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"Formulation and Evaluation of Citrus hystrix, Piper betel and Zingiber officinale Polyherbal Decoction for Antioxidant, Antimicrobial and in Vitro Anti-Arthritic Activity"

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Abstract: Arthritis comprises a group of inflammatory and degenerative joint disorders in which persistent inflammation, oxidative stress and progressive impairment of joint function contribute to disease burden. Medicinal plants are being investigated as sources of bioactive compounds with antioxidant, anti-inflammatory and antimicrobial properties. This narrative review discusses the phytochemical composition, traditional relevance and pharmacological potential of *Citrus hystrix* DC., *Piper betel* L. and *Zingiber officinale* Roscoe, with particular emphasis on their relevance to antioxidant, antimicrobial and anti-arthritic activity. *Citrus hystrix* contains flavonoids, phenolic compounds, coumarins and volatile constituents and recent evidence supports its anti-inflammatory activity. *Piper betel* contains phenols, flavonoids, alkaloids and essential-oil constituents and has been reported to possess antioxidant, antimicrobial and anti-inflammatory activities. *Zingiber officinale* contains gingerols, shogaols and related phenolic constituents and has substantial preclinical evidence for antioxidant and anti-inflammatory effects, including relevance to arthritic conditions. The combined phytochemical diversity of these plants provides a rationale for investigating their complementary activity in a polyherbal decoction. However, evidence for the specific three herb combination remains limited, and most available evidence is derived from individual plants or extracts rather than standardized clinical preparations. Standardization, toxicity assessment, mechanistic studies, formulation optimization and appropriately designed in vivo and clinical studies are required before therapeutic claims can be made.

Keywords: *Citrus hystrix*; *Piper betel*; *Zingiber officinale*; polyherbal formulation; decoction; antioxidant; antimicrobial; anti-arthritic; inflammation.

INTRODUCTION

Arthritis is a major group of musculoskeletal disorders characterized by pain, inflammation, stiffness and impaired joint function. In inflammatory forms of arthritis, excessive production of inflammatory mediators and reactive oxygen species can contribute to tissue damage and progression of disease. Oxidative stress and inflammation are closely interconnected: reactive oxygen species can activate inflammatory signaling, while inflammatory processes can further increase oxidative stress. This relationship has encouraged investigation of natural products capable of modulating both processes.

Medicinal plants contain chemically diverse secondary metabolites, including phenolics, flavonoids, terpenoids, alkaloids, coumarins and volatile constituents. Such compounds may contribute to

free-radical scavenging, modulation of inflammatory mediators and inhibition of microbial growth. *Piper betel* has been reviewed extensively for antioxidant, antimicrobial and anti-inflammatory properties, while ginger has been investigated for anti-inflammatory effects and applications relevant to osteoarthritis and rheumatoid arthritis. Recent systematic evidence also supports the anti-inflammatory potential of *Citrus hystrix*, although the evidence remains predominantly preclinical.

The present review therefore brings together the available evidence concerning *C. hystrix*, *P. betel* and *Z. officinale*, and discusses the scientific rationale for considering these plants in a polyherbal decoction intended for antioxidant, antimicrobial and anti-arthritic applications.

AIM:

The present study aims to formulate a polyherbal decoction using *Citrus hystrix*, *Piper betel*, and *Zingiber officinale*, and to evaluate its anti-arthritic potential through in vitro methods, with the following.

OBJECTIVES:

- To collect, authenticate, and process *Citrus hystrix*, *Piper betel*, and *Zingiber officinale* for formulation of the polyherbal decoction.
- To prepare the decoction by combining the selected plant materials in suitable proportions using standard decoction techniques.
- To perform preliminary phytochemical screening of the formulated decoction to detect bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds.
- To assess the in vitro anti-arthritic activity of the formulation using standard assays — protein (albumin) denaturation inhibition, HRBC membrane stabilization, and proteinase inhibition.
- To compare the anti-arthritic activity of the formulated decoction with a standard reference drug, such as diclofenac sodium.
- To identify the most effective concentration of the polyherbal decoction exhibiting significant

ARTHRITIS AND THE ROLE OF OXIDATIVE STRESS

Arthritis is not a single disease but a broad term covering conditions with different causes and pathological mechanisms. Osteoarthritis is predominantly associated with progressive changes in cartilage and joint structures, whereas rheumatoid arthritis is a systemic autoimmune inflammatory disorder. Other forms include gouty and infectious arthritis. In inflammatory arthritis, cytokines and mediators such as tumor necrosis factor-alpha, interleukins, cyclooxygenase-derived mediators and reactive oxygen species participate in the inflammatory response.

Oxidative stress occurs when the generation of reactive oxygen species exceeds antioxidant defense. Excessive oxidative stress can promote lipid, protein and cellular damage and may amplify inflammatory signaling. Consequently, antioxidant and anti-inflammatory activities are often considered complementary targets in research on plant-derived approaches to arthritic disorders.

MATERIALS AND METHODS

CITRUS HYSTRIX

Botanical and Phytochemical Profile

Citrus hystrix DC., commonly known as kaffir lime, is a citrus species traditionally used for culinary and medicinal purposes. Reported phytochemical groups include flavonoids, phenolic compounds, coumarins and volatile constituents. Essential-oil components reported from the plant include citronellal, citronellol, beta-pinene, sabinene, limonene and terpinene-4ol. The chemical profile can vary according to plant part, geographical origin, extraction procedure and processing.

Antioxidant and Anti-inflammatory Potential

Recent evidence provides stronger support for the anti-inflammatory potential of *C. hystrix*. A 2026 systematic review and meta-analysis identified nine eligible experimental studies and reported reductions in inflammatory markers including nitric oxide, TNF-alpha and IL-6. The review also found a pooled inhibitory effect on BSA denaturation, while noting substantial heterogeneity between studies. These findings support further investigation but do not establish clinical efficacy.

Relevance to Polyherbal Use

The presence of phenolics, flavonoids, coumarins and volatile constituents gives *C. hystrix* a plausible role as an antioxidant and anti-inflammatory component of a polyherbal preparation. However, evidence specifically examining its combination with *P. betel* and *Z. officinale* is not yet sufficient to claim a synergistic anti-arthritic effect.

PIPER BETEL

Botanical and Phytochemical Profile

Piper betel L. is an aromatic member of the Piperaceae family and its leaves have long been used in South and Southeast Asian traditional practices. Reviews describe a diverse phytochemical composition including phenolic compounds, flavonoids, alkaloids and essential oil constituents. Hydroxychavicol, eugenol, allylpyrocatechol and related compounds have been discussed as important bioactive constituents.

Antioxidant Activity

Experimental literature has reported free-radical scavenging and antioxidant activity of *P. betel* extracts. Phenolic constituents are particularly relevant because their chemical structures can support radical-scavenging activity. Differences in cultivar, extraction solvent and plant part can influence the measured antioxidant response, so standardized preparation is important when comparing studies.

Antimicrobial and Anti-inflammatory Potential

Published reviews describe antibacterial, antifungal and other antimicrobial activities of *P. betel*, together with anti-inflammatory effects. These properties make it potentially relevant to a polyherbal formulation where antioxidant and anti-inflammatory effects are the principal objectives and antimicrobial activity is an additional property.

Nevertheless, in vitro antimicrobial findings should not be interpreted as proof of clinical anti infective efficacy.

ZINGIBER OFFICINALE

Phytochemistry

Zingiber officinale Roscoe, commonly known as ginger, is widely used as a food and medicinal plant. Its rhizome contains phenolic constituents such as gingerols and shogaols, along with volatile oils and other secondary metabolites. The chemical composition changes with cultivar, maturity, processing and extraction conditions.

Antioxidant and Anti-inflammatory Activity

Ginger has demonstrated antioxidant and anti-inflammatory effects in experimental research. A 2025 comprehensive review discussed gingerols, shogaols and zingerone in relation to antioxidant and anti-inflammatory activity and summarized evidence relevant to conditions including osteoarthritis and rheumatoid arthritis. Earlier experimental work has also examined inhibition of protein denaturation, proteinase activity and membrane stabilization as indicators of anti-inflammatory potential.

Anti-Arthritic Relevance

The available evidence makes ginger the most directly relevant of the three selected plants to an anti-arthritic review. Its phytochemicals may influence oxidative stress and inflammatory pathways, providing a mechanistic rationale for further investigation. However, evidence from laboratory studies should be distinguished from evidence of clinical effectiveness in patients.

METHOD OF FORMULATION

The following method is used for the formulation of polyherbal decoction.

COLLECTION OF MATERIAL

Fresh Citrus hystrix fruits, Piper betel leaves and Zingiber officinale rhizomes should be collected from a reliable raw material source and authenticated by an appropriate taxonomist. Foreign materials and damaged plant portions should be removed before processing.

PREPARATION OF PLANT MATERIALS

The selected plant materials should be washed appropriately to remove adhering dirt and extraneous matter.

Piper betel leaves and **zingiber offivinale** rhizomes should be cut into small pieces and dried under controlled conditions, Preferably protected from excessive heat and direct sunlight.That materials all the directly involving in the **Hot Perculation** method.

For **Citrus Hystrix**, the plant part must be clearly standardized in the final manuscript. The fresh fruit juice using the project, the juice should be freshly prepared, filtered and added during the final formulation stage after the herbal decoction has cooled sufficiently. This approach helps avoid unnecessary prolonged heating of heat-sensitive constituents.

PREPARATION OF POLYHERBAL DECOCTION

- A predetermined quantity of powdered P. betel leaves and Z. officinale rhizome should heated gently until an appropriate volume reduction is obtained. The mixture should then be filtered through suitable muslin cloth or filter paper.
- The required quantity of freshly prepared C. hystrix fruit juice may subsequently be incorporated into the cooled decoction under controlled conditions. The final volume should be adjusted with purified water.
- The prepared formulation should be evaluated for appearance, colour, odour, taste, pH and other suitable physicochemical parameters.

Evaluation of Polyherbal Decoction

1. PHYTOCHEMICAL SCREENING

The formulation may be subjected to preliminary qualitative phytochemical screening for major groups such as:

- Alkaloids
- Flavonoids
- Phenolics
- Tannins
- Saponins
- Terpenoids
- Steroids
- Glycosides

The results should be reported as positive and negative according to the standard qualitative tests.

ANTIOXIDANT POTENTIAL OF THE THREE HERBS

The three plants provide different but potentially complementary sources of antioxidant constituents. *C. hystrix* contributes flavonoids, phenolics, coumarins and volatile compounds; *P. betel* contributes phenolic and volatile constituents including hydroxychavicol and eugenol; and *Z. officinale* contributes gingerols, shogaols and related phenolics. Antioxidant assays such as DPPH, ABTS and reducing-power assays can be useful for comparative screening, but results obtained by different assays should not be treated as directly interchangeable.

ANTI-ARTHRITIC POTENTIAL

Plant-derived anti-arthritic research commonly evaluates inhibition of protein denaturation, proteinase activity, membrane stabilization, inflammatory mediator production and oxidative stress. These models are useful preliminary screening approaches. The evidence summarized in the literature suggests that C. hystrix, P. betel and Z. officinale each possess one or more relevant antioxidant or anti-inflammatory properties. Ginger has particularly substantial literature related to inflammatory and arthritic conditions,

while recent evidence strengthens the case for C. hystrix. For P. betel, antioxidant and anti-inflammatory findings provide a supportive rationale, although direct anti-arthritic evidence is comparatively less established.

ANTIMICROBIAL POTENTIAL

P. betel has the clearest documented antimicrobial profile among the three plants, with reported antibacterial and antifungal effects. Citrus-derived constituents and ginger phytochemicals have also been investigated for antimicrobial effects. Agar diffusion methods can provide preliminary evidence, while quantitative dilution methods are more informative for determining minimum inhibitory concentrations. Because antimicrobial activity is strongly influenced by extract composition and test organism, comparisons require standardized methodology.

POSSIBLE RATIONALE FOR THE POLYHERBAL COMBINATION

Plant	Important phytochemical groups/constituents	Potential relevance
Citrus hystrix	Flavonoids, phenolics, coumarins, volatile constituents	Antioxidant and anti-inflammatory support
Piper betel	Phenolics, flavonoids, alkaloids, essential-oil constituents	Antioxidant, antimicrobial and anti-inflammatory support
Zingiber officinale	Gingerols, shogaols, zingerone, volatile constituents	Antioxidant and anti-inflammatory/anti-arthritic support

The combination is scientifically plausible because the selected plants contain overlapping and distinct classes of bioactive constituents. A rational polyherbal formulation could therefore be investigated for additive or synergistic effects. At present, however, the specific combination should be described as a promising research concept rather than as a clinically proven synergistic formulation.

SAFETY, LIMITATIONS AND FUTURE PERSPECTIVES

Although the selected plants have extensive traditional use and promising preclinical evidence, natural origin does not by itself establish safety or efficacy. Extract composition can vary substantially with species authentication, geographical source, plant part, harvesting stage, drying and extraction method. The evidence base for the exact three-herb combination is limited. Most findings are derived from individual plant extracts, isolated constituents or laboratory models. Future work should therefore focus on authenticated raw materials, standardized phytochemical markers, formulation optimization, toxicity evaluation, pharmacokinetic investigation, mechanistic studies, suitable in vivo models and, where justified, clinical evaluation

CONCLUSION

The literature reviewed supports the scientific interest of Citrus hystrix, Piper betel and Zingiber officinale as medicinal plants with antioxidant and anti-inflammatory potential, while P. betel also has notable reported antimicrobial activity and ginger has substantial relevance to arthritic research. Recent evidence strengthens the anti-inflammatory rationale for C. hystrix. Taken together, the three plants provide a reasonable basis for investigating a polyherbal decoction. Nevertheless, the specific combination requires systematic formulation, standardization and pharmacological validation. Current evidence should therefore be considered supportive and hypothesis-generating rather than proof of therapeutic efficacy.

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