Journal of Applied Pharmaceutical Science 2025. Article in Press
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2025.v15.i12.4
ISSN 2231-3354

Triphala, Trikatu, and Benjakul: an evidence-based review of their pharmacology, toxicology, and clinical potential in integrative medicine

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ARTICLE HISTORY
Received on: 03/03/2025
Accepted on: 18/07/2025
Available Online: XX

Key words:
Triphala formulation, Trikatu formulation, Benjakul formulation, pharmacological properties, toxicology.

ABSTRACT

Triphala, Trikatu, and Benjakul formulations are highly esteemed in various traditional medical systems in Thailand and have been utilized to treat a wide array of diseases. This review aims to provide a precise and up-to-date compilation of scientific information on the pharmacological activities and toxicology of Triphala, Trikatu, and Benjakul. It indicates that these formulations exhibit diverse pharmacological properties, as evidenced by previous experimental studies. Specifically, pharmacological investigations have verified their antimicrobial, antioxidant, antiinflammatory, immunomodulatory, antiaging, anti-obesity, and anticancer properties, as well as their clinical applications. Additionally, the toxic properties of these formulations have been assessed to ensure their safety and efficacy. This compilation serves as a valuable reference for scientists, researchers, and professionals working on these plants and facilitates the enhancement of knowledge in this domain. However, future studies are imperative to address the research gaps, particularly regarding the isolation of relevant phytochemical compounds, their preclinical and clinical characterization, and their evaluation. Additionally, the implementation of appropriate clinical trials and placebo-controlled studies is essential to establish a reliable role for Triphala, Trikatu, and Benjakul formulations in human health.

INTRODUCTION

Traditional medicine plays a crucial role in the holistic approach to maintaining good health, with a rich history of treating various diseases. Each region boasts its own distinct traditional medicines, known as local folk formulations, which draw upon theories, skills, knowledge, and experiences [1,2]. In Thailand, traditional herbal medicines continue to exert a considerable influence on medical treatment and are commonly utilized by local Thai people to treat a multitude of illnesses and disorders. Herbal remedies have gained recognition as alternative treatments in hospitals. Among traditional Thai herbal formulations, Triphala, Trikatu, and Benjakul hold prominent positions, motivating the scientific community to conduct extensive phytochemical and biological investigations. Triphala, renowned for its effectiveness in treating coughs and as a mucolytic agent [3], has also been traditionally employed for rejuvenation, detoxification, and rebalancing of body elements. It has applications in addressing various ailments, such as poor food assimilation, colon cleansing, persistent constipation, digestive disorders, and enhancing the immune system [4]. Trikatu, another celebrated herbal formulation, is particularly vital during rainy periods as it helps increase the body's warmth

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with its pungent flavor [5]. Its significance lies in its ability to enhance the bioavailability of phytoconstituents when combined with other formulations. However, it also offers numerous benefits when used independently, including the regulation of the balance of body elements and improvement in food digestion and absorption [6]. Benjakul, a popular Thai herbal formulation, is commonly used in folk medicine to support cancer treatment and restore balance among the body's elements. It is listed as a carminative and adaptogenic drug in the Thai National List of Essential Medicines (NLEM). Numerous investigations have focused on the biological properties of these herbal formulations. Therefore, this study aimed to provide a concise summary of the scientific evidence regarding the pharmacological activities and safety of Triphala, Trikatu, and Benjakul formulations.

MATERIALS AND METHODS

The articles were searched on Science Direct, Google Scholar, Scopus, Medline, Web of Science, and PubMed using the keywords, such as "Triphala," "Trikatu," "Benjakul," "phytochemical studies," and "ethnopharmacological studies" to retrieve relevant studies. Boolean operators were applied, with "OR" used to broaden the search within individual concepts and "AND" employed to narrow the results to the most pertinent literature. Titles and abstracts were then screened, and studies were excluded if they were unrelated to the topic, not written in English, review articles, or duplicates.

RESULTS

Triphala formulation

Triphala, a popular herbal combination originating from Ayurveda, boasts a history of over a thousand years in traditional medicine. Triphala has been an integral component of Ayurvedic medicine for over 2,000 years, with its historical usage deeply rooted in ancient Indian medical traditions. One of the earliest documented references to Triphala appears in the *Sushruta Samhita*, a foundational text of Ayurveda dated to approximately 1500 BCE [7,8]. The term "Triphala" stems from Sanskrit, signifying "three fruits." This formulation comprises *Emblica officinalis* from the Euphorbiaceae family, *Terminalia bellerica*, and *Terminalia chebula* from the Combretaceae family, in equal proportions (1:1:1) [8,9]. In Hindi, *E. officinalis* is referred to as Amla, Amlaki, or Amlakan; in Sanskrit, as Embelic Myrobalan or Indian Gooseberry; and in Thai, as Makham Pom. *T. bellerica* is known as Samor Pipek in Thai, Baheda in Hindi, Bibhitaki in Sanskrit, and Beleric Myrobalan in English. *T. chebula* is known as Samor Thai in Thai, Harad or Harra in Hindi, Abhaya in Sanskrit, and Chebulic Myrobalan in English [10]. Triphala is renowned for its health benefits and is available in forms such as powders, tablets, and teas. Traditionally, it served to support digestive health, detoxify, and act as an oral rinse thanks to its antibacterial, antiinflammatory, antioxidant, and immune-boosting effects [11,12]. Triphala has been recognized by the National Drug System Development Committee (2023) as one of the 50 Thai traditional medicines included in the NLEM of Thailand [13]. In traditional Thai medicine, the formulation of Triphala—a localized adaptation of the classical Ayurvedic Triphala—is prepared in three distinct ratios, each specifically designed to address seasonal imbalances and dominant bodily elements, in accordance with Thai medical theory [14]. In the Vata Samutthan formula, this formulation comprises *T. chebula* (1 part), *T. bellerica* (3 parts), and *P. emblica* (2 parts). It is typically administered during the rainy season when the Vata dosha becomes aggravated due to environmental instability and wind. According to Thai traditional texts, this formula helps relieve symptoms such as dizziness, muscle stiffness, and abdominal cramping, which are commonly associated with disturbances in air and movement-related body functions [15]. The Pitta Samutthan formula with a composition of *T. chebula* (3 parts), *T. bellerica* (2 parts), and *P. emblica* (1 part) is used during the summer season to counteract the rise in Pitta dosha, which governs heat and metabolism. It is employed to manage fevers, headaches, and other heat-related conditions. The high proportion of *T. chebula*, known for its purgative and detoxifying effects, facilitates cooling and antiinflammatory action [15]. In the Semha (Kapha) Samutthan formula, this variation uses *T. chebula* (2 parts), *T. bellerica* (1 part), and *P. emblica* (3 parts). Recommended for use in the winter season, it targets excess Kapha dosha, which is linked to mucus, heaviness, and fluid accumulation. Traditionally, it is prescribed to alleviate colds, runny nose, productive cough, and diarrhea, especially conditions involving respiratory congestion and digestive sluggishness [16]. Moreover, Triphala has been widely investigated for its broad spectrum of pharmacological activities. Evidence suggests that Triphala exhibits significant immunomodulatory effects [16], promotes wound healing [17], and demonstrates antiinflammatory activity [18]. In addition, Triphala is known for its strong antioxidant activity [19], which is believed to play a role in protecting against conditions associated with oxidative stress. Furthermore, it exhibits acetylcholinesterase inhibitory activity [20], indicating its potential neuroprotective benefits and possible application in the management of neurodegenerative disorders such as Alzheimer's disease [21]. The recommended dosage ranged from 1 to 2 g per 200 ml of warm water to tablets containing 300–600 mg extract to be taken three times daily [22]. The pharmacological activities of the Triphala formulation are listed in Table 1.

Phytochemical composition in the Triphala formulation

Triphala formulation primarily contains vitamin C, carotene, nicotinic acid, riboflavin, tannins, anthraquinones, polyphenols, gallic acid, tannic acid, and various glycosides [23,24]. Chebulinic acid, chebulic acid, and gallic acid constituted the main chemical compounds in *T. chebula* [24]. The primary bioactive compounds found in *T. bellerica* included glucosides, tannins, gallic acid, ellagic acid, ethyl gallate, gallyl glucose, and chebulanic acid [24]. Similarly, the main bioactive compounds in *P. emblica* were ellagic acid, chebulinic acid, and gallic acid [24,25]. Recently, researchers conducted phytochemical profiling of *P. emblica, T. chebula*, and *T. bellerica* using liquid chromatography–mass spectrometry with electrospray ionization. The results revealed that the *P. emblica* metabolome predominantly comprises phenylpropanoids and polyketides. Additionally, benzenoids, including benzene and its substituted derivatives, were also identified in *P. emblica*. In *T. chebula*, benzene

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Table 1. Pharmacological activities of the Triphala formulation.

Property	Activities	References
Antimicrobial	• Inhibited growth of various microorganisms (such as bacteria, fungi, viruses, and protozoa) • Showed synergistic effects with antibiotics • Supported gut microbiota balance	[28–40]
Antioxidant	• Displayed radical scavenging action against superoxide anions • Reduced primary reactive oxygen species responsible • Neutralized free radicals • Reduced malondialdehyde levels and lipid peroxidation • Enhanced activity of antioxidant enzymes	[9,18,40–49].
Antiinflammatory	• Inhibited or moderated proinflammatory cytokines, such as TNFα, IL-1β, IL-6, and IL-17 • Inhibited inflammatory enzymes, such as COX-2, XOD, and prostaglandins • Inhibited fibroblast formation • Reduced lipid peroxidation	[19,24,52–57].
Immunomodulatory	• Regulated immune response; exhibited both immunostimulant and immunosuppressive effects • Reduced proinflammatory cytokine expression • Enhanced IL-2, IFN-γ, and neutrophil function • Suppressed lymphocyte proliferation • Improved phagocytic activity of mononuclear cells, macrophages, and nonspecific immune responses	[16,50,53,58,59]
Antiaging	• Stimulated collagen and elastin synthesis • Inhibited enzymes related to aging (such as tyrosinase, collagenase, and elastase)	[40,60]
Anti-obesity	• Suppressed adipogenic gene expression • Improved lipid profiles (reduces LDL cholesterol and triglyceride levels; increases HDL cholesterol) • Reduced energy intake and percentage of body fat • Promoted metabolism regulation and weight loss • Improved HDL cholesterol levels and reduced blood sugar levels in volunteers	[4,61–69]
Protective/Preventive	• Reduced negative effects on oral mucosal epithelial cells induced by arecoline • Provided positive effects on cognitive and psychological resilience via modulation of 5-HT and BDNF receptors, gut microbiota, and antioxidant-related signaling pathways • Provided protective effects against hepatorenal damage induced by paracetamol in Swiss albino mice • Prevented acute liver injury induced by CCl4 by regulating levels of ALT, AST, MDA, TNF-α, and IL-6; enhancing levels of SOD and GSH-Px mRNA; and causing protein expression of Nrf-2, HO-1, and NQO-1	[67–70]
Anticancer	• Exhibited antiproliferative and antimetastatic properties • Inhibited cancer cell growth by targeting various signaling pathways, such as MAPK/ERK, PI3K/Akt/mTOR, and NF-κB/p53 • Induced apoptosis of tumors through the activation of p53 and ERK and ROS production	[71–74]
Toxicity	• Exhibited nontoxicity to human cells *in vitro* • Exhibited no signs of toxicity in the *in vivo* tests • Exhibited adverse effects in healthy volunteers taking 1,050 and 2,500 mg	[4,40,75,76].

5-HT = 5-hydroxytryptamine; BDNF = brain-derived neurotrophic factor; GSH = glutathione; PI3K/AKT = phosphatidylinositol 3-kinase/protein kinase B; XOD = xanthine oxidase.

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derivatives were significantly present, along with notable levels of phenols, prenol lipids, and steroids. Significant levels of flavonoids, carboxylic acids, and cinnamic acids have also been detected. For *T. bellerica*, the chemical composition showed less diverse, with a notable presence of benzene derivatives and organooxygen compounds [26].

Medicinal properties associated with infectious microorganisms

Research on the antimicrobial activity of Triphala revealed its efficacy in inhibiting the growth of various microorganisms, including its antibacterial, antifungal, antiviral, and antiprotozoal properties. Specifically, its broad-spectrum antibacterial properties were demonstrated through studies

using hydroalcoholic extracts, which showed effectiveness against a range of gram-positive and gram-negative bacterial strains, including *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus mirabilis*, with minimum inhibitory concentrations ranging from 12 to 25 mg/ml as determined by broth assays [27,28]. Additionally, both aqueous and ethanolic extracts exhibited broad-spectrum antibacterial activity against gram-positive and gram-negative bacteria, as assessed using the agar diffusion method [29–31]. Moreover, synergistic effects were observed when combining Triphala extract (in a ratio of 1-part Triphala to 3.34 parts water) with antimicrobial drugs such as gentamicin against multidrug-resistant gram-negative bacilli and oxacillin against methicillin-resistant *S. aureus* [32]. Triphala demonstrated the ability to inhibit microorganisms in the gastrointestinal tract, suppressing the growth of harmful gut microbes and promoting beneficial bacteria [8]. *In vivo* studies using Triphala extracted with dimethyl sulfoxide reported significant anti-infective properties against gram-positive and gram-negative pathogenic bacteria in the nematode host *Caenorhabditis elegans* N2-Bristol [33]. Furthermore, a Triphala decoction showed antiplaque and anti-halitosis effects in a randomized controlled clinical trial [34]. Regarding its antifungal properties, Triphala displayed good *in vitro* antifungal activity against *Candida albicans* in monospecies biofilm and exhibited *in vivo* anticandidal activity, though not significantly different from a denture cleanser. Additionally, it showed antifungal effects against *Aspergillus* species, reducing fungal growth by up to 37.96% [28,35–37]. The ethanolic extract of Triphala effectively inhibited dengue virus production and reduced the expression of IL-6 and CXCL-10, which are significant markers of cytokine storm effects in dengue virus infection [38]. Furthermore, both aqueous and ethanolic extracts exhibited significant *in vitro* anti-plasmodial activity against the *Plasmodium falciparum* K1 strain, with the aqueous extract demonstrating good *in vivo* antimalarial activity against the *Plasmodium berghei* ANKA strain [39].

Antioxidant properties of Triphala formulation

Triphala is renowned for its robust antioxidant properties, attributed to its rich content of vitamin C, polyphenols, and flavonoids [9,40]. Numerous studies highlighted its potent antioxidant capabilities using various colorimetric techniques, including 2,2-diphenyl-1-picrylhydrazyl free radical scavenging, ferric reducing antioxidant power (FRAP), superoxide anion radical scavenging activity [superoxide dismutases (SOD)], hydrogen peroxide (H_2O_2) scavenging, and nitric oxide (NO) radical scavenging assays [41,42]. In the DPPH assay, Triphala exhibited radical scavenging activity by accepting electrons or hydrogen radicals, leading to the formation of a stable diamagnetic molecule. The FRAP assay highlighted the ability of Triphala extract to neutralize free radicals and shield against oxidative damage by converting ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Triphala also demonstrated potent superoxide anion scavenging activity, indicating its effectiveness in reducing primary reactive oxygen species (ROS) responsible for cellular damage, oxidative stress-related ailments, and aging mechanisms. Its ability to scavenge H_2O_2 stemmed from electron donation and effectively neutralizing it into water. Moreover, Triphala's NO scavenging action mitigated oxidative damage caused by reactive nitrogen species, such as peroxynitrite [41,42]. In cell-based assays, Triphala exerted a protective effect on human dermal fibroblasts by preventing damage induced by H_2O_2. It mitigated cellular senescence triggered by H_2O_2 and protects cells from DNA damage [43]. Additionally, aqueous Triphala extract restored glutathione (GSH) levels, reduced malondialdehyde (MDA) levels, and enhanced the activity of antioxidant enzymes, such as superoxide dismutase, catalase, GSH peroxidase, and glutathione-S-transferase (GST), in an *in vitro* selenite-induced experimental model of cataract. At a dose of 25 mg/kg, Triphala prevented selenite-induced cataracts in rats through its antioxidant properties [40]. Further research in animal models revealed that Triphala, administered at doses of 150 and 300 mg/kg, alleviated colitis symptoms in rats by reducing MDA levels and restoring the activity of the antioxidant enzymes SOD and catalase (CAT) in the distal colon [44]. Moreover, Triphala, administered orally at a dosage of 1 g/kg, suppressed the increase in paw volume and lipid peroxidation while enhancing antioxidant levels in the plasma, liver, and spleen of mice induced with monosodium urate crystals [45]. Oral administration of Triphala at doses of 250 and 500 mg/kg enhanced antioxidant enzyme levels, including CAT, superoxide dismutase, GST, and GSH peroxidase while reducing lipid peroxidation in rats treated with bromobenzene [46]. Additionally, a 3-mg/kg oral dose of Triphala decreased lipid peroxidation and lactate dehydrogenase (LDH) levels in the livers of mice while increasing GSH levels and enhancing GST activity, indicating protection against peroxidative damage induced by 1,2-dimethylhydrazine dihydrochloride-induced endoplasmic reticulum stress [47]. Furthermore, Triphala exhibited preventative effects on lipid peroxidation and corticosterone levels induced by noise and cold stresses [48,49].

Antiaging properties of Triphala formulation

Beyond its antioxidant activity, Triphala has demonstrated notable antiaging properties. Its antioxidant action contributes to delaying cellular senescence and protecting against age-related oxidative damage. Notably, Triphala stimulated the expression of collagen-1 and elastin-synthesizing genes in human skin cells, which are critical for maintaining skin structure and elasticity [43,50].. Moreover, Triphala effectively inhibited the activities of skin-aging–related enzymes, including tyrosinase, collagenase, and elastase. By suppressing these enzymes, Triphala contributed to improvements in skin whitening and the reduction of wrinkles, supporting its potential application in dermatological and cosmetic formulations targeting skin aging [43,50].

Antiinflammatory effects of Triphala

Triphala exerted potent antiinflammatory effects, significantly reducing inflammatory markers and lipid peroxidation through various mechanisms, including the inhibition of proinflammatory enzymes and modulation of cytokines, along with the nuclear factor kappa B (NF-

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κB) pathway. Studies showed that Triphala inhibited the overproduction of TNF-α, IL-1β, IL-6, IL-17, inducible NO synthase, and cyclooxygenase-2 (COX-2) by suppressing the protein expression of NF-κB p65, phosphorylated NF-κB p65 nuclear translocation, and COX-2 protein in stimulated cells [18,51,52]. Additionally, Triphala inhibited the inflammatory mediator xanthine oxidase and stabilized red blood cell membranes, indicating *in vitro* antiinflammatory effects [53,54]. Moreover, green-synthesized silver nanoparticles derived from Triphala were found to stabilize human red blood cell membranes and inhibit protein denaturation [55]. Animal studies demonstrated the antiinflammatory properties of Triphala. Administration of the aqueous extract reduced ear edema by reducing swelling induced by ethyl phenylpropionate and prevented hind paw edema by suppressing prostaglandin release. Additionally, Triphala inhibited fibroblast formation during granuloma tissue formation, indicating its efficacy against chronic inflammation [56,57].

Immunomodulatory effects of Triphala formulation

Triphala exhibited immunomodulatory effects through various pathways, including immunostimulatory and immunosuppressive effects. *In vitro* studies revealed that Triphala inhibited the expression of cytokines, such as TNF-α, IL-1β, and IL-6 [18]. Additionally, Triphala suppressed lymphocyte proliferation while enhancing the phagocytic activity of mononuclear cells, macrophages, and nonspecific immune responses [24,58]. *In vivo* assays showed that administration of Triphala at a dosage of 1-g/kg/day enhanced IL-2, IFN-γ, and neutrophil function, elevated corticosteroid levels, and decreased Pan CD4+/CD8+ and IL-4 in a noise-induced stress model [16,59]. Furthermore, Triphala effectively suppressed both humoral and cell-mediated immune responses in delayed-type hypersensitivity reactions, indicating its immunosuppressive effects, which are particularly relevant in autoimmune diseases [58].

Anti-obesity effects of Triphala formulation

The anti-obesity effects of Triphala were demonstrated in several studies. *In vitro* experiments showed that the aqueous extract possessed antiadipogenic activity by reducing lipid accumulation within cells and suppressing the expression of adipogenic genes in both the early and late stages [60]. *In vivo* studies elucidated the anti-obesity properties of Triphala. Administration of the aqueous extract led to reductions in noradrenaline, leptin, interleukin-6, C-reactive protein, MDA, and total cholesterol levels and increased levels of high-density lipoprotein, adiponectin, superoxide dismutase, serotonin, and dopamine. Additionally, several genes associated with lipid metabolism were downregulated in albino Wistar rats [61]. Moreover, the aqueous extract decreased total cholesterol and triglyceride levels and increased high-density lipoprotein cholesterol levels in Sprague–Dawley albino rats receiving a high-fat diet [62]. Triphala also reduced energy intake and the percentage of body fat and improved the lipid profiles of mice fed a high-fat diet [63]. Furthermore, a randomized clinical trial demonstrated that taking Triphala tablets at a dose of 1,000 mg twice daily resulted in significant weight loss and a decrease in body fat percentage compared with a placebo after 3 months [64]. Additionally, Triphala extract treatment enhanced the synthesis of phenylalanine, tyrosine, and tryptophan during fecal batch culture fermentation in obese adults, aiding in the regulation of energy metabolism and supporting obesity management [65].

Protective and preventive effects of Triphala formulation

Triphala extract, at a concentration of 5 µg/ml, showed promising results *in vitro* by reducing β-galactosidase activity and suppressing the expression of senescence-related genes *p16* and *p21* while enhancing Ki-67 marker expression. These findings suggested that the extract mitigated the negative effects of arecoline-induced pathogenesis in oral mucosal epithelial cells [66]. Moreover, Triphala exhibited favorable effects on cognitive and psychological resilience by modulating the activity of 5-hydroxytryptamine and brain-derived neurotrophic factor receptors, which are associated with the regulation of antioxidant-related signaling pathways [67]. *In vivo* experiments further substantiated the protective effects of Triphala. The administration of Triphala at doses of 100 and 300 mg/kg demonstrated protective effects against paracetamol-induced hepatorenal damage in Swiss albino mice [68]. Triphala extract also demonstrated protective effects against acute liver injury induced by carbon tetrachloride, as evidenced by decreased levels of alanine transaminase (ALT), aspartate transaminase (AST), MDA, TNF-α, and IL-6, and increased levels of SOD, GSH-Px mRNA, and protein expression of Nrf-2, HO-1, and NQO-1 [69].

Anticancer activities of Triphala formulation

The anticancer properties of Triphala garnered significant attention. In particular, its aqueous extract has been documented as an antineoplastic agent with antiproliferative and antimetastatic properties. Studies demonstrated its efficacy in reducing tumor growth and inhibiting the migration of human gastric carcinoma cells *in vitro* [70]. Moreover, the aqueous extract demonstrated inhibitory effects on the growth of human ovarian cancer cells (SK-OV-3), cervical cancer cells (HeLa), and endometrial cancer cells (HEC-1-B) by targeting various signaling pathways, including MAPK/ERK, phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt)/mTOR, and NF-κB/p53 [71]. Triphala induced apoptosis in cancer cells. Studies indicated that Triphala triggered programmed cell death in pancreatic tumors both *in vitro* and *in vivo*, with activation of p53 at Ser-15 and ERK at Thr-202/Tyr-204 in Capan-2 cells, suggesting the involvement of ROS production in this process [72]. Additionally, the methanolic extract inhibited proliferation independent of p53 status in both HCT116 and human colon cancer stem cells. It induced p53-independent apoptosis in human colon cancer stem cells, reduced the levels of c-Myc and cyclin D1, and increased the Bax/Bcl-2 ratio, indicating its antiproliferative and apoptosis-inducing effects [73].

Toxic effects and safety of Triphala formulation

Triphala has demonstrated safety in various *in vitro* and *in vivo* toxicity studies. *In vitro* tests using human dermal

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fibroblasts and keratinocytes showed no toxicity, with half-maximal inhibitory concentration (IC_{50}) values of 204.90 ± 7.6 and 239.13 ± 4.3 µg/ml, respectively [43]. Studies investigating *in vivo* toxicity reported no signs of toxicity in acute, subacute, or chronic tests. Although alterations in blood chemistry and hematological parameters, such as glucose, blood urea nitrogen, red blood cells, hemoglobin (Hb), HCT, and mean cell volume, were detected, no histological abnormalities were observed [4,39,74,75]. Furthermore, the ethanolic extract of Triphala showed no adverse effects in healthy volunteers administered 1050 mg/day orally for 14 days [76]. Both aqueous and ethanol extracts of Triphala were safe in healthy volunteers at doses of 2,500 and 1,050 mg, respectively [4,76]. Additionally, oral administration of Triphala at a dose of 2,500 mg/day led to improvements in high-density lipoprotein cholesterol levels and reduced blood sugar levels in volunteers [4]. Collectively, these findings suggested that Triphala is well tolerated and safe for human consumption.

Trikatu formulation

Trikatu is also widely used in the Ayurvedic system of India, where it has been traditionally employed for the management of various conditions, including the common cold, asthma, pruritus (itching), musculoskeletal pain, and inflammatory disorders. Its broad spectrum of therapeutic benefits is attributed to its pharmacologically active constituents, which possess antiinflammatory, carminative, expectorant, and immunomodulatory properties [77]. It is composed of three herbs in equal quantities, the fruits of *Piper nigrum* L. and *Piper retrofractum* Vahl and the rhizomes of *Zingiber officinale* Roscoe [78]. In Thai traditional medicine, Trikatu formulation is a well-recognized traditional polyherbal formulation commonly utilized. This formulation closely resembles a traditional Ayurvedic preparation, with the primary distinction being the substitution of *Piper retrofractum* for *Piper longum* L. in Trikatu [79]. The National Drug System Development Committee has officially announced the approval to include Trikatu in the NLEMs. This inclusion is intended for its use in restoring the balance of the body's elements in accordance with seasonal changes [79]. These components are combined in varying proportions depending on the intended therapeutic application. The different formulations of Trikatu are prescribed to modulate the balance of bodily elements during the rainy season, addressing ailments believed to arise from the disturbance of the elemental forces of fire, wind, and water [80]. The formulation is thought to stimulate digestive fire, promote circulation, and facilitate detoxification, aligning with the traditional goal of restoring internal balance. In addition, Trikatu formulation is believed to possess immunomodulatory properties that help strengthen the immune system [81]. Collectively, these herbs enhance gastric digestion, stimulate bile secretion, and support liver detoxification processes [82]. Furthermore, Trikatu has been shown to modulate lipid metabolism, contributing to overall metabolic health [83]. Beyond its digestive and metabolic benefits, the Trikatu formulation has a long history of medicinal use for a variety of health conditions. Scientific and ethnopharmacological studies have reported its application as an anti-arthritic agent and its therapeutic role in managing respiratory tract infections, including asthma and the common cold. Additionally, it has been used to alleviate symptoms associated with rheumatoid arthritis, pruritus (itching), pain, and inflammatory conditions [5]. Trikatu may additionally influence the bioavailability of various substances, such as herbal remedies, nutrients, and pharmaceutical drugs [84]. Analysis of Trikatu extracts identified a range of bioactive constituents, including alkaloids, flavonoids, tannins, lignins, and steroids. These compounds contributed to a wide range of biological properties, highlighting the potential efficacy of Trikatu in managing diverse health conditions. The pharmacological activities of the Trikatu formulation are listed in Table 2.

Phytochemical composition in the Trikatu formulation

Phytochemical investigations of Trikatu led to the isolation of 6-shogaol (1), 6-gingerol (2), and piperine (3) from its powdered form [85]. Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oils of black pepper, Javanese long pepper, red ginger, and Trikatu revealed the presence of 11 common compounds, including linalool, caryophyllene, and beta-bisabolene. Principal component analysis highlighted distinct patterns of compound abundance among the samples. For instance, linalool appeared more abundantly in Trikatu samples, while gamma-bisabolene predominated in Javanese long pepper samples. Additionally, the GC-MS analysis confirmed 11 shared compound peaks across Trikatu, black pepper, Javanese long pepper, and red ginger essential oils [86].

Medicinal properties associated with infectious microorganisms

Aqueous, ethanolic, methanolic, diethyl ether, and acetone extracts demonstrated high effectiveness against various bacterial strains, including *E. coli*, *S. aureus*, *B. cereus*, *P. aeruginosa*, *Proteus vulgaris*, *S. epidermidis*, *Salmonella typhi*, *S. typhimurium*, and *Enterobacter aerogenes*, as observed using the agar well diffusion method [87–89]. Moreover, green-synthesized silver nanoparticles showed efficacy against both gram-negative bacteria, such as *E. coli*, and gram-positive bacteria, such as *S. aureus* [90]. Trikatu also demonstrated anthelmintic activity. Studies showed that the alcoholic extract induced paralysis and death in earthworms, particularly *Pheritima posthuma*, with Trikatu exhibiting a remarkable level of activity comparable with that of standard piperazine citrate [91,92]. Additionally, the ethanolic extract exhibited antimalarial activity, showing an IC_{50} value of 4.4 µg/ml [39].

Antioxidant activities of Trikatu formulation

Trikatu had antioxidant and alpha-glucosidase-inhibitory properties. However, Trikatu exhibited significantly lower antioxidant and alpha-glucosidase inhibitory activities, as measured by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH radical scavenging tests, compared to the combined extracts of the three ingredients [6,93]. In an *in vivo* study, the aqueous extract of Trikatu inhibited the activities of several antioxidant enzymes, including SOD, CAT, GSH peroxidase, GST, and GSH reductase, and reduced GSH levels in rats treated with monosodium urate crystals [84]. Additionally, co-administration of Trikatu with mercaptopurine

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Table 2. Pharmacological activities of the Trikatu formulation.

Property	Activities	References
Antimicrobial	• Exhibited effects against *E. coli*, *S. aureus*, *B. cereus*, *P. aeruginosa*, *P. vulgaris*, *S. typhi*, *S. typhimurium, S. epidermidis*, and *E. aerogenes* (aqueous, ethanolic, methanolic, diethyl ether, and acetone extracts); *E. coli* and *S. aureus* (silver nanoparticles)	[87–92]
	• Exhibited paralysis and death in earthworms	
	• Demonstrated antimalarial activity	
Antioxidant	• Demonstrated antioxidant and alpha-glucosidase inhibitory properties	[6,93]
Antiinflammatory	• Inhibited production and expression of proinflammatory cytokines and mediators, including TNF-α, IL-1β, IL-6, IL-17, MCP-1, RANKL, COX-2, and iNOS	[84,95–97]
	• Regulated AGE-RAGE, HIF-1, NF-kB, PI3K/Akt, PTGS2/COX-2, SRT1, and caspase-3 signaling pathways	
	• Reduced levels of circulating immune complexes and inflammatory mediators	
	• Reduced lipid peroxide levels	
	• Normalized levels of lysosomal enzymes	
Immunomodulatory	• Increased neutrophil adhesion	[82,98]
	• Stimulated cytokine production	
	• Showed immunosuppressive effects in an arthritic model	
Anti-obesity	• Demonstrated *in vitro* lipolytic activity, indicating a potential decrease in adipose mass	[5,99,100]
	• Exhibited *in vivo* hypolipidemic activity (increased HDL cholesterol and decreased LDL cholesterol and triglycerides)	
	• Improved serum cholesterol, triglycerides, VLDL, LDL, and HDL levels in clinical studies	
Protective/Preventive	• Improved liver markers and overall liver morphology in alcoholic disease through AGE-RAGE, HIF-1, NF-kB, and PI3K/Akt pathways	[97]
Anticancer	• Suppressed cholangiocarcinoma cells by inducing cell cycle arrest	[93]
Analgesic	• Increased reaction time in the hot plate method, indicating analgesic effects	[87]
Toxicity	• Nontoxic to cell lines; safe in acute and sub-acute toxicity studies in mice	[39,99]

AGE-RAGE = advanced glycation end product receptor for advanced glycation end products; HIF-1 = hypoxia-inducible factor; iNOS = inducible nitric oxide synthase; PI3K/Akt = phosphatidylinositol 3-kinase/protein kinase B; PTGS2 = prostaglandin-endoperoxide synthase 2; RANKL = receptor activator of NF-κB ligand; SRT1 = sirtuin 1.

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at a dosage of 2.5 mg/kg showed comparable efficacy to the therapeutic dose of mercaptopurine (5 mg/kg). Both treatments effectively decreased lipid peroxidation [94].

Antiinflammatory and analgesic activities of Trikatu formulation

Studies of the antiinflammatory effects of Trikatu yielded promising results. Administration of Trikatu reduced paw swelling and restored normal levels of lysosomal enzymes, glycoproteins, urinary markers (hydroxyproline and glycosaminoglycans), and bone collagen in a rat model of arthritis [95]. Moreover, Trikatu exhibited antiinflammatory by suppressing the production and expression of inflammatory cytokines and mediators via inhibition of the NF-κB signaling pathway. It effectively inhibited the production of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and monocyte chemoattractant protein MCP-1. Additionally, it reduced the mRNA expression levels of inflammatory mediators, including TNF-α, IL-1β, IL-6, IL-17, MCP-1, receptor activator of NF-κB ligand, COX-2, and inducible NO synthase, as well as transcription factors NF-κB-p65 and activator protein-1 in cultured AIA-fibroblast cells [96]. *In vivo* studies further confirmed the anti-inflammatory effects of Trikatu in a mouse model of alcoholic liver disease by modulating multiple signaling pathways, including the receptor for advanced glycation end products (RAGE), hypoxia-inducible factors, NF-κB, PI3K/Akt, COX-2, sirtuin 1, and caspase-3 [97]. Additionally, Trikatu exhibited antiinflammatory effects in arthritic rats by reducing levels of TNF-α and IL-1β [98]. Aqueous extract of Trikatu demonstrated strong antiinflammatory activity in a rat model of inflammation induced by monosodium urate crystals. Trikatu administration effectively reduced lipid peroxide levels to normal and suppressed the activities of acid phosphatase, β-glucuronidase, N-acetyl glucosaminidase, β-galactosidase, and cathepsin-D in plasma, liver, and spleen, indicating significant antiinflammatory effects [84]. Furthermore, the ethanolic extract of Trikatu demonstrated an analgesic effect in mice when administered at doses of 50, 100, 150, 200, and 250 mg/kg, as confirmed by increased reaction times in the hot plate method [87].

Immunomodulatory activities of Trikatu formulation

Trikatu reportedly possesses immunomodulatory properties, exhibiting a dose-dependent increase in the percentage of neutrophil adhesion, indicating its ability to enhance the

immune response to microbial infections. Additionally, Trikatu stimulated T-lymphocytes and macrophages to produce a large quantity of cytokines, including IL-12, IL-1, and TNF-α [98]. However, administration of 1,000 mg/kg led to reduced cell-mediated and humoral immune responses and decreased macrophage phagocytic indices in arthritic rats, indicating that Trikatu may also have immunosuppressive effects [82].

Hepatoprotective and protective against obesity effects of Trikatu formulation

Trikatu has been reported to possess hepatoprotective properties against alcoholic liver disease in rats by modulating various signaling pathways, including advanced glycation end products receptor for advanced glycation end products, hypoxia-inducible factors, NF-kB, and PI3K/Akt signaling. Network pharmacology analysis identified bioactive compounds such as piperine and gingerols as key modulators that activate PI3K, leading to phosphorylation of Akt. This activation promotes hepatocyte survival by enhancing anti-apoptotic proteins (e.g., Bcl-2), suppresses pro-apoptotic factors (e.g., Bax and caspase-3), and stimulates Nrf2-mediated antioxidant defenses (e.g., HO-1 and SOD). Additionally, Akt activation was found to inhibit NF-κB–mediated inflammatory responses and regulate lipid metabolism via downstream targets like mTOR and FOXO1, collectively mitigating oxidative stress, inflammation, steatosis, and fibrosis associated with ethanol-induced liver injury [97]. Regarding its anti-obesity properties, *in vitro* studies revealed that the ethanolic extract of Trikatu exhibited lipolytic activity, suggesting a reduction in adipose mass [5]. Furthermore, studies in mice demonstrated hypolipidemic activity following the administration of Trikatu at doses of 50 and 300 mg/kg, resulting in an increase in high-density lipoproteins (HDL) levels. Additionally, administration at a dose of 300 mg/kg decreased low-density lipoproteins (LDLs) levels on the 28th day after treatment in Charles Foster rats [99]. Other studies showed that Trikatu extract decreased triglycerides and LDL cholesterol while increasing HDL cholesterol [100]. A clinical study conducted within the Hindu community further supported these findings, showing that administering Trikatu at a dosage of 3 g twice a day led to significant improvements in serum cholesterol (9.67%), triglyceride (7.72%), very-low-density lipoprotein (12.92%), LDL (22.61%), and HDL (20.11%) [101].

Toxicity associated with anticancer properties and safety of Trikatu formulation

In anticancer studies, Trikatu suppressed the growth of human cholangiocarcinoma cells (KKU-214) and induced cell cycle arrest at the G2 phase. This arrest resulted from decreased levels of CDK2 and p53 alongside increased levels of p21 and p27 [93]. Additionally, *in vitro* toxicity testing showed that the aqueous extract of Trikatu exhibited no toxicity to the HepG2 and Vero cell lines [39]. Furthermore, Trikatu demonstrated safety in mice when administered at doses of 5, 50, 300, and 2,000 mg/kg, with no significant alterations observed in mortality, morbidity, gross pathology, weight gain, vital organ weight, hematological parameters, or biochemical parameters, including serum and lipid profiles, in acute and subacute toxicity studies [99].

Benjakul formulation

Benjakul consisted of equal parts of five plants: the fruits of *Piper chaba* Hunter (*P. chaba*), the roots of *Piper sarmentosum* Roxb. (*P. sarmentosum*), the stems of *Piper interruptum* Opiz. (*P. interruptum*), the roots of *Plumbago indica* L. (*P. indica*), and the rhizome of *Zingiber officinale* Roscoe (*Z. officinale*) [106]. This formulation served to maintain balance in the elements of the body and gained recognition for its carminative and adaptogenic properties, as listed in the Thai NLEMs [107]. In a survey involving 35 traditional Thai medicines, the Benjakul formulation emerged as a popular choice for adaptogenic purposes and the treatment of various ailments, such as allergies, fatigue, body aches, nasal congestion, and abnormal menstruation [108]. Moreover, Benjakul garnered scientific validation for its antiallergy, antiinflammatory, antipyretic, and antihypertensive effects and its role as an alternative treatment for patients with end-stage nonsmall cell lung cancer [109,110]. Notably, Benjakul influenced the bioavailability of various substances, such as herbal remedies, nutrients, and pharmaceutical drugs. Analysis of the Benjakul formulation revealed the presence of bioactive substances, such as piperine, 6-shogaol, 6-gingerol, myristicin, plumbagin, and methyl piperate, which contributed to its diverse range of biological properties [111]. The pharmacological activities of the Benjakul formulation were listed in Table 3.

Phytochemical composition in the Benjakul formulation

Qualitative phytochemical screening revealed that both aqueous and ethanolic extracts of Benjakul contained terpenoids, while tannins and saponins were predominantly found in the aqueous extract [33]. Additionally, the chemical fingerprints of the ethanolic extract of Benjakul were characterized using high-performance liquid chromatography. Five marker compounds were identified in Benjakul: myristicin, plumbagin, piperine, 6-gingerol, and 6-shogaol [105]. Furthermore, using the UPLC-MS method, six chemical constituents were detected in the Benjakul extract: pellitorine, piperine, piperlonguminine, plumbagin, 6-gingerol, and 6-shogaol [105].

Antiallergy properties of the Benjakul formulation

The 95% ethanol extract of the Benjakul formulation demonstrated antiallergic activity by suppressing β-hexosaminidase activity in RBL-2H3 cells. This study revealed that the Benjakul formulation extract exhibited stronger antiallergic effects than that of chlorpheniramine, a standard antihistamine drug. The Benjakul formulation showed an IC_{50} value of 12.69 ± 1.25 µg/ml, whereas chlorpheniramine had an IC_{50} value of 17.98 ± 0.78 µg/ml. Furthermore, IC_{50} values of 12.93 ± 1.28 µg/ml, 13.87 ± 0.66 µg/ml, 13.91 ± 1.54 µg/ml, 15.82 ± 2.00 µg/ml, and 21.74 ± 2.14 µg/ml were found in the plant components of the Benjakul formulation in *Z. officinale, P. sarmentosum, P. indica, P. chaba,* and *P. interruptum*, respectively. This suggested that the Benjakul components could serve as alternative antiallergic agents, with better outcomes potentially achieved when used in combination [109].

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Table 3. Pharmacological activities of the Benjakul formulation.

Property	Activities	References
Antiallergy	• Suppressed enzyme β-hexosaminidase in RBL-2H3 cells	[109]
Antiinflammatory	• Reduced the production of nitric oxide and inhibited the secretion of cyclooxygenase enzymes	[110,111,105]
	• Reduced the swelling of the white fungus-induced inflammation	
	• Attenuated TNF-α, IL-1β, and IL-6 release	
	• Inhibited the activity of PGE2 production	
Antipyretic	• Decreased body temperature after administration	[104]
Antihypertensive	• Reduced systolic pressure in rat-induced hypertension	[104]
Metabolic syndrome	• Attenuated ~~the~~ oral glucose tolerance, histopathological features, beta-galactosidase density, and MDA contents of the pancreas in a high-fat diet in rats	[112]
Antimalarial	• Inhibited the percentage of parasitemia	[38]
Anticancer	• Inhibited cell proliferation against nonsmall cell lung cancer cells	[113–115]
	• Increased the population of sub-G1 apoptotic cells	
	• Inhibited the colony formation of MCF-7 and MDA-MB-231 cells	
	• Promoted cell death through activating ROS formation, stimulating caspase 3 activity, and repressing mitochondrial function	
	• Reduced MMP-9 expression level	
Toxicity	• Exhibited no sign of toxicity in the *in vivo* test	[102,117,118]
	• Exhibited no adverse effects in healthy volunteers	

PGE2 = prostaglandin E2; ROS = reactive oxygen species.

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Antiinflammatory activity of the Benjakul formulation

The 95% ethanolic extract of the Benjakul formulation reduced NO production and inhibited COX-2 enzyme secretion, with IC_{50} values of 18.23 and 5.82 µg/ml, respectively [110]. This extract showed a potent inhibitory effect on NO with an IC_{50} value of 16.60 µg/ml but exhibited no activity on TNF-α in LPS-treated murine macrophage cells. Plumbagin and 6-shogaol, two compounds derived from the Benjakul formulation, exhibited higher potency than the extract, with IC_{50} values of 0.002 and 0.92 µg/ml, respectively [109]. Furthermore, 95% ethanol Benjakul extracts at doses of 1, 2, and 4 mg/20 µl/ear significantly reduced swelling induced by ethyl phenylpropiolate in white fungus-induced inflammation. Although this extract (at a solvent dose of 20 µl/ear) demonstrated a better reduction of swelling compared with that of phenylbutazone, the difference did not reach statistical significance. The EDI values of Benjakul and phenylbutazone were 60% and 58%, respectively. In LPS-induced inflammatory processes in colonic epithelial cells, the ethanolic extract of the Benjakul formulation attenuated the release of TNF-α, IL-1β, and IL-6 in a dose-dependent manner [111]. Additionally, Benjakul at doses of 300, 600, and 1,200 mg/kg body weight exhibited acute antiinflammatory effects in rats by reducing paw swelling induced by 1% carrageenan. These findings indicate that the mechanism of action may involve suppressing the synthesis and/or release of inflammatory mediators, including prostaglandins, histamine, kinins, and serotonin [104]. Moreover, studies on prostaglandin E2, both *in vitro* and *in vivo*, revealed that the ethanolic extract of the Benjakul formulation had high inhibitory activity on prostaglandin E2 production, with an IC_{50} value of 5.82 ± 0.10 µg/ml, while its water extract showed no activity. The ethanolic extract inhibited inflammation in rat models of ethyl phenylpropiolate-induced ear edema and carrageenan-induced hind paw edema [105].

Antipyretic effect of the Benjakul formulation

In a previous study, researchers induced fever in rats by subcutaneous injection of 25% brewer's yeast at a dose of 1-ml/100-g body weight and measured fever using rectal thermometers. Subsequently, the rats exhibited an increase in body temperature of 0.75°C after 18 hours post-injection. The rats were then treated with Benjakul at doses of 300-, 600-, and 1,200-mg/kg body weight. Aspirin served as the positive control and administered at a dose of 300-mg/kg body weight, whereas a 5% Tween 80 solution served as the control. The body temperature was recorded every 30 minutes for a duration of 2 hours. Rats administered the Benjakul formulation exhibited a decrease in body temperature: 300 mg/kg of body weight in 120 minutes, 600 mg/kg of body weight in 90 minutes, and 1,200 mg/kg of body weight at 30, 60, 90, and 120 minutes [104].

Antihypertensive and metabolic syndrome effects of the Benjakul formulation

The ethanolic extract of the Benjakul formulation, administered at doses of 100-, 500-, and 1,000-mg/kg body weight, effectively reduced systolic pressure in rats with hypertension induced by adrenaline [104]. An *in vivo* study demonstrated that rats fed a high-fat diet along with a low-dose Benjakul water extract or a high-dose Benjakul water extract exhibited attenuation of oral glucose tolerance, histopathological features, beta-galactosidase density, and

MDA content in the pancreas [112], demonstrating a protective effect against pancreatic abnormalities.

Antimalarial activity of the Benjakul formulation

In a previous study, both aqueous and ethanolic extracts of the Benjakul formulation were evaluated for their *in vitro* anti-plasmodial activity using the parasite LDH assay. The results revealed that the ethanolic extracts exhibited significant anti-plasmodial activity, with an IC_{50} value of 8.5 ± 1.1 µg/ml, while the aqueous extracts showed less potent activity with an IC_{50} value greater than 100 µg/ml. Additionally, the selectivity indices of both extracts were higher than two, indicating their nontoxicity to cells. These findings suggested that the ethanolic extract of Benjakul possessed promising anti-plasmodial activity and could be considered a potential candidate for the development of new antimalarial drugs [39].

Anticancer activity of the Benjakul formulation

A study on the anticancer effects of the Benjakul formulation revealed its ability to inhibit the proliferation of nonsmall cell lung cancer cells, with IC_{50} values ranging from 5.56 to 5.64 µg/ml. Notably, Benjakul exhibited the highest selectivity among the five plant ingredients, with selectivity index values ranging from 2.93 to 6.88. Plumbagin and 6-shogaol, the two bioactive compounds, displayed the highest cytotoxicity in NCI-H226 cells. Treatment with Benjakul and 6-shogaol led to a dose-dependent induction of G2/M phase arrest, whereas plumbagin induced S-G2/M phase arrest, with the highest percentage observed in the early incubation period at 12–24 hours. Additionally, Benjakul extract, 6-shogaol, and plumbagin at the highest dose increased the population of sub-G1 apoptotic cells, with the highest percentage observed after longer incubation periods of 60 to 72 hours [113]. In lung cancer cells, the ethanolic extracts of the Benjakul formulation exhibited specific inhibition against lung cancer cells, with an IC_{50} value of 19.8 µg/ml, whereas the water extracts showed no cytotoxicity. Three active ingredients isolated from the ethanolic extract of Benjakul, namely 6-gingerol, plumbagin, and piperine, displayed cytotoxic activity, particularly plumbagin, which exhibited the highest cytotoxic activity against COR-L23, HepG2, HeLa, and MRC-5 cells (IC_{50} = 2.55, 2.61, 4.16, and 11.54 µM, respectively) [114]. Regarding breast cancer cells, the Benjakul formulation extract demonstrated inhibition against MCF-7 and MDA-MB-231 cancer cells, with IC_{50} values for MCF-7 cells of 38.35 ± 5.89, 16.36 ± 1.22, and 14.54 ± 1.39 µg/ml, and IC_{50} values for MDA-MB-231 cells of 29.28 ± 1.93, 26.51 ± 1.88, and 21.16 ± 2.10 µg/ml at 24, 48, and 72 hours, respectively. The Benjakul extract also inhibited colony formation in MCF-7 and MDA-MB-231 cells, with IC_{50} values of 5.94 ± 0.73 and 13.16 ± 2.72 µg/ml, respectively. Mechanistically, Benjakul promoted cell death by activating ROS formation, stimulating caspase 3 activity, and repressing mitochondrial function. Moreover, Benjakul extract reduced MMP-9 expression in the culture medium, thereby suppressing cancer cell migration [115].

Toxicity effect of the Benjakul formulation

In the subacute toxicity evaluation of the five herbal formulations of the Benjakul formulation, Wistar rats were fed these formulations and randomly divided into four groups, each consisting of 10 male rats. The groups included a control group and groups receiving aqueous extracts at doses of 0.75-, 4.50-, and 27.00-g/kg body weight. The weight and food consumption of the rats were recorded, with changes noted every 2 days. The results revealed that male rats administered the extract exhibited decreased food consumption, likely due to the spiciness and viscosity of the extract. Some rats receiving the extract showed differences in hematological and serum biochemical parameters, such as white blood cells, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and albumin, compared with those of the control group [116].

In the chronic toxicity study, researchers evaluated the effect of the 95% ethanolic extract of the Benjakul formulation on the organs and systems of rats over 6 months. Wistar rats (30 rats per group) were administered the Benjakul formulation extract at doses of 90.75 mg/kg/day, 272.25 mg/kg/day, and 544.50 mg/kg/day. The results indicated no systemic symptoms from the administration of Benjakul extract; however, hypersalivation was observed after receiving the extract, likely because of its spiciness [104].

Clinical use of the Benjakul formulation

Toxicity effect of the Benjakul formulation in clinical trial phase 1

To assess the safety profile of the Benjakul extract in humans, tablets containing the Benjakul extract were administered to 20 volunteers, divided into two groups. Each group comprised 10 volunteers who orally received either 100 or 200 mg of the extract three times a day after meals for 14 days. Following the administration of the Benjakul extract tablets, the volunteers were observed and examined for liver function (LFT), renal function (RFT), lipid profile, blood sugar, hematology, and MDA levels. These assessments were conducted before and after administering the Benjakul extract tablets on days 0, 1, 7, and 14. The results revealed that the Benjakul extract tablets did not induce severe adverse effects or biochemical alterations in either group of volunteers [117].

Benjakul formulation extract for treating primary osteoarthritis (OA) of the knee

In a phase 2, double-blind, randomized, and controlled trial, the Benjakul formulation group received 300 mg of Benjakul extract per day, whereas the positive control group received 75 mg of diclofenac per day. All patients received the extract and were followed up for 14 and 28 days. The patients were evaluated for changes in the visual analog scale for pain, 100-m walking times, modified Thai WOMAC index scores, and global assessment. The visual analog scale pain score and 100-m walking time were reduced in all patients who were administered the extract. Furthermore, the modified Thai WOMAC scores of both groups were significantly reduced from baseline. The Benjakul formulation extract demonstrated

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clinical efficacy in relieving symptoms and may be used as an alternative agent for OA of the knee to diclofenac [102].

Benjakul cream for treating primary OA of the knee

In preliminary clinical studies, researchers assessed the effectiveness of Benjakul cream in 15 primary knee OA patients aged 40–80 years. Each patient received 2 g of Benjakul formulation extract cream three times per day for a duration of two weeks. The efficacy of the extract was measured using visual analog scales, modified Thai WOMAC Index scores, and time measurements after walking 100 m. The results indicated that Benjakul cream reduced knee pain experienced by patients after walking 100 m, resulting in reduced time. Furthermore, Benjakul cream significantly improved the quality of life, as evaluated by the WOMAC index scores [118].

Benjakul formulation extract for treating nonsmall cell lung cancer

In a case study, a 52-year-old man diagnosed with stage four nonsmall cell lung cancer received the Benjakul drug for a duration of 5.9 months but discontinued the treatment thereafter owing to the severity of the disease. The results revealed that the O_2 saturation level remained within the normal range, and RFT did not exhibit any changes. However, administration of the Benjakul formulation led to increased levels of AST and alkaline phosphatase (indicative of LFT disturbance), as well as elevation in cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and neutrophils [106]. In contrast, the Phase I clinical trial in healthy volunteers (section 4.3.9.1) demonstrated no significant adverse effects or biochemical alterations following short-term administration of Benjakul extract [117]. This discrepancy raises concern that extended administration of Benjakul, particularly in patients with severe underlying disease, may precipitate hepatotoxicity or metabolic disturbances. These findings underscore the need for cautious interpretation of preclinical and early phase data and highlight the importance of systematic safety monitoring and larger, controlled clinical trials to more accurately define the risk profile of Benjakul in vulnerable patient populations.

CONCLUSION

The study highlighted the extensive pharmacological and toxicological evidence supporting the therapeutic potential of Triphala, Trikatu, and Benjakul formulation. Triphala is particularly noted for its broad-spectrum antimicrobial properties, including antibacterial, antifungal, and antiviral activities, as well as its potent antiinflammatory, antioxidant, and immunomodulatory effects, making it effective against infections and oxidative stress. Trikatu demonstrated significant antimicrobial, anthelmintic, and antimalarial properties, alongside its ability to regulate oxidative stress and immune responses. It also showed hepatoprotective potential and efficacy in managing obesity through adipose reduction and lipid profile improvement. Benjakul exhibited diverse therapeutic benefits, including anticancer, antiinflammatory, antipyretic, and osteoarthritic properties, with its anticancer effects targeting critical pathways in cancer progression. Importantly, all three formulations demonstrated safety at recommended doses, with no toxicity observed in cell lines, animals, or healthy volunteers. Among the three, Triphala emerged as the most extensively studied formulation, reflecting its widespread therapeutic applications and well-documented safety profile. Future research on Triphala, Trikatu, and Benjakul should focus on elucidating the molecular mechanisms behind their pharmacological effects and identifying bioactive compounds. Furthermore, despite encouraging preclinical and limited clinical evidence supporting the pharmacological benefits of Benjakul, Triphala, and Trikatu, there remains a notable lack of chronic toxicity data; future long-term studies are therefore needed to comprehensively evaluate their cumulative safety profiles and inform evidence-based clinical use.

ABBREVIATIONS

ALT, alanine transaminase; AST, aspartate transaminase; CAT, catalase; COX-2, cyclooxygenase-2; FRAP, ferric reducing antioxidant power; GC-MS, gas chromatography-mass spectrometry; GSH, glutathione; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide scavenging; Hb, hemoglobin; HDL, high-density lipoproteins; IC_{50}, Half-maximal inhibitory concentration; LDH, lactate dehydrogenase; LDL, low-density lipoproteins; LFT, liver function; MDA, malondialdehyde; MMP9, matrix metallopeptidase 9**;** NO, nitric oxide; PI3K/Akt, phosphatidylinositol 3-kinase/protein kinase B; RFT, renal function; ROS, reactive oxygen species; SGPT, serum glutamic pyruvic transaminase; SOD, superoxide dismutases.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

FINANCIAL SUPPORT

This study did not receive any external funding.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon request.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:
Net-Anong S, Phuwajaroanpong A, Plirat W, Konyanee A, Chaniad P, Punsawad C. Triphala, Trikatu, and Benjakul: an evidence-based review of their pharmacology, toxicology, and clinical potential in integrative medicine. J Appl Pharm Sci. 2025. Article in Press.
http://doi.org/10.7324/JAPS.2025.v15.i12.4

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