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Comparative phytochemical profiling and evaluation of antioxidant, anti-inflammatory, and antibacterial activities of *Curcuma longa* L. extracts and curcumin

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Abstract

The study aimed for comparative evaluation of turmeric (*Curcuma longa* L.) rhizome extract and curcumin on phytochemicals content and *in vitro* functional properties such as antioxidant, anti-inflammatory and antibacterial activities for its application in bovine mastitis. Fresh turmeric rhizomes were shade dried and coarse powdered after its botanical authentication. The extraction was done using Soxhlet apparatus with ethanol and methanol solvents (80%). Qualitative phytochemical screening of turmeric extracts was done using standard protocols. Total phenol content (TPC) and total flavonoid content (TFC) in extracts and commercially available curcumin were estimated using Folin-Ciocalteu reagent and aluminium chloride methods, respectively. Antioxidant, anti-inflammatory and antimicrobial activities of extracts and curcumin were evaluated *in vitro* through DPPH assay, protein denaturation assay and disc-diffusion method, respectively. Extraction yield (%) was more in ethanol than methanol. Qualitative phytochemical screening of alcoholic extracts revealed the presence of alkaloids, phenol, flavonoids, tannin, saponin, terpenoids, flavonoids and glycosides with more phytoconstituents in methanolic (9) or ethanolic (7) than aqueous (5) extracts. TPC and TFC were significantly higher in curcumin (518-637 mg GAE/g sample) than alcoholic turmeric extracts (264-347 mg GAE/g sample) and concentration-dependent. The antioxidant and anti-inflammatory activity of curcumin was significantly higher than extracts in terms of IC₅₀ values DPPH scavenging and inhibition of protein denaturation (1032 vs 3091 µg/ml and 60 vs 205 µg/ml, respectively). Although, antimicrobial activity against *Staphylococcus epidermidis* (isolates from mastitic cows) was more in ethanolic extracts with higher concentration, curcumin inhibited the growth of *S. epidermidis* at lower concentration indicating its higher potency (zone of inhibition: 1.68 vs 1.48 cm). It is concluded that curcumin exhibited higher phenolic content and superior *in vitro* antioxidant, anti-inflammatory, and antibacterial activities compared to *C. longa* extracts. Further *in vivo* and clinical studies are needed to validate these findings for mastitis management.

1. Introduction

Mastitis in dairy animals is most common disease and causes adverse impact on dairy farmer's economy through reduction of milk yield, its quality, veterinary expenses, reproduction and animal welfare. Despite of several prevention strategies in practices, it is often difficult to eliminate or break the pathogens transmission chain, which resulted in continuous occurrence of mastitis in dairy farms. Meta-analysis reports in India revealed the prevalence of 41-46% subclinical mastitis (SCM) and 23-27% clinical mastitis in dairy animals (Bangar *et al.*, 2015; Krishnamoorthy *et al.*, 2017). Therefore, antimicrobials usage (AMU) for preventive or therapeutic purposes becomes inevitable tool in mastitis control programme. Globally,

mastitis is the primary reason for AMU in dairy animals and 60% of the antimicrobials were used for mastitis-affected animals under smallholder production system in Southern India (Pol and Reugg, 2007; Sharma *et al.*, 2022). Despite of more AMU in mastitis, the success rate remains less and cause antibiotic residue violation in milk which, not only affects human health but also affects dairy processing sector by interfering with starter culture (Nickerson and Ryman, 2019; Sachi *et al.*, 2019). An inappropriate AMU in dairy animals is one of the driving forces for antimicrobial resistance (AMR) development among animal and human pathogens. Isolation and identification of antibiotic-resistant pathogens from mastitis-affected animals are important evidence for such pathogen defence phenomenon (Al-harbi *et al.*, 2021).

Reduction of AMU and alternatives to antibiotics are important strategies to tackle AMR. The demand for alternative to antibiotics is also higher in organic dairy farming, where the udder health management is very challenging due to strict prohibition of AMU (Zwald *et al.*, 2004). In mastitis, alternative therapies would be at

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least useful for non-severe cases, in which antibiotics may not be required (Ruegg and Erskine, 2015). Among the various alternatives, the application of herbal therapy in veterinary medicines got important attention due to several advantages such as several pharmacological properties of phytochemicals, past history of antimicrobials development from herbal sources, traditional knowledge, and practice of using herbal plants, diverse chemical structures and mechanisms of action of plant molecules and increasing consumer demand for natural products. *C. longa*, *Aloe vera*, *Ocimum sanctum*, *Tinospora cordifolia* and *Terminalia chebula* are important herbal plants used for treating mastitis in India and other countries (Dilshad *et al.*, 2010; Punniamurthy *et al.*, 2017). *C. longa* and curcumin possess multiple pharmacological properties such as anticancer, anti-inflammatory, antioxidant and antimicrobial effects, which are essential for bovine mastitis management (Fuloria *et al.*, 2022). However, controlled clinical studies to proof their efficacy against mastitis are lacking in India. Medicinal plant extract contains several bioactive compounds, which acts synergistically to provide maximum therapeutic potential. Combined action of multiple bioactive compounds on multiple targets or pathways could be the reason for additive or synergistic effects of extract. Studies also shows a better effects plant extract than an equivalent dose of individual bioactive compound and it could be due to absence of interacting compounds present in the extract (Abreu *et al.*, 2012). On the other hand, recent finding suggests that only few bioactive compounds exert majority of the bioactivities. For example, more than 235 compounds identified in *C. longa* rhizome and curcuminoids (curcumin, demethoxycurcumin and bis-demethoxycurcumin) are major phytochemicals responsible for its functional properties. Curcumin in turmeric rhizome is major bioactive compound accountable for its therapeutic potential (Li *et al.*, 2021). Phytoconstituents and bioactivities are varying with varieties of same species and depends on climate, agronomical practices, and extraction methods (Ivanović *et al.*, 2021). The available studies were mostly focused on isolated way, to understand the beneficial effects of few bioactive compounds or whole extracts (Vaou *et al.*, 2022). Role of specific bioactive compound on overall beneficial effects of plant extract is not completely understood (Eloff, 2004). Therefore, comparative studies are required to investigate the effects of whole extract and bioactive compound for its application in bovine mastitis. We hypothesized that highly potent curcumin has equal functional properties with multiple bioactive compounds-mediated effects of *C. longa* rhizome extract. Accordingly, the study aimed for comparative evaluation of phytochemical contents and *in vitro* functional properties of *C. longa* rhizome extract and curcumin.

2. Materials and Methods

2.1 Procurement of plant material, authentication, and powder preparation

C. longa rhizomes were procured from the Thornavavi Village, Perundurai Taluk of Erode District (Tamil Nadu, India) and washed properly using tap water. Rhizomes were cut into smaller pieces and dried completely for 10-15 days under shade. Then, dried rhizomes pieces were grounded to coarse powder form by mixer grinder and stored in airtight containers. For each 500 g of dried turmeric rhizomes, about 495-496 g of turmeric powder was obtained. The botanical authenticity of *Curcuma longa* L. rhizome was done at Central Ayurveda Research Institute with Herbarium Number of

RRCBI-mus 277 and commercially available curcumin (M/s Sigma; 95% purity) was purchased for this study purposes.

2.2 Preparation of turmeric extracts, solvent evaporation and estimation of extract yield

Alcoholic extracts using ethanol, methanol was prepared by Soxhlet extraction method, and it took about 26-27 h for complete extraction. Extracts were collected in beakers and stored at 4°C for further use. Solvents were evaporated using rotary evaporator (Rotavapor R-300/100; M/s Buchi) and complete evaporation was done in 30-35 min for 150-160 ml of extracts. About 12 g of turmeric powder was dissolved in 150 ml of distilled water for preparation of aqueous extract using magnetic stirrer. Solution was kept at 40°C and 700-720 rpm for 24 h. The extract was filtered using Whatman filter paper and stored at 4°C for further use. The yield of alcoholic extracts was calculated by using the following formula:

Yield of extract (%) = [Final weight of the dried extract/Initial weight of the powder] × 100

2.3 Phytochemical screening of turmeric rhizome alcoholic extracts

The qualitative screening of phytochemicals such as alkaloids, saponin tannin, hydrolysable tannin, phlobatannin, phenols, flavonoids, steroids, terpenoids and glycosides in alcoholic extracts (in triplicate samples for each assay) were done using standard procedures.

2.3.1 Estimation of total phenol content (TPC) and total flavonoid content (TFC) in curcumin and *C. longa* rhizome extracts

The TPC in *C. longa* rhizome extracts and curcumin was estimated as per Herald *et al.* (2012) using Folin-Ciocalteu Reagent (FCR) which contains sodium molybdate and sodium tungstate. In this method, presence of phenolic compound in test material (*i.e.*, turmeric extracts and curcumin) cause reduction of FCR and forms molybdenum-tungsten blue colour complex, which can be measured using microplate reader. Phenolic compounds are reducing agent and thus when it reacts with redox reagents like FCR, it forms blue color complex by electron transfer reaction. TFC of ethanolic and methanolic extract of *C. longa* extracts and curcumin was estimated through aluminium chloride method using ELISA plate reader as per Herald *et al.* (2012). The TPC and TFC were estimated in extracts and curcumin using gallic acid as standard and expressed as mg of gallic acid equivalent per g of extracts or curcumin. TPC and TFC were estimated in three independent experiments in triplicates of each sample at different concentration of 50 to 300 µg/ml. A mixture of sample and solvents (ethanol and methanol) were used as control while solvent alone was used for blank.

2.3.2 Evaluation of *in vitro* antioxidant and anti-inflammatory activities of *C. longa* rhizome extracts and curcumin

Antioxidant activity was evaluated using DPPH free radical scavenging assay as per Moore and Yu (2007) using Trolox as the standard. Briefly, blank (200 µl ethanol or methanol solvent), control (100 µl solvent and 100 µl 0.2 mM DPPH) and standard (100 µl at different concentration of trolox at 7-42 µM and 100 µl DPPH) or samples (100 µl sample at different concentration of 50 to 300 µg/ml and 100 µl DPPH) were taken in triplicate in microplates. The reaction mixture

was gently shaken for 5 min, incubated for one h at 37°C and the absorbance was measured at 517 nm using microplate reader (Multiscan GO; M/s Thermo Fisher Scientific Co., USA).

Anti-inflammatory assay was done according to Khatun *et al.* (2021) using diclofenac sodium as standard. Briefly, 100 µl solvent (ethanol or methanol) as a blank, bovine serum albumin (BSA) with solvent as control, BSA with different concentration of standard or test samples (50-300 µg/ml) were added in respective wells. The reaction mixture was mixed properly and incubated for 20 min at 37°C. Then, the reaction mixture was kept in water bath at 70°C for 10 min and cooled down subsequently at room temperature for 20 min. The absorbance was taken at 660 nm using microplate reader (Multiscan GO; M/s Thermo Fisher Scientific Co., USA) and the percentage of inhibition of protein denaturation was calculated by using the following formula. The percentage of DPPH radical scavenging or BSA denaturation inhibition was measured at different concentrations and IC₅₀ was calculated by interpolating the concentration corresponding to 50% inhibition or scavenging using MS Excel.

% inhibition = [(Absorbance of control – Absorbance of sample)/ absorbance of control] × 100

2.3.3 Evaluation of antibacterial activities of *C. longa* rhizome extracts and curcumin

The antibacterial activity of samples were evaluated through disc diffusion method using Mueller-Hinton Agar (MHA). Initially, milk samples were collected aseptically from SCM affected cows (milk SCC of ≥ 200,000 cells/ml) and inoculated (10 µl by spread plating method) in selective media for *Staphylococcus* spp. and *Coliform* (*i.e.*, Mannitol salt agar and MacConkey agar, respectively) and incubated at 37°C for 24 h. Single colony of *Staphylococcus* spp. and *Coliform* positive samples were re-inoculated in selective media (twice) for preparation of pure culture and subsequently used for disc-diffusion assay. The DNA was extracted from these pure cultures using Quick-DNA™ Fungal/Bacterial Miniprep Kit (M/s Zymo Research Corp, USA) as per manufacturer's recommendation. Based

on the similarity (%) and completeness (%), it was identified the strain used for the disc-diffusion assay as *Staphylococcus epidermidis* and *Escherichia fergusonii* (94.6% similarities and 94.5% completeness), respectively, through 16s rRNA method. In this study, amoxicillin + clavulanic acid (20/10 µg) and gentamicin (10 µg) were used as positive control for *S. epidermidis* and *E. fergusonii*, respectively. Ethanol and methanol were used as blank control. The zone of inhibition of these drugs with ≥ 1.6 and ≥ 1.3 cm, respectively, were considered as sensitive and test samples (extracts and curcumin) with zone of inhibition, similar to positive control was considered as sensitive to these isolates. Assay was performed four times against each Gram-positive and Gram-negative isolates. All chemicals used in this culture study were purchased from M/s Himedia, Mumbai (India).

2.4 Statistical analysis

Data related to TPC, TFC, antioxidant and anti-inflammatory activities were analysed by univariate method considering test compounds, concentrations and their interactions as factors. Tukey post hoc test was used to understand the significant difference between groups. All the data were analysed by SPSS software (version 26.0.0.0) and *p* value ≤ 0.05 was considered as statically significant.

3. Results

3.1 Extraction yield and qualitative detection of phytochemicals in turmeric rhizome extracts

In this study, extraction of turmeric rhizome powder through Soxhlet apparatus provided slightly more extraction yield by ethanol (16%; range: 13 to 19% in 8 batches) than methanol (15% (Range: 14-17% in 3 batches) as a solvent. Qualitative detection of phytochemicals in *C. longa* rhizome extracts revealed the presence of alkaloids, phenol, flavonoids, tannin, saponin, terpenoids, flavonoids and glycosides in methanolic extract. Except saponin, all the other phytochemicals were present in ethanolic extract while aqueous extract had only alkaloids, phenol, flavonoids and tannin group of phytochemicals (Table 1).

Table 1: Phytochemical screening of *C. longa* rhizome alcoholic and aqueous extracts

S. No.	Phytochemical constituents	Ethanol (80%)	Methanol (80%)	Aqueous
1	Alkaloids	+	+	+
2	Saponin	-	+	-
3	Tannin	+	+	+
4	Phenol	+	+	+
5	Terpenoids	+	+	-
6	Flavonoids	+	+	+
7	Phlobatanin	-	-	-
8	Hydrolysable tannin	+	+	+
9	Glycosides	+	+	-
10	Cardiac glycosides	-	+	-

Each test was performed in triplicates. + indicates presence and – indicates absence of phytochemicals.

3.2 Total phenol content and total flavonoid content in turmeric rhizome extracts and curcumin

In this study, the overall TPC was higher in curcumin (518-637 mg GAE/g sample) than alcoholic turmeric rhizome extracts (264-347 mg GAE/g sample). Curcumin dissolved in ethanol had highest TPC, followed by curcumin dissolved in methanol and alcoholic turmeric

rhizome extracts (Figure 1a). Both curcumin and turmeric rhizome extracts were shown significantly ($p=0.001$) concentration-dependent increase of TPC (Figure 1b). Similar to TPC, TFC was also higher in curcumin (6779 -12879 mg GAE/g sample) than alcoholic turmeric extracts (1247-6072 mg GAE/g sample; Figure 2a) and both curcumin and extracts shown concentration dependent increase ($p=0.001$) of TFC (Figure 2b).(1b)

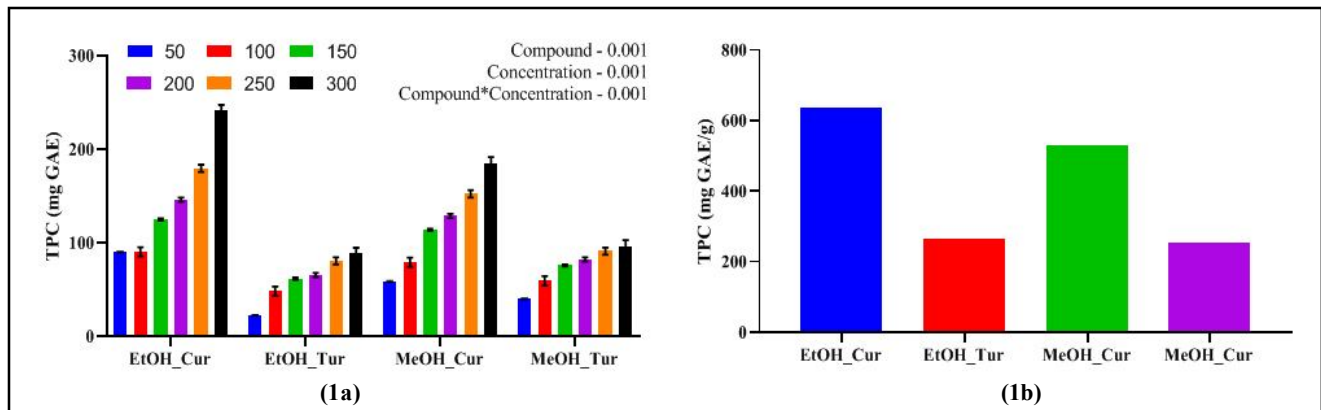


Figure 1: Total phenol content (TPC) in extracts and curcumin expressed as mg GAE (Figure 1a) and mg GAE/g sample (Figure 1b). EtOH_Cur and MeOH_Cur means curcumin dissolved in ethanol and methanol, respectively. EtOH_Tur and MeOH_Tur means ethanolic and methanolic extracts, respectively. $p \geq 0.05$ is significant.

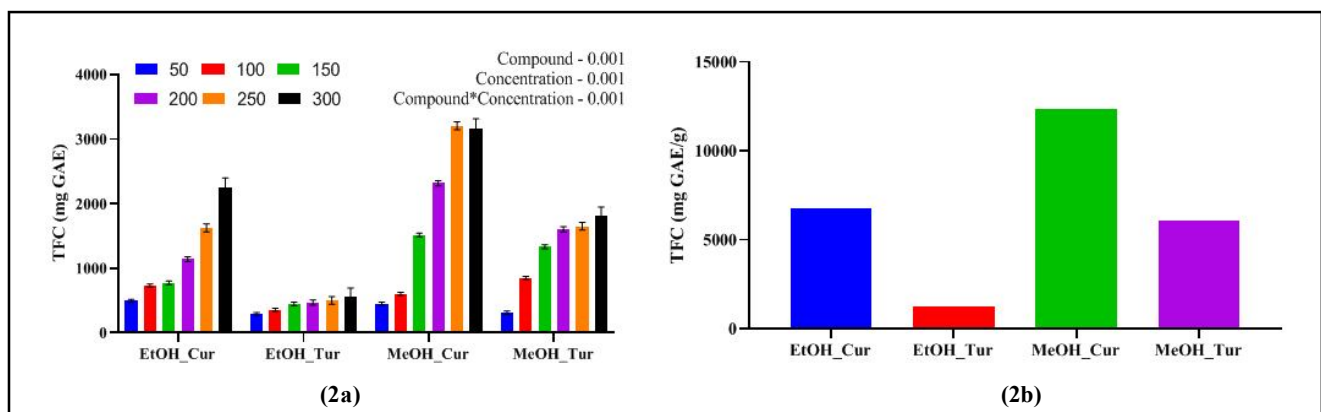


Figure 2: Total flavonoid content (TFC) in extracts and curcumin expressed as mg GAE (Figure 2a) and mg GAE/g sample (Figure 2b). EtOH_Cur and MeOH_Cur means curcumin dissolved in ethanol and methanol respectively, EtOH_Tur and MeOH_Tur means ethanolic and methanolic extracts, respectively. $p \geq 0.05$ is significant.

3.3 In vitro antioxidant and anti-inflammatory activities of turmeric rhizome extracts and curcumin

In this study, both curcumin and turmeric extracts were not shown any significant ($p=0.25$) concentration-dependent changes in their antioxidant activity (Figure 3a). The IC_{50} values of test compounds revealed higher potency of curcumin (dissolved in methanol) than alcoholic turmeric extracts (Figure 3b). Similarly, curcumin showed better anti-inflammatory activity than the alcoholic extracts (Figure 4a) and both curcumin and turmeric extracts shown significantly ($p = 0.001$) concentration-dependent increase in their anti-inflammatory activity (Figure 4b). In terms of IC_{50} values, the curcumin dissolved in ethanol was found to be four times more potent than ethanolic extract of turmeric rhizome (57 Vs 208 μ g/ml inhibition).

3.4 Antibacterial activity of turmeric rhizome extracts and curcumin

The results of antimicrobial activities of turmeric rhizome alcoholic extracts and curcumin against *S. epidermidis* are presented in Table 2. In this study, positive control of amoxicillin and clavulanic acid was shown as sensitive against *S. epidermidis* isolated from SCM mastitis affected cows. In comparison to positive control, the equivalent potency in terms of zone of inhibition were observed with the highest concentration of ethanolic and methanolic extracts (20 mg/ml), followed by lower concentration of curcumin (5 mg/ml) and lower concentration of ethanolic extract (5 mg/ml). Although, gentamicin as a positive control shown sensitive against *E. fergusonii* none of the test compounds shown inhibition of *E. fergusonii* growth in disc-diffusion method (data not presented).

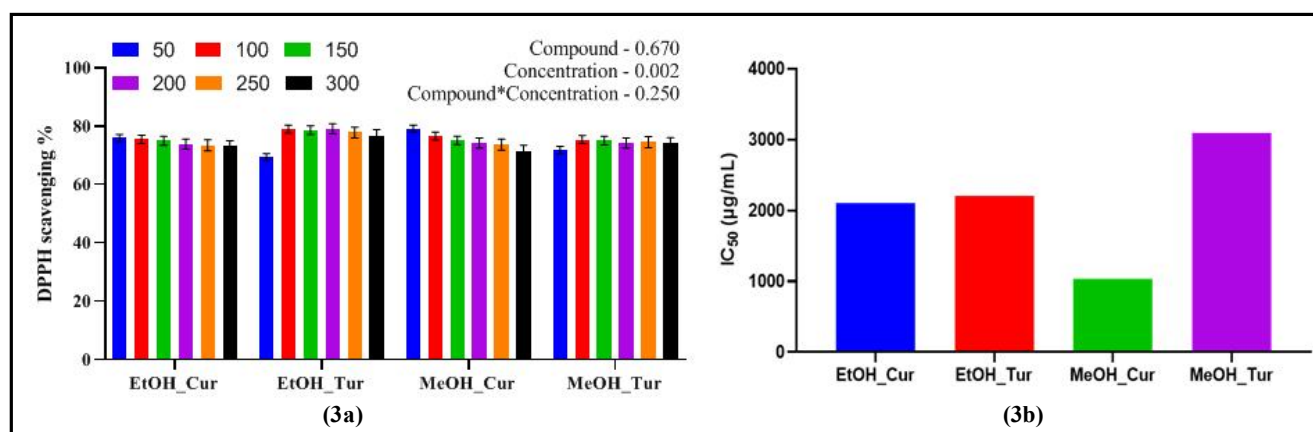


Figure 3: Antioxidant activities of extracts and curcumin at different concentration (Figure 3a) and its IC₅₀ values (Figure 3b). EtOH_Cur and MeOH_Cur means curcumin dissolved in ethanol and methanol, respectively, EtOH_Tur and MeOH_Tur means ethanolic and methanolic extracts, respectively. $p \geq 0.05$ is significant.

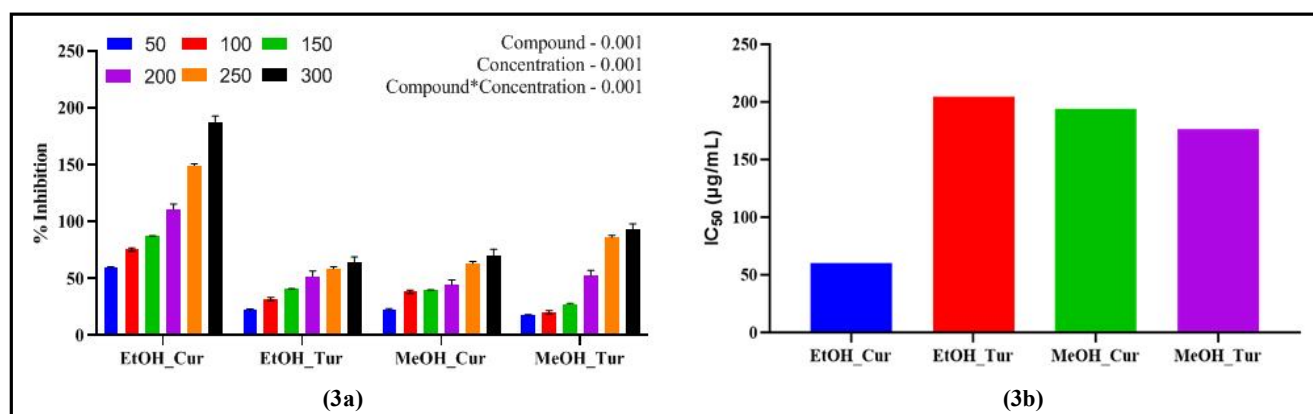


Figure 4: Anti-inflammatory activities of extracts and curcumin at different concentration (Figure 4a) and its IC₅₀ values (Figure 4b). EtOH_Cur and MeOH_Cur means curcumin dissolved in ethanol and methanol, respectively, EtOH_Tur and MeOH_Tur means ethanolic and methanolic extracts, respectively. $p \geq 0.05$ is significant.

Table 2: Antibacterial activity of *C. longa* rhizome alcoholic extracts and curcumin by disc diffusion method

S. No.	Sample	Zone of inhibition (cm) against <i>S. epidermidis</i>	Range (cm)
1	Amoxicillin + Clavulinic acid	1.78 ± 0.09	1.6-2.0
2	Ethanol	Nil	
3	Methanol	Nil	
4	Curcumin dissolved in ethanol (5 mg/ml)	1.48 ± 0.08	1.3-1.6
5	Curcumin dissolved in ethanol (10 mg/ml)	1.15 ± 0.03	1.1-1.2
6	Curcumin dissolved in ethanol (20 mg/ml)	1.13 ± 0.03	1.1-1.2
7	Curcumin dissolved in methanol (5 mg/ml)	1.20 ± 0.04	1.1-1.3
8	Curcumin dissolved in methanol (10 mg/ml)	Nil	
9	Curcumin dissolved in methanol (20 mg/ml)	Nil	
10	Ethanolic extract (5 mg/ml)	1.43 ± 0.14	1.2-1.8
11	Ethanolic extract (10 mg/ml)	1.18 ± 0.03	1.1-1.2
12	Ethanolic extract (20 mg/ml)	1.68 ± 0.19	1.3-2.2
13	Methanolic extract (5 mg/ml)	1.25 ± 0.06	1.1-1.4
14	Methanolic extract (10 mg/ml)	1.13 ± 0.05	1.0-1.2
15	Methanolic extract (20 mg/ml)	1.55 ± 0.06	1.4-1.7

Values are expressed as Mean ± SE of four samples each. Amoxicillin + Clavulinic acid of 30 µg (20/10 µg; M/s Himedia ABST disc) was used as positive control.

4. Discussion

4.1 Extraction yield and qualitative phytochemical screening of turmeric rhizome alcoholic extracts

The yield (%) observed in this study is also obtained by several researchers and they reported better yield of extract by ethanol than methanol (Sawant and Godghate, 2013; Do *et al.*, 2014; Sabir *et al.*, 2021). Several researchers reported that ethanol is most appropriate solvent for extraction of phytochemicals from *Curcuma* species (Arawande *et al.*, 2018; Ivanovi'c *et al.*, 2021). Ethanol is also more desirable solvent for extraction of phenol and other phytochemicals possessing antimicrobial properties and Soxhlet extraction method is superior to other methods for extraction of these compounds (Do *et al.*, 2014; Tanviret *et al.*, 2017). Similar to the present findings, presence of phytochemicals in ethanolic and methanolic extracts were also reported in several studies (Sawant and Godghate, 2013; Mehra and Jain, 2019; Maithilikarpagaselvi *et al.*, 2020). They also reported the absence of saponin in ethanolic extract and saponin and terpenoids in aqueous extract as also observed in this study (Abbasi and Shah, 2015; Deb *et al.*, 2013). It is observed greater number of phytochemicals in methanolic extract (*i.e.*, 7 of 7 phytochemicals tested), ethanolic (6 of 7) than aqueous (4 of 7) extracts. It could be due to inherent property of higher dielectric constant of methanol to extract most of the phytochemicals (Do *et al.*, 2014). Sawant and Godghate (2013) reported better yield by ethanol compare to methanol but, found a greater number of phytochemicals in methanol than ethanol as observed in this study.

4.2 Total phenol and flavonoid content in turmeric rhizome alcoholic extracts and curcumin

It is observed that curcumin dissolved in ethanol had higher TPC and it could be due to more potency of ethanol to extract phenols compounds from Zingiberaceae family plants including *C. longa* (Ivanovi'c *et al.*, 2021; Array *et al.*, 2018). Nisar *et al.* (2015) found more phenolic content in ethanolic than methanolic extract and found similar antioxidant activity as also observed in this study. Ezez and Tefera (2021) also reported significant effects of solvent on TPC in ginger. Similar to this finding, Dodamani (2025) reported significantly higher phenolic content in curcumin than turmeric extract in terms of TPC and TFC. They also reported higher TFC than TPC in both alcoholic extracts and curcumin as observed in this study. This supports the existing knowledge of turmeric as good source of natural flavonoids (Tanvir *et al.*, 2017).

4.3 *In vitro* functional properties of turmeric rhizome alcoholic extracts and curcumin

Although, solvent showed significant effects on TPC and TFC, we did not find any significant effects on antioxidant activities. The range of antioxidant activities across the different concentrations remain similar between turmeric extracts and curcumin. The observed IC_{50} values are also in similar range for ethanolic extracts and curcumin. Similarly, several studies reported lack of any significant effect of solvent on antioxidant activity of turmeric extract (Nisar *et al.*, 2015; Dodamani, 2025). Although, TPC in curcumin was higher than extracts, the equal antioxidant potential of ethanolic extract with curcumin (as per observed similar range of IC_{50}) could be due to multiple bioactive compounds-mediated synergistic effects of extract. In this study, both curcumin and alcoholic extracts were shown concentration-dependent increase of anti-inflammatory activities.

Similarly, several studies reported anti-inflammatory activities of turmeric extract (Anwar *et al.*, 2022) and curcumin (Asif *et al.*, 2023). The observed trend of IC_{50} values indicates that, curcumin is the most important phenolic compound responsible for anti-inflammatory activities and with higher potency. The observed trend of antioxidant and anti-inflammatory activities of extract and curcumin also confirms the influence of solvent on bioactivities (Maithilikarpagaselvi *et al.*, 2020).

S. epidermidis is most commonly involved pathogen in SCM (Reugg and Erskine, 2015). Although, the zone of inhibition by curcumin was lesser than ethanolic extract, the ability of curcumin to inhibit at its lower concentration indicates its higher potential. The observed lower or no zone of inhibition with higher concentration of curcumin could be due to its lesser solubility and difficulty in diffusion through agar medium. Therefore, the observed zone of inhibition may not be the true antimicrobial potential of curcumin. Studies also suggested that the disc diffusion method is not as sensitive as the microdilution method for detection of antimicrobial activities of extract or phytochemicals at lower concentration (Hossain 2024). In this study, based on 16s rRNA method, the species of *Coliform* was identified as *E. fergusonii*, an environmental pathogen similar to *E. coli*. Studies also reported the association of *E. fergusonii* with SCM affected cows (Al-Haddadi *et al.*, 2020). In this study, both turmeric extracts and curcumin were not shown inhibition of *E. fergusonii* growth indicating lack of sensitivity against *E. fergusonii* or *Coliform*. Several studies indicated that turmeric rhizome extract inhibited the growth of several Gram-positive organism like *Staphylococcus* spp, *Streptococcus* spp., but shown lesser or no inhibition against *E. coli* (Inchaisri *et al.*, 2005; Bhatt *et al.*, 2013). Other studies indicated that curcumin and ethanolic extract had higher sensitivity against Gram-positive than Gram-negative bacteria (Adameczak *et al.*, 2020; Muhamed *et al.*, 2019). Sedky *et al.* (2022) observed significant antibacterial activity against *Staphylococcus aureus*, *Streptococcus agalactiae* with 20 mg and 50 mg/ml concentrations as also used in our study and indicated that they can be used in bovine clinical mastitis. Mansouri *et al.* (2021) evaluated the antimicrobials activities of alcoholic extracts (20 mg/ml) against *Staphylococcus* spp. and found better activity by ethanolic than methanolic extracts as also observed in this study with similar concentration. Several studies reported the antimicrobials properties of ethanolic and methanolic turmeric rhizome extracts against *S. epidermidis* as observed in this study (Ungphaiboon *et al.*, 2005; Lawhavinit *et al.*, 2010). In general, outer membrane in Gram-negative bacteria acts barrier and permits only small hydrophilic molecules inside the cells and exclude the larger molecules. In addition, they also have membrane transport proteins which efflux some of antibacterial compounds across cell membrane (Marriott, 2010).

Based on the phytoconstituents and *in vitro* functional properties of extract and curcumin, it is evident that TPC and TFC are more and concentration-dependent in curcumin than alcoholic extracts. The observed concentration-dependent increase of anti-inflammatory properties in curcumin than extracts very well substantiate the above findings. The observed better antioxidant and antimicrobial properties of ethanolic extract at higher concentration indicates the possible synergistic action of many bioactive compounds present in extracts than curcumin alone. For example, turmeric rhizome contains 235 compounds of which 207 (88%) were identified as phenolic compounds and these compounds have significant activity on

immune system. The anti-inflammatory and antioxidant effects of turmeric and curcumin are primarily mediated through modulation of NF- κ B and Nrf-2 pathways (Yuandani *et al.*, 2021; Yusuf *et al.*, 2023). A part from functional property, ethanolic extract is also superior in safety point of view as ethanol solvent-based extract may be suitable for animal's application and generally regarded as safe for consumption. However, further *in vivo* studies in laboratory animal models to validate the functional properties, solvent optimization and evaluation of antimicrobial activity through more suitable method against diversified pathogens are required before application in bovine mastitis. Development of suitable formulation and evaluation of its safety and efficacy in target animals is also required for its further applications.

5. Conclusion

It is concluded that curcumin exhibited higher phenolic content and superior *in vitro* antioxidant, anti-inflammatory, and antibacterial activities compared to *C. longa* extracts. Further *in vivo* and clinical studies are needed to validate these findings for mastitis management.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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