REVIEW ARTICLE

Opioid-like Compounds Isolated From *Mitragyna speciosa*, *Picralima nitida* and *Clinacanthus nutans* as Potential Therapeutics for Peripheral Analgesia via Opioid and Non-opioid Mechanisms: A Review

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ABSTRACT

Growing evidence underscores nociceptor-immune system interactions in regulating chronic pain and inflammatory diseases. Opioid receptors play a role in modulating pain-associated inflammation via central and peripheral mechanisms. Concerns over central side effects have driven the exploration of plant-derived compounds mimicking opioids, aiming to relieve inflammation while mitigating issues like addiction. Traditionally, *Mitragyna speciosa*, *Picralima nitida* and *Clinacanthus nutans* are used for pain relief in conditions such as rheumatism, gastric pain and cancer. While their compounds primarily alleviate pain centrally by interacting with opioid receptors, their peripheral analgesic potential remains uncharted. The presence of opioid receptors and opioid-producing immunocytes at sensory neuron terminals hints at a peripheral opioid analgesic possibility. This review elucidates the peripheral role of the opioidergic system in pain management and explores the potential peripheral analgesic effects of compounds isolated from *M. speciosa*, *P. nitida* and *C. nutans* in pain management.

Keywords: Opioid; Peripheral analgesia; Anti-inflammatory; Non-opioid mechanism; Immunomodulation

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INTRODUCTION

In recent years, the rising prevalence of chronic pain has placed a significant socioeconomic burden on society. This is evident through a decrease in overall quality of life, higher mortality rates and escalating costs associated with pain management. Pain, which serves as a fundamental protective mechanism, can manifest as localised discomfort in specific regions or widespread sensation throughout the entire body [1]. Conventional opioids such as morphine and fentanyl which continue to serve as the standard of care for pain management, modulate the neurotransmission pathway by reducing the transmission of pain signals when they bind and activate opioid receptors in the central nervous system (CNS) [2]. In addition to their remarkable pain-relieving properties, opioids are associated with a range of unwanted side effects, including respiratory depression, sedation, the development of tolerance and the risk of addiction. These effects are primarily linked to their interaction with central opioid receptors [3].

Extensive research has established the intricate interplay between the peripheral nervous system (PNS) and the immune system in the context of pain sensitisation. Upon exposure to noxious stimuli, nociceptors release various inflammatory mediators at the terminals of sensory neurons. This, in turn, recruits immune cells to the affected area and amplifies subsequent sensitisation of nociceptors. Interestingly, apart from inducing pain, immune cells have demonstrated a significant role in releasing anti-inflammatory or analgesic mediators, including opioid peptides, at the injury site to alleviate pain. This underscores the presence of opioid receptors on nociceptive endings [4]. Both endogenous or exogenous opioids exhibit potent and clinically observable analgesia when these peripheral opioid receptors are activated. Intriguingly, this peripheral opioidergic action offers a novel approach to pain management that can potentially circumvent the common central side effects associated with opioids [3, 5]. Additionally, cyclooxygenase (COX) enzymes have been demonstrated to play a role in inflammatory pain through the production of prostaglandins from arachidonic acid. Besides heightening the inflammatory response during tissue injury, prostaglandins also can enhance neuronal pain signals by further sensitising peripheral nerves, making them more responsive to pain

signals. Nonsteroidal anti-inflammatory drugs (NSAIDs) are used to inhibit the COX enzymes, leading to reduced prostaglandin production. Consequently, the suppressed inflammatory response will desensitise the peripheral nerves, contributing to overall pain alleviation. NSAIDs serve not only as anti-inflammatory agents but also indirectly function as analgesic drugs [6].

Medicinal plants have a long history in traditional healing practices worldwide, offering various plant parts for therapeutic purposes including pain relief. For instance, the latex, seeds, stem and leaves of an ancient medicinal plants *Papaver somniferum* have been used to produce commercial narcotic analgesics like morphine and codeine. Throughout history, this plant has been employed in religious ceremonies and for medical purposes, primarily for its hypnotic and pain-relieving properties [7]. Besides opium poppy, *M. speciosa*, *P. nitida* and *C. nutans* have been traditionally consumed to alleviate inflammatory pain. The compounds isolated from these plants have demonstrated potent central analgesia by activating the opioidergic pathway, highlighting their potential as novel analgesics and anti-inflammatory agents [8-10]. In line with this, extensive research aims to discover plant-based compounds with high therapeutic efficacy and fewer side effects for pain management as an alternative to conventional opioids and nonsteroidal anti-inflammatory drugs. Many plant-based compounds are currently undergoing clinical development, highlighting the practicality of medicinal plants as sources of novel therapeutic candidates. This review aims to explore the potential of compounds isolated from *M. speciosa*, *P. nitida* and *C. nutans* in pain relief, with a specific focus on their capacity to induce peripheral analgesia through modulation of immune response. While numerous studies have previously discussed their central analgesic action, the exploration of compounds from these plants in mediating peripheral analgesia through activation of opioid receptors on immune cells remain limited. This review would also discuss the intricate relationship between the opioidergic and immune systems.

Modulation of the Immune Response in Pain Control

Inflammation represents a complex, localised biological reaction to cellular or tissue injuries caused by harmful stimuli. Paradoxically, it also serves as an essential immediate protective response to assist tissue recovery. Classic signs of inflammation include redness, warmth, swelling (edema), discomfort (pain) and a loss of tissue function [11]. Several underlying mechanisms contribute to these signs, including vasodilation which increases blood flow to the injured site; increased vascular permeability that allows direct entry of diffusible components to the affected area; chemotaxis-induced cellular infiltration with inflammatory cells migrating to the damaged region; tissue acidification and activation of immune and enzymatic systems [4]. Collectively, these mechanisms result in painful inflammation. In response to injury, immune cells like macrophages and dendritic cells release a range of pro-inflammatory mediators, including cytokines, chemokines, nerve growth factor, prostaglandins and adenosine triphosphate (ATP). Sometimes referred as 'inflammatory soup,' these mediators attract more leukocytes such as neutrophils and mast cells, to the inflamed site, amplifying local inflammatory responses. Additionally, tissue acidification on nociceptors also triggers the release of pro-algesic neurotransmitters into this 'inflammatory soup', further intensifying the pain sensations and hyperalgesia. As a results, endogenous analgesia mediators like opioid peptides, somatostatin, endocannabinoids and anti-inflammatory cytokines are simultaneously released within the inflamed tissue to counteract pain [12]. It is worth noting that inflammation, while providing protection from further damage by restoring tissue physiological function, can also become exacerbated when an imbalance in the release of pro- and anti-inflammatory mediators predisposes the injured tissue to an excessive inflammatory response [13].

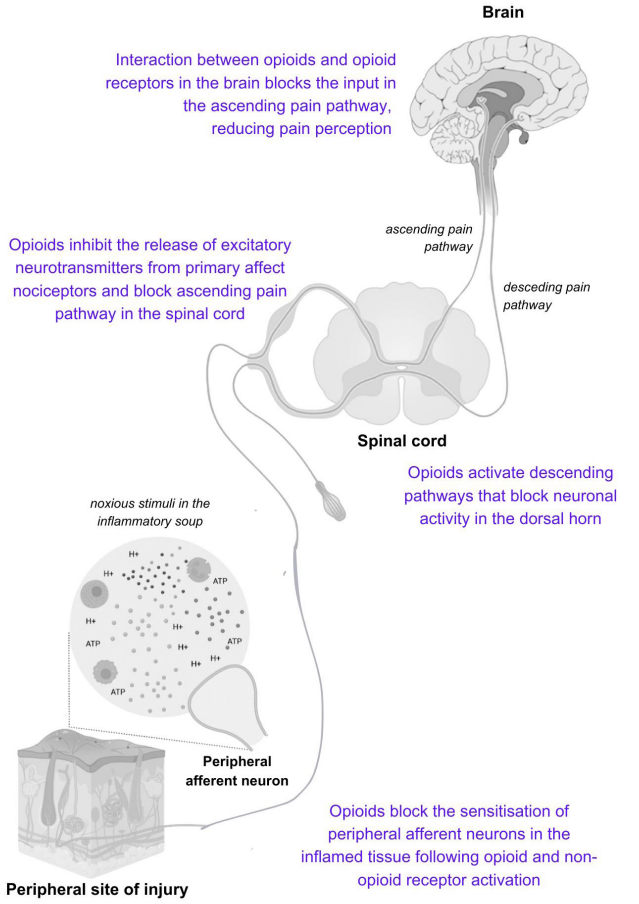

Figure 1 : Pathways of opioid-mediated central and peripheral analgesia.

Opioid Peripheral Analgesia in Modulation of Pain

Evidence from literature reveals that opioid receptor activation, resulting in analgesia occurs both in the CNS and peripheral sensory neurons (Fig. 1) [14-16]. The peripheral mechanism of opioid analgesia is linked to

tissue injury and involves bidirectional communication between the neuroendocrine and immune systems. During the acute stage of inflammation, both central and peripheral pathways play a role in intrinsic pain inhibition, with central opioid receptors taking the lead. However, in later phases of inflammation, anti-nociception is solely mediated by immune-derived opioid peptides that activate peripheral opioid receptors [16, 17]. In this context, immune cells are recognised as the primary producers of endogenous opioid peptides in the PNS [18]. Supporting this, a prior study has identified full-length pro-opiomelanocortin (POMC) transcripts from POMC genes involved in peptide signalling in rat mononuclear leukocytes [19]. The observed reduction in Met-enkephalin (MENK) release when genes coding for proenkephalin (PENK) are disrupted in the brain and immunocytes implies that enkephalin is synthesised from a similar precursor protein molecule in both neurological and immunological systems [20]. Inflammatory cells, including T- and B-lymphocytes, granulocytes (during early inflammation) and monocytes/macrophages (during late inflammation) have been shown to be involved in the production of peripheral opioid peptides [17, 21].

It is also worth noting that opioid peptides are significantly expressed in activated or memory cells, as evidenced by the abundant presence of beta-endorphin (β-END) in activated or memory T-lymphocytes in comparison to the naive cells in inflamed tissue [5, 22]. In line with this, another study revealed that non-inflamed paws do not produce any opioid peptides, emphasising immune cells as the source of opioid peptides in the periphery [23]. Additionally, previous studies have reported that in response to local inflammatory factors and stress-induced catecholamines, MENK, β-END and dynorphin A (Dyn A) are significantly produced in immunocytes within the inflamed area. Interestingly, these peptides remained undetected in non-inflamed sites. Their abundance presence in leukocytes highlights that opioid peptides are synthesised in circulating leukocytes that migrate to the inflamed tissue [5, 24, 25]. Opioid-producing leukocytes migrate from the circulation into inflamed tissue via a diapedesis mechanism. In this process, circulating leukocytes initially tether and roll along the endothelial cell wall, leading to attachment between leukocytes and endothelial cells. Subsequently, these leukocytes transmigrate towards the site of tissue damage by passing through the endothelial wall. They are then activated by chemokines released from both inflammatory and endothelial cells [18]. Upon stimulation by local inflammatory factors like corticotropin-releasing factor (CRF) and interleukin-1beta (IL-1β), which are abundantly found in immune cells, fibroblasts and vascular endothelium, opioid peptides are released from secretory granules into the inflamed tissue through exocytosis [26]. Studies have shown that the release of opioid peptides is associated with elevated level of intracellular calcium concentration and expression of noradrenaline-activated adrenergic receptors on opioid-containing immunocytes within peripheral inflamed tissue [18, 21]. Ligation of opioid peptides to opioid receptors located on peripheral sensory nerve terminals results in alleviation of inflammatory pain. The concept of opioid receptors being localised on primary sensory neurons and their role in pain suppression has garnered substantial support in the literature [17, 27]. These opioid receptors, namely mu-, kappa- and delta-opioid receptors (MOR, KOR, and DOR, respectively), belong to the classic family of G protein-coupled receptors (GPCRs). They are initially synthesised in the cell bodies of dorsal root ganglion (DRG) neurons and are subsequently transported to the terminal endings of sensory neurons within inflamed tissue through axonal transport. The interaction between opioid peptides and their corresponding opioid receptors leads to the generation of second messengers, ultimately mediating peripheral analgesic effects [28].

There are several underlying mechanisms that contribute to opioid peripheral analgesia. It has been established that the acidic environment within inflamed tissue significantly contributes to the effectiveness of opioid-mediated peripheral antinociception [29]. An earlier in vitro study revealed that the lower pH within the inflamed tissue is linked to higher opioid agonist efficacy, resulting from the reduced inactivation of G-proteins in neuronal membranes [30]. Inflammatory environment also promotes the sprouting of sensory nerve terminals, leading to increased permeability of the perineural barrier. This, in turn, enhances the accessibility of opioid ligands to opioid receptors on primary afferent neurons [31]. Opioid-induced peripheral analgesia is also most effective in an inflammatory milieu due to the abundant synthesis and expression of opioid receptors in the DRG [18]. Previous research has shown that the injection of complete Freund's adjuvant (CFA) and carrageenan into the rodent hind paw significantly increases the expression of opioid receptors in DRG neurons, mediating peripheral analgesia. However, the administration of capsaicin, a neurotoxin that acts on the primary afferent C-fibre in the DRG, inhibits the antinociceptive of opioid peptides in the inflamed hind paw. This underscores the crucial role played by DRG in the synthesis and expression of opioid receptors [17, 32, 33]. The release of IL-1β in the inflamed tissue also plays a significant role in peripheral opioid analgesia by enhancing the axonal transport of opioid receptors, making them more accessible to sensory neurons [32]. Prior studies have demonstrated that inflammation increases the axonal and site-directed trafficking of opioid receptors in the sciatic nerve, resulting in an increased receptor density in peripheral nerve terminals. However, this receptor up-regulation is abolished following sciatic nerve ligation, supporting the transport theory [34].

Opioid-like Plant Compounds for Peripheral Analgesia

It is well-established that peripherally restricted opioid agonists offer new insights into intrinsic mechanism of pain control, potentially circumventing the systemic side effects associated with currently available drugs especially the opioid analgesics. In the subsequent sections, we will look into several common folk medicinal plants used as analgesics in local communities, namely *M. speciosa*, *P. nitida* and *C. nutans*. Interestingly, certain compounds isolated from these plants have exhibited opioid-like properties, shedding light on their intriguing potential in immunomodulation-associated peripheral analgesia, akin to opioids.

Mitragyna speciosa

M. speciosa Korth, a member of the Rubiaceae family, is a tropical plant indigenous to the tropical and subtropical regions of Southeast Asian countries like Thailand, Malaysia, the Philippines, Myanmar and certain parts of Africa [35]. This plant is commonly known by various names such as 'kratom', kakuam, 'kraton', 'ithang' or 'thom' in Thailand, 'biak-biak' or 'ketum' in Malaysia and 'mambog' in the Philippines [36]. The leaves and smaller stems of *M. speciosa* are the parts traditionally consumed. This plant has been utilised as a folk remedy in treating fatigue, diarrhea, asthma, fever, cough and muscle pain. It has also been traditionally consumed by the labourers to enhance work productivity and increase tolerance for strenuous labour [37]. Notably, *M. speciosa* herbal preparations have been utilised as a substitute for opium or morphine in the treatment of drug addiction due to their unique opioid-like properties [38]. Over 40 alkaloids have been identified in *M. speciosa* leaves, with mitragynine being the major active constituent. Mitragynine exhibits a range of pharmacological effects including antidepressant, antinociceptive, anti-inflammatory, antidiarrheal, antioxidant, antibacterial and antitussive properties. In addition to mitragynine, 7-hydroxymitragynine (7-HMG), an active metabolite derived from mitragynine, has garnered significant attention among researchers for its potent analgesic properties in comparison to mitragynine [39, 40]. Other prominent alkaloids found in *M. speciosa* leaves include speciogynine, speciociliatine, paynantheine, mitragynaline, corynnatheidaline, mitragynalinic acid and corynantheidalinic acid [40, 41]. Nevertheless, previous literature on their pharmacological activities is scarce.

Over the years, numerous studies have yielded contrasting reports regarding the selectivity and binding affinity of primary alkaloids from *M. speciosa* alkaloids to different opioid receptors. Several previous investigations have indicated that mitragynine exhibits high selectivity and binding affinity to MOR while showing minimal interaction with KOR and DOR [42, 43]. Supporting this, Warner et. al conducted a prior review, affirming that mitragynine is a selective and full agonist of MOR, producing analgesic effects upon interaction with supraspinal MOR [35]. Similarly, 7-HMG has been shown to possess a higher binding affinity toward MOR than DOR and KOR in several studies [44-46]. It is suggested that the indole ring in these plant alkaloids contributes to their enhanced affinity for opioid receptors. In contrast to this, Bahalrudin et. al (2010) reported that mitragynine interacts with MOR, DOR and KOR, with higher affinities observed toward KOR. It acts as an agonist at MOR and an antagonist at DOR [43]. Additionally, Ya et. al suggested that mitragynine is a partial agonist at MOR and elicits competitive antagonistic activities at KOR and DOR intracellularly. Mitragynine is proposed as a superior option to morphine in terms of reduced adverse effects on opioid receptors. Morphine tends to induce respiratory depression by recruiting β-arrestin to MOR. Furthermore, β-arrestin also hinders G-protein signalling, which typically produces analgesia but may also lead to opiate tolerance. Although mitragynine is a G-protein-biased MOR agonist, it does not recruit β-arrestin due to its partial agonism at MOR. Therefore, respiratory depression can be avoided while maintaining analgesia [47]. A recent report by Obeng et al. highlights that mitragynine exhibits lower efficacy MOR agonism as compared to 7-HMG in vitro while concurrently displaying affinity for non-opioid receptors, resulting in its complex pharmacology. Nonetheless, mitragynine is considered a unique compound that holds promise as an effective therapeutic agent due to its low efficacy at MOR combined with other pharmacological mechanisms [44]. It is essential to note that despite the structural differences between mitragynine and morphine and other members of the opioid family, mitragynine is able to interact with MOR, KOR and DOR, thus, reflecting its broader receptor binding affinity [48]. On the other hand, the increased psychoactivity of mitragynine and 7-HMG may be attributed to their higher binding affinities for central DOR and KOR compared to morphine. Between these two compounds, 7-HMG demonstrates greater opioid receptor affinity with full agonist properties due to its increased polarity resulting from the presence of an additional hydroxyl group compared to mitragynine [35].

Accumulating evidence in the literature has consistently supported the idea that mitragynine and 7-HMG possess opioid-induced antinociceptive and anti-inflammatory activities, both in vitro and in vivo [44-46, 49]. Studies involving mice treated with *M. speciosa* extract have demonstrated central analgesic effects through the activation of the opioidergic system in rodent hot plate latency test. It is postulated that these extracts suppress the initial phase of edema and inhibit the synthesis, release or actions of hyperalgesic mediators in inflamed areas, consequently reducing sensitivity to pain

receptors [50]. In a study conducted using SK-N-SH neuroblastoma cells, mitragynine was found to inhibit the increase in forskolin-stimulated cAMP production following prior treatment with morphine in a dose-dependent manner. Additionally, mitragynine, even at lower concentrations, mitigated the downregulation of MOR mRNA expression [38]. Kruegel et al. previously demonstrated that mitragynine produced analgesic activity in mice through a MOR-dependent mechanism. This study also revealed that mitragynine is converted into 7-HMG in both mouse and human liver preparations, highlighting the importance of the route of administration in determining the metabolic activity of the compound. Nevertheless, the study concluded that both mitragynine and 7-HMG hold a significant potential as future therapeutics, warranting further investigations in animal and human studies [40]. In contrast, a recently published pharmacokinetic and pharmacodynamic study suggested that 7-HMG plays a negligible role in the antinociceptive effects of mitragynine in mice. The study reported that the exposure of this compound in the brain after an antinociceptive dose of mitragynine was approximately 3.7-4.0-fold lower in both male and female mice compared to after an antinociceptive dose of 7-HMG. These findings suggest that the analgesic effect of mitragynine is not solely attributed to the potent 7-HMG metabolites; rather, various other factors could influence these results, including species differences in metabolism, brain penetration levels, and the presence of a free fraction of the compounds [51]. In terms of anti-inflammatory effects, one study demonstrated that the methanolic extract of *M. speciosa* leaves able to inhibit carrageenan-induced hind paw edema in rats by up to 60% following the administration of the extracts [52]. An in-vitro study conducted by Utar et al. revealed that mitragynine peripherally inhibits the production of prostaglandin E2 (PGE2) by suppressing cyclooxygenase-2 (COX-2) mRNA expression but not cyclooxygenase-1 (COX-1) mRNA expression in RAW 264.7 macrophages, thereby ameliorating inflammatory conditions [53]. In addition to this, Mat et al. (2023) have also reported the reduced expression of the transient receptor potential cation channel subfamily V member 1 (TRPV1) in the rats' brains following treatment with mitragynine at various concentrations, denoting the analgesic effects of this indole alkaloid through a non-opioid receptor mechanism [54]. This finding indirectly suggests that mitragynine produces analgesic and anti-inflammatory effects through a mechanism similar to conventional pain therapy. From an immunopharmacology perspective, the anti-inflammatory and antinociceptive activities of mitragynine and 7-HMG may result from a combination of factors, including the inhibition of pro-inflammatory mediator release, reduction of vascular permeability, enhancement of immune response and stimulation of tissue repair. These processes involve the regulation of a key inflammatory mediator, nuclear factor-kappaB (NF-κB), at the transcription level. Although mitragynine exhibits opioid-like properties, it remains unclear whether the regulation of NF-κB translocation and the subsequent release of pro-inflammatory factors in macrophages involve opioid receptor activation. Previous studies have suggested the involvement of opioid-mediated immunological functions, hinting at the NF-κB signalling pathway as one of the potential mechanisms underlying the immunomodulatory effects of opioid receptor activation during inflammatory pain [55, 56]. Despite a recent study outlining the significant inhibition of mitragynine on the expression of TRPV1receptors which highlights the involvement of non-opioid mechanism in pain alleviation [54], the specific effects of this compound at the transcriptional level, including on NF-κB regulation and the subsequent release of pro-inflammatory cytokines, remain unknown and necessitate further investigation.

Picralima nitida

Picralima nitida is a deciduous plant that sparsely populates the forest of Western Africa and holds a significant ethnomedicinal applications in African folk medicine. Belonging to the Hunterian tribe of the Apocynaceae family, this plant goes by various names, including 'picralima,' 'akuamma,' or 'pile plant.' Various parts of *P. nitida* such as the leaves, seeds, stem bark and roots, have traditionally been used to treat conditions like fever, hypertension, jaundice, gastrointestinal disorders and malaria infections. Notably, the seeds are processed into dried powder, encapsulated and used as a pain relief remedy [57]. The traditional uses of this plant are supported by scientific studies, highlighting its potential as a therapeutic agent. *P. nitida* has demonstrated significant analgesic and anti-inflammatory properties similar to opioids. An orally administered dose range of 100 - 400 mg/kg of the aqueous ethanolic extract of *P. nitida* has shown a dose-dependent anti-inflammatory effect in Wistar rats [58]. Consistently, a 400 mg/kg dose of the aqueous extract of dried *P. nitida* seeds and leaves blocked centrally mediated nociception, partly dependent on the action at the opioid receptors. Importantly, this analgesic effect did not result in sedation or a lack of locomotor activity, suggesting that these effects are not responsible for the antinociceptive effect of a blend of seeds and leaves of *P. nitida* [59]. Ezeamuzie et al. have demonstrated a dose-dependent anti-inflammatory effect of the methanolic extract of *P. nitida* fruit when administered intraperitoneally in a rodent model of carrageenan-induced paw edema. In the same study, the extract also exhibited a higher antipyretic effect compared to a 200 mg/kg aspirin dose in rabbits with LPS-induced fever [60]. Substantial evidence suggests that all these effects result from the interactions between the indole alkaloids of *P. nitida* and the opioid receptors, as well as non-opioid receptors in the opioidergic system of the body [8, 57, 61-63]. Major alkaloids associated with opioid analgesic and anti-inflammatory activities include akuammine, akuammidine, akuammicine,

akuammigine and pseudoakuammigine, all of which are isolated from the seeds of *P. nitida* [61]. Additionally, several other alkaloids such as picraphylline, picracine, picraline, picralicine, picratidine, picranitine, burnamine, percalline and pericine, have been identified, but their pharmacological actions are not well-documented [57].

In one of the earliest studies conducted by Lewin et al., displacement studies and assessments of relative affinities between *P. nitida* alkaloids and opioid receptors were undertaken. The findings revealed that akuammine displayed micromolar affinities for KOR and MOR but exhibited a significantly lower affinity, 10 times less, for DOR. On the other hand, dihydroakuammine, derived from the reduction of akuammine, demonstrated micromolar affinities for KOR but did not exhibit any binding affinity for DOR [64]. Another study by Menzies et al. explored the binding affinities of five major alkaloids isolated from *P. nitida* (akuammine, akuammidine, akuammicine, akuammigine and pseudoakuammigine) toward primary opioid receptors using isolated tissue bioassays and radioligand binding assays. Intriguingly, the data revealed that these alkaloids possessed differential agonist and antagonist activities at the opioid receptors. For example, akuammidine exhibited preferential binding to MOR in the guinea pig ileum preparation, which was later confirmed by its reversal action when antagonised by naloxone and a MOR-selective antagonist in the vasa deferentia of the mouse and the rabbit. Akuammicine selectively bound to KOR in the guinea pig ileum preparation but exhibited only partial binding to this opioid receptor in the vasa deferentia of the mouse and the rabbit [61].

In a previous in vivo study, the analgesic and anti-inflammatory properties of pseudoakuammigine were investigated using a carrageenan-induced rat paw edema model. The findings demonstrated that this alkaloid reduced total paw swelling in the rats and increased the baseline latent reaction time when rat tails were immersed in warm water. Compared to standard analgesics, pseudoakuammigine was found to be 3.5 and 1.6 times less potent than morphine and indomethacin, respectively [62]. The study suggested that pseudoakuammigine could block the induction of peripheral inflammation by inhibiting the release of various prostanoids, which mediate the increase in COX-2 expression and induce peripheral inflammation in the animal model [65]. This aligns with a subsequent study which reported that *P. nitida* seeds extract suppressed PGE2 production and inhibited the elevation of COX-2 expression in SK-N-SH neuronal cells after stimulation with IL-1β in a dose-dependent manner [63]. Ajayi et al. attributed the anti-inflammatory properties of the aqueous extract of *P. nitida* seeds and leaves to their effects on inhibiting the release of inflammatory mediators and nitrite levels. These effects result in reduced vascular permeability, decreased migration of inflammatory cells and less activation and generation of oxidative stress at the inflammation site. Furthermore, the reduction in nitric oxide levels compromises the generation of reactive oxygen species, subsequently reducing lipid peroxidation and preventing the depletion of reduced glutathione. As a result, this herbal extract suppresses the formation of hydrogen peroxide and superoxide anions, possibly due to its free radical scavenging properties [59]. The underlying mechanism of this effect is linked to the interference of the NF-κB signaling pathway through the inhibition of IL-1β-mediated phosphorylation of p38 in a dose-dependent manner by the seeds extract. However, the exact involvement of opioid receptors in regulating NF-κB signal transcription remains unknown and requires further investigation. Previous reports indicating the interaction of compounds from *P. nitida* with opioid receptors, however, suggest the possibility that these compounds could potentially produce a peripheral analgesia via classical NSAIDs-like mechanisms.

Clinacanthus nutans

Clinacanthus nutans, native to tropical countries such as Indonesia, Malaysia, Africa, Brazil and Central America, has been traditionally used in Southeast Asia for various medicinal purposes, including treating skin inflammation, snakebites, diabetes mellitus and fever. The plant, belonging to the Acanthaceae family, primarily utilises its leaves for ethnopharmacological applications due to its antibacterial, anti-inflammatory, anti-dengue, anti-mutagenic, antioxidant, anti-proliferative and anti-tumour properties. Recent research has shown that *C. nutans* extracts possess anti-nociceptive and anti-inflammatory properties, making it a potential candidate for pain relief [66, 67]. Abdul Rahim et al. demonstrated that the methanolic extract of *C. nutans* produced antinociception at both peripheral and central levels using multiple animal experimental models, such as the acetic acid-induced abdominal constriction, hot-plate test and formalin-induced paw licking test [68]. Furthermore, their investigation extended to evaluating the effects of the petroleum ether fraction derived from the methanolic extract of *C. nutans*. The results revealed that this petroleum ether fraction also possessed anti-nociceptive activity, as evidenced by its ability to inhibit abdominal constriction, prolong the latency of responses in the hot-plate test and reduce the latency for paw licking in both the first and second phases of the formalin-induced pain test [10]. What's particularly noteworthy is that these studies indicated that the anti-nociceptive effects of *C. nutans* are linked to the activation of opioid receptors. This assertion finds support in the observation that the anti-nociceptive activity of the petroleum fraction of *C. nutans* could be reversed by pre-treatment with opioid antagonists such as β-FNA, NALT and nor-BNI. The activity of the methanolic extract was completely reversed by naloxone, a non-selective opioid receptor antagonist and partially reversed by a nitric oxide (NO) precursor,

an NO synthase inhibitor or their combinations. These findings collectively suggest that the anti-nociceptive actions of *C. nutans* occur through the central opioid system and the modulation of the L-arg/NO-mediated pathway, which operates independently of cGMP [10, 68]. To note, the L-arg/NO/cGMP pathway has previously been associated with central and peripheral anti-nociception induced by analgesic drugs [69].

The body of evidence supporting the involvement of opioid-mediated anti-nociceptive properties of *C. nutans* is further substantiated by multiple findings indicating the interaction between the plant extracts and opioid receptors (MOR, DOR and KOR) through a non-selective binding mechanism. In addition to their interactions with opioid receptors, *C. nutans* extracts demonstrate the ability to influence a variety of other pathways, expanding their potential therapeutic effects. These pathways include dopaminergic, cholinergic, glutamatergic and TRPV1 receptors as well as the α2-noradrenergic and β-adrenergic receptor systems. These interactions are notable as they can contribute to the plant's overall pharmacological effects, which are interconnected with the opioidergic pathway [10]. Interestingly, the extracts have demonstrated an ability to modulate Toll-like receptor 4 (TLR4) activity. Research conducted by Mai et al. revealed that the phenolic compounds and flavonoids found in this plants' extracts could inhibit the production of cytokines and TLR4-related inflammatory proteins induced by lipopolysaccharides (LPS) in HEK-Blue™-hTLR4 cells. This suggests that this plant may possess anti-inflammatory properties attributed, at least in part, to their ability to influence TLR4 activity [70]. This observation is supported by a recent systematic review that connected the anti-inflammatory effects of *C. nutans* extracts with their capacity to inhibit TLR4 activation [71].

Previous evidence suggest that opioids play a role in modulating the TLR4 signaling pathway [72, 73]. Additionally, in silico docking analysis has indicated that human MD-2, which is a potential binding site for opioids in the TLR4/MD-2 complex, may also be involved in the opioid signalling pathway. Subsequent research has supported this discovery both structurally and functionally [74]. Recently, Sauer et al. revealed that the activation of TLR4 by its primary agonist, LPS, leads to the release of β-END from monocytes. This highlights the possibility of TLR4 regulating peripheral endogenous opioid-mediated analgesia during inflammation [75]. Furthermore, classic opioids such as morphine and fentanyl have previously demonstrated inhibitory activity against TLR4 signalling through non-competitive binding with LPS [76]. Tantowi et al. identified several compounds in the leaf extract of *C. nutans* that are responsible for its anti-inflammatory properties, such as apigenin and apigenin-C-glycosides, namely schaftoside, isoscaftoside, vitexin, and isovitexin [77]. Previous studies collectively reported that *C. nutans* exhibit potent anti-inflammatory characteristics through multiple mechanisms, which include inhibiting the activation of TLR4 [70, 71], suppressing the production of inflammatory cytokines, preventing the activation of the TNF-α gene expression [78], restraining the nuclear translocation of NF-κB/p65 [79], suppressing the mRNA expression of pro-inflammatory cytokines [80] and inhibiting neutrophil migration and responsiveness [81]. Taken together, these collective findings underscore the possibility that opioid-like compounds found in *C. nutans* may produce analgesic effects via activation of both opioid and non-opioid mechanisms. The effects of the above-mentioned medicinal plants on opioid system is summarised in Table I.

CONCLUSION

Accumulating research has brought to light the presence of opioid receptors on immune cells and the existence of peripheral analgesia, thereby highlighting this pathway as a viable drug target. Considering the properties of peripheral opioid analgesia, targeting this pathway will be beneficial in promoting opioid receptor down-regulation and desensitisation which helps mitigating issues associated with opioid tolerance and dependence. In such scenarios, there is potential to extend the treatment duration of peripherally acting endogenous opioids while minimising the incidence of opioid tolerance in cases of inflammatory pain. Nevertheless, drugs that exclusively and selectively activate peripheral opioid receptors without affecting the central opioid system are still unavailable to date. Most opioid medications, including those used for pain relief and diarrhea, exhibit effects on both peripheral and central opioid receptors. For instance, loperamide is an opioid receptor agonist that acts primarily on MOR in the gastrointestinal tract at therapeutic doses, resulting in reduced gastrointestinal motility during diarrhea episodes. However, when consumed at excessive doses, this drug crosses blood-brain barrier, producing the unwanted central side effects [82]. Achieving selectivity for peripheral opioid receptors whilst avoiding the detrimental opioid central effects poses significant challenges and extensive research is currently ongoing in investigating novel compounds and improvising drug delivery methods to enhance the peripheral selectivity. Since opioid and non-opioid receptor activation have intricate connections with the regulation of NF-κB at peripheral nerve endings leading to immunodulation and analgesia, it is worthwhile to further investigate the potential of major isolated compounds from *M. speciosa*, *P. nitida* and *C. nutans* to achieve these effects. The findings from these studies are hopeful to unveil novel therapeutics targeting analgesia with minimal risks of central opioid-associated adverse effects via opioid and non-opioid mechanisms. Therefore, there is a pressing need for more comprehensive research in this

Table I : The effects of compounds derived from *M. speciosa*, *P. nitida* and *C. nutans* on the opioid system

Plant	Extracts/Compounds	Effects on opioid system	References
M. speciosa	Mitragynine	Inhibits electrically induced contraction of guinea-pig ileum through opioid receptor activation	[49]
		Reduces the up-regulation of cAMP level in forskolin-indued human neuroblastoma SK-N-SH cells	[38]
		Cotreatment of morphine with mitragynine reduces the downregulation of MOR mRNA expression	[38]
	7-hydroxymitragynine	Inhibits electrical-induced contraction of guinea-pig ileum through MOR	[45,46]
		Shows high affinity towards MOR in homogenates of guinea pig brain	[45]
		Antinociceptive effects in tail-flick and hot-plate tests in mice through MOR agonism	[40,45]
	Methanolic extract	Inhibits formalin-induced nociception in rats via activation of opioid system	[50]
		Increases the response latency time to thermal stimulation in hot plate test in mice through central opioid action	[50]
P. nitida	Aqueous extract of the seeds	Produces analgesic actions comparable to morphine	[83]
	Pseudoakuammigine	Inhibits swelling in carrageenan-induced edema in rats	[62]
		Shows equal binding affinity towards MOR and DOR in opioid binding assays of guinea pig brain homogenates	[61]
	Akuammidine	Inhibits contraction of electrically stimulated mouse isolated vas deferens via MOR activation	[61]
	Akuammine	Displays high binding affinity towards MOR in opioid binding assays of guinea pig brain homogenates	[61]
	Akuammicine	Full agonist at KOR in the guinea pig ileum preparation but partial KOR agonist in the vasa deferentia of the mouse and the rabbit	[61]
C. nutans	Methanolic extract of the leaves	Produces antinociceptive response in rodent acetic acid-induced abdominal constriction test, formalin-induced paw licking test and hot plate tests via activation of opioid receptors	[68]
	Petroleum ether fraction	Produces central antinociceptive activity that could be reversed by β-FNA, NALT and nor-BNI	[68]

field to address pertinent questions and shed light on the potential novel mechanisms by which these medicinal plants regulate inflammatory pain.

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