THE REVIEW ON BIOLOGICAL ACTIVITIES OF ENT-KAURANE DITERPENOIDS EXTRACTED FROM CROTON TONKINENSIS

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ABSTRACT

Objective: *Croton tonkinensis* has been a focal point of medicinal research due to its rich phytochemical composition, particularly ent-kaurane diterpenoids. These compounds exhibit diverse biological activities, including anticancer, anti-inflammatory, anti-microbial, and osteogenic effects, which are explored in this review. We summarize extraction techniques, structural properties, and recent findings on their pharmacological potential, offering a foundation for future research on therapeutic applications.

Method: The study focuses on retrieving articles from PubMed, Scopus, and Web of Science using the keyword *"Croton tonkinensis"*. The collected articles are analyzed to extract and summarize critical information related to the extraction processes, chemical structures, and biological activities of ent-kaurane diterpenoids isolated from *Croton tonkinensis*. Based on these findings, the research evaluates potential practical applications of these compounds and explores future research directions for *Croton tonkinensis*.

Results: Ent-kaurane diterpenoids extracted from *Croton tonkinensis* have emerged as a versatile class of bioactive compounds with significant therapeutic potential. Their extraction typically employs organic solvents such as methanol or ethanol, followed by chromatographic techniques for isolation and purification. Advanced analytical methods, including high-performance liquid chromatography and nuclear magnetic resonance spectroscopy, are crucial for elucidating their molecular structures. These diterpenoids exhibit notable biological activities across diverse therapeutic areas. As anticancer agents, they demonstrate potent efficacy in both monotherapy and combination therapies with radio- or chemotherapy, modulating key apoptotic and signaling pathways across various cancer types. Additionally, their strong anti-inflammatory properties, mediated by the regulation of nitric oxide production, oxidative stress, and NF-κB activation, underscore their potential in managing inflammatory diseases. With low IC_{50} values and high efficacy in cell-based assays, these compounds also represent promising candidates for antimycobacterial therapies, particularly against drug-resistant tuberculosis. Furthermore, their osteogenic properties are evident in their ability to significantly enhance alkaline phosphatase (ALP) activity, upregulate osteoblastic gene promoter activity, and, in some cases, increase mRNA levels of ALP and collagen type I alpha, thus promoting osteoblast differentiation and bone matrix production. These findings highlight the therapeutic versatility of ent-kaurane diterpenoids, warranting further in vivo investigations to advance their clinical applications.

Conclusion: Ent-kaurane diterpenoids from *Croton tonkinensis* exhibit multifaceted biological activities, including anticancer, anti-inflammatory, antitubercular, and osteogenic effects. These findings highlight their potential as lead compounds for novel therapeutic agents.

Keywords: *Croton tonkinensis, ent-kaurane diterpenoids, anticancer, anti-inflammation, antibaterial effect.*

I. INTRODUCTION

Croton tonkinensis, a tropical shrub indigenous to Northern Vietnam locally known as 'kho sam cho la', has been extensively utilized in traditional Vietnamese medicine. It is employed in the treatment of various ailments, including abscesses, impetigo, and gastrointestinal conditions such as gastric, duodenal ulcers and stomachaches [1-2]. Additionally, it has been applied therapeutically for managing malaria, urticaria, leprosy, psoriasis, and genital prolapse. These diverse medicinal applications highlight its significance in traditional healthcare systems [1].

Building upon the extensive history of *C. tonkinensis* in traditional medicine, modern analytical techniques have been employed to identify and isolate bioactive compounds for

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potential therapeutic applications. As a member of the *Euphorbiaceae* family, the plant is notable for its abundance of diterpenoids, particularly ent-kaurane diterpenoids. These compounds are distinguished by their complex ring structures and functional groups, making them suitable for pharmacological targeting. This review examines the recent findings on the biological effects of ent-kaurane diterpenoids extracted from *C. tonkinensis*, focusing on anticancer, anti-inflammatory, antimicrobial, and osteogenic properties.

II. OVERVIEW OF ENT-KAURANE DITERPENOIDS EXTRACTED FROM *C. TONKINENSIS*

2.1. Extraction and structural characterization

Extraction methods for ent-kaurane diterpenoids often employ organic solvents (e.g., methanol, ethanol) combined with chromatographic techniques for isolation

and purification. Advanced methods, including high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy, are critical for elucidating the molecular structures of these diterpenoids.

Extraction techniques

The extraction and purification of bioactive compounds from *Croton tonkinensis* involve similar methodologies across various studies, employing solvent-based extractions, partitioning, and chromatographic techniques to isolate target compounds of diterpenoids. Initially, methanol (MeOH) or ethanol (EtOH) was universally employed as the primary extraction solvent due to their effectiveness in solubilizing a wide range of bioactive constituents. The resulting extracts were concentrated under reduced pressure to obtain crude materials for further processing. Fractionation followed, utilizing solvents of increasing polarity or the mixture of them, such as n-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and n-butanol (n-BuOH), to separate hydrophobic and hydrophilic components, with the n-hexane and CH_2Cl_2 fractions often demonstrating significant bioactivity. Chromatographic purification was achieved through silica gel chromatography with gradient elution systems (e.g., n-hexane-EtOAc) for initial separation, complemented by preparative high-performance liquid chromatography (HPLC) for final purification of target compounds. Across this method, the primary focus was the isolation of ent-kaurane diterpenoids, which were consistently obtained in milligram quantities, underscoring their pharmacological relevance [3-7].

Structural attributes

The ent-kaurane diterpenoids have a characteristic four-ring core structure. Variations in functional groups across these rings lead to diverse biological activities. Specific modifications, such as hydroxylation and oxidation states, have been shown to enhance bioactivity [3-7]. Among the key compounds, CrT1 (ent-18-acetoxy-7β-hydroxy kaur-15-oxo-16-ene) and other ent-kaurane diterpenoids with a 15-oxo-16-ene moiety have been shown to possess significant cytotoxic activity on various cancer cell lines [7-8].

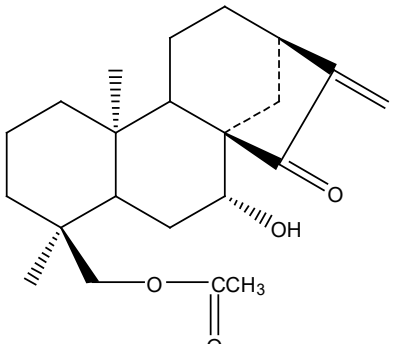

Figure 1. **Structure of *ent*-18-acetoxy-7*β*-hydroxy kaur-15-oxo-16-ene (CrT1) [8]**

2.2. Anticancer properties

Ent-kaurane diterpenoids derived from *Croton tonkinensis*, have garnered attention for their promising anticancer properties. These compounds have shown broad anticancer activity across multiple cancer types, including hepatocellular carcinoma (HCC), colorectal cancer, breast cancer, ovarian cancer, and head and neck squamous cell carcinoma (HNSCC), highlighting their potential as therapeutic agents. The molecular mechanisms underlying the anticancer effects of ent-kaurane diterpenoids include apoptosis induction, reactive oxygen species (ROS) generation, and modulation of critical signaling pathways, such as AMPK, JNK, and ERK1/2.

The anticancer efficacy of ent-kaurane diterpenoids has been extensively tested in various cancer cell lines, including HepG2 and Hep3b for HCC, Caco-2 and LS180 for colorectal cancer [9-11], MCF-7 and MDA-MB-231 for breast cancer [10], SKOV3 for ovarian cancer [12], and OML1 and OML1-R for HNSCC [13]. Among the key compounds, CrT1 (ent-18-acetoxy-7β-hydroxy

kaur-15-oxo-16-ene) and other ent-kaurane diterpenoids with a 15-oxo-16-ene moiety have been shown to possess significant cytotoxic activity, with inhibitory concentrations (IC_{50}) ranging from sub-micromolar to micromolar levels [11,15].

The mechanisms by which ent-kaurane diterpenoids exert their anticancer effects are diverse. A key mechanism involves the induction of apoptosis, which is facilitated through the activation of caspases (caspase-3, -7, -8, -9) and poly(ADP-ribose) polymerase (PARP), alongside the modulation of apoptotic regulators such as Bax, p53, and Bcl-2 [9,11,14]. In colorectal cancer, ROS generation is critical for activating JNK through MKK4 phosphorylation, ultimately leading to apoptosis [10]. Additionally, the activation of AMP-activated protein kinase (AMPK) has been linked to the anticancer effects of CrT1, where it modulates the mTOR/p70S6K pathway, driving tumor suppression and apoptosis in HCC cells. Moreover, CrT1's cytotoxicity is accompanied by the inhibition of tumor growth *in vivo*, offering potential as a chemotherapeutic agent [10]. Furthermore, in HNSCC, ent-kaurane diterpenoids inhibit the PI3K/AKT/mTOR pathway, enhancing radio-sensitization and reducing cell viability [15]. The activation of ERK1/2, particularly in ovarian cancer, is also a crucial pathway through which these compounds exert their effects, inducing apoptosis and inhibiting migration and invasion [14].

Moreover, ent-kaurane diterpenoids exhibit synergistic effects when combined with other therapies. In HCC, subtoxic concentrations of these compounds have been shown to sensitize cells to doxorubicin, enhancing apoptosis [8]. In HNSCC, these diterpenoids potentiate the effects of radiotherapy by inhibiting key survival pathways, demonstrating their potential as radiosensitizers [15].

These studies collectively emphasize the anticancer efficacy of *Croton tonkinensis*, particularly its ent-kaurane diterpenoids. The findings support further exploration of these compounds as promising leads for developing novel anticancer agents, with a focus on their structure-activity relationships and effectiveness against drug-resistant cancer types. Future studies should focus on elucidating additional molecular targets, optimizing the pharmacokinetics and bioavailability of these compounds, and conducting clinical trials to assess their safety and efficacy in humans. Combining ent-kaurane diterpenoids with other chemotherapeutic agents or targeted therapies could provide a powerful strategy for cancer treatment.

The anticancer effects of **ent-kaurane diterpenoids** derived from *Croton tonkinensis is summarized in following table*

***Table 1. The anticancer effects of* ent-kaurane diterpenoids *derived from* Croton tonkinensis**

Aspect	Details
Key compound	CrT1 (ent-18-acetoxy-7β-hydroxy kaur-15-oxo-16-ene) and others with 15-oxo-16-ene moiety
Cancer types	HCC (HepG2, Hep3b), Colorectal (Caco-2, LS180), Breast (MCF-7, MDA-MB-231), Ovarian (SKOV3), HNSCC (OML1, OML1-R)
IC50	Sub-micromolar to micromolar levels [11,15]
Molecular mechanisms	**- Apoptosis Induction**: Caspase activation (-3, -7, -8, -9) and PARP cleavage [9,11,14] **- ROS Generation**: JNK activation via MKK4 phosphorylation (colorectal cancer) [10] **- AMPK Activation**: Modulates mTOR/p70S6K pathway (HCC) [10] - **PI3K/AKT/mTOR Pathway Inhibition**: Enhances radio-sensitivity (HNSCC) [15] - **ERK1/2 Activation**: Induces apoptosis, inhibits migration/ invasion (ovarian cancer) [14]
Synergistic effects	- Sensitizes HCC cells to doxorubicin, enhancing apoptosis [8] - Potentiates radiotherapy effects in HNSCC [15]

2.3. Anti-inflammatory activity

Ent-kaurane diterpenoids, isolated from *Croton tonkinensis*, have shown significant anti-inflammatory properties, which make them potential candidates for the treatment of various inflammatory diseases. Their bioactive effects

have been studied in several experimental models, particularly focusing on nitric oxide (NO) production, superoxide anion generation, and the modulation of the NF-kappaB signaling pathway.

A range of ent-kaurane diterpenoids have been evaluated for their anti-inflammatory activities, have demonstrated potent inhibition of LPS-induced NO production, a critical inflammatory mediator. This inhibition was shown to occur at low IC_{50} values of less than 5 μM for several compounds [14]. This suggests that these diterpenoids may serve as effective anti-inflammatory agents by suppressing NO production, which is often upregulated during inflammatory responses.

Additionally, ent-18-acetoxykaur-16-en-15-one displayed strong inhibition of superoxide anion generation and elastase release, both of which are key contributors to the inflammatory response. These effects were noted to be concentration-dependent, with significant suppression of oxidative stress and inflammation markers in the tested cell models [16]. The ability of ent-kaurane diterpenoids to modulate oxidative stress highlights their dual role in reducing both the inflammatory mediator levels and the cellular damage caused by oxidative species.

The molecular mechanisms underlying the anti-inflammatory effects of these diterpenoids have been further elucidated in studies targeting the NF-kappaB signaling pathway. NF-kappaB is a critical regulator of inflammation and immune responses. In a study of four specific ent-kaurane compounds, including both known and newly isolated diterpenoids, it was found that they inhibited LPS-induced NF-kappaB activation in murine macrophage RAW264.7 cells with IC_{50} values ranging from 0.07 to 0.42 μM [17]. This inhibition correlated with reduced production of pro-inflammatory cytokines and NO, indicating that these compounds interfere with the early stages of the inflammatory cascade.

These findings underscore the potential of ent-kaurane diterpenoids as modulators of inflammatory pathways. Their ability to target key molecular players, such as NF-kappaB and reactive oxygen species (ROS), positions them as promising candidates for the development of novel anti-inflammatory drugs. However, despite their potent in vitro effects, further research is required to fully understand the pharmacological profiles of these compounds in vivo. Specifically, studies investigating their bioavailability, toxicity, and long-term effects in animal models will be critical to advancing their clinical applications. Moreover, the synergistic effects of these compounds with existing anti-inflammatory drugs should be explored to enhance therapeutic efficacy in treating chronic inflammatory diseases.

2.4. Antibacterial activity

Recent studies have highlighted the significant antibacterial properties of ent-kaurane diterpenoids, particularly against *Mycobacterium tuberculosis* (M. tuberculosis), the causative agent of tuberculosis (TB). These diterpenoids, which are rich in bioactive functional groups, exhibit potent activity against both drug-susceptible and drug-resistant strains of M. tuberculosis, marking a noteworthy contribution to the search for novel antituberculosis agents.

Among the various diterpenoids tested, ent-1β,7α,14β-triacetoxykaur-16-en-15-one (cpp604) demonstrated the highest antituberculosis activity, with minimum inhibitory concentrations (MIC) of 0.78 μg/ml, 1.56 μg/ml, and 3.12-12.5 μg/ml against the H37Ra, H37Rv, and several resistant strains of M. tuberculosis, respectively. These results indicate that cpp604 possesses broad-spectrum activity against both sensitive and resistant strains, which is a critical challenge in the treatment of TB [17]. The antibacterial activity of these diterpenoids is likely linked to the structural features of their molecular scaffolds. The mechanism of action behind the antibacterial effects of ent-kaurane diterpenoids is not yet fully elucidated but is believed to involve disruption of the mycobacterial cell wall or interference with essential metabolic pathways. Their potency against both susceptible and resistant strains indicates that they may target bacterial systems that are less prone to the resistance mechanisms typically encountered with conventional antibiotics, such as rifampicin and isoniazid [18].

Given the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of M. tuberculosis, the discovery of novel agents with distinct mechanisms of action is urgently needed. The ent-kaurane diterpenoids from *Croton tonkinensis* show great promise in this regard. However, further research is necessary to explore their full pharmacological profiles, including their bioavailability, toxicity, and the specific molecular

targets they affect within the bacterial cell. In vivo studies will be essential to evaluate the therapeutic potential of these compounds in animal models, and clinical trials will be required to establish their safety and efficacy in humans.

In conclusion, ent-kaurane diterpenoids from *Croton tonkinensis* represent a promising class of compounds for the development of new antimycobacterial agents, particularly in the context of drug-resistant tuberculosis. Their potent activity, coupled with their structural diversity, offers valuable insights into the design of novel antibiotics that could address the growing global threat of tuberculosis. Further investigations into their molecular mechanisms, SAR optimization, and preclinical studies are crucial steps in advancing these compounds from laboratory research to clinical application.

2.4. Osteogenic Activity

One of the intriguing aspects of ent-kaurane diterpenoids is their ability to promote osteoblast differentiation, a critical process in bone formation. A study indicates the osteogenic activity of ent-kaurane diterpenoids isolated from *Croton tonkinensis* and their potential as therapeutic agents for bone diseases such as osteoporosis. Four compounds were assessed using C2C12 cells, a model known for studying osteoblast differentiation. These compounds significantly enhanced alkaline phosphatase (ALP) activity, a key marker of osteoblast function, and upregulated osteoblastic gene promoter activity. Among them, three compounds further increased the mRNA levels of ALP and collagen type I alpha, indicating their role in osteoblast differentiation at the molecular level. These findings suggest that the diterpenoids act by directly stimulating the molecular pathways involved in bone matrix production. Future studies should focus on elucidating the precise molecular targets, exploring the signaling pathways involved, and conducting in vivo assessments to confirm their therapeutic potential and safety for clinical use in treating bone-related disorders [19].

III. CONCLUSION AND FUTURE DIRECTIONS

In summary, ent-kaurane diterpenoids extracted from *Croton tonkinensis* exhibit a range of pharmacological activities, from anticancer, anti-inflammatory and antibacterial effects to promoting bone health. These properties make them a valuable focus for drug development and integrative medicine. Despite promising findings, several gaps remain. For instance, the bioavailability and potential toxicity of ent-kaurane diterpenoids in humans require further exploration. Additionally, understanding the specific molecular mechanisms of action, particularly in multi-target pathways, will be essential for developing effective therapeutic agents based on these compounds.

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