and immunomodulatory activities. Mechanistic evidence suggests that these effects involve modulation of oxidative stress, inflammatory signaling, microbial sterol biosynthesis, biofilm-related proteins, and metabolic enzymes such as α-glucosidase and pancreatic lipase. Among the reported compounds, pachypodol, crocatin derivatives, β-sitosterol, and stigmasterol show promising pharmacological profiles and may serve as lead structures for further development. Overall, the chemical diversity and multitarget activities of *P. crocatum* support its potential as a source of drug discovery candidates. Future studies should prioritize bioassay-guided isolation, molecular target validation, pharmacokinetic evaluation, and structure–activity relationship analysis.

Keywords: *Piper crocatum*, phytochemistry, pharmacological activity, natural product drug discovery, multitarget therapy, molecular mechanisms

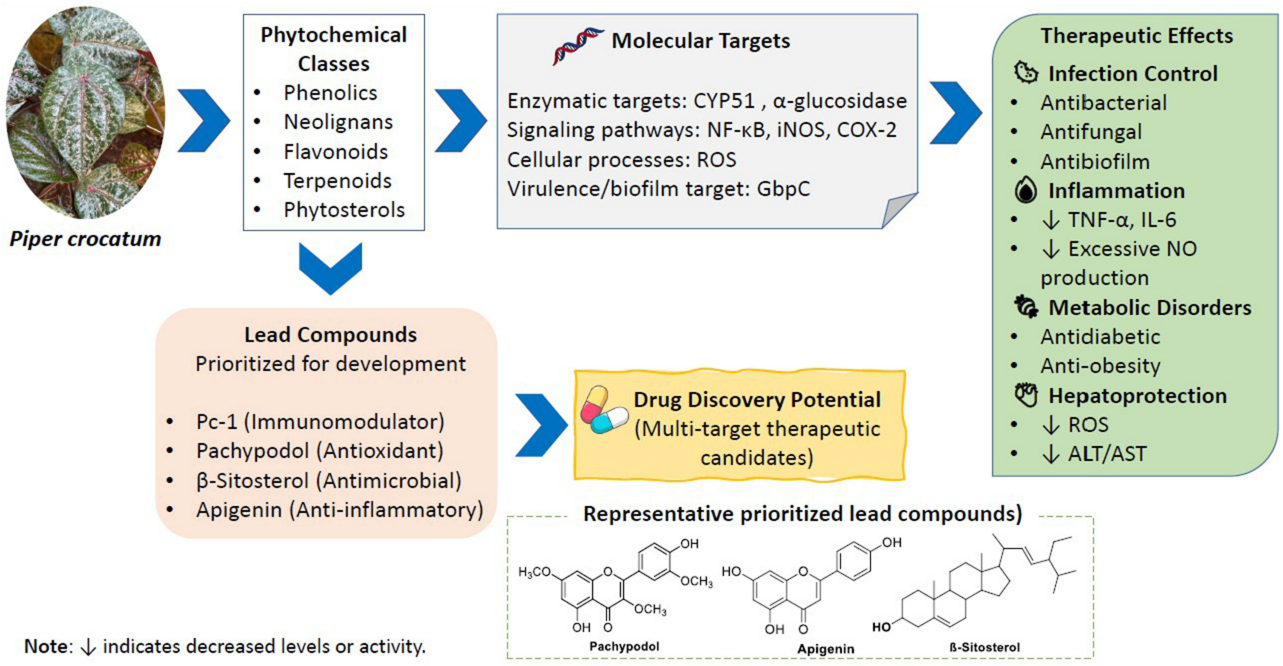
Introduction

Natural products continue to play a vital role in modern drug discovery by providing structurally diverse molecules that may serve as scaffolds for the development of new therapeutic agents. Most currently approved drugs are derived from natural products or inspired by natural product scaffolds, primarily for the treatment of anti-infective, anticancer, and metabolic diseases.[1,2] Thus, medicinal plants rich in chemically diverse secondary metabolites represent a valuable source of bioactive compounds with potential pharmaceutical applications.

Piper crocatum Ruiz & Pav., commonly known as red betel, is a medicinal plant of the Piperaceae family that has attracted increasing scientific attention owing to its diverse applications in traditional medicine. *P. crocatum* is widely

https://doi.org/10.2147/DDDT.S611626
Received: 24 March 2026
Accepted: 10 June 2026
Published: 20 June 2026

Graphical Abstract



used in traditional medicine in Southeast Asia, particularly in Indonesia, where its leaves are used to manage infections, inflammation, metabolic disorders, oral conditions, and gynecological complaints.[3–6] These traditional uses have encouraged pharmacological research aimed at identifying the bioactive compounds responsible for its therapeutic effects.

Phytochemical studies have shown that *P. crocatum* contains various secondary metabolites, including phenolic compounds, lignans, flavonoids, terpenoids, and phytosterols. These constituents have been associated with antimicrobial, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, immunomodulatory, and analgesic activities.[7–13] Many of these effects involve molecular targets related to oxidative stress, inflammatory signaling, microbial growth, and metabolic enzyme regulation, supporting the relevance of these constituents in drug discovery and therapeutic development.[14]

Although studies on the biological properties of *P. crocatum* have increased, the available evidence remains fragmented across phytochemical, pharmacological, and computational studies. Most previous reports have focused on individual extracts, isolated compounds, or specific biological activities, without linking these findings into a broader drug discovery context. As a result, the relationship between the chemical constituents of *P. crocatum*, their biological activities, and their molecular targets remains insufficiently synthesized. Unlike previous reviews that mainly summarized traditional uses, phytochemical profiles, or selected pharmacological effects, this review integrates phytochemistry, multitarget pharmacology, molecular mechanisms, and lead compound prioritization within a drug discovery framework.

Therefore, following a PRISMA-guided literature screening approach, this review aimed to systematically summarize the phytochemical constituents and pharmacological activities of *P. crocatum* and to integrate evidence from in vitro, in vivo, and in silico studies. Specifically, this review sought to: (i) compile the major classes of compounds reported from *P. crocatum*; (ii) summarize their reported biological activities and possible mechanisms of action; (iii) identify links between selected compounds and molecular targets; and (iv) evaluate their relevance as potential lead structures for future drug discovery and development.

Methods

This systematic review was conducted in accordance with the PRISMA 2020 guidelines.[15] We conducted a systematic literature search in Scopus and PubMed to identify studies published between 2000 and 2026 related to the phytochemical constituents and pharmacological activities of *P. crocatum*. The search terms included combinations of keywords related to the plant name (*Piper crocatum*, red betel, red betel leaf/leaves), phytochemical classes (phenolic compounds, flavonoids, lignans, neolignans, terpenoids, steroids, phytosterols, alkaloids, tannins, quinones, and saponins), biological activities (antibacterial, antifungal, antidiabetic, antioxidant, anti-inflammatory, and anticancer), and drug discovery-related approaches (in vitro, in vivo, in silico, molecular docking, drug design, pharmacological mechanism, absorption, distribution, metabolism, excretion, and toxicity [ADMET], and structure–activity relationship [SAR]).

A total of 180 records were initially identified from Scopus (n = 161) and PubMed (n = 19). Before manual screening, 19 duplicate records were removed, leaving 161 records for title and abstract screening. After title and abstract screening, 44 records were excluded because they did not meet the inclusion criteria, lacked relevance to *P. crocatum*, or did not report relevant phytochemical or pharmacological information, leaving 117 reports for full-text retrieval. Of these, one report could not be retrieved, leaving 116 full-text articles for eligibility assessment. Full-text evaluation resulted in the exclusion of 14 articles, including seven articles that were not relevant to the research scope and seven review articles. Consequently, 102 studies were included in the final analysis.

The inclusion criteria were as follows: (1) peer-reviewed original research articles published in English; (2) studies investigating extracts, fractions, or isolated compounds from *P. crocatum*; (3) studies reporting biological activities supported by in vitro, in vivo, or in silico approaches; and (4) studies providing phytochemical or compound identification data, where applicable, such as nuclear magnetic resonance (NMR), mass spectrometry (MS), fourier-transform infrared spectroscopy (FTIR), or gas chromatography–mass spectrometry (GC–MS). The exclusion criteria included duplicate records, review articles, non-scientific publications, studies lacking clear species identification, and reports without relevant phytochemical or pharmacological data, such as purely ethnobotanical descriptions. For several compounds reported from *P. crocatum*, additional bioactivity data were retrieved from studies in which the same compounds were isolated from other natural sources or synthesized. These complementary data were used to assess the pharmacological and drug development potential of the compounds rather than to represent the direct activity of *P. crocatum* extracts alone. A detailed overview of the literature search and study selection process is presented in the PRISMA 2020 flow diagram (Figure 1).

Phytochemistry of P. crocatum Ruiz & Pav

P. crocatum Ruiz & Pav. is a plant native to Peru, South America, that has spread to several regions of the world, including Indonesia.[16] It is a climbing shrub with elliptical leaves, dark green upper surfaces, and reddish-purple lower surfaces (Figure 2).[17] Phytochemical screening revealed that red betel contains various secondary metabolites including alkaloids, tannins, phenolics, flavonoids, quinones, terpenoids, steroids, and saponins.[18] According to previous studies, forty compounds have been isolated from red betel and characterized using spectroscopic and spectrometric techniques, including ultraviolet–visible (UV–Vis), FTIR, NMR, and mass spectrometry. These compounds comprise seventeen phenolic compounds, ten lignans, seven terpenes, three flavonoids, two steroids, and one hydroxylamine.

Phenolic Compounds

Most phenolic compounds isolated from red betel are phenolic glycosides (**1**–**15**) with C6-C1 (**5, 6, 12**–**15**), C6-C2 (**4, 9**–**11**), and C6-C3 (**1**–**3, 7**, and **8**) skeletons. These structural types indicate the contribution of simple phenolic and phenylpropanoid-derived metabolites to the phytochemical profile of this species. In addition to phenolic glycosides, phenolic amide derivatives have also been reported. Compound **16** was isolated from the methanol extract, whereas piperyamide A (**17**) was detected in the ethyl acetate extract of red betel leaves.[19,20] The structures of the phenolic compounds isolated from *P. crocatum* are shown in Figure 3.

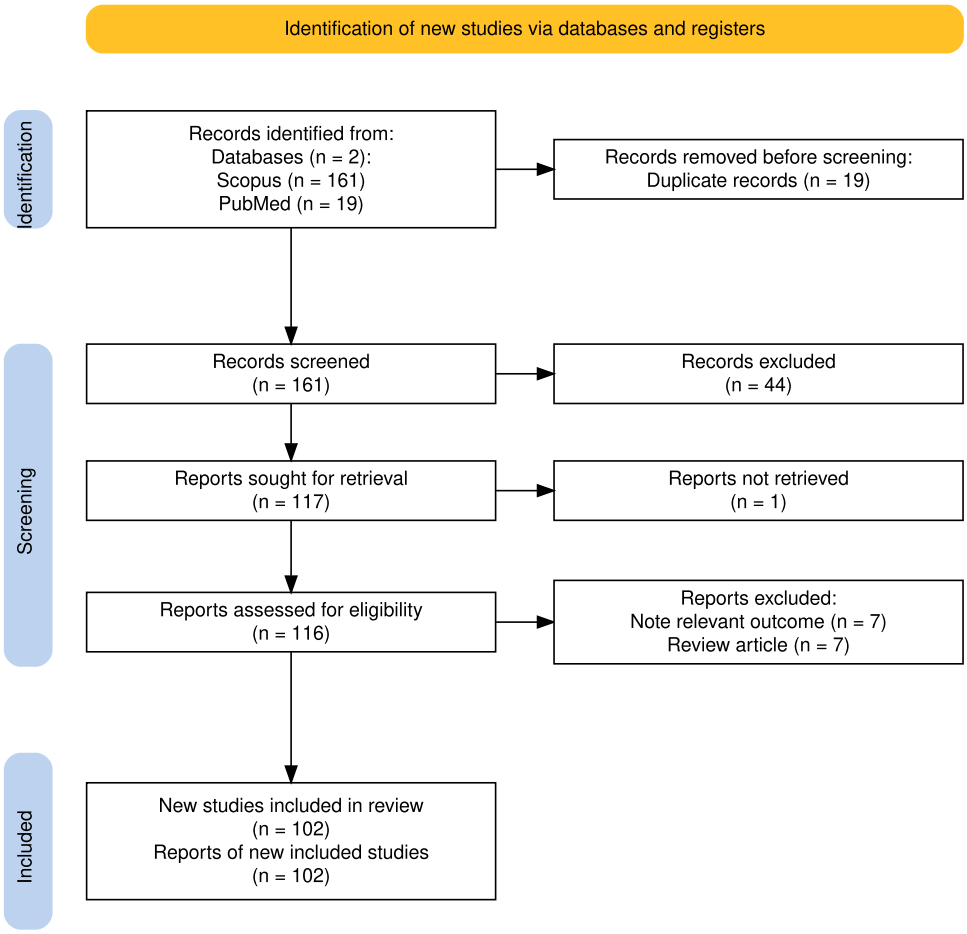


Figure 1 PRISMA 2020 flow diagram of the study selection process.

Figure 2 *P. crocatum* plants.

Lignans

In addition to phenolic compounds, red betel leaves contain lignans.[21] Lignans are natural products formed through the oxidative dimerization of two phenylpropanoid units.[22,23] Based on the linkage pattern between the phenylpropanoid units, lignans can be broadly classified into classical lignans and neolignans. Two classical lignans belonging to the 2,6-diarylfurofuran subtype (**18** and **19**) have been isolated from the methanol extract of red betel leaves.[24,25] In addition, five

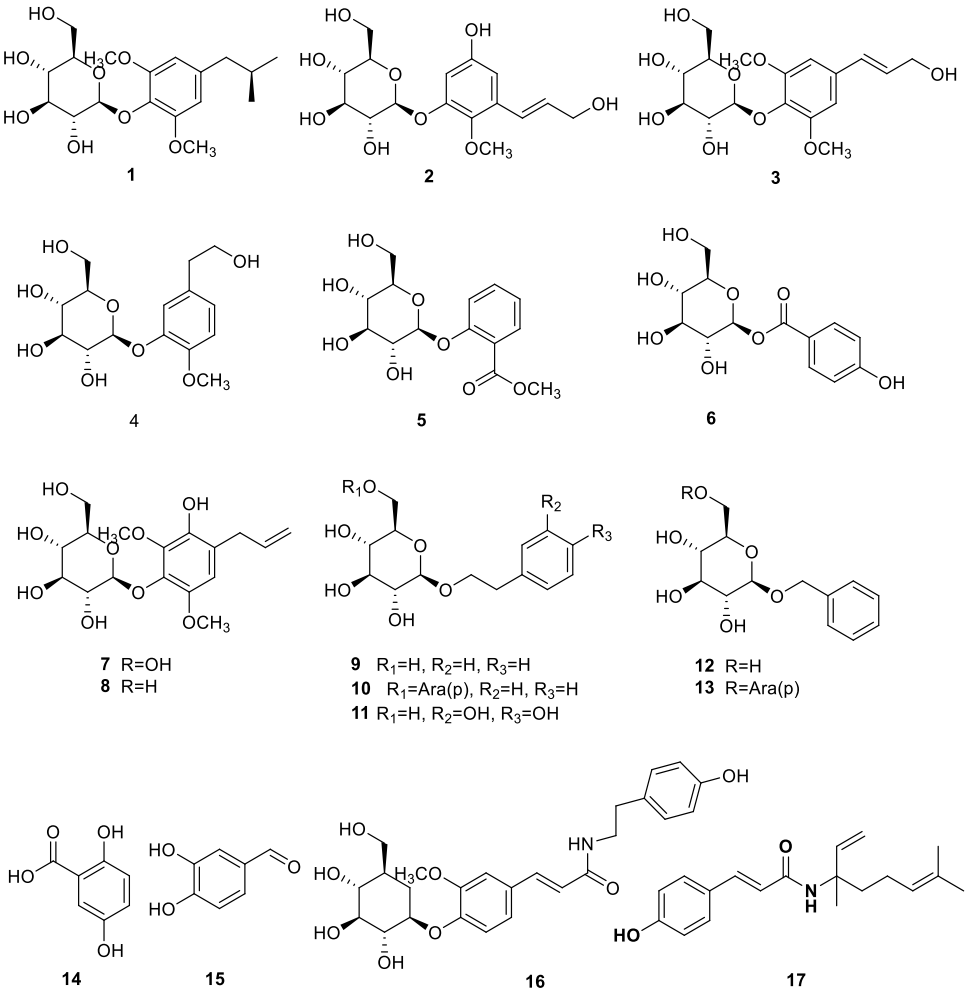


Figure 3 Phenolic compounds of *P. crocatum*: (8*R*)-8-(4-hydroxy-3,5- dimethoxy)-propane-8-ol-4-*O*-β-D-glucopyranoside (**1**); 3-[(1*E*)-3-hydroxy-1-propen-1-yl]-2,5-dimethoxyphenyl-D-glucopyranoside (**2**); syringin (**3**); cimidahurinin (**4**); methylsalicylate-2-*O*-β-D-glucopyranoside (**5**); 4-Hydroxybenzoic acid-D-glucosylester (**6**); 4-Allyl -2,6-dimethoxy-3-hydroxy-1-D-glucopyranoside (**7**); erigeside II (**8**); β-phenylethyl-β-D-glucoside (**9**); icariside D1 (**10**); Hydroxytyrosol-1glucopyranoside (**11**); benzyl-β-D-glucoside (**12**); phenylmethyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**13**); gentisic acid (**14**); catechaldehyde (**15**); *N*-trans-feruloyltyramine-4′-O-β-D-glucopyranoside (**16**); and piperyamide A (**17**).

bicyclo[3.2.1]octane-type neolignans (**20**–**24**) and three other lignan-related compounds (**25**–**27**) were reported from the methanol extract.[26–28] These findings indicate that lignans and neolignans represent one of the major compound groups in *P. crocatum*. Their structures are shown in Figure 4.

Flavonoids

Although phytochemical screening has indicated the presence of flavonoids in red betel, only three flavonoids (**28**–**30**) have been successfully isolated and structurally characterized.[24,29] These compounds belong to the flavone subclass (Figure 5). The relatively small number of isolated flavonoids compared with phenolic compounds and lignans suggests that flavonoids are present as minor but pharmacologically relevant constituents in *P. crocatum*.

Terpenoids

Terpenoid-related compounds have also been identified from *P. crocatum*. These include one monoterpenoid derivative (**31**), five norisoprenoids (**32**–**36**), and one diterpenoid (**37**) which were reported from the ethyl acetate fraction of red betel leaves.[19,30] Their structures are shown in Figure 6. The presence of these terpenoid derivatives expands the chemical diversity of *P. crocatum* beyond phenolic and lignan-based metabolites.

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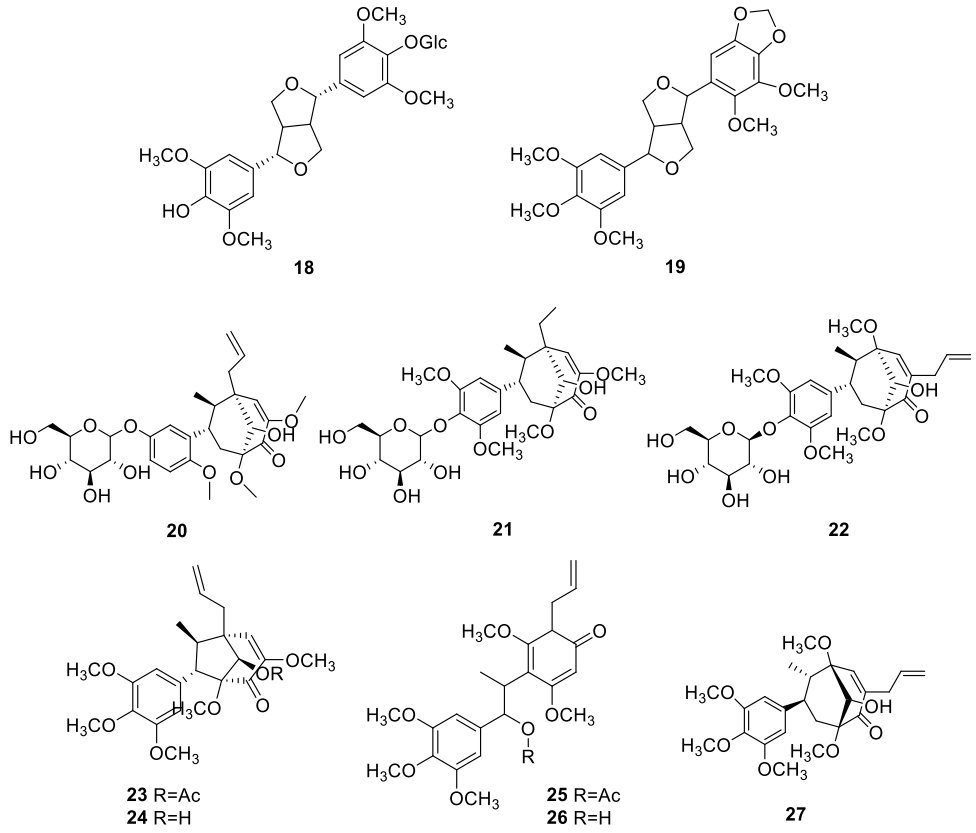

Figure 4 Structure of isolated lignans from *P. crocatum*; syringaresinol-β-D-glucoside (**18**); 2-(5',6'-dimethoxy-3',4'-methylenedioxyphenyl)-6-(3',4',5-trimethoxyphenyl)-dioxabiclo [3,3,0] octane (**19**); pipcroside A (**20**); pipcroside B (**21**); pipcroside C (**22**); crocatin A (**23**); crocatin B (**24**); pipercrocatin (**25**); deacethyl pipercrocatin (**26**), and nectamazin A (**27**).

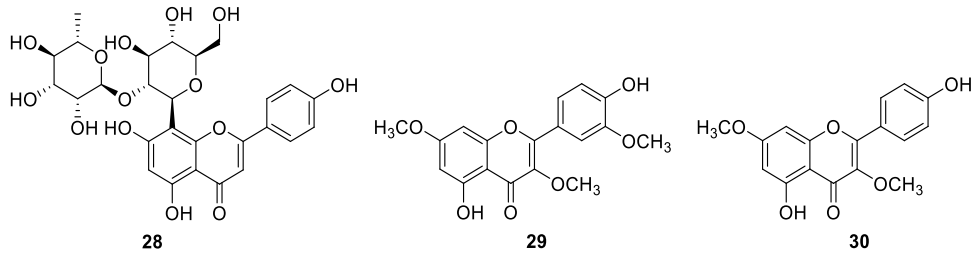

Figure 5 Flavonoid of *P. crocatum*; vitexin 2″- O-rhamnoside (**28**); pachypodol [4′,5-dihydroxy-3,3′,7- trimethoxyflavone] (**29**); and kumatakenin [4′,5-dihydroxy-3,7-dimethoxyflavone] (**30**).

Steroids

Two phytosterols, β-sitosterol (**38**) and stigmasterol (**39**), have been isolated from red betel (Figure 7).[20,24] Steroids are characterized by a tetracyclic carbon framework consisting of three six-membered rings and one five-membered ring. The identification of these phytosterols is important because sterol-type compounds are commonly associated with membrane-related biological effects and may contribute to the pharmacological profile of *P. crocatum*.

Other Compounds

A hydroxylamine derivative, piperyamine A (**40**) was isolated from the methanol extract of red betel leaves (Figure 8).[20] This compound has a unique structure with an unsaturated side chain. Although its biosynthetic origin has not been fully established, the presence of this hydroxylamine derivative highlights the structural diversity of metabolites produced by

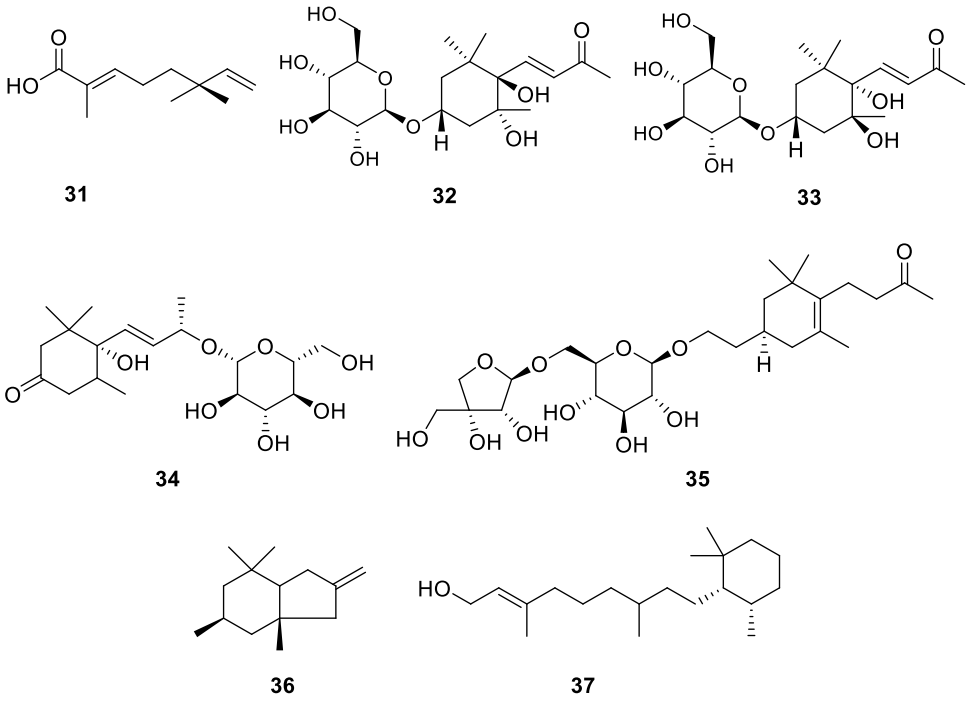

Figure 6 Monoterpens and sesquiterpenes of *P. crocatum*; (*S*)-menthiafolic acid (**31**); 5β,6β-dihydroxy-3α-(β-D-glucopyranosyloxy)-7*E*-megastigmen-9-one (**32**); (3*E*)-4-[(1*S*,2*S*,4*S*)-4-(β-D-glucopyranosyloxy)-1,2-dihydroxy-2,6,6-tri-methylcyclohexyl] 3-buten-2-one (**33**); (6*S*,9*S*)-roseoside (**34**); cuneataside E (**35**), ioliolide (**36**); and cassipourol (**37**).

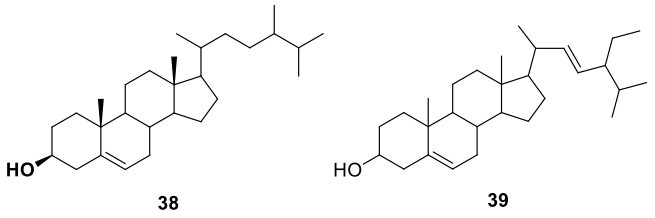

Figure 7 Steroid structure of *P. crocatum*; β-sitosterol (**38**) and stigmasterol (**39**).

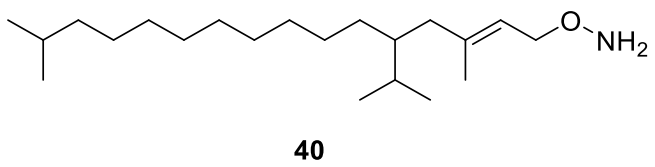

Figure 8 Structure of Piperyamine A (**40**).

P. crocatum. Hydroxylamine-containing natural products are relatively uncommon, and their occurrence in red betel provides an additional example of the chemical uniqueness of this species.

Overall, these compound classes provide different structural and physicochemical features that may influence their biological behavior. Phenolic compounds and flavonoids contain hydroxylated and methoxylated aromatic systems that may support redox-related and enzyme-modulating activities, whereas lignans and neolignans provide phenylpropanoid-derived frameworks relevant to antimicrobial and anti-inflammatory potential. In contrast, terpenoids and phytosterols contribute more lipophilic scaffolds that may influence membrane-related interactions but may also present limitations in solubility and bioavailability.

https://doi.org/10.2147/DDDT.S611626

Pharmacological Activities of P. crocatum Ruiz & Pav

Antimicrobial and Antibiofilm Activity

P. crocatum has shown antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria, fungi, and selected protozoa, indicating its potential relevance in the management of infectious diseases. The reported activities included crude extracts, fractions, and isolated compounds, with varying degrees of effectiveness, depending on the chemical class and purity level (Table 1).

Antibacterial Activity

The ethanolic extract showed inhibitory effects against *S. aureus, S. epidermidis, S. haemolyticus, S. warneri*, and *S. xylosus* with inhibition zones ranging from 13.0 to 20.3 mm at a concentration of 200 mg/mL.[48] Activity was also observed against gram-negative pathogens including *E. coli, P. aeruginosa*, and *K. pneumoniae*.[54] Despite the relatively high concentration of this extract, this indicates the presence of bioactive constituents capable of inhibiting bacterial growth. More potent activity was observed for the isolated compounds. β-Sitosterol (**38**) showed significant activity against *S. aureu*s (minimum inhibitory concentration [MIC] = 7.8 µg/mL) and *K. pneumoniae* (MIC 31.2 µg/mL) and showed moderate inhibition against oral pathogens such as *S. mutans* and *S. sanguinis* (MIC 312–625 µg/mL).[41,45] Crocatin B (**24**) also showed inhibitory activity against *S. mutans* and *E. faecalis* with MIC of 156.3 and 187.5 µg/mL,

Table 1 Antibacterial and/or Antifungal Activity of Extract, Fraction, and Isolated Compounds

No	Compound/Fraction/ Extract (Solvent)	Presence in *P. crocatum*	Source of Bioactivity Data	Microorganism	Method	Key Findings	Reference
1	Syringin (**3**)	Methanol extract	*Carduus leptacanthus*	*S. aureus* *S. epidermis* *S. agalactiae*	Broth microdilution	MIC = 13.3 mg/mL MIC = 16 mg/mL MIC = 16 mg/mL	[31]
2	Gentisic acid (**14**)	Methanol extract	Synthesis	*E. coli* *B. cereus* *L. monocytogenes* *S. cerevisiae* *F. culmorum*	Microtitration plates	MIC = 2.5 mmol/l MIC = 2.5 mmol/l MIC = 2.5 mmol/l MIC = 10 mmol/l MIC = 5 mmol/l	[32]
3	Catechaldehyde (**15**)	Methanol extract	*Trichomanes chinense*	*E. coli* *V. cholera* *S. aureus* *S. thypimurium*	Disc diffusion	Inhibition zone 5.5 mm in 2.5 µg/mL Inhibition zone 6.0 mm in 2.5 µg/mL Inhibition zone 5.5 mm in 2.5 µg/mL Inhibition zone 8.0 mm in 2.5 µg/mL	[33]
			Maple syrup	*P. mirabilis* *P. aeruginosa* *C. jejuni*	Broth microdilution	MBC = 1.25 mg/mL MBC = 1.25 mg/mL MBC = 3.1 mg/mL	[34]
			Synthesis	*L. monocytogenes* *S. enterica*	Microtitration plates	MIC = 3.3 mg/mL MIC = 2.7 mg/mL	[35]
4	Piperyamide A (**18**)	Methanol extract	*P. crocatum*	*C. albicans*	Broth microdilution	MIC = 4.6 mg/mL MFC = 18 mg/mL	[20]
5	Crocatin B (**24**)	Ethyl acetate fraction	*P. crocatum*	*S. mutans* *S. sanguinis* *E. faecalis* *C. albicans*	Broth microdilution	MIC = 156.3 µg/mL MIC = 973.5 µg/mL MIC = 187.5 µg/mL MIC = 781.3 µg/mL	[36]
6	Nectamazin A (**27**)	Ethyl acetate fraction	*P. crocatum*	*S. pyogenes* *S. mutans* *S. sanguinis* *C. albicans*	Broth microdilution	MIC = 156.3 µg/mL MIC = 625 µg/mL MIC = 1250 µg/mL MIC = 312.5 µg/mL	[37]

(*Continued*)

Table 1 (Continued).

No	Compound/Fraction/ Extract (Solvent)	Presence in *P. crocatum*	Source of Bioactivity Data	Microorganism	Method	Key Findings	Reference
7	Vitexin 2"-O-rhamnoside (**28**)	*n*-Hexane fraction	*P. crocatum*	*S. aureus* *E. coli* *P. aeruginosa* *E. faecalis*	Broth microdilution	MIC = >1000 μg/mL MIC = 500 μg/mL MIC = 500 μg/mL MIC = 1000 μg/mL	[38]
8	Loliolide (**36**)	Ethyl acetate fraction	*Sonchus oleraceus*	*B. subtilis* *E. coli* *S. aureus* N. *gonorrhoea*	Agar plate diffusion	Inhibition zone 16 mm in 10 μg Inhibition zone 16 mm in 10 μg Inhibition zone 15 mm in 10 μg Inhibition zone 15 mm in 10 μg	[39]
			Penstemon campanulatus	*S. aureus* *S. epidermidis* *P. aeruginosa* *E. cloacae* *K. pneumoniae* *E. coli* *C. albicans* *C. tropicalis* *C. glabrata*	Agar dilution	MIC = 1.0 mg/mL MIC = 0.9 mg/mL MIC = 1.5 mg/mL MIC = 1.3 mg/mL MIC = 1.0 mg/mL MIC = 1.5 mg/mL MIC = 4.4 mg/mL MIC = 3.8 mg/mL MIC = 3.8 mg/mL	[40]
9	Cassipourol (**37**)	Methanol extract	*P. crocatum*	*C. albicans*	Broth microdilution	MIC = 625 μg/mL	[30]
10	β-sitosterol (**38**)	Methanol extract	*Kalanchoe tomentosa*	*S. aureus* *K. pneumonia*	Broth microdilution	MIC = 7.8 μg/mL MIC = 31.2 μg/mL	[41]
			Ocimum basilicum	*S. sanguinis* *E. faecalis*	Broth microdilution	MIC = 25 mg/mL MIC = 25 mg/mL	[42]
			Odontonema strictum	*S. aureus*	Broth microdilution	MIC = 1.2 mg/mL	[43]
			Ginkgo biloba	*S. enterica*	Broth microdilution	MIC = 31.1, MFC = 125 μg/mL	[44]
			P. crocatum	*S. mutans* *S. sanguinis* *C. albicans*	Broth microdilution	MIC = 312 μg/mL MIC = 625 μg/mL MIC = 625 μg/mL	[45]
11	Stigmasterol (**39**)	Methanol extract	*Ginkgo biloba*	*S. aureus* MRSA *S. aureus* *S. faecalis* *E. coli* *P. fluorescens* *C. albicans* *C. krusei*	Broth dilution	MIC = 125 μg/mL MIC = 6.2 μg/mL MIC = 12.5 μg/mL MIC = 12.5 μg/mL MIC = 25 μg/mL MIC = 25 μg/mL MIC = 25 μg/mL MIC = 12.5 μg/mL	[44]
			P. crocatum	*S. mutans* *S. sanguinis* *E. faecalis* *C. albicans*	Broth microdilution	MIC = 625 μg/mL MIC = 312.5 μg/mL MIC = 312.5 μg/mL MIC = 625 μg/mL	[46]
12	Piperyamine A (**40**)	Methanol extract	*P. crocatum*	*C. albicans*	Broth microdilution	MIC = 0.62%, MFC 2.5%	[20]
13	Isolated triterpenoid	Chloroform fraction	*P. crocatum*	*M. tuberculosis*	Broth dilution	MIC = 5 μg/mL	[47]

(*Continued*)

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Table 1 (Continued).

No	Compound/Fraction/ Extract (Solvent)	Presence in *P. crocatum*	Source of Bioactivity Data	Microorganism	Method	Key Findings	Reference
14	Extract	Ethanol extract (70%)	*P. crocatum*	*S. aureus* *S. haemolyticus* *S. warneri* *S. epidermidis* *S. xylosus*	Disc diffusion	Inhibition zone 20.3 mm in 200 mg/mL Inhibition zone 16.2 mm in 200 mg/mL Inhibition zone 13.0 mm in 200 mg/mL Inhibition zone 15.0 mm in 200 mg/mL Inhibition zone 13.2 mm in 200 mg/mL	[48]
15	Fraction	Chloroform fraction	*P. crocatum*	*M. tuberculosis*	Broth dilution	MIC = 3.1 mg/mL	[49]
16	Extract	Ethanol extract (70%)	*P. crocatum*	*L. acidophilus* *L. bifidus*	Broth dilution	MIC = 6.2 mg/mL, MBC = 12.5 mg/mL MIC = 6.2 mg/mL, MBC = 12.5 mg/mL	[50]
17	Fraction	Fraction *n*-hexane: ethyl acetate (9:1)	*P. crocatum*	*E. faecalis*	Disc diffusion	Inhibition zone 7.6 mm in 40 mg/mL	[11,51]
18	Extract	Ethanol extract (70%)	*P. crocatum*	*T. vaginalis*	Broth microdilution	MIC = 50 mg/mL	[52]
19	Extract	Ethanol extract (96%)	*P. crocatum*	*C. albicans*	Broth microdilution	MIC = 23 mg/mL	[53]
20	Extract	Ethanol extract (70%)	*P. crocatum*	*E. coli* *P. aeruginosa* *S. aureus* *C. albicans*	Disc diffusion	Inhibition zone 10.7 mm in 200 mg/mL Inhibition zone 12.6 mm in 200 mg/mL Inhibition zone 13.4 mm in 200 mg/mL Inhibition zone 14.4 mm in 200 mg/mL	[54]
21	Extract	Mehanol extract	*P. crocatum*	*S. aureus*	Microdilution checkerboard	MIC vancomycin to be 4fold reduction	[55]

Notes: *Bacillus cereus, Bacillus subtilis, Campylobacter jejuni, Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Fusarium culmorum, Klebsiella Pneumoniae, Lactobacillus acidophilus, Lactobacillus bifidus, Listeria monocytogenes, Mycobacterium tuberculosis, Methicillin-Resistant Staphylococcus aureus, Neisseria gonorrhoeae, Propionibacterium acnes, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Saccharomyces cerevisiae, Salmonella enterica, Salmonella typhi, salmonella typhimurium, Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus xylosus, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus faecalis, Streptococcus mutans, Streptococcus sanguinis, Streptococcus pyogenes, Trichomonas vaginalis, Vibrio cholera.*

respectively.[36] In addition, nectamazin A (**27**) exhibited MIC of 156.3 µg/mL against *S. pyogenes*.[37] Stigmasterol (**39**) showed activity against *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and *E. coli*, with MIC values as low as 6.2 µg/mL in some models.[44] These findings suggested that the phytosterol skeleton may significantly contribute to its antibacterial potential. Phenolic compounds such as gentisic acid (**14**) and catechaldehyde (**15**) exhibit moderate inhibitory activity against several microorganisms, including *E. coli, B. cereus, L. monocytogenes, S. enterica, P. aeruginosa*, and *V. cholerae*.[32–35,44] The presence of hydroxyl and aldehyde groups may facilitate interactions with membranes and induce oxidative stress, thereby contributing to the bacteriostatic or bactericidal effects. The triterpenoid isolated from the chloroform fraction is of particular interest, as it exhibits activity against *M. tuberculosis* (MIC 5 µg/mL),[47] highlighting its potential relevance in the discovery of antituberculosis drugs. Considering the urgent need for new anti-TB agents, these findings require further mechanistic and in vivo validation.

Significantly, the methanol extract showed a synergistic interaction with vancomycin, resulting in a fourfold decrease in the MIC values against *S. aureus*.[55] This indicates potential resistance-modifying properties, possibly through membrane disruption or efflux pump interference. Combination therapies of this type may be useful in overcoming antimicrobial resistance.

Antibiofilm and Oral Pathogen Relevance

The antimicrobial profile of *P. crocatum* is particularly prominent in oral pathogens. β-Sitosterol and stigmasterol have been shown to inhibit *S. mutans, S. sanguinis*, and *E. faecalis*, which are closely associated with dental plaque formation and endodontic infections.[45,46] The results of clinical applications further reinforce this relevance. Water extracts have been evaluated as root canal irrigants, and 30% extracts effectively removed residual layers in a clinical setting. Red betel leaf gel increased clinical attachment levels by 31.4% when utilized as adjunctive periodontal therapy, and a mouthwash formulation containing ethanol extract reduced dental plaque accumulation (Table 2).[7,56–58] These findings indicate not only antimicrobial properties but also antibiofilm properties, which are crucial for treating chronic oral infections.

Molecular docking studies have provided a possible mechanism for the antibiofilm potential. β-sitosterol was predicted to bind to glucan-binding protein C (GbpC) and sortase C (SrtC), both of which are involved in biofilm formation and bacterial adhesion.[45] Suppression of these targets could interrupt extracellular polysaccharide synthesis and bacterial colonization, suggesting a plausible explanation for the observed reduction in plaque formation.

Antifungal Activity

Several compounds exhibit antifungal activity, particularly against *C. albicans* and other related species. Among the compounds that showed inhibitory activity against *C. albicans*, piperyamide A, piperyamine A, cassipourol, stigmasterol, β-sitosterol, and nectamazin A showed the same MIC value of 625 μg/mL, whereas crocatin B showed an MIC of 312 μg/mL. In silico studies suggest that several of these compounds may interfere with fungal membrane sterol formation by targeting enzymes involved in ergosterol biosynthesis. Piperyamide A, stigmasterol, and piperyamine A interact with ERG1, ERG2, ERG11, and ERG24 with inhibition constants (Ki) in the submicromolar to micromolar range.[20] This mechanism is consistent with antifungal strategies targeting lanosterol 14α-demethylase (CYP51) as summarized in Table 3. Cassipourol, β-sitosterol, crocatin A, crocatin B, and nectamazin A have been predicted to bind to CYP51, further supporting their multitarget antifungal potential.[30,36,37,45] Although ADMET predictions indicate limitations in the oral bioavailability of some compounds, the convergence of ergosterol biosynthesis highlights a promising route for targeted antifungal agents.

Overall, these findings highlight ergosterol synthesis as a potential molecular target for mediating antifungal activity of *P. crocatum*. This mechanism-based insight is particularly relevant for traditional therapeutic practices where red betel leaf preparations have been used to manage symptoms related to vaginal infections, including fluor albus. A decoction of *P. crocatum* leaves effectively reduced the symptoms of Fluor albus in women of reproductive age, both in adults and adolescents. There were significant differences in pH levels before and after the application of boiled red betel leaf water, with pH averaging 2.00 before and 1.21 after treatment.[3,60]

However, despite its broad spectrum of activity, the following limitations must be acknowledged: the majority of studies on the extract have been carried out using high concentrations (>100 mg/mL), which directly limits its clinical relevance (1); inconsistent standardization of extract composition (2); poor reporting of bacterial killing kinetics or post-antibiotic effects (3); inconsistency in biofilm inhibition testing (4); and lack of pharmacokinetic data (5). Thus, despite

Table 2 Application of *P. crocatum* Extract

No	Extract	Infection	Purpose	Method	Key Findings	Reference
1	Aqueous extract	Oral infection	Root canal irrigant solution	Clinical study	30% of the extract is effective in cleaning the smear layer in the root canal	[56]
2	Boiled water of red betel	Oral infection	Reduce gingivitis	Clinical study	Boiled water of red betel reduces gingivitis in pregnant women	[59]
3	Red betel leaf gel	Oral infection	Adjunctive therapy	Clinical study	Red betel leaf gel increases the clinical attachment level by 31.4%	[7]
4	Red betel leaf solution and ethanol extract	Oral infection	Mouthwash	Clinical study	Red betel leaf solution decreases plaque	[58]

https://doi.org/10.2147/DDDT.S611626

Table 3 In silico Study of *P. crocatum* Compounds and Protein-Related Bacteria or Fungi

No	Compound	Protein Target	Method	Key Findings	Reference
1	Piperyamide A (**17**)	ERG1 ERG2 ERG11 ERG24	Molecular docking ADMET Drug-likeness (Lipinski)	Ki = 1.8 μM Ki = 0.2 μM Ki = 0.7 μM Ki = 122.9 μM Not favorable for oral drug use Yes	[20]
2	Crocatin A (**23**) Crocatin B (**24**)	DNA ligaseCYP51 DNA ligaseCYP51	Molecular docking ADMET Drug-likeness (Lipinski) Molecular docking ADMET Drug-likeness (Lipinski)	Ki = 4.7 μM Ki = 0.2 μM Favorable for oral drug use Yes Ki = 36.4 μM Ki = 1.6 μM Favorable for oral drug use Yes	[36]
3	Nectamazin A (**27**)	PBP SrtA CYP51 ERG11 GtfB GtfP	Molecular docking Molecular dynamic (to ERG11) ADMET Drug-likeness (Lipinski)	Ki = 5.3 μM Ki = 98.7 μM Ki = 7.2 μM Ki = 0.15 μM Ki = 967.5 μM Ki = 58.9 μM Stable Favorable for oral drug use Yes	[37]
4	Cassipourol (**37**)	NMT SAP5 CGTase DHFR CYP51	Molecular docking ADMET Drug-likeness (Lipinski)	Ki = 24.9 μM Ki = 33.2 μM Ki = 1.9 μM Ki = 4.3 μM Ki = 1.3 μM Not favorable for oral drug use Yes	[30]
5	β-sitosterol (**38**)	GbpC SrtC Sap5 CYP51	Molecular docking ADMET Drug-likeness (Lipinski)	Ki = 54.7 μM Ki = 33210 μM Ki = 201.6 μM Ki = 0.8 μM Potential to be used as an oral drug Yes	[45]
6	Stigmasterol (**39**)	ERG1 ERG2 ERG11 ERG24 LMD Exo-β-(1,3)-glucanase	Molecular docking ADMET Drug-likeness (Lipinski)	Ki = 6.8×10^{-3} μM Ki = 4.0×10^{-4} μM Ki = 1.6×10^{-3} μM Ki = 8.9 μM Ki = 1.8×10^{-2} μM Ki = 4.7 μM not favorable for oral drug use No	[20,46]
7	Piperyamine A (**40**)	ERG1 ERG2 ERG11 ERG24	Molecular docking ADMET Drug-likeness (Lipinski)	Ki = 10.9 μM Ki = 0.3 μM Ki = 2.4 μM Ki = 1060 μM not favorable for oral drug use Yes	[20]

the emergence of sterols and triterpenoids as promising frameworks, most antimicrobial evidence remains at the preliminary level.

Redox-Related and Enzyme-Modulating Bioactivity

P. crocatum leaves have demonstrated antioxidant activity through various experimental approaches, including free-radical chemistry systems, lipid peroxidation models, enzyme inhibition assays, and in silico studies of target proteins associated with oxidative stress. In general, extracts and compounds isolated from this plant can scavenge free radicals,

inhibit lipid peroxidation, reduce transition metal ions, and modulate the activity of enzymes associated with oxidative stress.

The 70% ethanol extract exhibited 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with an IC_{50} value of 85.2 µg/mL. Another study comparing several solvents reported that water extract had the strongest activity (IC_{50} 81.2 µg/mL), followed by ethanol extract (185.3 µg/mL), ethyl acetate extract (202.8 µg/mL), and *n*-hexane extract (552.2 µg/mL).[61,62] This indicates that more polar components likely contribute dominantly to the antioxidant activity. At the pure compound level, pachypodol (**29**) and kumatakenin (**30**) showed stronger activities than the extracts. In the DPPH assay, pachypodol had an IC_{50} of 58.3 µg/mL and kumatakenin 204.8 µg/mL (Table 4). In the superoxide dismutase (SOD) mimetic activity test, both showed excellent activity, with pachypodol reaching an IC_{50} of 7.1 µg/mL and kumatakenin 42.3 µg/mL. Density Functional Theory (DFT) studies showed that the hydrogen atom transfer (HAT) mechanism is thermodynamically favored by both flavonoids, with the C4′–OH group identified as the most reactive site for hydrogen donation.[29] These data indicate that flavonoids play an important role in the free radical scavenging capacity of *P. crocatum*.

In addition, *P. crocatum* extract was also tested in a lipid peroxidation model using the Thiobarbituric acid reactive substances (TBARS) method, which measures malondialdehyde (MDA) levels as an indicator of lipid oxidative damage. The 70% ethanol extract inhibited lipid peroxidation by 80.4% at 200 µg/mL.[61] In the TBARS-based in vivo model, the

Table 4 Redox-Related and Enzyme-Modulating Bioactivities of *P. crocatum* Extracts, Fractions, and Isolated Compounds

No	Compound/ Fraction/Extract (Solvent)	Biological Model/Test System	Assay Type	Detection Method	Key Findings	Reference
1	Water fraction	Cell-free enzymatic system	Tyrosinase inhibition	Spectrophotometry (λ = 475 nm)	IC_{50} = 6.3 µg/mL	[63]
2	Ethanol extract (70%)	Cell-free lipid peroxidation model Cell-free radical system	TBARS (MDA inhibition) DPPH radical scavenging	Spectrophotometry (λ = 532 nm) Spectrophotometry (λ = 517 nm)	Inhibition = 80.4% (conc. 200 µg/mL) IC_{50} = 85.2 µg/mL	[61]
3	Ethanol extract (70%)	Cell-free radical system	DPPH radical scavenging H_2O_2 Scavenging ABTS-Reducing Activity	Spectrophotometry (λ = 517 nm) Spectrophotometry (λ = 510 nm) Spectrophotometry (λ = 745 nm)	IC_{50} = 4.0 µg/mL IC_{50} = 186.3 µg/mL IC_{50} = 38.4 µg/mL	[9]
4	Ethanol extract (70%) Ethyl acetate fraction *n*-Hexane fraction Water fraction	Cell-free chemical redox system (Cu(II)/Cu(I))	CUPRAC antioxidant capacity assay	Spectrophotometry (microplate reader, λ = 450 nm; Trolox equivalents)	21.3 ± 4.3 µmol Tr/g extract 26.2 ± 5.9 µmol Tr/g extract 23.0 ± 5.0 µmol Tr/g extract 17.3 ± 2.1 µmol Tr/g extract	[64]
5	Ethanol extract Ethyl acetate extract n-Hexane extract Water extract	Cell-free radical system Cell-based lipid peroxidation model (in vivo)	DPPH radical scavenging TBARS (MDA inhibition)	Spectrophotometry (λ = 517 nm) Fluorometric detection (Ex 520 nm/Em 550 nm)	IC_{50} = 185.3 µg/mL IC_{50} = 202.8 µg/mL IC_{50} = 552.2 µg/mL IC_{50} = 81.2 µg/mL Decrease MDA level significantly at dose 8 mg	[62]
6	*n*-Hexane extract Ethyl acetate extract Methanol extract	Cell-free chemical redox system (Cu(II)/Cu(I))	CUPRAC antioxidant capacity assay	Spectrophotometry (microplate reader, λ = 459 nm; Trolox equivalents)	IC_{50} = 31.8 µg/mL IC_{50} = 17.3 µg/mL IC_{50} = 14.8 µg/mL	[65]
7	Pachypodol (**29**) Kumatakenin (**30**) Pachypodol (**29**) Kumatakenin (**30**)	Cell-free radical system	DPPH radical scavenging SOD radical scavenging	Spectrophotometry (λ = 517 nm) Spectrophotometry (λ = 560 nm)	IC_{50} = 58.3 µg/mL IC_{50} = 204.8 µg/mL IC_{50} = 7.1 µg/mL IC_{50} = 42.3 µg/mL	[29]

Abbreviations: TBRAS, Thiobarbituric Acid Reactive Substances; MDA, Malondialdehyde, HMG-CoA reductase, methylglutaryl-CoA reductase; CUPRAC, cupric reducing antioxidant capacity; SOD, Superoxide dismutase.

https://doi.org/10.2147/DDDT.S611626

administration of 8 mg significantly reduced MDA levels.[62] These results indicate that the antioxidant activity of *P. crocatum* is not limited to the chemical radical system, but is also capable of suppressing the formation of lipid oxidation products.

Based on the results of the cupric reducing antioxidant capacity (CUPRAC) method, 70% ethanol extract, ethyl acetate fraction, *n*-hexane fraction, and water fraction showed reduction capacities in the range of 17–26 µmol Trolox equivalent per gram of extract.[64] These findings indicate that *P. crocatum* compounds contribute significantly to metal reduction capacity. The antioxidant activity related to enzyme modulation was reported using a tyrosinase inhibition test. The water fraction showed strong tyrosinase inhibitory activity (IC_{50} of 6.3 µg/mL).[63] Tyrosinase is an enzyme that plays a role in the oxidation of phenols and the formation of melanin; therefore, its inhibition is often associated with antioxidant activity and potential applications in dermatology. As shown in Table 5, in silico studies showed that *P. crocatum* compounds, such as piperine, piperamide-C7:2, and trichostachine, interact with tyrosinase with fairly good binding affinity (range −6.8 −7.0 kcal/mol). Molecular dynamics simulations revealed the stability of the ligand–protein complex, and ADMET analysis and drug-likeness parameters (Lipinski) indicated a profile supporting oral utilization.[63] These results confirm the experimental data that, in addition to radical-scavenging activity, several *P. crocatum* compounds can inhibit enzyme activity related to oxidative processes.

Although *P. crocatum* exhibits strong antioxidant activity in vitro, current evidence is largely derived from chemical assays. Without mechanistic validation of cellular systems and pharmacokinetic characterization, classification as a viable source of antioxidant drugs remains premature. Nevertheless, pachypodol has emerged as a promising redox-active framework with the potential for further optimization.

Immunomodulatory and Anti-Inflammatory Activity

Modulation of Innate Immune Response

The immunomodulatory activity of *P. crocatum* has been demonstrated through its ability to modulate macrophage functions. In an *L. monocytogenes* infection model in BALB/c mice, the macrophage phagocytic index and nitric oxide (NO) production were used as indicators of innate immune activation. Administration of the methanol extract at a dose of 450 mg/kg significantly improved macrophage activity and NO levels, showing an efficacy equivalent to that of isolated neolignans (Pc-1 and Pc-2) delivered at a dose of 5 mg/kg.[28] This approximately 90-fold difference in dose indicates a much higher potential for the pure compound to stimulate innate immune responses.

Nitric oxide production in stimulated macrophages is regulated by inducible nitric oxide synthase (iNOS), implying that the immunostimulatory effect may involve classical macrophage signaling pathways, such as nuclear factor-kappa

Table 5 In silico Study of *P. crocatum* Compounds and Protein-Related Antioxidant Activity

No	Compound	Compound Identification Method	Protein Target	Method	Key Findings	Reference
1	Piperine	UHPLC	Tyrosinase	Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski)	Binding affinity = −7.04 kcal/mol Stable Favorable for oral drug use Yes	[63]
2	Piperamide-C7:2 (2E,6E)	UHPLC	Tyrosinase	Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski)	Binding affinity = −6.87 kcal/mol Stable Favorable for oral drug use Yes	[63]
3	Trichostachine	UHPLC	Tyrosinase	Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski)	Binding affinity = −6.79 kcal/mol Stable Favorable for oral drug use Yes	[63]
4	Pachypodol (**29**) Kumatakenin (**30**)	FTIR, NMR, and MS		DFT	Favorable for the HAT mechanism	[29]

B (NF-κB) or the mitogen-activated protein kinase (MAPK) cascade. During microbial infection, these pathways are typically triggered by pattern recognition receptor signals and mediate the transcription of genes associated with antimicrobial defense. However, upstream receptor interactions, particularly the possible involvement of Toll-like receptors (TLRs), have not been directly investigated, resulting in unresolved molecular mechanisms.

Although they had similar immunostimulatory efficacy, significant toxicological differences were observed between the isolated compounds and the crude extracts. Pc-2 caused hydropic degeneration in the liver tissue, indicating hepatocellular stress, whereas Pc-1 and crude extracts did not cause significant histopathological changes and maintained the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels within acceptable limits. These differences highlight an essential translational concern: higher potency does not necessarily translate into a greater therapeutic potential. Crude extracts may offer a buffering effect that reduces toxicity through antagonistic or balanced synergistic interactions between the phytochemicals. Such interactions may stabilize the immune response and prevent overstimulation. For drug development, Pc-1 appeared to offer the best balance of efficacy and safety, whereas Pc-2 had stronger efficacy, but caused concerns about hepatotoxicity. The crude extract, which was less potent, showed better bioavailability.

Suppression of Pro-Inflammatory Signaling

Most anti-inflammatory studies of *P. crocatum* use lipopolysaccharide (LPS)-induced RAW 264.7 macrophages, which represents activation of the TLR4–NF-κB pathway. LPS stimulates TLR4; induces IκB kinase (IKK) phosphorylation, IκB degradation, and NF-κB translocation to the nucleus; and subsequently increases the transcription of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), IL-6, and iNOS, which generates NO. Experimental data showed that extracts and isolated compounds from *P. crocatum* suppressed mediators at several points in this pathway, particularly at cytokine and iNOS levels (Table 6). The ethanol extract reduced TNF-α by 51.6%, IL-1β by 21.4%, IL-6 by 68.7%, and NO by 81.2% at 50 μg/mL, suggesting the upstream modulation of inflammatory signaling.[14] However, direct measurements of key molecular events, such as IKK phosphorylation or NF-κB nuclear translocation, have rarely been performed; therefore, the exact intervention site remains unknown.

At a single compound level, Lely et al reported that a neolignan, crocatin B (**24**), significantly attenuated inflammatory responses in LPS-stimulated RAW 264.7 cells at 5 μg/mL.[66] Measurements using the sandwich enzyme-linked immunosorbent assay (ELISA) method demonstrated a reduction in TNF-α and intercellular adhesion molecule-1 (ICAM-1) concentrations compared with those in the control LPS-treated group, reflecting the inhibition of pro-inflammatory cytokine secretion and adhesion molecule expression. These dual activities imply modulation of upstream regulatory pathways, such as the NF-κB signaling cascade, which controls the transcriptional regulation of TNF-α and ICAM-1 in activated macrophages. Cell toxicity assessments showed that the viable cell counts remained above 80% over the tested concentration range, indicating that the observed biological activity was not due to general cellular toxicity. Preliminary safety data highlighted the selectivity of the compound toward inflammatory targets. In terms of drug design, the neolignan skeleton, which is rich in phenolic and methoxy moieties, likely contributes to key molecular interactions through hydrogen bonding and improved membrane permeability. Such structural features may underlie the observed bioactivity and offer a rational approach for further structure-activity relationship analysis. Overall, these findings suggest that neolignans from *P. crocatum* are prospective multitarget anti-inflammatory agents. However, further in vivo and in silico validation is required to confirm their target engagement and translational potential.

NO and iNOS Regulation

P. crocatum methanol extract inhibited NO production by approximately 70–75% and decreased iNOS protein expression by approximately 70–75% at 60 μg/mL. Meanwhile, cell viability remained above 90%, indicating that the inhibition of inflammatory mediators was not caused by cellular toxicity. Catechaldehyde (**15**) showed similar effects at lower concentrations (20 μg/mL), indicating that the potential of the single compounds was stronger than that of the extracts.[67] The decrease in iNOS expression indicated that the activity was not limited to NO scavenging but was also implicated in the regulation of enzyme transcription or translation. However, most studies have not evaluated the

https://doi.org/10.2147/DDDT.S611626

Table 6 Anti-Inflammation Activity of *P. crocatum* Extracts, Fractions, and Isolated Compounds

No	Compound/Fraction/ Extract (Solvent)	Biological Model/Test System	Assay Type	Detection Method	Key Findings	Reference
1	Crocatin B (**24**)	In vitro RAW 264.7 murine macrophages, LPS-induced In vitro RAW 264.7 murine macrophages, LPS-induced In vitro RAW 264.7 macrophages	TNF-α quantification (sandwich ELISA) ICAM-1 quantification (sandwich ELISA) Cell viability (MTT assay)	Spectrophotometry (ELISA microplate reader λ = 450 nm) Spectrophotometry (ELISA microplate reader λ = 450 nm) Spectrophotometry (λ = 570 nm)	TNF-α levels (ng/L): 5 μg/mL = 439.3 ± 17.2; LPS control = 802.7 ± 26.0; Normal = 26.4 ± 4.2 ICAM-1 levels (ng/L): 5 μg/mL = 374.0 ± 45.1; LPS control = 821.3 ± 21.6; Normal = 50.6 ± 11.2% Viability: 5 μg/mL = 89.2 ± 6.7; (non-cytotoxic ≥ 80%)	[66]
2	Catechaldehyde (**15**)	In vitro LPS-stimulated RAW 264.7 macrophages In vitro IgE-sensitized RBL-2H3 mast cells, DNP-BSA-stimulated In vitro RAW 264.7 and RBL-2H3 cells	NO production inhibition Protein expression (iNOS) Mast cell degranulation Cell viability/ cytotoxicity	Spectrophotometry (ELISA microplate reader λ = 570 nm) Chemiluminescence (ECL), densitometry Spectrophotometry (ELISA microplate reader λ = 405 nm) Spectrophotometry (ELISA microplate reader λ = 490 nm)	NO level: ~50–55% inhibition at 20 μg/mL iNOS protein level: reduced ~70–75% at 20 μg/mL β-Hexosaminidase release: reduced ~50–55% at 20 μg/mL % Viability >90%	[67]
3	Syringin (**3**) Cimidahurinin (**4**) Methyl salicylate 2-O-β-D-glucopyranoside (**5**) Erigeside II (**8**)	In vitro, cell-free enzyme system (recombinant human sEH)	sEH enzyme inhibition assay	Fluorescence microplate reader (Excitation 330 nm/Emission 465 nm)	% inhibition = 17.4 ± 0.6% inhibition = 8.8 ± 2.5% inhibition = 18.9 ± 1.5% inhibition = 92.9 ± 0.5 (conc. 100μM) IC_{50} = 58.5 ± 0.5 μM	[25]
4	Methanol extract	In vitro LPS-stimulated RAW 264.7 macrophages In vitro IgE-sensitized RBL-2H3 mast cells, DNP-BSA-stimulated In vitro RAW 264.7 and RBL-2H3 cells	NO production inhibition Protein expression (iNOS) Mast cell degranulation Cell viability/ cytotoxicity	Spectrophotometry (ELISA microplate reader λ = 570 nm) Chemiluminescence (ECL), densitometry Spectrophotometry (ELISA microplate reader λ = 405 nm) Spectrophotometry (ELISA microplate reader λ = 490 nm)	NO level: ~70–75% inhibition at 60 μg/mL iNOS protein level: reduced ~70–75% at 60 μg/mL β-Hexosaminidase release: reduced ~70–75% at 60 μg/mL % Viability >90%	[67]
5	Ethanol extract (70%)	In vivo rheumatoid arthritis (RA) mouse model – *Mus musculus*	Immunophenotyping assay (flow cytometry-based lymphocyte profiling)	Flow cytometry (FACS Calibur) using FITC-conjugated anti-mouse CD4 and PE-conjugated anti-mouse CD8 antibodies; hemocytometer with trypan blue for lymphocyte counting	Extract reduced $CD4^+$ by 59.4% and $CD8^+$ by 60.4% at a 200 mg/kg BW dose	[68]
6	Ethanol extract (96%) in gel formulation	In vivo (Wistar rats; post-excision wound model)	IL-10 VEGF biomarker analysis Collagen density Wound area measurement	ELISA/IHC; light microscopy + ImageJ	IL-10: ~250 pg/mL at conc. 50% (vs ~320 pg/mL, negative control); VEGF: peak ~55 pg/mL at conc. 25% Collagen density improved (~2 vs ~1, at conc. 12.5%) Wound area reduced from ~75% (negative control) to ~11% (conc. 50%)	[69]

7	Ethanol extract (96%)	In vitro, cell supernatant, LPS-induced	Sandwich ELISA cytokine assay NO production inhibition	Spectrophotometry (ELISA microplate reader λ = 450 nm) Spectrophotometry (ELISA microplate reader λ = 540 nm)	TNF-α levels (ng/L): reduced 51.6% at 50 µg/mL IL-1 (ng/L): reduced 21.4% at 50 µg/mL IL-6 (ng/L): reduced 68.7% at 50 µg/mL NO (ng/L): reduced 81.2% at 50 µg/mL	[14]
8	*n*-Butanol fraction	In vivo (Wistar rats; high-cholesterol diet-induced atherosclerosis)	Serum cytokine quantification (TNF-α, IL-6; colorimetric assay)	UV–Vis spectrophotometry (546 nm)	TNF-α levels (ng/L): reduced 45.6% at 150 mg/BW IL-1 (ng/L): reduced 15.4% at 150 mg/BW	[70]
9	Extract	In vivo rheumatoid arthritis (RA) mouse model – *Mus musculus*, CFA/IFA-induced paw inflammation	Anti-inflammatory cytokine assay Edema measurement	Indirect ELISA (IL-1β, serum, absorbance-based microplate reader) Vernier caliper measurement (paw edema thickness, mm)	IL-1β: Extract 200 mg/mL showed a *trend* toward lower IL-1β but not significantly different Edema thickness: Extract 400 mg/mL significantly reduced paw edema	[71]

Abbreviation: sEH, soluble epoxide hydrolase.

https://doi.org/10.2147/DDDT.S611626

activation of signal transducer and activator of transcription 1 (STAT1) or the MAPK pathway, which also contribute to iNOS expression; therefore, the mechanism remains partially undefined.

Enzyme-Targeted Anti-Inflammatory Activity

Several compounds from *P. crocatum* also target the enzymes associated with inflammatory signaling. Erigeside II (**8**) showed a 92.9% inhibition of soluble epoxide hydrolase (sEH) at 100 μM (IC_{50} 58.5 μM).[25] sEH is an attractive target as it regulates the degradation of anti-inflammatory fatty acid epoxides into pro-inflammatory diols. Although the percentage of inhibition is high, the IC_{50} value in the tens of micromolar range represents moderate potency compared to the latest generation of synthetic sEH inhibitors (typically in the nanomolar range). Thus, erigeside II could be viewed as a more reasonable initial scaffold than a direct candidate.

In silico studies further support this anti-inflammatory potential (Table 7). Docking simulation showed that apigenin had an affinity of −9.9 kcal/mol for Cyclooxygenase-2 (COX-2), higher than kaempferitrin and rutin (−8.7 kcal/mol).[72,73] Structurally, apigenin is a flavonoid aglycone with a lower molecular weight and more balanced lipophilicity than the

Table 7 In silico Study of *P. crocatum* Compounds and Protein-Related Anti-Inflammation Activity

No	Compound	Compound Identification Method	Protein Target	Method	Key Findings	Reference
1	Kaempferitrin Afzelin Rutin	LCMS	COX-2	Molecular docking ADMET Molecular docking ADMET Molecular docking ADMET	Binding affinity = −8.7 kcal/mol Not favorable for oral drug use Binding affinity = −8.0 kcal/mol Not favorable for oral drug use Binding affinity = −8.7 kcal/mol Not favorable for oral drug use	[72]
2	Apigenin	HPLC	COX-2	Molecular docking ADMET	Binding affinity = −9.9 kcal/mol Favorable for oral drug use	[73]
3	Sesamin		NF-κB	Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski)	Binding affinity = −6.9 kcal/mol Stable Favorable for oral drug use Yes	[74]
4	Kaempferitrin Apigenin Catechin		NF-κB TNFR1 IκK NF-κB TNFR1 IκK NF-κB TNFR1 IκK	Molecular docking ADMET Molecular docking ADMET Molecular docking ADMET	Binding affinity = −7.6 kcal/mol Binding affinity = −9.8 kcal/mol Binding affinity = −8.2 kcal/mol Not favorable for oral drug use Binding affinity = −6.2 kcal/mol Binding affinity = −7.7 kcal/mol Binding affinity = −8.3 kcal/mol Favorable for oral drug use Binding affinity = −6.1 kcal/mol Binding affinity = −8.2 kcal/mol Binding affinity = −7.9 kcal/mol Favorable for oral drug use	[75]
5	Apigenin Chavibetol Hydroxychavicol Luteolin	LC-MS	NF-κB	Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski) Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski) Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski) Molecular docking Molecular dynamics ADMET Drug-likeness (Lipinski)	Binding affinity = −6.3 kcal/mol Stable Favorable for oral drug use Yes Binding affinity = −4.7 kcal/mol Stable Favorable for oral drug use Yes Binding affinity = −4.8 kcal/mol Stable Favorable for oral drug use Yes Binding affinity = −6.0 kcal/mol Stable Favorable for oral drug use Yes	[76]
6	β –amyrin	LC-MS	mPGES-1	Molecular docking ADMET	Binding affinity = −8.4 kcal/mol Favorable for oral drug use	[77]

Abbreviations: COX-2, Cyclooxygenase-2, TNFR1, Tumor Necrosis Factor Receptor 1; IκK, Inhibitor kappa B kinase; mPGES-1, microsomal Prostaglandin E Synthase-1.

glycoside forms. Glycosylated flavonoids (rutin and kaempferitrin) show high affinity; however, their ADMET profiles do not support their oral use, likely because of their large molecular size and high polarity. These results indicate that flavonoid aglycones are feasible COX-2 modulator candidates.

Docking simulations were performed for NF-κB, TNFR1, and IKK. The results showed that kaempferitrin had a strong affinity for TNFR1 (−9.8 kcal/mol), while apigenin and catechin showed values of −7 to −8 kcal/mol with better ADMET profiles.[74–76] Sesamin displayed complex stability in molecular dynamics simulations and met Lipinski's criteria, rendering it a more drug-like candidate than the glycosylated flavonoids. However, docking does not always correspond directly to biological activity in living cells, and thus requires further biochemical validation.

In vivo Evidence and Immunomodulation

In vivo studies provide additional evidence of systemic anti-inflammatory effects. In a rat atherosclerosis model, the *n*-butanol fraction reduced TNF-α levels by 45.6% at 150 mg/kg BW, indicating systemic activity.[69] Other studies have demonstrated that concentrated extracts reduced edema in a rheumatoid arthritis model, while 70% ethanol extract at 200 mg/kg reduced $CD4^+$ and $CD8^+$ lymphocyte populations by approximately 60%.[68] These findings suggest that *P. crocatum* may influence both the innate and adaptive immune responses. However, the relatively high doses used and absence of pharmacokinetic data raise questions regarding the clinical feasibility and potential risk of non-selective immunosuppression.

Conceptual Integration: Immune Homeostasis Regulation

Although the immunostimulatory and anti-inflammatory effects initially seem contradictory, they are likely to be derived from the same modulation of the macrophage signaling networks. During microbial infection, moderately activated macrophage pathways strengthen phagocytosis and pathogen elimination via NO production. Conversely, overstimulation of the same pathways under sterile inflammatory conditions induces the excessive production of cytokines and inflammatory mediators. Thus, the pharmacological profile of *P. crocatum* indicates dependent regulation of macrophage signaling, rather than simple immune stimulation or suppression. Both activities accumulate on the TLR4–NF-κB–iNOS signaling axis. Activation of this pathway promotes host defense in infection models, whereas suppression of downstream mediators, such as TNF-α, IL-6, and NO, limits inflammatory damage in LPS-induced systems. The dual physiological role of NO—as an antimicrobial agent and inflammatory mediator illustrates this regulatory balance. A dual modulation model was proposed to conceptualize context-dependent immunological behavior (Figure 9). This model emphasizes that *P. crocatum* does not act as a simple immunostimulant or immunosuppressant but rather as a context-dependent regulator of immune homeostasis. Such dual functionality is particularly relevant for therapeutic strategies targeting infections accompanied by uncontrolled inflammation.

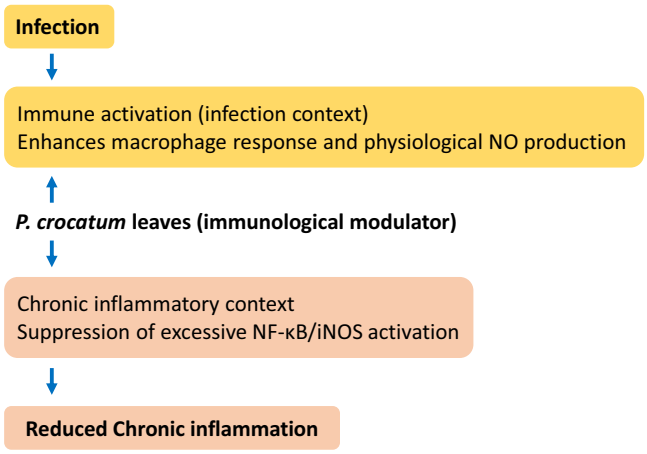

Figure 9 Dual immunological modulation of *P. crocatum* in infection and inflammatory contexts.

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Overall, our findings indicate that *P. crocatum* functions as a bifunctional regulator of immune homeostasis, enhancing innate immune responses during infection while suppressing excessive inflammatory signaling. However, the existing studies have largely relied on a limited number of experimental models that commonly use higher concentrations or doses. Further research integrating molecular pathway analysis, pharmacokinetic characterization, and multi-model validation is required to clarify the potential of *P. crocatum* as a source of immunoregulatory agents.

Anticancer Activity

Extract-Level Cytotoxicity in Breast and Cervical Cancer Models

The cytotoxic profile of *P. crocatum* extract showed significant variation between the cancer subtypes and extraction solvents (Table 8). The most prominent activity was observed in MCF-7 breast cancer cells, where the 96% ethanol extract reached an IC_{50} of 6.7 μg/mL, indicating strong growth inhibition.[8] This potency contrasts significantly with the activity of the methanol extract on 4T1 cells (IC_{50} = 120 μg/mL) and HeLa cells (LC_{50} up to 0.8 mg/mL), highlighting a striking difference in selectivity.[78,79] In T47D cells, the methanol extract suppressed proliferation (IC_{50} = 44.25 μg/mL) but led to minimal apoptosis, indicating a cytostatic rather than cytotoxic effect.[80] Inhibition of extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation suggests disruption of the MAPK pathway, a key regulator of proliferation and viability in breast cancer. Further suppression of insulin-induced ERK activation implies the upstream regulation of receptor-mediated signaling. These results indicate that *P. crocatum* extract may act primarily through the blockade of proliferation signaling, rather than through the direct induction of apoptosis. However, the lack of mechanistic analysis beyond ERK phosphorylation limits the pathway identification. Additionally, the IC_{50} values of the extracts above 40 μg/mL (except for MCF-7) represent a barrier to translation unless isolated and enriched active constituents are identified.

In silico Targeting of Oncogenic Proteins

Docking analysis showed that β-asarone, methylpiperonyl ketone, and p-coumaric acid target human epidermal growth factor receptor 2 (HER-2), matrix metalloproteinase-9 (MMP-9) and TNF-α, which play key roles in tumor progression,

Table 8 Anticancer Activity of *P. crocatum* Extracts, Fractions, and Isolated Compounds

No	Compound/ Fraction/Extract (Solvent)	Biological Model/Test System	Assay Type	Detection Method	Key Findings	Reference
1	Methanol extract	In vitro, 4T1 breast cancer cells	Cytotoxicity (MTT)	Spectrophotometry (ELISA microplate reader λ = 595 nm)	IC_{50} = 120 μg/mL	[79]
2	Ethanol extract	In vitro, HeLa cervical cancer cells	Cytotoxicity assay (trypan blue exclusion)	Light microscopy + hemocytometer	LC_{50} = 0.8 ± 0.3 mg/mL	[78]
3	Methanol extract	In vitro, T47D human breast adenocarcinoma cells	Cell viability (MTT) Apoptosis & cell cycle analysis (DAPI staining PI flow cytometry) Signaling pathway analysis (Western blot, ERK1/2)	Spectrophotometry (570 nm, MTT) Fluorescence microscopy (DAPI) Flow cytometry (PI, Sub-G1) Immunoblot detection (ECL)	IC_{50} = 44.25 μg/mL (24 h); dose-dependent reduction in cell viability (5–50 μg/mL) No significant apoptotic induction (Sub-G1 ≤ 4.23%) Inhibition of ERK1/2 phosphorylation, including suppression of insulin-stimulated ERK activation, indicating MAPK pathway involvement	[80]
4	Methanol extract	In vitro, HeLa cervical cancer cells	Cytotoxicity assay (trypan blue exclusion)	Light microscopy + hemocytometer	IC_{50} = 34.2 ± 1.5 mg/mL	[5]
5	Ethanol extract (96%)	In vitro, MCF-7 human breast cancer cells	Cytotoxicity (MTT)	Spectrophotometry (ELISA microplate reader λ = 565 nm)	IC_{50} = 6.7 μg/mL	[8]

Note: Values refer to 24 h exposure unless otherwise stated.
Abbreviations: IC_{50}, concentration required to inhibit 50% of cell viability; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide; ERK, extracellular signal-regulated kinase.

invasion, and modulation of the inflammatory microenvironment. Among these, β-asarone consistently exhibited strong binding scores, particularly to MMP-9 (−216.1 kcal/mol), indicating potential anti-metastatic activity through the inhibition of extracellular matrix degradation.[81] p-Coumaric acid showed the strongest predicted MMP-9 interaction (−220.9 kcal/mol), despite its relatively simple phenylpropanoid structure. Importantly, all three compounds met Lipinski's criteria and exhibited favorable ADMET predictions, positioning them as viable small-molecule candidates for oral administration.

Antidiabetic and Antihyperuricemic Activities

Cellular and Enzymatic in vitro Evidence

Two lignans (Pipcroside B and C) from the ethyl acetate fraction showed moderate potential in cellular pyruvate dehydrogenase (PDH) assays ($IC_{50} \approx 80–100$ μM), indicating the potential for enhanced mitochondrial glucose oxidation (Table 9).[27] However, these micromolar concentrations represent a moderate intrinsic potential compared with clinically relevant metabolic modulators. On the other hand, a few extracts showed significant α-glucosidase inhibition at low μg/

Table 9 Antidiabetic Activity of *P. crocatum*

No	Compound/Fraction/ Extract (Solvent)	Biological Model/ Test System	Assay Type	Detection Method	Key Findings	Reference
1	Pipcroside B (**21**) Pipcroside C (**22**)	Human AD-293 cells (HEK293-derived, in vitro)	Cellular PDH activity assay	Fluorescence high-content imaging	IC_{50} = 99.8 μM IC_{50} = 80.2 μM	[27]
2	*n*-Hexane extract Ethyl acetate extract Methanol extract	In vitro, β-glucosidase (cell-free enzymatic system)	Enzyme inhibition assay	Colorimetric spectrophotometry UV-Vis (λ = 405 nm)	IC_{50} = 9.8 ± 0.1 μg/mL IC_{50} = 5.3 ± 0.0 μg/mL IC_{50} = 6.4 ± 0.0 μg/mL	[65]
3	*n*-Hexane extract Ethyl acetate extract Methanol extract	In vitro, α-glucosidase (cell-free enzymatic system)	Enzyme inhibition assay	Colorimetric spectrophotometry using a microplate reader (λ = 410 nm)	Inhibition: 8.9 ± 0.6% Inhibition: 31.9 ± 1.1% Inhibition: 43.9 ± 1.8% (conc. 10 mg/mL)	[82]
4	Methanol extract	In vitro, α-glucosidase and α-amylase (cell-free enzymatic system)	Enzyme inhibition assay	Colorimetric spectrophotometry (λ = 410 nm for α-glucosidase and λ = 536 nm for α-amylase)	IC_{50} = 10.0 ± 0.1 mg/mL for α-glucosidase IC_{50} = 8.5 ± 0.3 mg/mL for α-amylase	[83]
5	Ethanol extract (96%)	In vivo, Wistar rats induced with alloxan	Diabetes induction with alloxan Blood glucose measurement	Glucometer Light microscopy	Extract improved histopathological appearance (350 mg/kgBW dose) Extract decreased blood glucose levels	[84]
6	Ethanol extract (96%)	In vivo, Alloxan-induced diabetic mice	Blood glucose measurement	Glucometer	Extract reduced the blood glucose level by 41.1%	[85]
7	Ethanol extract (96%)	In vivo, Wistar rats induced with streptozotocin and nicotinamide	Blood glucose measurement	Glucometer	Extract reduced blood glucose by 48.8% (at 125 mg/kgBW dose)	[86]
8	Ethanol extract (70%)	In vivo, Wistar rats induced with alloxan	Blood glucose measurement Renal histopathology	Glucometer Light microscopy	Extract reduced fasting blood glucose by 37.7% (at 200 mg/kg BW dose) Extract significantly reduced kidney tissue damage	[87]
9	Ethanol extract (70%)	In vivo, Wistar rats induced with streptozotocin	Fasting Blood Sugar (FBS) measurement Insulin expression analysis	Glucometer Immunohistochemistry (IHC) staining with anti-insulin antibody	Reduced FBS level by ~0.4% at a dose of 50 mg/kgBW Increased insulin expression level: ~10-fold	[88]

(*Continued*)

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Table 9 (Continued).

No	Compound/Fraction/ Extract (Solvent)	Biological Model/ Test System	Assay Type	Detection Method	Key Findings	Reference
10	Decoction	In vivo (human clinical model – adult patients with diabetes mellitus)	Blood glucose measurement	Glucometer	Decoction reduced blood glucose by 31.2% (at 200 mg/kg BW dose)	[89]
11	Ethanol extract (50%)	In vivo, Wistar rats, potassium oxonate- and chicken liver–induced hyperuricemia model	Antihyperuricemic activity assay	Blood uric acid measurement from the tail vein using an enzymatic colorimetric assay	Extract (46 mg/kg BW) reduced blood uric acid levels by 50.9% on the fourth day of treatment at 120 minutes of treatment	[90]
12	Ethanol extract (50%)	In vivo, Wistar rats, potassium oxonate- and chicken liver–induced hyperuricemia model	Uricosuric activity assay	Urinary uric acid quantification by enzymatic colorimetric assay measured with micro-lab instrument at 546 nm	Extract (46 mg/kg BW) increased urinary uric acid excretion by 36.8% at 46 mg/kg dose.	[91]

mL ranges (ethyl acetate IC_{50} = 5.3 µg/mL),[65] suggesting that semi-polar phytochemicals may act as competitive enzyme inhibitors. Marked differences between mg/mL and µg/mL IC_{50} reports among studies emphasize the need for standardization and methodological consistency.

In vivo Antidiabetic Activity

Studies in rats have consistently shown glucose-lowering effects (37–49%) in streptozotocin (STZ)- and alloxan-induced models.[85–87] Interestingly, an approximately ten-fold increase in insulin expression was observed in rats treated with STZ and exposed to 70% ethanol extract, suggesting a potential protective or regenerative effect on β-cells. The improved renal histopathology in diabetic rats further supports the protective effect against diabetic nephropathy. Collectively, these findings suggest multitarget modulation, including maintenance of pancreatic β-cells, reduction of oxidative stress, improved peripheral glucose utilization, and suppression of intestinal glucose absorption. Clinical studies reporting a 31.2% decrease in glucose levels reinforce translational relevance,[89] although controlled and randomized clinical trials are required.

Antihyperuricemic Activity

The ethanol extract of *P. crocatum* also demonstrated antihyperuricemic activity by reducing uric acid levels by 50.9% and increasing uricosuria by 36.8% in rats.[90,91] This indicates the presence of additive metabolic regulatory effects, which likely occur through the inhibition of xanthine oxidase or the modulation of renal urate transporters. It is worth noting that this dual antihyperglycemic–antihyperuricemic profile is highly relevant regarding the strong clinical correlation between type 2 diabetes and hyperuricemia.

Hepatoprotective Effect

The potential hepatoprotective effects of *P. crocatum* extract were assessed in HepG2 human hepatocellular carcinoma cells exposed to acetaminophen (APAP)-induced damage (Table 10). APAP-induced liver damage is primarily mediated through the CYP2E1-dependent bioactivation of N-acetyl-p-benzoquinone imine (NAPQI), resulting in decreased glutathione levels, oxidative stress in the mitochondria, and secretion of inflammatory cytokines. In HepG2 cells, *P. crocatum* extract exhibited significant hepatoprotective effects at 25 µg/mL, with reductions in lactate dehydrogenase (LDH) (45–50%), AST (35–40%), and ALT (20–25%) levels.[92] Interestingly, reactive oxygen species (ROS) levels declined by up to 70% and TNF-α levels were reduced by approximately 50%.[93] These findings suggest that hepatoprotection is mediated by attenuation of oxidative stress, inhibition of inflammatory signaling, and reduction of necrotic cell death. However, mechanistic investigations into nuclear factor erythroid 2-related factor 2 (Nrf2) induction,

Table 10 Hepatoprotective Effect of *P. crocatum*

No	Compound/Fraction/ Extract (Solvent)	Biological Model/ Test System	Assay Type	Detection Method	Key Findings	Reference
1	Extract	HepG2 cells induced with APAP	MTS cytotoxicity assay Bradford protein assay LDH/ALT/AST enzyme assays	Spectrophotometry (490 nm, 595 nm), colorimetric enzyme kits	Extract is non-toxic up to 100 µg/mL Extract reduced LDH by~ 45 −50%, ALT by ~ 20–25%, and AST by~ 35–40% (extract conc. 25 µg/mL)	[92]
2	Ethanol extract	HepG2 cells induced with APAP	Cell viability assay Intracellular oxidative stress assay Inflammatory mediator assay Necrosis assessment	Colorimetric (MTT/ CCK-8-type) Spectrophotometric ROS/antioxidant readouts, ELISA for inflammatory markers, Microscopy for necrosis	Extract decreased TNF-α level by ~ 45–50%, ROS level by ~ 65 −70%, and the percentage of death and necrotic cells (extract conc. 25 µg/mL)	[93]
3	Ethanol extract	In vivo rat model – Wistar rats induced with CCl_4	Hepatoprotective assay Liver function biomarker analysis Histopathological evaluation	Serum enzyme assays for ALT, AST, and ALP Liver histology using hematoxylin–eosin staining and light microscopy	Ethanol extract (600 mg/kg BW) reduced ALT, AST, and ALP levels and improved liver histoarchitecture, increasing the number of normal hepatocytes.	[94]
4	Ethanol extract	In vivo rat model – Wistar albino rats induced with CCl_4	Liver enzyme assays (ALT, AST, ALP)	Spectrophotometry	Ethanolic extract (600 mg/kg bw) reduced ALT by 77.4%, AST by 53.7%, ALP by 62.5%	[95]

Abbreviations: HepG2, Human hepatocellular carcinoma; APAP, acetaminophen.

mitochondrial membrane potential, and caspase involvement have not been conducted, thus limiting the identification of pathways.

The ethanol extract (600 mg/kg body weight) significantly reduced liver enzyme levels (ALT, AST, and alkaline phosphatase [ALP]) and restored liver structure in a CCl_4-induced rat model. The reduction in ALT levels (77.4%) indicated substantial attenuation of liver cell leakage. Histological improvements further imply functional recovery rather than limited biochemical modulation.[94,95] However, the high dose required (600 mg/kg) prompts translation-related issues regarding equivalent human dosing, bioavailability of active compounds, and long-term safety profiles.

Docking analysis revealed allyl pyrocatechol and hydroxychavicol as compounds with the potential to bind CYP2E1 (−7.3 kcal/mol).[96] Considering the role of CYP2E1 in the bioactivation of APAP and CCl_4, partial inhibition may reduce the production of reactive intermediates. However, no enzyme inhibition assays confirmed the suppression of CYP2E1 activity, the docking affinity indicated moderate interaction, and selectivity toward other CYP isoforms was not evaluated. Therefore, CYP2E1 inhibition remains hypothetical.

Other Activities

Adulticidal and Larvicidal Activity

As shown in Table 11, the ethanol extract exhibited adult mosquito-killing effects against female Aedes aegypti mosquitoes with moderate toxicity (a maximum mortality rate of 43.3% and an LC_{50} value of 9901 ppm).[97] Notably, the lack of statistically significant differences among the various concentrations (ANOVA, $p = 0.541$) indicates a weak dose-response relationship. From a developmental perspective, a mortality rate of less than 50% in adult mosquitoes at practical concentrations represents suboptimal potential. Adults have a thicker cuticle and more mature detoxification enzymes (eg, cytochrome P450 monooxygenase), which may reduce the availability of the extract compared to larvae. Therefore, the crude ethanol extract is not suitable as a standalone adulticide without further purification or formulation optimization.

Table 11 Other Activities of *Piper crocatum*

No	Compound/Fraction/ Extract (Solvent)	Activity	Biological Model/Test System	Assay Type	Detection Method	Key Findings	Reference
1	Ethanol extract (96%)	Adulticidal	In vivo - *Aedes aegypti* adult female mosquitoes	Adulticidal vapor-phase bioassay; LC_{50} /LC_{90} (probit)	Visual mortality at 24 h post-exposure	Max mortality 43.3% at 1.25%; LC_{50} = 9.901 ppm (95% CI 9.139–10.653); LC_{90} = 19.524 ppm (95% CI 17.287–23.217); ANOVA p = 0.541	[97]
2	Essential oil	Larvacidal	In vivo - *Aedes aegypti* larvae (instar III)	WHO larvicidal bioassay; LC_{50}/LT_{99} (probit)	Visual mortality count (24 h); probit analysis	24 h mortality: 29% (0.01%), 84% (0.05%), 100% (0.1–0.15%); LC_{50} = 0.02425%; LC_{99} = 0.0831%; 0.1% caused 100% mortality within 3 h	[98]
3	Ethanol extract	Nephroprotective	In vivo - Male Wistar rats with gentamicin-induced nephrotoxicity	Renal function assays Antioxidant assay Histopathological analysis	Spectrophotometry TBA-based colorimetric Light microscopy	Extract (500–750 mg/kg BW) reduced serum creatinine and BUN toward normal levels. Extract reduced plasma and renal MDA levels. Extract reduced tubular necrosis and glomerular atrophy	[99]
4	Ethanol extract *n*-Hexane fraction Ethyl acetate fraction Water fraction	Acetylcholinesterase inhibitor/anti-dementia agents	Cell-free enzymatic system (AChE)	AChE inhibition assay (Ellman)	Colorimetric spectrophotometry (≈408 nm)	IC_{50} = 11.1 µg/mL IC_{50} = 23.7 µg/mL IC_{50} = 16.8 µg/mL IC_{50} = 41.0 µg/mL	[100]
5	Water extract	Acetylcholinesterase	Cell-free enzymatic system (AChE)	AChE inhibition assay (Ellman)	Colorimetric spectrophotometry (≈408 nm)	IC_{50} range = 40.8–58.4 µg/mL	[101]
6	Ethanol extract (70%)	Antidepressant	In vivo murine model - Mice	Forced Swim Test (FST)	Behavioral observation and manual timing of immobility	% Antidepressant activity = 51.1% (200 mg/kg BW dose)	[102]
7	Ethanol extract (70%)	Pancreatic Lipase Inhibitor	Cell-free enzymatic system	Lipase inhibition assay	Spectrophotometry (microplate reader λ = 427 nm)	% inhibition = 49 ± 9.1%	[103]

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In contrast, *P. crocatum* essential oil exhibited significant larvicidal activity with 100% mortality at concentrations ≥0.1% within 3 h. The LC_{50} and LC_{99} values were 0.02425% and 0.0831%, respectively, suggesting promising larvicidal potential according to the World Health Organization standard test.[98] The rapidly lethal effect points to possible neurotoxicity or disruption of membrane integrity, in line with other *Piper* species reported to contain essential oils rich in monoterpenoids and phenylpropanoids. These include acetylcholinesterase inhibition, octopamine receptor interference, mitochondrial membrane depolarization, and respiratory failure due to cuticle penetration. Unfortunately, no molecular or enzymatic assays have been performed to verify its mechanism of action.

Nephroprotective Activity

Gentamicin-induced nephrotoxicity involves the formation of reactive oxygen species and disruption of tubular epithelium. Treatment with ethanol extract (500–750 mg/kg body weight) improved renal function biomarkers (creatinine and blood urea nitrogen [BUN]), reduced MDA levels, and restored histological architecture.[99] The reduction in lipid peroxidation indicates antioxidant-driven protection. However, no mechanistic investigations on Nrf2 activation, mitigation of mitochondrial dysfunction, or regulation of inflammatory cytokines have been conducted. The high effective dose poses translation-related considerations regarding bioavailability and determination of an equivalent dose in humans.

Acetylcholinesterase (AChE) Inhibition

P. crocatum also shows potential as an anti-dementia agent for the treatment of Alzheimer's disease through AChE inhibition. Among the fractions tested, the ethanol extract exhibited the strongest AChE inhibition (IC_{50} = 11.1 µg/mL), approaching moderate potency when compared to known plant-derived cholinesterase inhibitors.[100] Docking results (Table 12) indicate that flemiphilippinin A (−11.2 kcal/mol) and columbin (−9.2 kcal/mol) interact with the active site via sufficiently strong binding energies.[101] However, these results indicate that the anti-dementia potential is still preliminary because of the absence of kinetic inhibition studies (competitive/non-competitive), the prediction of blood–brain barrier (BBB) penetration has not been experimentally verified, and the selectivity toward butyrylcholinesterase has not been evaluated.

Table 12 In silico Study of *P. crocatum* Compounds and Protein-Related Other Activities

No	Compound	Compound Identification Method	Protein Target	Method	Key Findings	Reference
1	2-(3,4-Dimethoxyphenyl) -6 ethoxy-7-methoxy-1-naphthol Columbin Flemiphilippinin A	LC-MS	Acetylcholinesterase (AChE)	Molecular docking ADMET Drug likeness Molecular docking ADMET Drug likeness Molecular docking ADMET Drug likeness	Binding affinity = −8.8 kcal/mol Favorable for oral drug use Yes Binding affinity = −9.2 kcal/mol Favorable for oral drug use Yes Binding affinity = −11.2 kcal/mol Favorable for oral drug use Yes	[101]
2	Calanolide A Myricanone (-)-8-prenylnaringenin	LC-MS	Pancreatic Lipase	Molecular docking Molecular dynamic ADMET Drug likeness Molecular docking Molecular dynamic ADMET Drug likeness Molecular docking Molecular dynamic ADMET Drug likeness	Binding affinity = 10.4 kcal/mol Stable Favorable for oral drug use Yes Binding affinity = 10.0 kcal/mol Stable Favorable for oral drug use Yes Binding affinity = 9.5 kcal/mol Stable Favorable for oral drug use Yes	[103]

Abbreviation: HMG-CoA, 3-hydroxy-3-methyl-glutaryl coenzyme A.

Antidepressant Activity

In a study on antidepressant activity, the ethanol extract of *P. crocatum* reduced the immobility time by 51.1% in the forced swim test (FST), indicating central nervous system activity.[102] However, mechanistic attribution remains speculative because of the lack of investigation into monoaminergic pathways; the absence of measurements of serotonin, dopamine, or brain-derived neurotrophic factor (BDNF); and the possibility that confounding effects from sedatives cannot be ruled out.

Anti-Obesity: Pancreatic Lipase Inhibition

A 70% ethanol extract of *P. crocatum* inhibited pancreatic lipase activity by 49 ± 9.1% in a cell-free enzymatic assay, implying moderate potential to block the digestion of dietary lipids.[103] Pancreatic lipase is the main enzyme involved in the hydrolysis of triglycerides in the digestive tract and its inhibition is a well-established pharmacological strategy for reducing intestinal fat absorption. The reported inhibitory activity demonstrated that *P. crocatum* may promote lipid metabolism regulation and weight management. This mechanism is highly relevant in the context of metabolic disorders, where overconsumption and accumulation of lipids are closely related to insulin resistance caused by obesity and the development of type 2 diabetes mellitus. In line with these metabolic relationships, several studies have reported the antidiabetic effects of *P. crocatum* extract, including improvements in glycemic parameters and markers of oxidative stress. Therefore, the inhibition of pancreatic lipase may represent one of the complementary mechanisms through which *P. crocatum* mediates its metabolic regulatory effects, linking its anti-obesity potential to broader antidiabetic activity.

Systems-Level Therapeutic Integration of *P. crocatum*

The pharmacological activities of *P. crocatum* appear to be interconnected rather than isolated. Across antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, metabolic, and hepatoprotective studies, several recurring pathways are evident. Multiple constituents appear to converge on shared biological processes, supporting the view that *P. crocatum* acts through a multitarget pharmacological network rather than through a single mechanism (Figure 10).

Integrated evidence further indicated that the pharmacological effects of *P. crocatum* are organized around a defined set of shared molecular targets, as shown in Figure 10. Multiple bioactive constituents converge on key nodes, including microbial-associated proteins (GbpC), sterol biosynthesis enzymes (CYP51), inflammatory regulators (NF-κB, COX-2, iNOS), redox mediators (ROS, MDA, NO), metabolic enzymes (α-glucosidase, PDH), and hepatic biomarkers (LDH, ALT, AST). This convergence suggests that the observed biological activities arise from the coordinated modulation of interconnected pathways, thus providing a mechanistic basis for the system-level pharmacological profile of *P. crocatum*.

Infection–Inflammation Axis

One of the most consistent and mechanistically coherent domains is the infection–inflammation axis, which integrates antibacterial, antifungal, and antibiofilm activities with immunomodulatory and anti-inflammatory responses. Extracts and isolated compounds exhibit activity against a broad spectrum of pathogens and interfere with biofilm-associated targets, such as GbpC.[40,54] Antifungal activity is primarily associated with disruption of ergosterol biosynthesis via CYP51 inhibition, leading to impaired membrane integrity.[20,45] These antimicrobial mechanisms are functionally linked to host immune responses in which macrophage activation enhances NO-mediated pathogen clearance. However, the excessive activation of these pathways may lead to inflammatory damage. In this context, *P. crocatum* demonstrates the ability to suppress downstream inflammatory mediators, including TNF-α and NO, primarily through modulation of the NF-κB–iNOS signaling axis. This dual activity indicates that *P. crocatum* functions not only as an antimicrobial or anti-inflammatory agent but also as a regulator of infection-associated inflammation, maintaining a balance between host defense and tissue protection.

Redox–Inflammation Axis

Oxidative stress is a central node that is associated with multiple pharmacological effects. Antioxidant studies have demonstrated that extracts and flavonoids such as pachypodol effectively scavenge ROS and inhibit lipid peroxidation, as reflected by reduced MDA levels.[29,67] Importantly, redox modulation is closely associated with inflammatory signaling.

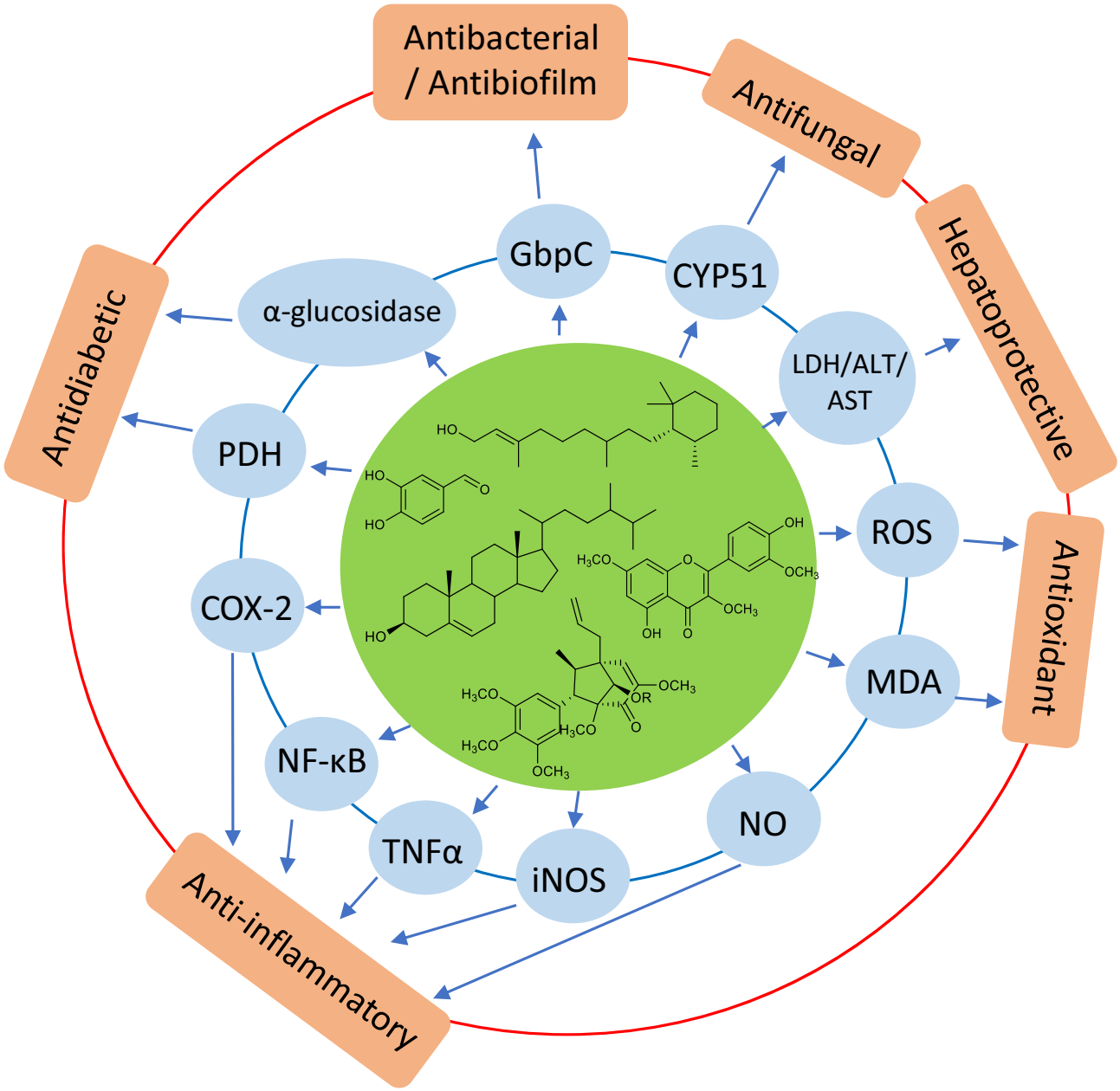

Figure 10 Multi-target pharmacological network of *P. crocatum*.

The observed suppression of ROS was accompanied by the downregulation of iNOS expression and decreased production of pro-inflammatory mediators, suggesting coordinated control of redox-sensitive pathways. Given that ROS contribute to NF-κB activation, its attenuation may indirectly limit the transcription of inflammatory cytokines such as TNF-α. Therefore, the antioxidant activity of *P. crocatum* extends beyond chemical radical scavenging and plays a functional role in regulating inflammation, reinforcing its role as a redox modulator of inflammation.

Metabolic Regulation Axis

The metabolic effects of *P. crocatum* integrate its antidiabetic and antiobesity activities into a unified framework. Inhibition of α-glucosidase suggests reduced intestinal glucose absorption, whereas modulation of PDH indicates potential regulation of mitochondrial energy metabolism. In parallel, inhibition of pancreatic lipase supports reduced lipid digestion and absorption, linking metabolic control to obesity-related pathways. These mechanisms are supported

https://doi.org/10.2147/DDDT.S611626

by in vivo evidence of improved glycemic regulation and metabolic parameters.[88,90] Collectively, these findings suggest that *P. crocatum* modulates interconnected metabolic pathways rather than single enzymatic targets, which is consistent with a systems-level approach to metabolic diseases.

Hepatic Protection and Detoxification Axis

The hepatoprotective activity of *P. crocatum* is closely associated with modulation of oxidative stress and inflammatory responses. Experimental studies have demonstrated reductions in key liver damage biomarkers including LDH, ALT, and AST, accompanied by decreased ROS levels.[94] These effects suggest attenuation of hepatocellular injury and improved cellular integrity. Although the molecular targets remain partially defined, the integration of redox regulation, inflammatory suppression, and metabolic modulation indicates that hepatoprotection arises from multipathway interactions rather than a single mechanism. This reinforces the position of *P. crocatum* as a system-level modulator of liver-associated disorders.

Conceptual Framework: Multitarget Homeostatic Regulation

Collectively, these interconnected axes support a unified conceptual model, in which *P. crocatum* functions as a regulator of physiological homeostasis. Rather than producing isolated pharmacological effects, its bioactive constituents modulate shared signaling pathways, particularly those involving NF-κB, ROS, metabolic enzymes, and membrane-associated targets. This system-level behavior reflects a form of context-dependent pharmacology, where immune activation is enhanced under infectious conditions, whereas excessive inflammation is suppressed in chronic states. This bidirectional modulation is consistent with the emerging drug discovery strategies that emphasize network regulation over single-target inhibition. However, despite this promising profile, its translation into clinical application remains limited by several factors, including high effective doses in vivo, lack of pharmacokinetic and bioavailability data, variability in the extract composition, and insufficient molecular target validation. Addressing these limitations is essential to advance *P. crocatum* from a pharmacological resource to a viable scaffold for drug development.

Lead Compound Prioritization

To facilitate the application of the research findings, the bioactive compounds identified from *P. crocatum* were systematically prioritized based on their pharmacological potential, mechanism of action, safety profile, and drug development suitability. This integrated assessment revealed varying levels of developmental relevance ranging from high-priority drug candidates to preliminary scaffolds (Table 13).

Table 13 Lead Compound Prioritization for Drug Development

Compound	Main Activity	Target/Mechanism	Potency	ADMET Insight	Development Priority
Pc-1 (Neolignan)	Immunomodulatory	NO production/macrophage activation	High (significant dose reduction vs extract)	Favorable safety profile	High-Priority Lead
Pc-2 (Neolignan)	Immunostimulant	NO/immune activation	Very high	Hepatotoxic signal observed	Preliminary Scaffold
Pachypodol	Antioxidant	Radical scavenging (HAT-dominant, DFT-supported)	Strong	Favorable	High-Priority Lead
Pipcroside B or C	Antidiabetic	PDH modulation (mitochondrial metabolism)	Moderate (μM)	Requires optimization	Promising Lead
Allylpyrocatechol	Hepatoprotective	CYP2E1 inhibition (predicted)	Moderate (in silico)	Promising	Promising Lead
Crocatin B	Antibacterial		Moderate	Promising	Promising Lead
β-sitosterol	Antimicrobial	Membrane interaction + biofilm protein targeting	Strong (μg/mL)	Drug-like	High-Priority Lead
Apigenin	Anti-inflammatory	COX-2/NF-κB (predicted)	Moderate–strong (in silico)	Favorable	Promising Lead

Among the evaluated compounds, Pc-1 (neolignan) and pachypodol have been identifed as the most promising high-priority candidates. Pc-1 demonstrates a good balance between immunomodulatory efficacy and safety, particularly when compared with Pc-2, which, despite its higher potency, exhibits signs of hepatotoxicity.[28] This highlights a key principle in drug development: potency alone does not determine translational value. In parallel, pachypodol exhibited strong antioxidant activity, as supported by experimental tests and DFT analysis, which confirmed a thermodynamically favorable HAT mechanism. Its consistent activity across various redox-related assays, along with a favorable ADMET profile, further reinforces its potential as a redox-active pharmacological framework.[29] β-Sitosterol also qualifies as a high-priority lead compound because of its relatively strong antimicrobial potential at the μg/mL level and its multitarget mode of action, which involves membrane interactions and disruption of biofilm-associated proteins.[30,41,44] The reported drug-like characteristics of these prioritized compounds further support their suitability for drug development.

Compounds such as pipcroside B or C, allyl pyrocatechol, and apigenin have been identified as promising candidates. Pipcroside derivatives exhibit moderate activity in modulating pyruvate dehydrogenase, suggesting a role in mitochondrial glucose metabolism. However, their potency remains in the micromolar range and requires further optimization. Allylpyrocatechol showed potential hepatoprotective activity via predicted CYP2E1 interactions; however, this mechanism has not yet been experimentally validated. Similarly, apigenin exhibits strong binding affinity for inflammatory targets, such as COX-2 and NF-κB, in silico, supported by favorable ADMET properties and a relatively drug-like physicochemical profile. However, there has been no direct validation in a *P. crocatum*-specific model.

In contrast, Pc-2 is more appropriately classified as a modifiable scaffold rather than a direct lead candidate, despite its strong immunostimulatory potential, because of its hepatotoxic effects. This emphasizes the importance of safety profiles in the early-stage prioritization process and suggests that Pc-2 may serve as a structural template for further modification rather than as a direct development candidate.

Overall, this prioritization confirms that the therapeutic potential of *P. crocatum* lies not only in its chemical diversity but also in the varying translational potential of its constituents. The identification of high-priority compounds and modifiable scaffolds provides a rational basis for drug development. However, this prioritization also highlights an important gap: several structurally characterized metabolites remain poorly explored or have not yet been pharmacologically evaluated.

Underexplored Compounds and Research Gaps

Several compounds isolated from *P. crocatum* have not yet been studied in sufficient pharmacological detail. This gap is important because some of these metabolites share structural features with compounds that have already shown biological activity. For example, 4-allyl-2,6-dimethoxy-3-hydroxy-1-D-glucopyranoside (**7**) and erigeside II (**8**) are structurally related to syringin (**3**), as they contain a phenylpropanoid glycoside framework with hydroxylated and methoxylated aromatic groups. Erigeside II has been reported to inhibit soluble epoxide hydrolase, whereas compound **7** remains less explored. This structural relationship suggests that compound **7** may warrant further evaluation in assays related to inflammatory enzyme modulation and redox-associated pathways.

A similar consideration applies to the pipcroside group. Pipcroside A (**20**), pipcroside B (**21**), and pipcroside C (**22**) share structural similarities with nectamazin A (**27**), particularly in their lignan-derived glycosidic framework and methoxylated aromatic substituents. Nectamazin A has shown antibacterial, antifungal, and multitarget in silico activities against proteins involved in microbial growth and biofilm formation. Therefore, the pipcroside derivatives may also have related biological potential, although this assumption still requires direct experimental validation. The reported pyruvate dehydrogenase-modulating activity of pipcroside B and C further suggests that this subgroup may have pharmacological relevance beyond metabolic regulation.

These examples indicate that the pharmacological profile of *P. crocatum* remains incomplete. Several structurally identified but less-explored metabolites may contribute to the overall activity of this plant or provide additional scaffolds for lead optimization. Future studies should prioritize bioassay-guided evaluation of these compounds, followed by target-based assays, pharmacokinetic assessment, and structure–activity relationship analysis to clarify their relevance in drug discovery.

Limitations, Regulatory Considerations, and Future Directions

Despite the growing pharmacological evidence for *P. crocatum*, several limitations remain. Most studies are still based on crude extracts, fractions, or preliminary in vitro and in silico models. In vivo validation, pharmacokinetic characterization, and standardized toxicity evaluation are still limited. Differences in extraction methods, plant origin, dosage, and chemical standardization also make direct comparison among studies difficult. These limitations need to be addressed before *P. crocatum*-derived compounds can be considered reliable drug candidates.

From a regulatory perspective, further development of *P. crocatum*-based products requires plant authentication, raw material standardization, quality control of marker compounds, reproducible extraction procedures, and comprehensive safety evaluation. For isolated compounds intended for drug development, pharmacokinetic profiling, toxicological assessment, mechanism validation, and efficacy testing in relevant preclinical models are also required.

Although several metabolites identified from *P. crocatum* show pharmacological potential, their commercial development remains limited. Some widely distributed plant metabolites, including phytosterols and selected flavonoids, are already used as ingredients in nutraceutical or cosmetic products from other botanical sources. However, there is currently limited evidence that compounds specifically isolated from *P. crocatum* have been developed into standardized commercial drug products. This highlights the need for further formulation development and regulatory assessment before *P. crocatum*-derived metabolites can be translated into commercial therapeutic products.

Based on the available evidence, oral and local routes appear to be the most relevant routes of administration for *P. crocatum*-based preparations. Oral administration in the form of decoctions or extracts may be suitable for systemic applications, including metabolic, inflammatory, and hepatoprotective effects. In contrast, local dosage forms, such as mouthwash, gel, and root canal irrigant solutions, may be more appropriate for oral infections, periodontal conditions, and antibiofilm applications because they allow direct contact with the target site. For isolated compounds with limited solubility or oral bioavailability, formulation strategies such as nanoformulations, topical delivery systems, or prodrug approaches may be required. However, the selection of an appropriate dosage form should be supported by pharmacokinetic, toxicity, and target-site validation studies.

Future research should therefore move beyond descriptive bioactivity screening toward mechanism-driven studies, target validation, and pharmacokinetic evaluation. Several pathways repeatedly appear in the available studies, including the TLR4–NF-κB–iNOS pathway in inflammation, CYP2E1-mediated bioactivation in hepatotoxicity, and mitochondrial enzymes in metabolic regulation. These pathways provide a rational basis for focused validation using enzyme assays, gene expression analysis, and pathway-specific reporter systems.

From a medicinal chemistry perspective, several compounds identified in this review may serve as starting points for structural optimization. Neolignans such as Pc-1 show a favorable balance between efficacy and safety in immunomodulatory models, whereas pachypodol provides a redox-active scaffold with a clearer antioxidant mechanism. In contrast, Pc-2 highlights the need for structural modification to reduce toxicity despite its potent immunostimulatory activity. Strategies such as functional group modification, scaffold simplification, hybrid molecule development, prodrug design, nanoformulation, and delivery system modification may help improve selectivity, potency, bioavailability, and pharmacokinetic properties. However, comprehensive ADMET profiling and in vivo pharmacokinetic studies are still needed to connect in vitro activity with clinical feasibility.

The multitarget pharmacological profile of *P. crocatum* suggests that its therapeutic value may not rely on a single target. Instead, its constituents may act through multiple pathways that are relevant to complex conditions such as metabolic syndrome, chronic inflammation, and infection-related disorders. Future studies using systems biology approaches, multiparametric analysis, and tissue-level models may help clarify these interactions. Overall, development of *P. crocatum* as a source of drug candidates will require standardized materials, validated mechanisms, improved pharmacokinetic data, and careful safety evaluation.

Conclusion

Current scientific evidence highlights the potential of *P. crocatum* as a valuable source of bioactive compounds for drug discovery and therapeutic development. Phytochemical studies have identified a variety of secondary metabolites,

including phenolics, neolignans, flavonoids, terpenoids, and phytosterols, several of which exhibit meaningful biological activities. Pharmacological studies further show that *P. crocatum* has antimicrobial, antibiofilm, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, and immunomodulatory effects. These activities appear to involve the modulation of several molecular pathways, including oxidative stress regulation, inflammatory signaling, microbial membrane disruption, and inhibition of key metabolic enzymes. This multi-target pharmacological profile supports the traditional use of this plant and highlights its potential relevance for developing therapeutic agents targeting infectious diseases, metabolic disorders, and inflammatory conditions.

Despite these promising findings, several limitations remain. Most studies still rely on crude extracts evaluated at relatively high concentrations, whereas pharmacokinetic data, toxicity evaluation, and detailed mechanism-of-action studies are limited. Inconsistencies in extraction methods, experimental models, and compound standardization also complicate direct comparisons among studies. Future studies should prioritize bioassay-guided isolation, structural optimization of key compounds, molecular-level mechanism validation, and comprehensive pharmacokinetic evaluation. Overall, *P. crocatum* represents a chemically diverse and pharmacologically relevant natural source, but its development as a source of drug candidates will require interdisciplinary research integrating natural product chemistry, pharmacology, medicinal chemistry, and translational validation.

Acknowledgments

The authors are grateful to Universitas Padjadjaran for supporting this work through the Beasiswa Program Doktor Padjadjaran (BPDP) 2024 (grant number 1727/UN6.3.1/PT.00/2024), Penelitian Disertasi Doktor 2026 (grant number 1548/UN6.3.1/PT.00/2026), and Penelitian Fundamental Reguler (PFR) Grant (grant number 1549/UN6.3.1/PT.00/2025).

This publication charge is funded by Universitas Padjadjaran through the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science, and Technology and managed under the EQUITY Program (Contract No. 4303/B3/DT.03.08/2025 and 3927/UN6. RKT/HK.07.00/2025).

Disclosure

The authors report no conflicts of interest in this work.

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