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REVIEW

Cleistocalyx operculatus (Roxb.) Merr. & Perry: Phytochemistry, biological activities, and potential applications

Pham Thi Quyen[1]; Le Pham Tan Quoc[2] *

[1] Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, 700000, Vietnam.
[2] Faculty of Health Sciences, Industrial University of Ho Chi Minh City, Ho Chi Minh City, 700000, Vietnam.

* Corresponding author: lephamtanquoc@iuh.edu.vn (L. P. T. Quoc).

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Abstract
Cleistocalyx operculatus (Roxb.) Merr. & Perry is a medicinal plant widely distributed in Vietnam and several Asian countries, traditionally used in herbal tea and folk medicine. Owing to the growing scientific interest in this species, this study combines bibliometric analysis and a comprehensive literature review to evaluate research trends, phytochemical constituents, biological activities, and potential applications of *C. operculatus*. Bibliometric analysis of scientific publications revealed a marked increase in research output during the last decade, with phytochemistry, antioxidant activity, antimicrobial effects, and bioactive compounds emerging as major research themes. Published studies have identified a wide range of phytochemicals, including flavonoids, chalcones, terpenoids, phenolic compounds, phloroglucinol derivatives, and essential oils. Among them, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) has been recognized as a key bioactive compound responsible for several pharmacological effects. Extracts and isolated compounds from *C. operculatus* exhibit diverse biological activities, including antioxidant, antimicrobial, antiviral, anti-inflammatory, antidiabetic, anti-obesity, anticancer, hepatoprotective, nephroprotective, and neuroprotective properties in both *in vitro* and *in vivo* studies. Furthermore, the plant shows promising applications in functional foods, edible coatings, green synthesis of nanoparticles, wastewater treatment, and biomedical materials. Despite these advances, further studies on standardization, toxicity, clinical validation, and large-scale cultivation are required to facilitate industrial and pharmaceutical applications. Overall, *C. operculatus* represents a valuable natural resource and an emerging research subject with considerable potential for future development in food, pharmaceutical, and biotechnological sectors.

Keywords: *Cleistocalyx operculatus*; phytochemistry; biological activities; flavonoids; DMC; medicinal plant; nanoparticles; functional food.

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1. Introduction

Cleistocalyx operculatus (Roxb.) Merr. & Perry is a plant species belonging to the Myrtaceae family, predominantly distributed in Southeast Asian countries such as Vietnam, Thailand, and China (Mai et al., 2009). In Vietnam, this species is widely known as "Vối," whose flower buds and leaves are traditionally used for herbal tea preparation and as folk remedies to support digestion, reduce body heat, and improve general health (Đỗ, 2004). Owing to its distinctive aroma and long-standing ethnomedicinal value, *C. operculatus* has gained increasing scientific attention as a promising source of functional food ingredients and natural therapeutic agents (Gallo, 2023).

Phytochemical investigations have demonstrated that *C. operculatus* contains a wide range of bioactive constituents, including chalcones, flavonoids, polyphenols, anthocyanins, terpenoids, and essential oils (Thanh et al., 2024a). Among these compounds, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) has been recognized as a characteristic phytochemical and is considered one of the major contributors to the biological activities of the species (Huang et al., 2012). In addition to DMC, numerous flavonoids and meroterpene derivatives isolated from the flower buds, leaves, and fruits of the plant have also exhibited considerable pharmacological potential.

In recent years, extensive *in vitro* and *in vivo* studies have revealed a broad spectrum of biological activities associated with *C. operculatus*. Extracts and isolated compounds from this species have demonstrated anti-inflammatory, antiviral, antimicrobial, antidiabetic, lipid-regulating, hepatoprotective, neuroprotective, and anticancer properties (Dao et al., 2010; Le et al., 2025). Notably, several studies have highlighted the ability of *C. operculatus* constituents to inhibit key enzymes associated with metabolic syndrome, including α-glucosidase, α-amylase, pancreatic lipase, and protein tyrosine phosphatase 1B (PTP1B) (Mai et al., 2024). Furthermore, its strong antioxidant activity, mediated through free radical scavenging and modulation of endogenous antioxidant defense systems, is considered a fundamental mechanism underlying many of its cytoprotective effects.

Although numerous studies have reported the remarkable pharmacological potential of *C. operculatus*, information regarding its phytochemical composition, biological activities, and mechanisms of action remains fragmented and has not yet been comprehensively summarized. Therefore, this review aims to systematically compile and critically discuss the current knowledge on the phytochemical characteristics, major biological activities, and potential applications of *C. operculatus* in the fields of functional foods and natural pharmaceuticals. In addition, current research limitations and future perspectives are highlighted to facilitate more effective exploitation and development of this valuable plant resource.

2. Methodology

2.1. Literature search and study selection

A comprehensive literature review was conducted to collect and analyze scientific publications related to *C. operculatus*. Relevant studies were retrieved from Web of Science. The search strategy employed combinations of keywords such as "*Cleistocalyx operculatus*", "phytochemistry", "bioactive compounds", "antioxidant", "antibacterial", "antimicrobial", "anti-inflammatory", "antidiabetic", "anticancer", and "pharmacological activities".

Peer-reviewed articles published in English were considered for inclusion. Additional publications were identified through manual screening of references cited in relevant articles. Studies focusing on phytochemical composition, biological activities, mechanisms of action, and potential applications of *C. operculatus* were included. Duplicate records, studies lacking sufficient experimental information, conference abstracts, and publications unrelated to the scope of this review were excluded.

The selected literature was critically evaluated and organized according to major research themes, including phytochemical constituents, antioxidant activity, antimicrobial effects, antidiabetic potential, and other pharmacological properties.

2.2. Bibliometric analysis

A bibliometric analysis was performed to evaluate the research trends and knowledge structure of *C. operculatus*. Bibliographic data was retrieved from the Scopus database using the following search query: *("Cleistocalyx operculatus") AND (antioxidant OR antibacterial OR phytochemical OR flavonoid OR phenolic OR extract).* Only research articles were included in the analysis. After screening and removing irrelevant records, a total of 90 publications were retained for bibliometric evaluation. The bibliographic information, including authors, affiliations, countries, keywords, publication years, and source titles, was exported from Scopus in CSV format. Bibliometric analyses were conducted using the Bibliometrix package in R software. The annual scientific production was analyzed to identify publication trends over time. Country collaboration networks were generated to visualize international research cooperation. Keyword co-occurrence analysis was performed to identify major research hotspots and thematic relationships. In addition, thematic mapping and trend-topic analyses were applied to explore the conceptual structure of the field and reveal emerging research directions.

3. Annual scientific production

The bibliometric overview of *C. operculatus* research is presented in Figure 1, which includes the thematic distribution of publications (Figure 1a) and the annual scientific production (Figure 1b).

The thematic distribution of publications reveals that Applied Chemistry & Environmental Science field accounts for the largest proportion with 29 articles (32.2%), followed by Food Chemistry & Pharmacology field with 22 articles (24.4%), and Nanotechnology & Materials Science field with 16 articles (17.8%). Meanwhile, Phytochemistry & Natural Products field contributed 15 articles (16.7%), while Plant Biology & Biotechnology field represented the smallest share with only 8 articles (8.9%) (Figure 1a). This distribution indicates that research on *C. operculatus* has progressively shifted from traditional phytochemical characterization toward applied fields, particularly environmental chemistry, food science, and nanotechnology.

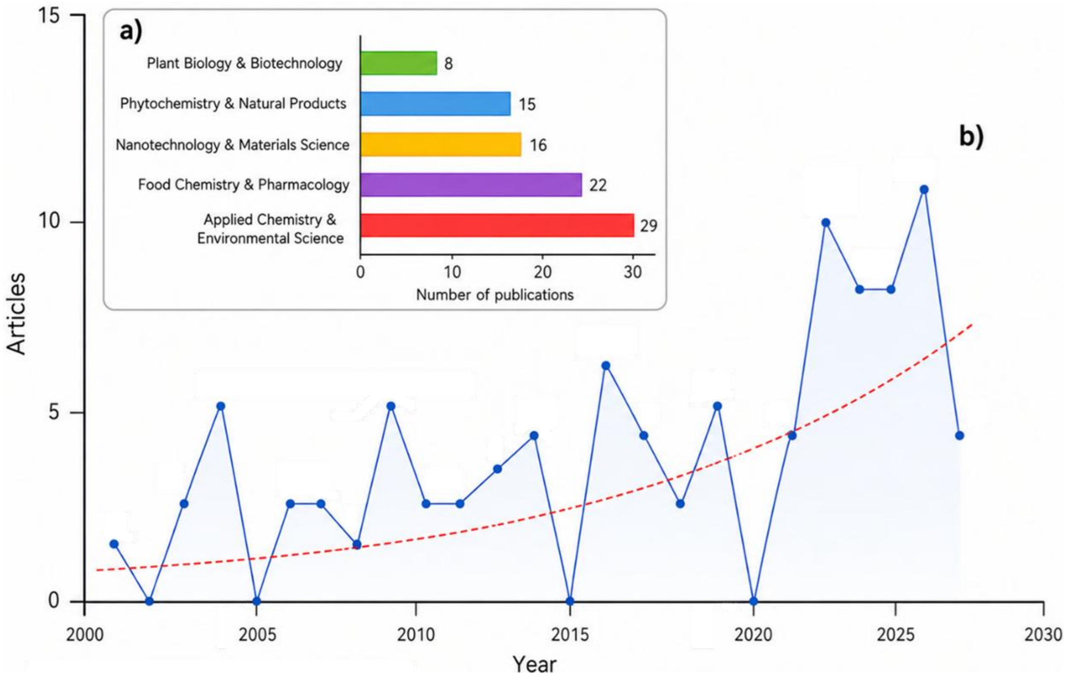

Figure 1. Overview of *C. operculatus* research: (a) Thematic distribution of publications and (b) Annual scientific production (2002–2026).

Regarding the annual scientific production (Figure 1b), the number of publications on *C. operculatus* remained relatively low and fluctuated during the period from 2002 to 2021, with fewer than six publications per year in most cases. Several years, including 2003, 2006, 2015, and 2020, recorded no publications, indicating limited and intermittent research activity during the early stages of investigation. A notable increase in publication output was observed from 2022 onward, reaching 10 articles in 2022 and peaking at 11 articles in 2025. This growing trend reflects increasing scientific interest in *C. operculatus*. The lower number of publications in 2026 is likely due to the incomplete coverage of the current year at the time of data collection.

4. Number of publications by country

The country collaboration analysis revealed that Vietnam was the leading contributor to research on *C. operculatus*, with 131 publications, followed by China (67), South Korea (38), and Thailand (29) (Figure 2). This distribution reflects the geographical occurrence and traditional utilization of the plant in Southeast and East Asia, where research efforts have primarily focused on its phytochemical composition and biological activities.

In contrast, contributions from countries outside Asia were relatively limited, with the United States (7), India (6), Japan (6), and several European countries reporting only a small number of publications. The dominance of Asian countries suggests that research on *C. operculatus* remains regionally concentrated, while the relatively low participation of Western countries highlights opportunities for broader international collaboration and further exploration of its pharmacological and industrial potential.

5. Thematic map

The thematic map indicates that "extract", "mechanisms", and "essential oil" are positioned within the Motor Themes quadrant, suggesting that these topics are both highly developed and central to the research field (Figure 3). This finding highlights that studies on extraction processes, bioactive mechanisms, and essential oils have become the main driving forces in *C. operculatus* research. In addition, the cluster comprising "green synthesis", "silver nanoparticles", and "antibacterial" is in the Niche Themes quadrant, indicating a well-developed but relatively specialized research area with limited connections to the broader field.

The Basic Themes quadrant is dominated by keywords such as "flavonoids", "*Cleistocalyx operculatus*", and "Roxb. Merr.", reflecting the fundamental role of phytochemical characterization in the existing literature. Meanwhile, "phenolic compounds" and "radical scavenging activity" appear in the Emerging or Declining Themes quadrant, suggesting either newly developing research directions or topics receiving decreasing attention. The cluster containing "apoptosis", "inhibition", and chalcone-related compounds occupies an intermediate position with relatively high centrality but moderate development, indicating growing interest in the pharmacological and anticancer potential of specific bioactive constituents from *C. operculatus*.

6. Keyword co-occurrence

The keyword co-occurrence network analysis identified six thematic clusters representing the intellectual structure and evolving research landscape of *C. operculatus* studies (Figure 4).

The largest and most central domain (Cluster 1, red) encompasses core terms including "*Cleistocalyx operculatus*", "flavonoids", "myrtaceae", "essential oil", "antibacterial activity", "apoptosis", "molecular docking", and "tumor". The prominence of this cluster underscores that phytochemical characterization and pharmacological bioactivities—particularly antibacterial and anticancer properties—remain the predominant research focus. Notably, the inclusion of "molecular docking" signals a methodological shift toward computational biology and structure-based mechanistic investigations.

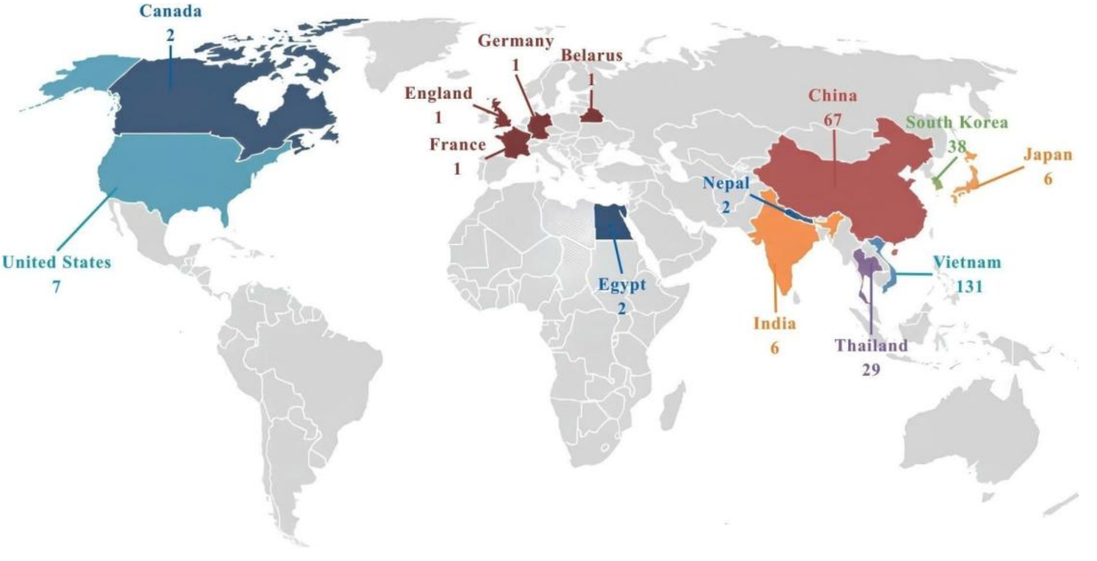

Figure 2. Number of publications on *C. operculatus* by country.

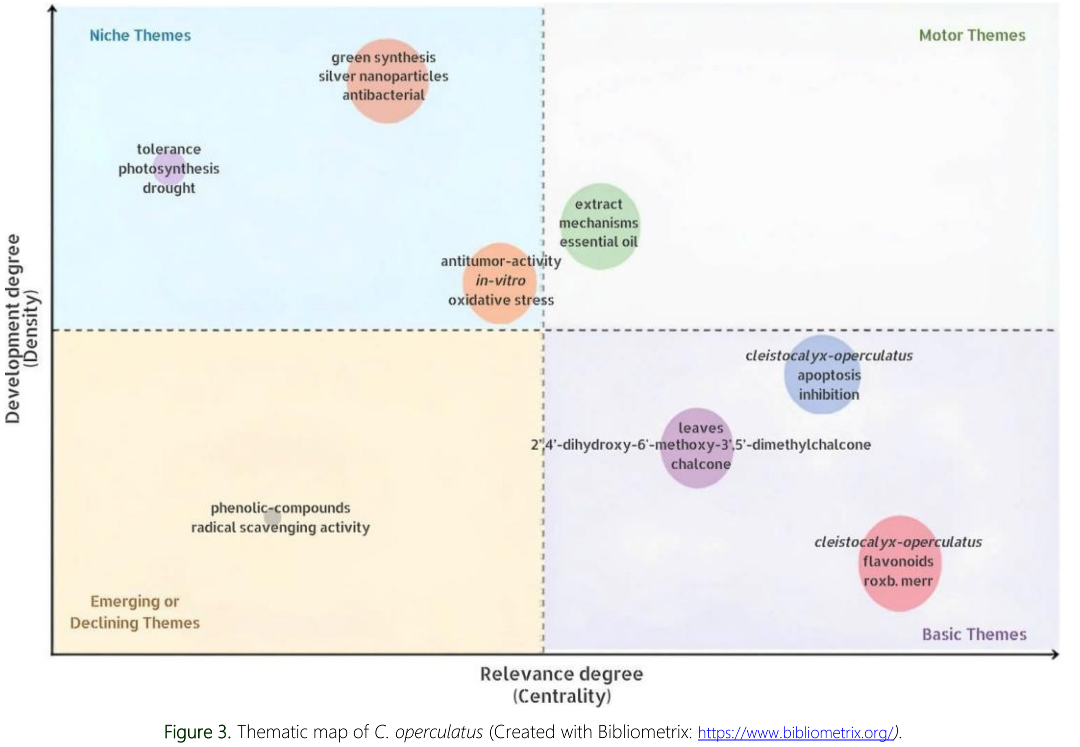

Figure 3. Thematic map of *C. operculatus* (Created with Bibliometrix: https://www.bibliometrix.org/).

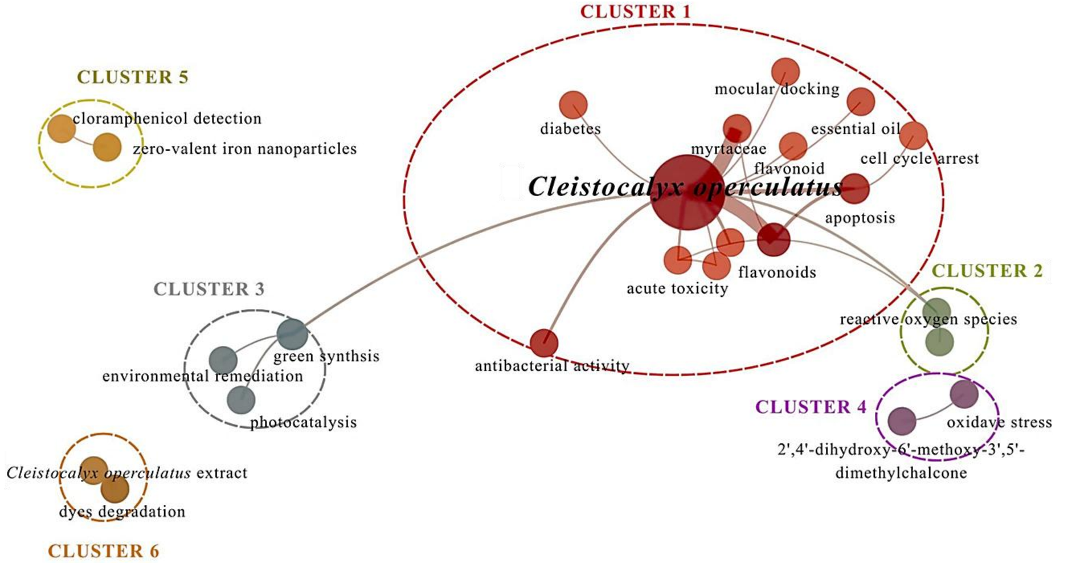

Figure 4. Keyword co-occurrence network of *C. operculatus* research.

Another well-established research theme (Cluster 2, green), comprising "reactive oxygen species" and "hydrogen peroxide", reflects a sustained interest in oxidative stress pathways and the antioxidant mechanisms of *C. operculatus* bioactives. This cluster highlights the growing recognition of the species as a potential source of natural antioxidants. In contrast to the traditional phytochemical focus, an emerging research direction (Cluster 3, blue) involving "photocatalysis" and "environmental remediation" demonstrates the expanding application of *C. operculatus* extracts in materials science and green chemistry. This shift indicates that the research scope has broadened considerably beyond conventional phytochemistry. More specifically, the presence of the chalcone "2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone" alongside "oxidative stress" in Cluster 4 (purple) suggests that this particular compound has attracted significant attention for its potential role in oxidative stress modulation, reflecting a trend toward identifying individual bioactive constituents with specific mechanisms of action.

Alongside these major themes, a highly specialized niche (Cluster 5, orange) has emerged, linking "zero-valent iron nanoparticles" with "chloramphenicol detection". This cluster reflects the integration of nanotechnology with analytical chemistry, particularly in biosensor development for antibiotic residue detection.

Finally, the presence of Cluster 6 (brown), featuring "*Cleistocalyx operculatus* extract" and "dyes degradation", reinforces the growing interest in the environmental applications of plant extracts, specifically in the photocatalytic degradation of organic pollutants.

Overall, the co-occurrence network reveals a progressive thematic evolution from traditional phytochemical investigations toward multidisciplinary applications, including nanomaterial synthesis, environmental remediation, and target-specific pharmacological research. The coexistence of well-established and emerging clusters indicates a dynamic and rapidly diversifying research field with increasing interdisciplinary connectivity.

7. Phytochemical constituents of *C. operculatus*

Phytochemical investigations have demonstrated that *C. operculatus* possesses a diverse secondary metabolite profile comprising numerous biologically valuable compounds, including flavonoids, chalcones, flavanones, phloroglucinol derivatives, terpenoids, phytosterols, and polyphenolic constituents (Table 1). Among the investigated plant parts, the flower buds and leaves have received the greatest attention due to their high phenolic contents and pronounced biological activities. The structural diversity of the isolated compounds highlights the considerable potential of this species in natural product chemistry and the development of novel functional bioactive compounds.

Most phytochemical studies have focused on flavonoids and chalcones, which are considered characteristic constituents of *C. operculatus*. Ye et al. (2004) and Min et al. (2008) isolated several novel flavonoids from the flower buds, including chalcones and flavanones bearing uncommon formyl substituents (Figure 5). In particular, compounds such as 3-formyl-4,6,4'-trihydroxy-2-methoxy-5-methylchalcone and (2S)-8-formyl-6-methylnaringenin exhibited unusual structural features compared with typical plant flavonoids. The presence of methoxy, methyl, and formyl groups on the flavonoid skeleton may contribute to enhanced biological activities by modifying hydrophobicity, enzyme-binding affinity, and antioxidant properties of the molecules.

In addition to these newly identified flavonoids, several common flavonols, including Myricetin-3′-methylether 3-O-β-D-galactopyranoside; myricetin-3′,5′-dimethylether 3-O-β-D-galactopyranoside, quercetin, kaempferol, and tamarixetin, were also isolated from the flower buds of *C. operculatus* (Figure 6) (Min et al., 2010). These compounds have been widely reported to exhibit antioxidant, anti-inflammatory, and cytoprotective effects in previous studies.

Table 1
Phytochemical constituents identified from different parts of *C. operculatus*

Study	Plant part	Major compounds identified	Compound class / remarks
Ye et al. (2004)	Dried buds	3′-formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone; (2S)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone	Two novel flavonoids identified using UV, IR, EIMS, NMR, and HMBC analyses
Min et al. (2008)	Buds	3-formyl-4,6,4-trihydroxy-2-methoxy-5-methylchalcone; 3-formyl-6,4-dihydroxy-2-methoxy-5-methylchalcone 4-O-β-D-glucopyranoside; (2S)-8-formyl-6-methylnaringenin; (2S)-8-formyl-6-methylnaringenin 7-O-β-D-glucopyranoside	Four new flavonoids characterized by spectroscopic analyses and 2D NMR
Min et al. (2010)	Buds	Myricetin-3′-methylether 3-O-β-D-galactopyranoside; myricetin-3′,5′-dimethylether 3-O-β-D-galactopyranoside; quercetin; kaempferol; tamarixetin	Flavonoids and flavonol derivatives
Song et al. (2019)	Buds	Cleistocaltone A; Cleistocaltone B	Rare cinnamyl-β-triketone-containing conjugates
Chariyakornkul et al. (2021)	Dried fruit extract	2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC); β-selinene; α-selinene; γ-selinene; o-cymene; 24(Z)-methyl-25-homocholesterol	Major flavonoid, terpenoids, and phytosterol identified in dichloromethane extract
Ha et al. (2016)	Leaves	Two new acetophenones (1–2); one new flavanone (3); six known compounds (4–9)	Bioactivity-guided isolation from neuraminidase inhibitory fractions
Su et al. (2018)	Leaves	Four novel phloroglucinol derivatives (1–4); biosynthetic precursors (5–6)	Phloroglucinol dimers and phloroglucinol-terpene adducts with unusual polycyclic skeletons; compound 1 showed anti-HSV-1 activity

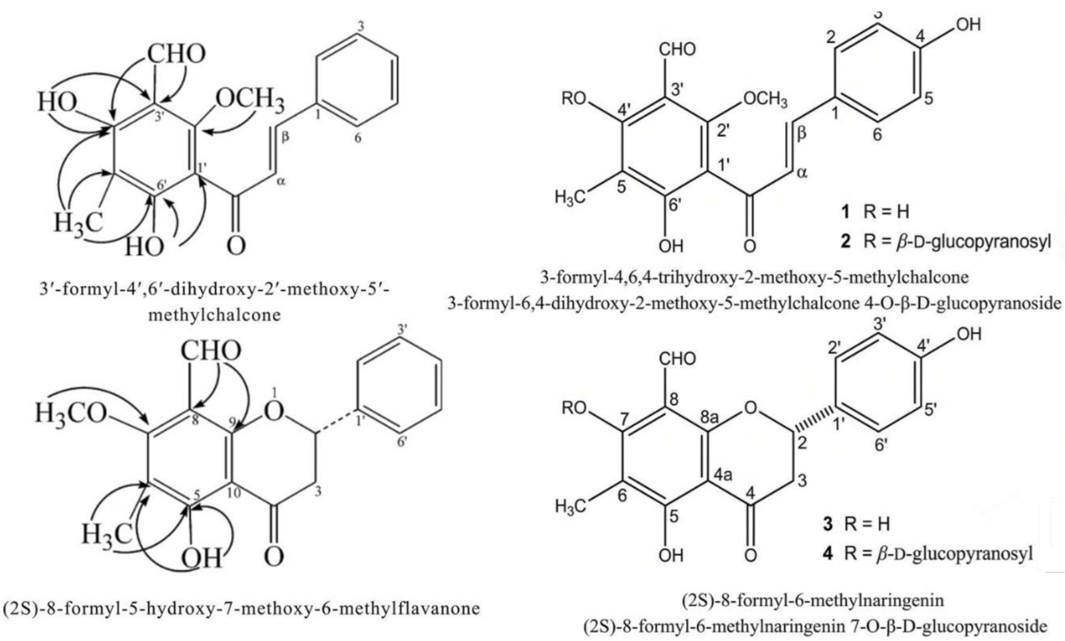

Figure 5. Chemical structures of flavonoids isolated from *C. operculatus* buds reported by Ye et al. (2004) and compounds 1–4 reported by Min et al. (2008).

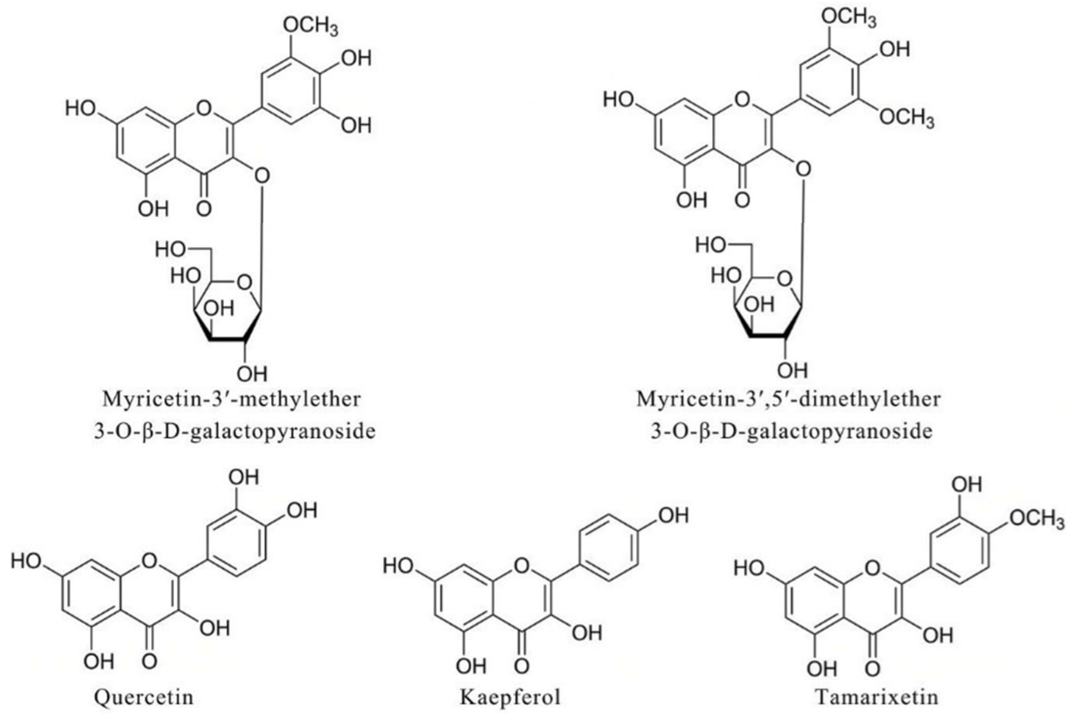

Figure 6. Chemical structures of characteristic flavonoids isolated from the flower buds of *C. operculatus*. Adapted from the isolation and identification by Min et al. (2010).

The simultaneous occurrence of both aglycone and glycosylated flavonoids suggests that this species possesses a rich and complex flavonoid biosynthetic pathway, which may partly explain its broad spectrum of pharmacological activities.

Among the identified compounds, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) is considered the most prominent constituent and has frequently been reported as a major compound in extracts derived from the flower buds or fruits of *C. operculatus*. Numerous studies have associated DMC with the anti-inflammatory, antioxidant, anticancer, and antidiabetic activities of this species (Thanh et al., 2024b). The consistent detection of DMC at relatively high levels across different studies indicates that this compound may serve as a potential marker compound for quality standardization and the development of functional products derived from *C. operculatus*.

A summary of isolated compounds from various studies is presented in Table 1. Notably, from the leaves of *C. operculatus*, Ha et al. (2016) conducted a bioactivity-guided investigation targeting neuraminidase inhibitory activity. This led to the isolation of two new acetophenones (1–2), one new flavanone (3), along with six known compounds (4–9). The chemical structures of these isolated compounds are presented in Figure 7.

Besides flavonoids, recent studies have also identified several structurally unique and chemically complex metabolites. Song et al. (2019) isolated Cleistocaltone A and B, which contain the rare 2,2,4-trimethyl-cinnamyl-β-triketone moiety, whereas Su et al. (2018) reported novel phloroglucinol dimers and phloroglucinol–terpene adducts possessing unprecedented polycyclic skeletons (Figure 8). These findings suggest that *C. operculatus* is not only a rich source of conventional polyphenols but also capable of biosynthesizing structurally distinctive secondary metabolites with high chemical value. Such compounds may represent promising lead structures for future natural drug discovery and pharmaceutical research.

In addition, the terpenoid and phytosterol compositions of *C. operculatus* have also been investigated. Chariyakornkul et al. (2021) identified β-selinene, α-selinene, γ-selinene, and *o*-cymene as major terpenoid constituents, while 24(Z)-methyl-25-homocholesterol was reported as the principal phytosterol in the dichloromethane extract. These terpenoid compounds are believed to contribute to the characteristic aroma of the plant as well as its anti-inflammatory and antimicrobial properties. Furthermore, Le et al. (2025) reported that the leaves of *C. operculatus* contain relatively high levels of total polyphenols, further supporting the potential of this plant part as a natural source of antioxidant compounds.

Collectively, current studies indicate that *C. operculatus* possesses a rich and diverse phytochemical composition containing numerous biologically active metabolites. However, most existing investigations have primarily focused on compound isolation and structural elucidation, whereas the relationships between chemical structures and biological activities of many newly identified compounds remain insufficiently understood. Therefore, further studies on mechanisms of action, bioavailability, and practical applications are necessary to fully exploit the medicinal and functional potential of *C. operculatus*.

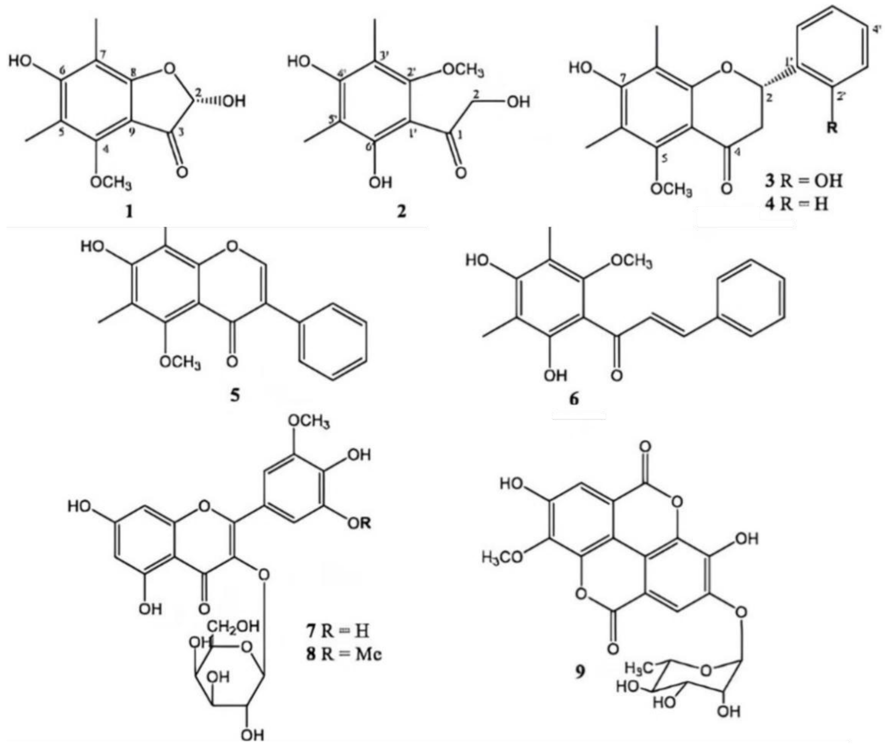

Figure 7. Chemical structures of compounds isolated from *C. operculatus* leaves by Ha et al. (2016). Two new acetophenones (1–2), one new flavanone (3), and six known compounds (4–9).

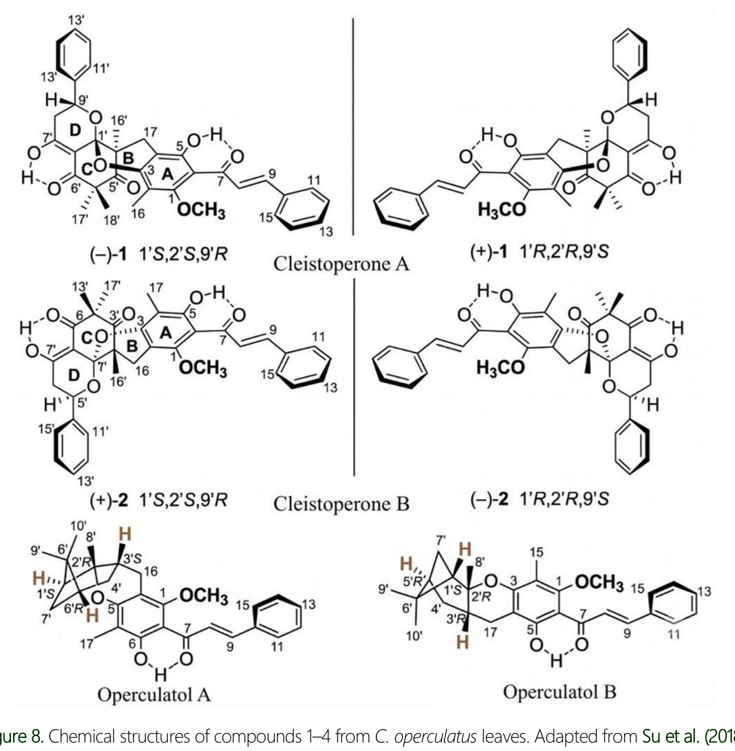

Figure 8. Chemical structures of compounds 1–4 from *C. operculatus* leaves. Adapted from Su et al. (2018).

8. Chemical composition of *C. operculatus* essential oil

Table 2 demonstrates considerable variations in the chemical composition of the essential oils of *C. operculatus* among different studies, particularly regarding dominant constituents and the relative proportions of terpene compounds. Nevertheless, the essential oils of this species are generally characterized by the predominance of monoterpene hydrocarbons and sesquiterpenes, with ocimene and myrcene consistently reported as major constituents in several investigated samples.

In leaf samples collected from Vietnam, trans-β-ocimene/(E)-β-ocimene was identified as the dominant constituent, accounting for as much as 52.9% of the total oil composition in the study by Thuy et al. (2021). In contrast, Tran et al. (2023) reported allo-ocimene (31.6%) and (Z)-β-ocimene (30.4%) as the two major components in leaf essential oil from the same region. The predominance of ocimene derivatives suggests that the leaf essential oil of *C. operculatus* belongs to an unsaturated monoterpene-rich chemotype. Ocimene compounds have previously been associated with antimicrobial, anti-inflammatory, and plant defense activities, in addition to contributing substantially to the characteristic aroma of the essential oil.

Conversely, the study conducted by Dosoky et al. (2015) using leaf samples collected in Nepal revealed a markedly different chemotype, in which myrcene accounted for up to 69.70% of the total essential oil composition, significantly exceeding the levels reported in Vietnamese samples. This oil also contained considerable amounts of (E)-β-ocimene (12.24%) and linalool (4.08%). Such pronounced differences in chemical composition among geographical regions may be attributed to climatic conditions, soil characteristics, altitude, harvesting period, and other ecological factors influencing terpene biosynthesis in the plant.

In addition to monoterpenes, several sesquiterpenes, including caryophyllene, humulene, α-selinene, γ-murolene, α-cadinol, and aromadendrene, have also been identified in *C. operculatus* essential oils. Caryophyllene was detected at a relatively high proportion in the study of Thuy et al. (2021) (8.1%), whereas (+)-aromadendrene was reported as a major constituent in flower bud essential oil by Lien & Cuong (2017).

Table 2
Chemical composition of *C. operculatus* essential oil

Compound	Leaves (Thuy et al., 2021)	Leaves (Dosoky et al., 2015)	Leaves (Tran et al., 2023)	Buds (Lien & Cuong, 2017)
Trans-β-Ocimene / (E)-β-Ocimene	52.9	12.24	-	-
Cis-β-Ocimene / (Z)-β-Ocimene	10.9	4.79	30.4	-
allo-Ocimene	-	-	31.6	-
Caryophyllene / (E)-caryophyllene	8.1	1.11	-	0.07
Humulene	2.5	-	-	-
Epiglobulol	2.1	-	-	-
γ-Murolene	2.0	-	-	-
α-Selinene	1.1	-	-	-
Caryophyllene oxide	1.1	0.23	-	-
β-Selinene	1.0	-	-	-
γ-Elemene	1.0	-	-	-
α-Cadinol	0.7	-	-	1.28
β-Cubebene	0.6	-	-	-
Cis-lanceol	0.5	-	-	-
β-Myrcene / Myrcene	0.4	69.70	-	-
γ-Cadinene	0.4	-	-	-
Cedrene	0.3	-	-	-
β-Pinene	0.2	-	-	-
(+)-Aromadendrene	-	-	-	4.73
γ-Terpinene	-	0.82	-	0.09
Torreyol	-	-	-	0.19
Carotol	-	-	-	0.26
δ-Cadinol	-	-	-	0.19
β-Guaiene	-	-	-	0.12
α-Thujene	-	0.53	-	-
α-Phellandrene	-	0.69	-	-
p-Cymene	-	2.71	-	-
β-Phellandrene	-	1.20	-	-
Terpinolene	-	0.26	-	-
Linalool	-	4.08	-	-
(E)-Nerolidol	-	1.57	-	-

These sesquiterpenes are commonly associated with anti-inflammatory, antioxidant, and antimicrobial properties and may additionally contribute to the stability and complexity of the essential oil aroma profile.

Notably, the chemical composition of the essential oil also varies depending on the plant organ analyzed. Leaf essential oils are predominantly rich in monoterpenes such as ocimene and myrcene, whereas flower bud oils contain higher proportions of sesquiterpenes and terpene alcohols, including α-cadinol, torreyol, and carotol. These findings indicate substantial differences in secondary metabolic pathways among the various organs of *C. operculatus*.

Overall, current evidence suggests that the essential oil of *C. operculatus* represents a rich source of volatile terpenes with highly variable chemical profiles across geographical regions and plant parts. Such variability may play an important role in guiding the selection of appropriate plant materials for specific biological and industrial applications.

9. Antimicrobial activity of *C. operculatus*

Studies summarized in Table 3 indicate that *C. operculatus* possesses a broad spectrum of antimicrobial activities against Gram-positive bacteria, gastrointestinal pathogenic bacteria, phytopathogenic bacteria, and several fungal species. These activities have been reported for various sample types, including methanolic extracts, bioactive fractions, essential oils, and purified compounds, highlighting the versatile application potential of this species in the fields of food preservation, pharmaceuticals, and plant protection.

Nguyen et al. (2017) reported that methanolic leaf extracts of *C. operculatus* exhibited inhibitory effects against several bacterial and yeast strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Candida maltosa*, *Streptococcus mutans*, *Staphylococcus epidermidis*, and *Staphylococcus haemolyticus*. The inhibition zone diameters ranged from 7 to 16 mm, with the strongest activity observed against the *S. aureus* North German epidemic strain, which showed an inhibition zone of 16 mm. These findings suggest that polar compounds present in the leaves possess effective antimicrobial activity against common pathogenic microorganisms.

Regarding gastrointestinal pathogens, particularly *Helicobacter pylori*, Thanh et al. (2024a) demonstrated that the hexane extract from *C. operculatus* flower buds exhibited notable antibacterial activity against *H. pylori* ATCC51932 and ATCC43504, with MIC values of 0.06 and 0.13 mg/mL, respectively. In addition, the extract strongly inhibited *H. pylori* urease activity, with an IC_{50} value of 4.51 µg/mL. Since urease plays a critical role in enabling *H. pylori* survival under acidic gastric conditions, inhibition of this enzyme suggests promising applications of *C. operculatus* in the management of *H. pylori* infections and related gastric disorders.

In another study, Thanh et al. (2024b) further demonstrated that the hexane fractions derived from flower buds (BHF) and leaves (LHF) exhibited potent antibacterial activity against *H. pylori*, with MIC values of 125 µg/mL. Notably, the isolated compound 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) showed substantially greater activity than the crude fractions, with MIC values ranging from 25 to 50 µg/mL.

Table 3
Antimicrobial activities of extracts, essential oils, and isolated compounds from *C. operculatus*

Study	Plant part / sample	Tested microorganisms	Main findings
Nguyen et al. (2017)	Methanolic leaf extract	*Staphylococcus aureus*, *Bacillus subtilis*, *Candida maltosa*, *Streptococcus mutans*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*	Inhibition zones ranged from 7–16 mm; strongest activity against *S. aureus* North German epidemic strain (16 mm)
Thanh et al. (2024a)	Flower bud extracts	*Helicobacter pylori* ATCC51932 and ATCC43504, *Salmonella enterica* serovar Typhimurium	Hexane extract showed strongest antibacterial activity with MIC values of 0.06–0.40 mg/mL; strong urease inhibition against *H. pylori* (IC_{50} = 4.51 µg/mL)
Thanh et al. (2024b)	Flower bud hexane fraction (BHF), leaf hexane fraction (LHF), isolated DMC	*H. pylori* ATCC51932 and ATCC43504	BHF and LHF showed strong antibacterial activity (MIC = 125 µg/mL); DMC exhibited higher activity (MIC = 25–50 µg/mL)
Dosoky et al. (2015)	Leaf essential oil	*Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans*	MIC values ranged from 625–2500 µg/mL; strongest activity against *S. aureus* and *A. niger*
Bajpai et al. (2010)	Bud essential oil and extracts	*Xanthomonas* spp.	Inhibition zones ranged from 7–23 mm; MIC values ranged from 31.25–500 µg/mL; strong *in vivo* antibacterial activity observed on infected melon plants

These findings indicate that DMC may represent one of the principal compounds responsible for the antibacterial activity of *C. operculatus*. Moreover, DMC was reported to inhibit biofilm formation and increase bacterial membrane permeability, suggesting a multitarget antimicrobial mechanism.

The leaf essential oil of *C. operculatus* has also demonstrated antibacterial and antifungal properties. Dosoky et al. (2015) reported that leaf essential oil collected in Nepal inhibited *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus niger*, and *Candida albicans*, with MIC values ranging from 625 to 2500 µg/mL. These activities were attributed primarily to the high abundance of monoterpenes such as myrcene and ocimene in the oil. Volatile terpene compounds are generally known to disrupt microbial cell membrane integrity and interfere with intracellular metabolic processes.

Beyond biomedical applications, the antimicrobial activity of *C. operculatus* also shows potential for agricultural and plant protection purposes. Bajpai et al. (2010) reported that the essential oil and extracts from flower buds inhibited several *Xanthomonas* species pathogenic to crops, producing inhibition zones ranging from 7 to 23 mm. The MIC and MBC values ranged from 31.25 – 500 µg/mL and 125 – 1000 µg/mL, respectively. Importantly, the essential oil also demonstrated *in vivo* antibacterial efficacy against *Xanthomonas* infection in oriental melon plants under greenhouse conditions, suggesting its potential as an environmentally friendly plant-derived antimicrobial agent.

The presence of flavonoids, chalcones, polyphenols, saponins, and terpenes in the extracts and essential oils may largely explain the diverse antimicrobial activities of *C. operculatus*. In particular, flavonoid- and phenolic-rich fractions consistently exhibited stronger biological activities, emphasizing the important role of these compounds in the antimicrobial mechanisms of this species.

10. Antioxidant activity and oxidative stress protection of *C. operculatus*

Studies summarized in Table 4 demonstrate that *C. operculatus* exhibits remarkable antioxidant activity through multiple mechanisms, including free radical scavenging, metal ion reduction, protection against oxidative cellular damage, and modulation of endogenous antioxidant enzyme systems. These activities are primarily associated with the abundant presence of polyphenols, flavonoids, chalcones, and anthocyanins in plant parts such as flower buds, leaves, seeds, and fruits.

Several studies have identified the flower buds of *C. operculatus* as a rich source of phenolic and flavonoid compounds with potent biological activity. Thanh et al. (2024a) reported that the aqueous extract of flower buds exhibited the highest total polyphenol content (TPC), reaching 328.9 mg GAE/g extract, whereas the ethanolic extract demonstrated the strongest DPPH radical scavenging activity with an IC_{50} value of only 25.2 µg/mL. These findings indicate that extraction solvents significantly influence the recovery efficiency of antioxidant compounds. Polar solvents such as water and ethanol generally provide superior extraction efficiency due to their high solubility for phenolic constituents.

In addition to crude extracts, several purified compounds isolated from *C. operculatus* have also exhibited significant antioxidant activity. Ye et al. (2013) demonstrated that DMC (2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone) possessed strong ABTS radical scavenging and ferric reducing capacities, with values of 176.5 and 213.3 µmol Trolox equivalents, respectively. Moreover, this compound protected human umbilical vein endothelial cells (HUVECs) against H_2O_2-induced oxidative damage by reducing intracellular and extracellular ROS levels. These findings suggest that DMC functions not only as a direct radical scavenger but also as a cytoprotective agent capable of preserving cellular structure and function under oxidative stress conditions.

Several flavonoids isolated from the flower buds of *C. operculatus* have also shown neuroprotective and enzyme inhibitory potential. Min et al. (2010) reported that compounds such as quercetin, kaempferol, and tamarixetin inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), with AChE IC_{50} values ranging from 19.9 to 37.8 µM. Such activities suggest potential applications in neuroprotection and the prevention of neurodegenerative disorders associated with oxidative stress. Furthermore, Min et al. (2008) isolated several novel chalcones and flavanones with notable biological activities, thereby expanding current knowledge regarding antioxidant-related phytochemicals in this species.

In addition to flower buds, the leaves of *C. operculatus* have also been recognized as a valuable source of polyphenols. Le et al. (2025) reported that the leaves contained total polyphenol levels of 141.65 mg GAE/g dried leaves. Meanwhile, Giap et al. (2023) demonstrated that enzyme-assisted extraction using laccase and Celluclast® substantially improved the recovery efficiency of antioxidant compounds, resulting in antioxidant activity reaching 80.32%. These findings suggest that biotechnological extraction approaches may represent promising strategies for enhancing the utilization of bioactive compounds from plant materials.

Moreover, combining *C. operculatus* with other medicinal plants may further improve sensory properties while maintaining biological activity. Minh et al. (2023) reported that a mixture of *C. operculatus* and Mentha arvensis at a ratio of 7:3 retained strong DPPH radical scavenging activity (IC_{50} = 35.6 µg/mL) and significantly inhibited lipid peroxidation in rat brain tissue. This finding highlights the potential application of herbal combinations in the development of functional beverages and health-promoting foods.

Collectively, current evidence indicates that the antioxidant activity of *C. operculatus* extends beyond simple free radical neutralization in chemical assays and involves broader cytoprotective mechanisms, including regulation of antioxidant enzymes and attenuation of oxidative damage in both *in vitro* and *in vivo* models. These properties strongly support the potential application of this species in functional foods, herbal medicines, and health products aimed at preventing oxidative stress-related disorders.

Table 4
Antioxidant and other biological activities of *Cleistocalyx* species (extracts and isolated compounds)

Plant part	Compound / Extract	Method / Model	Key results	References
Buds	Water extract	Isolated rat heart perfusion; Na^+/K^+-ATPase, Ca^{2+}-ATPase assays	↑ contractility, ↓ frequency; inhibits Na^+/K^+-ATPase and Ca^{2+}-ATPase	Woo et al. (2002)
Buds	3-formyl-4,6,4'-trihydroxy-2-methoxy-5-methylchalcone (1); (2S)-8-formyl-6-methylnaringenin 7-O-β-D-glucopyranoside (4)	Bioactivity assay	IC_{50}: (1) 22.8 mM; (4) 27.1 mM	Min et al. (2008)
Buds	Myricetin-3'-methylether 3-O-β-D-galactopyranoside (1); Quercetin (3); Kaempferol (4); Tamarixetin (5)	AChE/BChE inhibition	AChE IC_{50}: 19.9–37.8 µM; BChE IC_{50}: 62.5–177.8 µM	Min et al. (2010)
Buds	C-methylated flavonoids	Neuraminidase inhibition (H1N1 WT, H274Y)	IC_{50}: WT = 8.15 µM; H274Y = 3.31 µM	Dao et al. (2010)
Buds	DMC	*In vivo* – BEL-7402/5-FU liver tumor xenograft + 5-FU	Tumor inhibition 72.2%; ↑ caspase-3, no significant toxicity	Huang et al. (2012)
Buds	DMC	ABTS, FRAP, ·OH scavenging, H_2O_2-induced HUVEC	ABTS: 176.5 TE; FRAP: 213.3 TE; ·OH IC_{50}: 243.7 µM; protects HUVEC	Ye et al. (2013)
Buds	Water, ethanol, methanol, hexane, chloroform, butanol, ethyl acetate extracts	TPC, TSC, TTC, TFC, DPPH (IC_{50})	Highest TPC: water (328.9 mg GAE/g); best DPPH: ethanol (IC_{50} 25.2 µg/mL)	Thanh et al. (2024a)
Buds	Ethanolic extracts, DMC	H. pylori urease, α-amylase, biofilm, membrane permeability	DMC: urease IC_{50} = 3.2 µg/mL; α-amylase IC_{50} = 83.8 µg/mL; no human cell toxicity	Thanh et al. (2024b)
Leaves	Ethanol extract	Cytotoxicity on HaCaT keratinocytes	IC_{50}: 119.98 µg/mL; 62.5 µg/mL stimulated viability	Nguyen et al. (2017)
Leaves	Enzyme-assisted extract (laccase + celluclast)	TPC, antioxidant activity (%)	TPC: 23.95 mg GAE/g dry solid; activity: 80.32%	Giap et al. (2023)
Leaves	Herbal mixture	DPPH, lipid peroxidation (rat brain)	DPPH IC_{50}: 35.6 µg/mL; inhibits lipid peroxidation	Minh et al. (2023)
Leaves	Ethanol/water extract	TPC	TPC: 141.65 mg GAE/g dried leaf	Le et al. (2025)
Flower buds	Water, ethanol, methanol extracts	TPC, DPPH	As above (duplicate entry – can merge or keep separate if different collection)	Thanh et al. (2024a)
Seeds (waste)	Phenolic extract (microwave-assisted: 450 W, 213 s, 51% ethanol)	TPC (experimental)	TPC: 75.13 ± 0.58 mg GAE/g DW	Narkprasom et al. (2015)
Whole plant	Aqueous extract	*In vivo* – rat liver (4 weeks, oral gavage)	Low dose (100 mg/kg): pro-oxidant (↑ MDA); High dose (500 mg/kg): ↑ heme oxygenase-1	Taya et al. (2009)

Notes: TPC (total polyphenol content), TFC (total flavonoid content), TSC (total tannin content), TTC (total triterpenoid content), GAE (gallic acid equivalents), QE (quercetin equivalents), CE (catechin equivalents), OAE (oleanolic acid equivalents), TE (Trolox equivalents), GSH (glutathione), GPx (glutathione peroxidase), CAT (catalase), MDA (malondialdehyde), HUVEC (human umbilical vein endothelial cells), DMC (2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone), IC_{50} (half-maximal inhibitory concentration), 5-FU (5-fluorouracil).

11. Anti-inflammatory, antiviral, and antimicrobial activities of *C. operculatus*

Recent studies have demonstrated that *C. operculatus* is a rich source of compounds with promising antiviral potential, particularly against influenza viruses and respiratory syncytial virus (RSV) (Table 5). Ha et al. (2016) reported that two flavonoids isolated from the leaves of *C. operculatus* strongly inhibited neuraminidase enzymes from several influenza strains, including H1N1, H9N2, and oseltamivir-resistant H1N1 carrying the H274Y mutation. The IC_{50} values ranged from 5.07 – 9.34 µM, indicating relatively potent activity compared with many other natural compounds. Notably, these flavonoids acted through a non-competitive inhibition mechanism, suggesting interactions with allosteric sites rather than the catalytic active site of the enzyme. This finding is important for the development of next-generation neuraminidase inhibitors to overcome antiviral drug resistance.

Song et al. (2024) further expanded the antiviral potential of *C. operculatus* by isolating a series of compounds, cleistoperones A–D, from the leaves. Among them, cleistoperone A exhibited the strongest activity against RSV, with an IC_{50} value of only 1.71 ± 0.61 µmol/L. Mechanistic studies revealed that this compound inhibited viral replication through modulation of the Akt/mTOR/p70S6K signaling pathway, which plays a crucial role in intracellular viral proliferation and replication. These findings suggest that *C. operculatus* may serve as a promising natural source for the discovery of novel antiviral agents.

In addition to its antiviral effects, *C. operculatus* also exhibits significant anti-inflammatory activity in both *in vitro* and *in vivo* models. Tran et al. (2019a) demonstrated that ethanol extracts from flower buds and the chalcone compound DMC strongly inhibited lipopolysaccharide (LPS)-induced inflammatory responses in macrophages and in an experimental septic shock mouse model. The extracts reduced the production of pro-inflammatory mediators and attenuated lung and liver damage in treated animals. The anti-inflammatory mechanism was associated with activation of the Nrf2/HO-1 signaling pathway, an important cellular defense system against oxidative stress and inflammation.

Similarly, Wang et al. (2016) reported that cleistocalyxic acid E significantly inhibited the production of IL-6 and TNF-α in LPS-stimulated RAW264.7 macrophages. The suppression of these pro-inflammatory cytokines suggests that compounds from *C. operculatus* may contribute to the regulation of excessive inflammatory responses associated with chronic diseases. The leaf essential oil of *C. operculatus* has also shown considerable potential in wound healing applications. Tran et al. (2019b) observed that mice treated with the essential oil exhibited a wound contraction rate of 100% after 20 days, significantly higher than that of the control group. Histological analysis revealed complete epidermal regeneration, well-organized collagen structures, and reduced infiltration of inflammatory cells. These findings indicate that the essential oil not only promotes tissue regeneration but also modulates inflammatory responses at the wound site.

Table 5
Anti-inflammatory, antiviral, and antimicrobial activities of *C. operculatus*

Plant part	Sample / Compound	Biological target / Model	Key findings	References
Leaves	Compounds 6 and 8 (flavonoids)	Neuraminidase inhibition against H1N1, H9N2, oseltamivir-resistant H1N1 (H274Y)	Strong NA inhibition with IC_{50} values of 5.07–9.34 µM; non-competitive inhibition; reduced H1N1-induced cytotoxicity in MDCK cells	Ha et al. (2016)
Leaves	Cleistoperone A–D	Respiratory syncytial virus (RSV)	Cleistoperone A showed potent antiviral activity (IC_{50} = 1.71 ± 0.61 µmol/L); inhibited viral replication via Akt/mTOR/p70S6K pathway	Song et al. (2024)
Flower buds and leaves	Ethanolic extracts, DMC	*Helicobacter pylori* urease, biofilm formation, membrane permeability	DMC inhibited urease (IC_{50} = 3.2 µg/mL), suppressed biofilm formation, increased membrane permeability, and damaged bacterial cell membrane	Thanh et al. (2024a)
Flower buds	Ethanol extract, DMC	LPS-induced inflammation in macrophages and septic mice	Reduced inflammatory mediators; alleviated lung and liver damage; activated Nrf2/HO-1 signaling pathway	Tran et al. (2019a)
Essential oil	Essential oil from leaves	Burn wound healing in mice	Enhanced wound contraction (100% after 20 days); improved epidermal regeneration and collagen organization	Tran et al. (2019b)
Leaves	Cleistocalyxic acid E	LPS-induced RAW264.7 macrophages	Inhibited IL-6 production by 68.1% and TNF-α by 53.7% at 2 µM	Wang et al. (2016)
Leaves	Essential oil rich in myrcene	RAW264.7 macrophages; cancer cell lines	Reduced IL-1β, TNF-α, iNOS, COX-2, and NFκB expression; inhibited proliferation of PC-3, A431, A549, and MCF-7 cells	Pandey et al. (2025)

Furthermore, Pandey et al. (2025) demonstrated that myrcene-rich leaf essential oil markedly reduced inflammatory markers such as IL-1β, TNF-α, iNOS, COX-2, and NFκB in RAW264.7 cells. Myrcene, which accounted for approximately 56% of the essential oil composition, was considered the major contributor to this activity. The study also reported antiproliferative effects of the essential oil against several cancer cell lines, suggesting a close relationship between anti-inflammatory and anticancer properties.

The antimicrobial activity of *C. operculatus* has been particularly notable against *Helicobacter pylori*, a bacterium strongly associated with gastric ulcers and gastric cancer. Thanh et al. (2024a) showed that DMC strongly inhibited *H. pylori* urease activity, with an IC_{50} value of only 3.2 µg/mL. Urease is an essential enzyme that enables the bacterium to survive in the acidic gastric environment. In addition, DMC inhibited biofilm formation, increased membrane permeability, and caused severe deformation of bacterial cell structures. Scanning electron microscopy further revealed substantial membrane damage following treatment with DMC and bioactive hexane fractions.

Importantly, the tested fractions and isolated compounds exhibited no significant toxicity toward human cell lines, highlighting their potential safety for developing supportive therapies against *H. pylori* infection. In the context of increasing antibiotic resistance, natural compounds from *C. operculatus* may represent promising candidates for the development of novel antimicrobial agents with multitarget mechanisms of action.

12. Antidiabetic and metabolic regulatory activities

Current studies indicate that *C. operculatus* possesses considerable potential in the management of diabetes and metabolic disorders through multiple biological mechanisms, including inhibition of carbohydrate- and lipid-digesting enzymes, regulation of glucose uptake, improvement of oxidative stress, and protection of pancreatic β-cells (Table 6).

One of the most extensively investigated mechanisms is the inhibition of enzymes involved in carbohydrate and lipid metabolism. Zhang & Lu (2012) demonstrated that extracts from the flower buds of *C. operculatus*, as well as the chalcone compound DMC, strongly inhibited pancreatic lipase and α-amylase activities. Notably, DMC exhibited significant inhibitory effects against both enzymes, suggesting that this chalcone may contribute to reducing postprandial lipid and carbohydrate absorption. Enzyme kinetic analysis further revealed distinct inhibition mechanisms, with pancreatic lipase inhibited in a non-competitive manner, whereas α-amylase inhibition followed a competitive mode. These findings reflect the diverse interactions between phytochemicals and biological targets.

In addition, the study by Mai & Chuyen (2007) further confirmed the antihyperglycemic potential of *C. operculatus* flower buds through the inhibition of α-glucosidase, maltase, and sucrase activities. The reduction of postprandial blood glucose levels in streptozotocin (STZ)-induced diabetic rats was consistent with the *in vitro* findings. Importantly, the hypoglycemic effect of the flower bud extract at a dose of 500 mg/kg was reported to be comparable to that of acarbose in certain experiments, highlighting its potential as a natural source for controlling postprandial hyperglycemia.

Animal studies have also demonstrated the broad metabolic protective effects of *C. operculatus*. Mai et al. (2009) reported that aqueous extracts from flower buds reduced plasma glucose, total cholesterol, triglycerides, and sorbitol accumulation in the lenses of diabetic rats. Furthermore, the extract restored the activities of antioxidant enzymes such as superoxide dismutase and glutathione S-transferase, thereby helping to alleviate oxidative stress, a major factor associated with diabetic complications. Subsequently, Mai et al. (2010) showed that flower bud extracts protected pancreatic β-cells, maintained stable insulin levels, and delayed the development of diabetic cataracts.

In addition, solvent-dependent extraction effects have been shown to significantly influence the recovery of bioactive compounds and resulting biological activities, as demonstrated by Nguyen et al. (2023), who reported notable variations in phenolic content and *in vitro* bioactivities of *Bidens pilosa* depending on the extraction solvent used.

Beyond crude extracts, several isolated compounds from *Cleistocalyx* have also exhibited remarkable metabolic activities. Mai et al. (2024) identified chalcone–monoterpene hybrids with potent inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), an important therapeutic target in type 2 diabetes and insulin resistance. The low micromolar IC_{50} values suggest promising potential for the development of natural lead compounds for antidiabetic therapies. In parallel, Ponce-Zea et al. (2024) reported that several isolated compounds stimulated glucose uptake in differentiated 3T3-L1 adipocytes, indicating glucose-regulating effects at the cellular level.

Table 6
Antidiabetic and metabolic regulatory activities of *Cleistocalyx* species and isolated compounds

Plant part	Species / Sample	Extract / Compound	Experimental model / assay	Main findings	Reference
Flower buds	*C. operculatus*	Chalcone-monoterpene hybrids (1–3), chalcone (9), synthetic derivatives	PTP1B inhibitory assay, molecular docking	Compounds 4, 9, 13, and 16b strongly inhibited PTP1B (IC_{50} = 0.9–3.9 µM); active compounds showed mixed-type inhibition and favorable docking interactions	Mai et al. (2024)
Flower buds	*C. operculatus*	Water, ethanol, petroleum ether, ethyl acetate extracts; DMC	Pancreatic lipase and α-amylase inhibition	DMC, petroleum ether, ethyl acetate, and 95% ethanol extracts strongly inhibited pancreatic lipase; water extract and DMC strongly inhibited α-amylase	Zhang & Lu (2012)
Leaves	*C. operculatus*	Essential oil constituents (allo-ocimene, (Z)-β-ocimene)	Molecular docking and molecular dynamics with α-glucosidase	Allo-ocimene and (Z)-β-ocimene showed stable binding affinity toward α-glucosidase (−5.358 and −5.330 kcal/mol)	Tran et al. (2023)
Fruits	*C. nervosum*	Water, ethanol, hexane extracts	α-Amylase and α-glucosidase inhibition; glucose uptake assays	Ethanolic extract showed strongest α-amylase (IC_{50} = 0.42 mg/mL) and α-glucosidase inhibition (IC_{50} = 0.23 mg/mL); enhanced glucose utilization in HepG2 and L6 cells	Chukiatsiri et al. (2022)
Flower buds	*C. operculatus*	Aqueous extract (COB)	STZ-induced diabetic rats; antioxidant and lipid profile analyses	Reduced plasma glucose, cholesterol, triglycerides, sorbitol accumulation, and lipid peroxidation; restored antioxidant enzyme activities	Mai et al. (2009)
Flower buds	*C. operculatus*	COB aqueous extract	STZ-induced diabetic rats	Improved insulin stability, preserved pancreatic β-cells, reduced glucose and HbA1c, and delayed cataract formation	Mai et al. (2010)
Flower buds	*C. operculatus*	Flower bud extract	α-Glucosidase, maltase, sucrase inhibition; STZ diabetic rats	α-Glucosidase inhibition reached 68.2%; maltase IC_{50} = 0.70 mg/mL; sucrase IC_{50} = 0.47 mg/mL; reduced postprandial glucose in diabetic rats	Mai & Chuyen (2007)

Essential oils and volatile compounds from *C. operculatus* leaves have also shown promise in metabolic enzyme regulation studies. Tran et al. (2023) used molecular docking and molecular dynamics simulations to demonstrate that allo-ocimene and (Z)-β-ocimene could stably bind to α-glucosidase. Although this investigation was conducted in silico, the results broaden current understanding of the potential role of monoterpenes in glycemic control. Overall, current evidence suggests that the antidiabetic activity of *C. operculatus* involves multitarget mechanisms, including digestive enzyme inhibition, glucose metabolism regulation, antioxidant effects, and protection of target organs. These findings provide an important scientific basis for future studies aimed at developing functional foods, health-supporting products, and natural lead compounds derived from this medicinal plant.

13. Anticancer and cytoprotective activities

Studies on the anticancer and cytoprotective activities of the genus *Cleistocalyx* indicate that *C. operculatus* is the most extensively investigated species, mainly due to the presence of the characteristic chalcone 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC). Most studies have focused on its apoptosis-inducing effects, oxidative stress modulation, and cytoprotective mechanisms, whereas studies on *C. nervosum* var. *paniala* are primarily included as supplementary comparisons to broaden the understanding of biological potential within the genus (Table 7).

In *C. operculatus*, DMC is considered the most prominent bioactive compound because of its selective cytotoxicity against several cancer cell lines. Tuan et al. (2019) demonstrated that DMC strongly inhibited the proliferation of pancreatic cancer cells PANC-1 and MIA PaCa-2, with IC_{50} values of 10.5 and 12.2 µM, respectively. The mechanism involved activation of caspase-3 and caspase-9, PARP cleavage, upregulation of the pro-apoptotic protein Bak, and downregulation of Bcl-2, indicating mitochondria-mediated apoptosis. Similarly, Ye & Lai (2016) reported that DMC exhibited significant cytotoxicity toward human hepatocellular carcinoma SMMC-7721 cells while showing much lower toxicity toward normal cells, suggesting relatively high biological selectivity. Their study further revealed that reactive oxygen species (ROS) played a central role in DMC-induced apoptosis through mitochondrial membrane potential disruption and regulation of apoptosis-related proteins such as p53, PUMA, and caspases.

In addition to chalcones, Wang et al. (2016) isolated several novel pentacyclic triterpenoids from the leaves of *C. operculatus*, including cleistocalyxic acids and cleistocalyxolides. Some compounds exhibited potent cytotoxicity against HepG2, NCI-N87, and MCF-7 cell lines, with IC_{50} values ranging from 3.2 to 6.5 µM. Notably, cleistocalyxic acid E did not exhibit direct cytotoxicity but strongly inhibited IL-6 and TNF-*α* production in LPS-stimulated RAW264.7 macrophages, suggesting potential anti-inflammatory effects associated with cancer prevention and progression control.

Besides direct anticancer activity, several studies have demonstrated the cytoprotective properties of *C. operculatus* against oxidative stress and tissue injury. Tran et al. (2019a) showed that ethanol extracts from flower buds and DMC reduced LPS-induced endotoxin shock through activation of the Nrf2/HO-1 signaling pathway, thereby suppressing inflammatory mediators and alleviating liver and lung damage in mice. Tissue-protective effects were also reported by Tran et al. (2019b), where *C. operculatus* essential oil significantly accelerated burn wound healing compared with tamanu oil and untreated controls, while improving epidermal regeneration and reducing inflammatory cell infiltration.

Compared with *C. operculatus*, studies on *C. nervosum* var. *paniala* have mainly emphasized antioxidant and organ-protective properties rather than direct anticancer cytotoxicity.

Chariyakornkul et al. (2021) demonstrated that dichloromethane extracts reduced preneoplastic lesions in the liver and colon of experimental animals by enhancing phase II detoxifying enzymes such as glutathione S-transferase (GST) and UDP-glucuronyltransferase. Meanwhile, Chariyakornkul et al. (2022) and Panritdum et al. (2024) highlighted the hepatoprotective role of anthocyanins, particularly cyanidin-3-glucoside, in reducing oxidative stress, enhancing endogenous antioxidant enzyme activities, and suppressing hepatocyte apoptosis.

Table 7
Anticancer and cytoprotective activities of *Cleistocalyx* species and isolated compounds

Plant part	Species / Sample	Extract / Compound	Experimental model / assay	Main findings	Reference
Flower buds	*C. operculatus*	DMC (2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone)	Human pancreatic cancer cells (PANC-1, MIA PACA2)	Cytotoxicity against PANC-1 and MIA PACA2 cells with IC_{50} values of 10.5 and 12.2 µM; induced apoptosis via caspase-3/-9 activation, PARP cleavage, ↑ Bak and ↓ Bcl-2	Tuan et al. (2019)
Flower buds	*C. operculatus*	DMC	Human hepatocellular carcinoma SMMC-7721 cells	Induced ROS-mediated apoptosis with IC_{50} = 32.3 µM; affected mitochondrial membrane potential, caspase activation, p53 and PUMA pathways	Ye & Lai (2016)
Leaves	*C. operculatus*	Cleistocalyxic acids A–K and cleistocalyxolides A–B	Phytochemical isolation and cytotoxicity screening	Isolation of thirteen new pentacyclic triterpenoids with cytotoxic potential	Wang et al. (2016)
Fruit pulp	*C. nervosum* var. *paniala*	Anthocyanin-rich extract	APAP-induced hepatotoxicity in rats	Improved hepatocyte architecture, reduced MDA, increased glutathione, GPx and UDP-glucuronosyltransferase activity	Chariyakornkul et al. (2022)
Fruits	*C. nervosum*	Dichloromethane extract	DEN/DMH-induced hepatocarcinogenesis and colon carcinogenesis in rats	Reduced preneoplastic lesions and PCNA-positive cells; enhanced GST and UDP-glucuronyltransferase activities	Chariyakornkul et al. (2021)
Fruits	*C. nervosum* var. *paniala*	Ethanol extract, cyanidin-3-glucoside	H_2O_2-induced oxidative stress in HepG2 cells	Increased cell survival and antioxidant enzyme activity; inhibited Bax, cytochrome c, caspase-3/-9 activation	Panritdum et al. (2024)
Fruits	*C. nervosum* var. *paniala*	Berry extract	Glutamate-induced oxidative stress in HT22 neuronal cells	Reduced ROS and ER stress-mediated apoptosis; enhanced SOD, CAT, GPx and GST expression	Sukprasansap et al. (2017)
Fruits	*C. nervosum* var. *paniala*	Fruit extract	*Caenorhabditis elegans* aging and neuroprotection model	Extended lifespan, reduced ROS and lipofuscin accumulation, improved stress resistance and neuroprotection	Prasanth et al. (2019)
Fruits	*C. nervosum*	Fruit extract	DEN/PB-induced hepatocarcinogenesis in rats	Reduced GST-P positive foci and oxidative stress; increased GPx and catalase activity	Taya et al. (2014)
Fruits	*C. nervosum* var. *paniala*	Fruit extract	Cadmium-induced nephrotoxicity in rats	Improved renal function and antioxidant status; reduced oxidative damage markers	Poontawee et al. (2016)

Neuroprotective and anti-aging effects were also predominantly reported for *C. nervosum*. Sukprasansap et al. (2017) demonstrated that fruit extracts protected HT22 neuronal cells from glutamate-induced oxidative stress by reducing ROS production and regulating endoplasmic reticulum stress-related proteins. Likewise, Prasanth et al. (2019) found that fruit extracts prolonged lifespan and improved oxidative stress resistance in *Caenorhabditis elegans* through pathways associated with longevity-related genes such as *daf-16*, *skn-1*, and *sir-2.1*.

Furthermore, Charoensin et al. (2012) and Poontawee et al. (2016) reported that *C. nervosum* exhibited relatively high biological safety, with no significant acute toxicity or genotoxicity, while also protecting against cadmium-induced nephrotoxicity through enhancement of endogenous antioxidant defense systems. These findings further support the potential application of *Cleistocalyx* species in functional foods and preventive health products.

Overall, current evidence suggests that *C. operculatus* is particularly prominent for its direct anticancer potential, especially through DMC and characteristic chalcone/triterpenoid derivatives, whereas *C. nervosum* var. *paniala* demonstrates stronger antioxidant and organ-protective properties. These differences reflect the chemical and biological diversity within the genus and provide a basis for species-specific exploitation in future pharmaceutical and functional food applications.

14. Applications and industrial potential of *Cleistocaly operculatus*

Table 8 and Figure 7 summarize the diverse applications of *C. operculatus*, a medicinal plant widely distributed in tropical Asian countries, particularly Vietnam and Thailand. The reported applications can be categorized into food and beverage, food preservation, green nanoparticle synthesis, wastewater treatment, agriculture, and biomedical fields.

Regarding traditional and food-related applications, the flower buds of *C. operculatus* have long been used as key ingredients in herbal teas and tonic beverages in southern China and Vietnam (Woo et al., 2002; Tran et al., 2019b). A 12-week clinical intervention in Vietnamese patients with type 2 diabetes demonstrated that regular consumption of bud tea significantly reduced fasting blood glucose and HbA1c levels while increasing serum insulin concentrations; postprandial hyperglycemia was also suppressed (Mai et al., 2013). These findings support the traditional use of the plant in diabetes management. In addition, polyphenol-rich leaf extracts have been applied in food preservation. A chitosan/gum arabic edible coating containing *C. operculatus* polyphenols successfully extended banana shelf life up to 21 days at room temperature through antioxidant activity and formation of a protective surface layer (Le et al., 2021).

Another prominent application of *C. operculatus* is in green nanoparticle synthesis. Extracts from flower buds and leaves have been used as both reducing and stabilizing agents for the biosynthesis of silver nanoparticles (AgNPs) with particle sizes ranging from 20–70 nm, exhibiting strong antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Nguyen et al., 2023; Le et al., 2025). Leaf extracts were also employed to synthesize spherical selenium nanoparticles (Se NPs) with diameters of 50–200 nm that demonstrated antimicrobial activity against *Escherichia coli* and *S. aureus*, while showing lower acute oral toxicity in rats compared with SeO_2 (Vu et al., 2022). Furthermore, zinc oxide nanoparticles (ZnO NPs) (Le et al., 2026), nanoscale zero-valent iron particles (nZVI) (Le et al., 2022; Hoang et al., 2022a; Hoang et al., 2022b), Fe_3O_4 nanoparticles (Nguyen & Nguyen, 2025), and copper–chitosan nanoparticles (CuNPs-CTS) (Pham et al., 2024) have also been successfully synthesized using green methods based on *C. operculatus* leaf extracts. Notably, the Ag/Fe_3O_4/graphene nanocomposite (GF31A) synthesized from leaf extracts achieved removal efficiencies exceeding 70% for COD, TN, and PO_4^{3-} and eliminated *E. coli* in wastewater treatment, while also displaying antibacterial and antifungal activities with IC_{50} values ranging from 1.5 to 11.1 mg/L (Le et al., 2022). More recently, *C. operculatus* extract-loaded chitosan nanoparticles prepared using ionic gelation combined with ultrasound treatment achieved an encapsulation efficiency of 88%, particle sizes of 178–270 nm, antibacterial activity above 95%, and radical scavenging activity up to 94% (Do et al., 2025). To further support its role in food preservation and functional material development, edible coating systems incorporating plant extracts have also been highlighted as an effective strategy for extending fruit shelf life through the combined effects of antioxidant and antimicrobial protection (Pham et al., 2023). In environmental applications, *C. operculatus* leaves have been directly utilized as biosorbents for Pb^{2+} removal from aqueous solutions, achieving a removal efficiency of 86.82% after 60 min at pH 6 and 303 K. The maximum adsorption capacity based on the Langmuir model reached 64.59 mg/g, while approximately 70% efficiency was retained after three adsorption–desorption cycles (Vu & Nguyen 2026). Fe_3O_4 nanoparticles synthesized from leaf extracts also exhibited a high adsorption capacity toward Congo Red dye (137.9 mg/g), highlighting their potential in textile wastewater treatment (Nguyen & Nguyen, 2025).

Table 8
Applications of *C. operculatus*

Application field	Plant part / Species	Preparation / Material	Key findings	Reference
Food & beverage	Flower buds – *C. operculatus*	Herbal tea, tonic drinks	Used as ingredient in herbal tea and tonic drinks in tropical countries (Vietnam, Southern China)	Tran et al. (2019b); Woo et al. (2002)
Food & beverage	Flower buds – *C. operculatus*	COB tea	12-week intervention in type 2 diabetic patients: ↓ fasting blood glucose, ↓ HbA1c, ↑ serum insulin; postprandial glucose rise inhibited	Mai et al. (2013)
Food preservation	Leaves – *C. operculatus*	Polyphenol-enriched extract in chitosan/gum arabic edible coating	Polyphenol-enriched extract/chitosan/gum arabic coating extended banana freshness up to 21 days at room temperature; smooth surface vs. wrinkled/cracked uncoated fruit	Le et al. (2021)
Nanoparticle synthesis – Ag	Buds – *C. operculatus*	Silver nanoparticles (AgNPs)	Dual role: reducing and stabilizing agent; UV-Vis peak at 432 nm	Nguyen et al. (2023)
Nanoparticle synthesis – Ag	Leaves – *C. operculatus*	AgNPs (optimized by Box–Behnken model)	Optimal: 1.543 mM $AgNO_3$, 7.514 volume ratio, 48.9°C, 51 min; particle size 20–70 nm, UV-Vis peak 445 nm; inhibits *S. aureus* (12 mm) and *P. aeruginosa* (16 mm)	Le et al. (2025)
Nanoparticle synthesis – Ag	Leaves – *C. operculatus*	Ag/TiO_2 nanocomposites	Aqueous leaf extract as reductant for green synthesis	Nguyen et al. (2022)
Nanoparticle synthesis – Ag	Leaves – *C. operculatus*	Ag nanoparticles on graphene nanoplatelets	AgNPs 20–40 nm, uniformly distributed; excellent antibacterial activity against *E. coli*	Thi et al. (2022)
Nanoparticle synthesis – Se	Leaves – *C. operculatus*	Selenium nanoparticles (Se NPs)	Spherical, 50–200 nm; antimicrobial against *E. coli* and *S. aureus*; acute oral toxicity LD_{50} = 7.75 mg/kg (lower than SeO_2)	Vu et al. (2022)
Nanoparticle synthesis – ZnO	Leaves – *C. operculatus*	Zinc oxide nanoparticles (ZnO NPs)	Green synthesis using leaf extract as reducing and capping agent	Le et al. (2026)
Nanoparticle synthesis – nZVI	Leaves – *C. operculatus*	Zero-valent iron nanoparticles (nZVI)	Spherical, ~100 nm; synthesized using leaf extract as reductant	Le et al. (2022)
Nanoparticle synthesis – nZVI	Leaves – *C. operculatus*	nZVI (microfluidic reactor)	Plate-like structure, ~5 nm thickness; >90% Rhodamine B removal in 30 min	Hoang et al. (2022a)
Nanoparticle synthesis – nZVI	Leaves – *C. operculatus*	nZVI@graphene nanoplatelets (GNPs)	Spherical 30–100 nm on GNPs; >98% removal of RhB and Cr^{6+} in 30 min	Hoang et al. (2022b)
Nanoparticle synthesis – bio-nZVI	Leaves – *C. nervosum*	Bio-nZVI (core–shell: Fe^0 core, Fe_2O_3 shell, organic capping)	Spherical 80–350 nm; optimal 20–40 mg/L improved peanut seed germination and *Basella alba* growth	Nguyen et al. (2025)
Nanoparticle synthesis – magnetic	Leaves – *C. operculatus*	Fe_3O_4 nanoparticles (microwave-assisted)	Spherical, spinel cubic, 12.3 nm; Congo Red adsorption capacity 137.9 mg/g	Nguyen & Nguyen (2025)
Nanoparticle synthesis – magnetic	Leaves – *C. operculatus*	Ag/Fe_3O_4/graphene (GF31A)	GO:Fe_3O_4 = 3:1 + 5% Ag; >70% COD, TN, PO_4^{3-} removal; 100% *E. coli* removal; antibacterial/antifungal (IC_{50} 1.5–11.1 mg/L)	Le et al. (2022)
Nanoparticle synthesis – Cu	Leaves – *C. operculatus*	Copper-chitosan (CuNPs-CTS)	COLE as reductant, chitosan as stabilizer; CuNPs 10–30 nm; inhibition zone: *S. aureus* (20 mm), *E. coli* (18 mm) – better than $NaBH_4$-synthesized	Pham et al. (2024)
Nanoparticle encapsulation	Leaves – *C. operculatus*	COE-loaded chitosan nanoparticles (ionic gelation + ultrasound)	60% ethanol extract: TPC 362.48 mg GAE/g, TFC 19.51 mg QE/g; encapsulation efficiency 88%; particle size 178–270 nm; >95% bacterial inhibition; 94% radical scavenging	Do et al. (2025)
Wastewater treatment – heavy metal	Leaves – *C. operculatus*	Biosorbent (raw leaves)	Porous structure with N/O functional groups; Pb^{2+} removal 86.82% in 60 min (pH 6, 303 K); Langmuir capacity 64.59 mg/g; ~70% after 3 cycles; real wastewater: 63.5–71.4% removal	Vu & Nguyen (2026)
Wastewater treatment – dye	Leaves – *C. operculatus*	Fe_3O_4 nanoparticles	Congo Red adsorption: 137.9 mg/g	Nguyen & Nguyen (2025)
Agricultural application – pesticide	Whole plant – *C. operculatus*	Ethanol extract (major: 4',5'-dimethoxy-2'-hydroxy-4-methylchalcone 41.7%)	Antifungal against *Cercospora nicotianae* (leaf spot pathogen); MIC 9.54 mg/mL; supported by docking simulation	Hai et al. (2024)
Medical – burn treatment	– *C. operculatus*	Essential oil	Used for burn treatment	Tran et al. (2018)
Medical – anti-diabetic	Flower buds – *C. operculatus*	COB tea	Improved fasting glucose and HbA1c in type 2 diabetic patients after 12 weeks	Mai et al. (2013)

In agriculture, bio-nZVI synthesized from *C. nervosum* leaf extracts at optimal concentrations of 20–40 mg/L significantly improved peanut seed germination and promoted the growth of *Basella alba*, whereas higher concentrations did not provide additional benefits (Nguyen et al., 2025). Moreover, ethanol extracts from the whole plant of *C. operculatus*, rich in chalcone compounds such as 4′,5′-dimethoxy-2′-hydroxy-4-methylchalcone (41.71 %), exhibited strong antifungal activity against *Cercospora nicotianae*, the causal agent of leaf spot disease, with a minimum inhibitory concentration of 9.54 mg/mL. This activity was further supported by molecular docking analysis (Hai et al., 2024).

In biomedical and skincare applications, *C. operculatus* essential oil has traditionally been used for burn treatment (Tran et al., 2018). In addition, methanol extracts from mature leaves of *C. nervosum* showed high levels of polyphenols and flavonoids (511.44 µg GAE/mg and 262.96 µg QE/mg, respectively), together with strong antioxidant activity (SC_{50} = 0.02 mg/mL), tyrosinase inhibition (IC_{50} = 0.02 mg/mL), and MMP-2 inhibition up to 91.14% at 0.1 mg/mL, without cytotoxicity toward human dermal fibroblasts (Manosroi et al., 2014). These findings suggest considerable potential for the development of natural anti-aging and skin-whitening products from *C. operculatus* and related species.

Overall, *C. operculatus* is not only a traditional medicinal plant but also a sustainable and environmentally friendly source of bioactive materials for advanced technological applications, ranging from green nanomaterial synthesis and environmental remediation to food preservation, biomedical products, and agriculture. The rich diversity of phytochemicals, particularly polyphenols, flavonoids, and chalcones, appears to play a crucial role in its biological activities as well as its reducing and stabilizing capacities in nanoparticle synthesis. Future studies should focus on optimizing extraction and synthesis processes, evaluating long-term safety, and scaling up production systems to facilitate the practical commercialization of *C. operculatus*-derived products.

15. Challenges and future perspectives of *C. operculatus*

Although *C. operculatus* has demonstrated a wide range of important biological activities, including antioxidant, antimicrobial, anti-inflammatory, metabolic regulatory, and anticancer effects, the large-scale exploitation and industrial application of this species still face several major challenges, as summarized in Figure 9.

First, the current raw material supply is mainly obtained from household-scale harvesting or small cultivation areas in Vietnam and southern China, while stable large-scale commercial cultivation systems have not yet been established. Variations in climate, soil conditions, harvesting time, and plant parts used may lead to significant fluctuations in the contents of bioactive compounds, particularly flavonoids, chalcones, and essential oils (Lien & Cuong, 2017; Thuy et al., 2021; Thanh et al., 2024a). Such variability creates difficulties in raw material standardization and maintaining batch-to-batch consistency for industrial products.

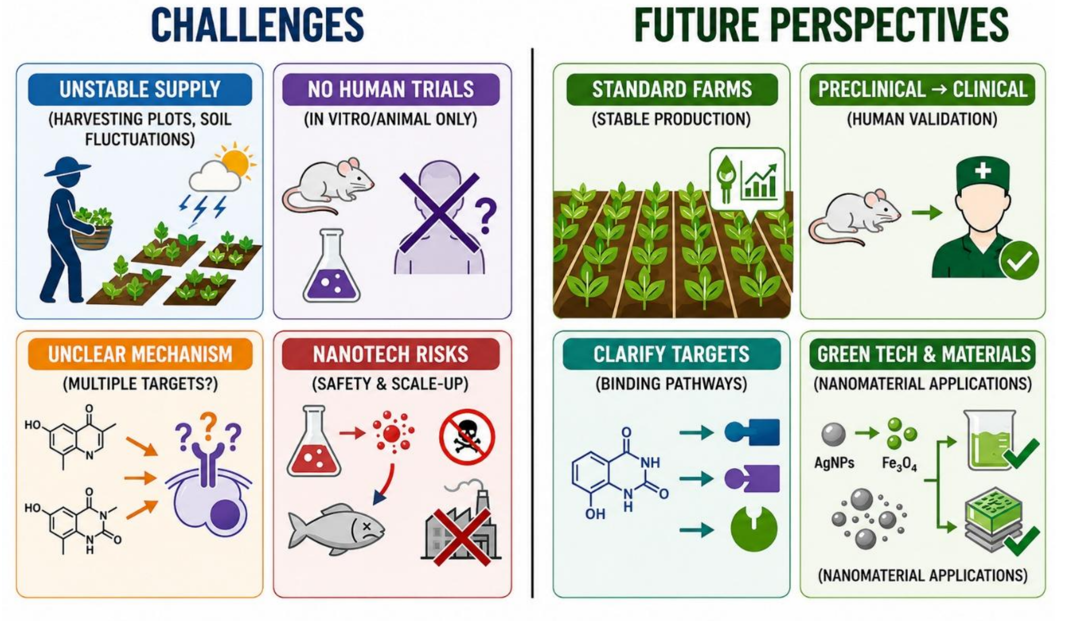

Figure 9. Challenges and future perspectives for research and application of *C. operculatus*.

In addition, most existing studies remain limited to *in vitro* experiments or animal models. Although compounds such as DMC have shown promising anti-inflammatory, anti-*Helicobacter pylori*, and anticancer activities (Tran et al., 2019a; Tuan et al., 2019; Thanh et al., 2024a), clinical evidence in humans is still insufficient. This limitation hinders the translation of current findings into high-value commercial products such as functional foods, pharmaceuticals, and cosmeceuticals.

Another major challenge is that the biological mechanisms of many compounds have not yet been fully elucidated. Most studies have primarily focused on compound isolation and activity screening, whereas investigations on bioavailability, metabolism, long-term toxicity, and synergistic interactions among phytochemicals remain limited. For instance, chalcones and flavonoids isolated from flower buds have demonstrated strong inhibitory activities against α-amylase, PTP1B, and neuraminidase, but the relationship between chemical structure and biological activity still requires further clarification (Dao et al., 2010; Zhang & Lu 2012; Mai et al., 2024).

Furthermore, although the application of *C. operculatus* in nanotechnology and biomaterials is highly promising, comprehensive evaluations of environmental safety, bioaccumulation potential, and large-scale practical performance are still lacking. Recent studies have demonstrated that leaf extracts can be successfully utilized for the green synthesis of AgNPs, Fe_3O_4 nanoparticles, ZnO nanoparticles, and nanoscale zero-valent iron particles (Le et al., 2022; Vu et al., 2022; Nguyen et al., 2023; Le et al., 2025). However, the commercialization potential of these nanomaterials still depends on production costs, physicochemical stability, and long-term biosafety.

In the future, research on *C. operculatus* should adopt a more integrated approach to enhance both scientific understanding and practical applications of this species. Establishing standardized cultivation systems and stable raw material production areas will be essential to ensure consistent phytochemical quality across seasons and growing regions. At the same time, extraction, isolation, and preservation techniques should be optimized to improve the recovery efficiency of biologically active compounds such as chalcones, flavonoids, and terpenoids. Beyond *in vitro* and animal studies, more preclinical and clinical investigations are necessary to validate efficacy and safety in humans. In addition to pharmaceutical and functional food applications, *C. operculatus* also shows considerable promise in green nanotechnology, biomaterials, and environmental remediation due to its ability to mediate nanoparticle synthesis and adsorb environmental pollutants. With its rich reservoir of natural compounds and broad spectrum of biological activities, *C. operculatus* is considered a promising resource for the development of high-value products in the future.

16. Conclusions

This review highlights *C. operculatus* as a valuable medicinal and functional plant possessing diverse phytochemical constituents and broad-spectrum biological activities. Numerous studies have identified flavonoids, chalcones, terpenoids, phenolics, and essential oil components as major contributors to its pharmacological potential. Among these compounds, DMC has emerged as a key bioactive molecule with remarkable antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer properties. Experimental evidence demonstrated that extracts and isolated compounds from *C. operculatus* exhibit significant activities against oxidative stress, microbial pathogens, metabolic disorders, inflammation, and cancer-related processes through multiple molecular mechanisms. Furthermore, the plant has shown considerable promise in applications related to functional foods, herbal beverages, green nanotechnology, environmental remediation, and biomedical materials. However, most current studies remain limited to *in vitro* assays and animal models, while clinical investigations and standardized cultivation systems are still insufficient. Variations in phytochemical composition caused by geographic origin, extraction methods, and plant parts also present challenges for quality control and commercialization. Therefore, future research should focus on standardization of raw materials, elucidation of molecular mechanisms, safety assessment, and clinical validation to support the development of high-value products derived from *C. operculatus*. Overall, the accumulated evidence indicates that this species has substantial potential as a sustainable source of bioactive compounds for food, pharmaceutical, and biotechnological applications.

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Conflicts of Interest
There are no conflicts of interest

Contribution of the authors
Research concept and design: L.P.T.Q.; Collection and assembly of data: P.T.Q. and L.P.T.Q.; Data analysis and interpretation: P.T.Q.; Writing the article: P.T.Q. and L.P.T.Q.; Critical revision of the article: P.T.Q.; Final approval of the article: L.P.T.Q. All authors have read and agreed to the published version of the manuscript.

ORCID

P. T. Quyen https://orcid.org/0000-0003-3695-3703

L. P. T. Quoc https://doi.org/0000-0002-2309-5423

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