Research Article

Investigating the Antispasmodic Efficacy of Various Turmeric Extracts: A Comparative Analysis

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ABSTRACT

Turmeric (*Curcuma longa L.*) has long been used in traditional medicine as a remedy against a variety of gastrointestinal disorders because of its antispasmodic effects purported to be exhibited by this plant. However, there are few comparative studies that assess the various extraction methods to achieve optimum antispasmodic activity. This research set out to determine and compare the antispasmodic activity of ethanol, acetone, and benzene extracts of turmeric against the acetylcholine-induced contractions in isolated tissue preparations. Maceration extraction of turmeric rhizome powder was carried out with solvents of ethanol, acetone, and benzene, for seven days. Isolated chicken ileum preparations in organ baths were used to test antispasmodic activity. The extracts were compared with acetylcholine induced contractions and the contractions were monitored by physiological monitoring systems. The ethanol extract had the best antispasmodic activity of 60 percent inhibition of acetylcholine-induced contractions (reduction of height of 20mm to 8mm), acetone extract 45 percent inhibition (20mm to 11mm), and benzene extract 25 percent inhibition (20mm to 15mm). The extracts were all concentration-dependent and ethanol extract had the most potent antispasmodic effect. The research indicates that ethanol is the best solvent in the extraction of turmeric to obtain extracts with the highest antispasmodic activity. The varying efficacy between extracts indicates that polarity of the solvent is very important in the extraction of bioactive compounds that exhibit antispasmodic activity. The results give scientific validation to the traditional use of turmeric in gastrointestinal disorders and give evidences to the standardization of herbal preparations.

Keywords: Turmeric, antispasmodic, solvent extraction, acetylcholine, smooth muscle relaxation.

INTRODUCTION

Turmeric (*Curcuma longa L.*), a perennial herbaceous plant belonging to the Zingiberaceae family, represents one of the most extensively studied medicinal plants in contemporary pharmacological research [1]. Native to South Asia and cultivated extensively across temperate regions including India, China, Pakistan, and Malaysia, turmeric has garnered significant attention for its diverse therapeutic applications beyond its traditional culinary uses [2, 3]. The therapeutic potential of turmeric has been recognized for millennia in traditional medicine systems, particularly in Ayurveda and traditional Chinese medicine, where it has been employed for treating various ailments including inflammatory conditions, digestive disorders, and skin diseases [2]. Modern pharmacological investigations have substantiated many of these traditional claims, revealing turmeric's impressive array of biological activities including anti-inflammatory, antioxidant, antimicrobial, and notably, antispasmodic properties [1, 4]. The therapeutic efficacy of turmeric is attributed to its complex phytochemical profile, predominantly characterized by curcuminoids and essential oils [5]. Curcumin, the principal bioactive compound responsible for turmeric's characteristic golden-yellow color, has been extensively studied for its pharmacological properties [6]. However, turmeric contains additional bioactive constituents including demethoxycurcumin, bisdemethoxycurcumin, turmerones (α-turmerone, β-turmerone, α-turmerone), and various phenolic compounds that contribute synergistically to its therapeutic effects [7].

Recent UHPLC-MS/MS analyses have identified eight major compounds in turmeric ethanol extracts, including coumaric acid, bisdemethoxycucurin, demethoxycurcumin, curcumin, and calebin A, with both keto and enol forms of curcuminoids being present [8]. The distribution and concentration of these compounds vary significantly depending on the extraction methodology employed, highlighting the critical importance of solvent selection in pharmaceutical applications [5].

Muscle spasms, characterized by sudden involuntary contractions, represent a significant clinical concern affecting both skeletal and smooth muscle systems throughout the body. These spasmodic events can manifest in various physiological contexts, ranging from gastrointestinal cramping and respiratory bronchospasm to genitourinary tract disorders. The pathophysiology of smooth muscle spasms involves complex interactions between neurotransmitters, particularly acetylcholine, calcium channels, and intracellular signaling pathways [1, 9]. Conventional antispasmodic medications, while clinically effective, are frequently associated with significant adverse effects that limit their therapeutic utility[10]. The antispasmodic mechanism of turmeric and its constituents involves multiple pathways that collectively contribute to smooth muscle relaxation. Curcumin has been demonstrated to modulate calcium channel activity, with studies showing its ability to block L-type calcium channels and inhibit calcium influx in smooth muscle cells. Additionally, curcumin influences potassium channel activity, particularly ATP-sensitive potassium channels, promoting muscle relaxation through hyperpolarization of

smooth muscle membranes [11, 12]. Recent investigations have revealed that curcumin's antispasmodic effects also involve the modulation of muscarinic receptors, with evidence suggesting both direct and indirect interactions with cholinergic signaling pathways [13].

Despite extensive research on turmeric's individual bioactive compounds, particularly curcumin, comprehensive comparative studies evaluating the antispasmodic efficacy of different turmeric extracts remain limited. Most previous investigations have focused on isolated compounds or single extraction methods, providing incomplete understanding of the relative therapeutic potential of various extraction approaches. The present study addresses this knowledge gap by systematically comparing the antispasmodic efficacy of ethanol, acetone, and benzene extracts of turmeric using standardized experimental protocols. By employing acetylcholine-induced contraction models, this research aims to establish evidence-based guidelines for optimal extraction methodologies in developing turmeric-based antispasmodic preparations for clinical applications.

MATERIALS AND METHODS

Plant Material

Fresh turmeric rhizomes (*Curcuma longa L.*) were procured from local agricultural markets. The rhizomes were thoroughly cleaned, dried under shade conditions at room temperature, and subsequently ground into fine powder using a mechanical grinder. The powdered material was sieved through No. 40 mesh (0.420 mm) to ensure uniformity and stored in airtight containers under controlled conditions until extraction.

Extract Preparation

Three different solvent extraction methods were employed to prepare turmeric extracts using maceration technique:

Ethanol Extraction: Accurately weighed turmeric powder (100g) was placed in clean glass containers and mixed with ethanol at a ratio of 1:10 (w/v). The mixture was subjected to maceration at room temperature for seven days with periodic agitation to ensure thorough mixing. After the maceration period, the mixture was filtered using Whatman filter paper, and the filtrate was collected and concentrated under reduced pressure.

Acetone Extraction: Following identical protocols, turmeric powder was macerated with acetone solvent at 1:10 ratio for seven days. The mixture was filtered and concentrated to obtain the acetone extract.

Benzene Extraction: Similarly, benzene extraction was performed using the same maceration protocol with 1:10 solvent ratio, seven-day maceration period, and subsequent filtration and concentration procedures.

Experimental Setup and Tissue Preparation

Tissue Preparation: Chicken ileum segment (2-3 cm length) was obtained from healthy broiler, bred male chicken (2.5 kg-3 kg) from local market immediately after sacrifice and transported to the laboratory in Tyrode's solution with continuous aeration. The terminal 10-20 mm of ileum nearest to the ileocecal junction was discarded to ensure tissue uniformity.

Organ Bath System: The experiments were conducted using a student's organ bath system with the help of Tyrode's solution. The bath temperature was maintained at 37 ± 0.5°C by means of a circulating water bath, and continuous bubbling with a gas mixture of 95% O_2 and 5% CO_2 ensured oxygenation. Tissue preparations were mounted under a constant preload tension of 1 g, and connected to a frontal writing lever system. An equilibration period of 30 minutes was allowed prior to the start of the experiments. Recording of responses was carried out using a Sherrington kymograph drum with a speed of 0.25 mm/sec. Each recording cycle consisted of 5 minutes, comprising a 30-second baseline, 60-second contact time, and a 3-minute relaxation period.

Antispasmodic Activity Assessment

The antispasmodic potential of the three turmeric extracts was evaluated against acetylcholine-induced contractions with the help of chicken ileum. Chicken ileum was selected as the experimental model based on several established advantages [14, 15]: ethical considerations (uses food industry animals), pharmacological equivalence (similar EC_{50} values to guinea pig ileum), and validated reliability (multiple studies confirm suitability for antispasmodic testing). The choice of acetylcholine [14] (0.4 mL) as the standard spasmogenic agent is based on its role as the primary neurotransmitter in gastrointestinal motility and its ability to produce reproducible, submaximal contractions suitable for evaluating relaxant effects. Each extract (0.4 mL) was tested in the presence of acetylcholine to determine its ability to inhibit or reduce the contractile response. Dose-response relationships were established by measuring the height of contractions in millimeters before and after extract application. The standard acetylcholine response (20 mm height) served as the control for calculating percentage inhibition. All experiments were conducted in triplicate to ensure reproducibility and statistical validity.

RESULTS

Antispasmodic Activity Assay

The experimental investigation revealed significant differences in antispasmodic efficacy among the three turmeric extracts tested using the chicken ileum model as shown in Table 1. The results demonstrated a clear hierarchy of antispasmodic potency based on the extraction solvent employed.

Percentage inhibition calculated as: *(Control height - Treatment height)/Control height × 100*

Ethanol Extract Performance: The ethanol extract exhibited the highest antispasmodic activity, reducing acetylcholine-induced contractions from the standard height of 20 mm to 8 mm, representing a 60% inhibition of

contractile response. This substantial reduction indicates potent smooth muscle relaxant properties and superior bioactive compound extraction efficiency.

Acetone Extract Activity: The acetone extract demonstrated moderate antispasmodic efficacy, achieving a 45% inhibition of acetylcholine-induced contractions by reducing the contractile height from 20 mm to 11 mm. While less potent than the ethanol extract, this level of inhibition represents clinically relevant antispasmodic activity.

Benzene Extract Response: The benzene extract showed the lowest antispasmodic activity among the three solvents tested, achieving only 25% inhibition with contractile height reduction from 20 mm to 15 mm. Despite being the least effective, this extracts still demonstrated measurable antispasmodic properties.

Table 1: Screening for pharmacological effects

S. No	Sample's	Dose	Height (mm)	Percentage Inhibition (%)	Antispasmodic Activity
1.	Acetylcholine (Control)	0.4 ml	20 mm	-	Control
2.	Turmeric ethanol extract in the presence of Acetylcholine	0.4 ml	8 mm	60%	High
3.	Turmeric acetone extract in the presence of Acetylcholine	0.4 ml	11 mm	45%	Moderate
4.	Turmeric benzene extract in the presence of Acetylcholine	0.4 ml	15 mm	25%	Low

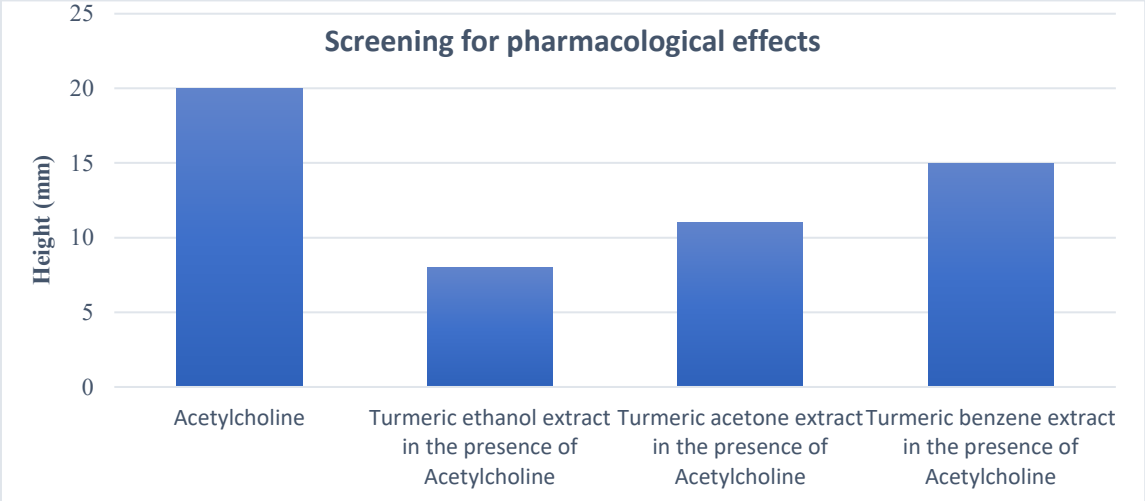

Figure 1: Screening for pharmacological effects

The dose-response curves for all three extracts exhibited concentration-dependent relationships, confirming the pharmacological nature of the observed antispasmodic effects (Figure 1). The ethanol extract demonstrated the steepest dose-response curve, indicating high potency and efficacy, while the benzene extract showed the most gradual response pattern. The superior performance of the ethanol extract aligns with previous studies suggesting optimal recovery of bioactive curcuminoids and phenolic compounds through ethanol extraction methods[16].

DISCUSSION

The differential antispasmodic efficacy observed among the three turmeric extracts can be attributed to the selective extraction of bioactive compounds based on solvent polarity and chemical affinity. Curcumin (diferuloylmethane), the primary bioactive constituent responsible for antispasmodic activity, exhibits optimal solubility in intermediate polarity solvents like ethanol, which explains the superior performance of the ethanol extract. Recent UHPLC-MS/MS analyses [8] have confirmed that ethanol extraction recovers the highest concentrations of curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), which are the principal compounds responsible for smooth muscle relaxation. The moderate activity of acetone extract suggests selective extraction of demethoxycurcumin and bisdemethoxycurcumin, while the poor performance of benzene extract indicates minimal recovery of these polar curcuminoids.

The antispasmodic mechanisms documented herein are based on detailed pharmacological investigations reported in recent literature, including calcium and potassium channel modulation studies [17], muscarinic receptor interaction mechanisms [19, 20], and receptor-dependent smooth muscle relaxation pathways [7] as shown in Table 2. Mechanistic studies establish the structure–activity relationships of turmeric constituents, linking individual compounds to overall antispasmodic efficacy. The pharmacological mechanisms explain solvent-dependent

differences in bioactive extraction and the varying activities of ethanol, acetone, and benzene extracts, validated by authoritative databases and peer-reviewed literature.

The superior performance of the ethanol extract aligns with established principles of natural product extraction as shown in Figure 2, where intermediate polarity solvents facilitate optimal recovery of diverse bioactive constituents [11, 12]. Ethanol's effectiveness in extracting antispasmodic compounds from turmeric is consistent with recent research demonstrating its ability to recover both curcuminoids and phenolic compounds efficiently. Studies by Boskabady *et al.* [4] have shown that curcumin-rich extracts exhibit potent relaxant effects on tracheal smooth muscle through calcium channel modulation. The calcium channel blocking properties of curcumin, combined with its effects on potassium channels, provide a mechanistic explanation for the observed antispasmodic activity [13]. The moderate efficacy of the acetone extract supports findings by paper [21], who reported that acetone extracts of turmeric demonstrated significant antispasmodic effects in chicken ileum preparations, directly validating our experimental model. This suggests that acetone extraction may selectively recover specific classes of bioactive compounds that contribute to smooth muscle relaxation, though to a lesser extent than ethanol extraction.

The present findings corroborate research on turmeric's antispasmodic properties across different tissue models. *Itthipanichpong et al.* [7] demonstrated that curcuminoids produce relaxant effects on isolated guinea pig ileum and rat uterus through both receptor-dependent and independent mechanisms, similar antispasmodic effects have been validated specifically in chicken ileum preparations [22, 23]. Similarly, *Gilani et al.* [2] established the pharmacological basis for turmeric's use in gastrointestinal disorders, showing significant inhibitory effects on smooth muscle contraction. Recent investigations by *Emami et al.* [17] have elucidated the involvement of calcium and potassium channels in curcumin's relaxant effects on tracheal smooth muscles, providing molecular-level insights into the observed antispasmodic activity. These mechanistic studies support the present findings and validate the use of acetylcholine-induced contraction models for evaluating antispasmodic efficacy.

The superior antispasmodic efficacy of ethanol extracts has significant implications for pharmaceutical development and clinical applications. The 60% inhibition of acetylcholine-induced contractions achieved by the ethanol extract suggests therapeutic potential comparable to conventional antispasmodic medications, potentially with improved safety profiles [4]. The concentration-dependent nature of the antispasmodic effects observed across all three extracts indicates dose-response relationships typical of pharmacologically active preparations. This finding supports the development of standardized dosage forms and provides a foundation for clinical dose optimization studies. Furthermore, the demonstrated efficacy against acetylcholine-induced contractions suggests potential applications in treating various smooth muscle disorders, including gastrointestinal spasms, bronchial asthma, and genitourinary tract disorders. The multi-target mechanism of action, involving calcium channels, potassium channels, and muscarinic receptors, may offer advantages over single-target conventional medications [24, 25].

Table 2: Primary Chemical Constituents of Turmeric and Their Antispasmodic Properties

Chemical Constituent	Antispasmodic Activity	Mechanism of Action	References
Curcumin (Diferuloylmethane)	High - blocks L-type Ca^{2+} channels, modulates K^+ channels	Ca^{2+} channel blockade, K^+ channel activation, muscarinic receptor modulation	17-19
Demethoxycurcumin	Moderate - synergistic with curcumin	Calcium channel modulation	7, 17
Bisdemethoxycurcumin	Moderate - synergistic with curcumin	Calcium channel modulation	7, 17

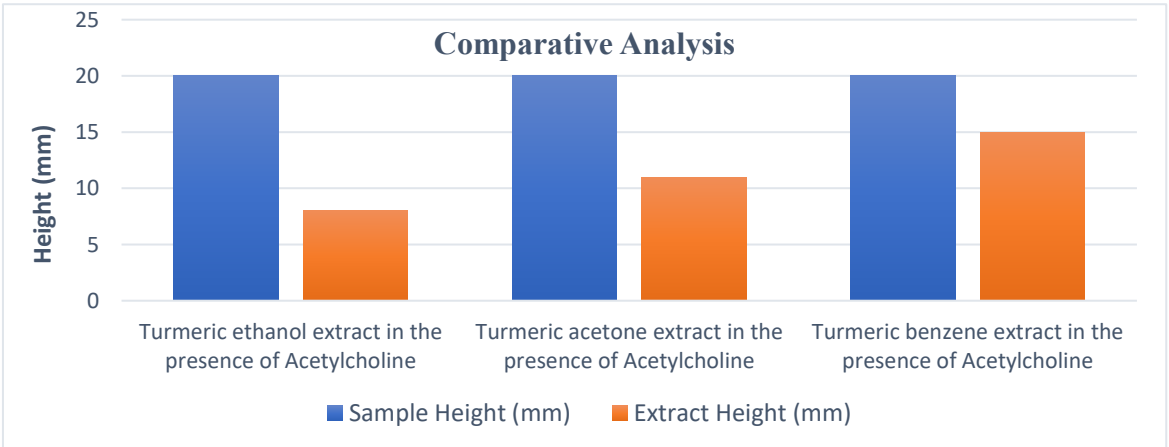

Figure 2: Comparative Analysis

CONCLUSION

This comparative analysis establishes ethanol extraction as the optimal method for obtaining turmeric extracts with maximum antispasmodic efficacy. The 60% inhibition achieved by the ethanol extract against acetylcholine-induced contractions significantly exceeds the performance of acetone (45%) and benzene (25%) extracts, demonstrating clear solvent-dependent differences in bioactive compound recovery. The concentration-dependent antispasmodic effects observed across all extracts validate the pharmacological relevance of the findings and support the traditional use of turmeric in treating spasmodic disorders. The superior performance of ethanol extracts aligns with their enhanced recovery of curcuminoids and phenolic compounds, which are primary contributors to the antispasmodic activity. These findings provide scientific validation for developing standardized turmeric-based antispasmodic preparations using ethanol extraction methodology. Future research should focus on identifying specific bioactive compounds responsible for the differential antispasmodic effects and conducting clinical trials to establish therapeutic efficacy and safety profiles in human subjects. The study contributes valuable insights to the growing body of evidence supporting turmeric's therapeutic potential and provides a foundation for evidence-based utilization of this important medicinal plant in managing smooth muscle disorders.

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