Tamarind (*Tamarindus indica*) Concentrate as a Natural Antimicrobial and Insecticidal Agent

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Abstract

Tamarind (*Tamarindus indica* L.) is a multipurpose tropical tree of the family Fabaceae, native to tropical Africa and widely naturalized across Asia and Latin America. Tamarind concentrate is a yellow brown to tan paste with a distinctive aroma, obtained by solvent extraction and characterized by the presence of furfural, 5-hydroxymethylfurfural, ethyl oleate, ethyl palmitate, palmitic acid, and elaidic acid. These constituents contribute to its bioactive properties, which have attracted increasing attention for natural antimicrobial and insecticidal applications. The aim of this study was to evaluate the antimicrobial activity of tamarind concentrate against selected bacterial strains and its insecticidal activity against two beetle species infesting stored products. Antimicrobial activity was determined using the disc diffusion method and by assessing the minimum inhibitory concentration (MIC_{50} and MIC_{90}) against six bacterial strains: *Bacillus cereus* CCM 2010, *Enterobacter aerogenes* CCM 2531, *Klebsiella pneumoniae* CCM 2318, *Listeria innocua* CCM 4030, *Staphylococcus epidermidis* CCM 4418, and *Citrobacter koseri* CCM 2535. An antibiotic disc (ABT) was used as a positive control. Insecticidal activity was evaluated against *Callosobruchus maculatus* and *Megabruchidius dorsalis* at concentrations ranging from 3.125 % to 100 %. Disc diffusion results revealed inhibition zones ranging from 9.33 to 14.33 mm, with the strongest activity observed against *B. cereus* (14.33 mm) and *L. innocua* (13.67 mm). MIC_{50} values ranged from 0.770 mg/mL (*S. epidermidis*) to 2.625 mg/mL (*C. koseri*), while MIC_{90} values ranged from 0.779 mg/mL (*L. innocua*) to 3.757 mg/mL (*E. aerogenes*). At the highest concentration (100 %), insecticidal activity reached 92.67 % mortality for *C. maculatus* and 88.67 % for *M. dorsalis*. Tamarind concentrate demonstrated noteworthy antimicrobial activity, particularly against Gram-positive bacteria, along with considerable insecticidal effects against both tested beetle species. These findings underscore its potential as a plant-derived bioactive material for food protection applications.

Keywords: *Tamarindus indica*; plant-derived compounds; antibacterial properties; grain pests; eco-friendly insecticide; post-harvest protection.

1. Introduction

Tamarindus indica L. is a long-lived, semi-evergreen tree belonging to the family Fabaceae, native to the dry savanna regions of tropical Africa, most likely originating from Sudan, and subsequently introduced into South Asia through early human migration [1]. Today, it is widely cultivated across the tropical belt, including India, Southeast Asia, Latin America, and the Caribbean, with India remaining the world's largest producer. The fruit is characterised by a brown, brittle pod containing a dark-brown, acidic pulp surrounding hard seeds. This pulp is one of the most widely used natural souring agents in Asian and Latin American cuisines, incorporated into chutneys, curries, beverages, confectionery, and preserved products. In addition to its culinary importance,

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tamarind holds a significant role in traditional medicine across Africa, Asia, and the Americas, where different plant parts have been used for the treatment of fever, gastrointestinal disorders, inflammatory conditions, snake bites, and as antiseptic agents [2].

The phytochemical composition of *Tamarindus indica* is complex and varies among plant tissues. The fruit pulp is particularly rich in organic acids primarily tartaric, citric, malic, and succinic acids which contribute to its pronounced acidity and may exert antimicrobial effects through local pH reduction [3,4]. Phenolic compounds, including procyanidins, catechin, epicatechin, and various phenolic acids, have been identified in the pulp, pericarp, and seeds [5,6]. Gas chromatography–mass spectrometry (GC-MS) analyses of methanolic extracts have identified 5-hydroxymethylfurfural (5-HMF) as one of the dominant volatile components. This furan derivative, formed during thermal degradation of hexose sugars, has demonstrated antibiofilm and antibacterial activity [7,8]. Additional volatile constituents reported in tamarind extracts include furfural, α-pinene, β-pinene, myrcene, linalool, and carvacrol, which collectively contribute to the fumigant insecticidal potential of the material [1]. Furthermore, fatty acid esters such as ethyl oleate and ethyl palmitate, characteristic of concentrate-type extracts, are amphiphilic molecules capable of interacting with bacterial membranes and potentially enhancing antimicrobial effects [9,10].

The antimicrobial activity of *Tamarindus indica* has been investigated in several studies using different extraction approaches. Ghaly et al. [11] demonstrated that ethanolic extracts exhibited inhibitory activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and reported synergistic interactions with conventional antibiotics. The proposed mechanism involved disruption of cell membrane integrity leading to bacterial lysis, as confirmed by scanning electron microscopy. Fagbem et al. [7] reported antibacterial activity of methanolic fruit pulp extracts, with *Plesiomonas shigelloides* identified as the most susceptible strain (MIC = 0.22 mg/mL), whereas *Bacillus cereus* and *Klebsiella pneumoniae* required higher concentrations (MIC = 3.51 mg/mL). Similarly, Bonin et al. [12] demonstrated strong antibacterial activity of tamarind seed extracts against Gram-positive bacteria, including *S. aureus* and *B. cereus*, at MIC values of 62.5 and 125 µg/mL, respectively. Despite these findings, no study has yet evaluated tamarind concentrate the full-spectrum aromatic extract obtained by non-polar solvent extraction for antimicrobial or insecticidal activity under standardised laboratory conditions.

Postharvest losses caused by bruchid beetles in stored leguminous commodities remain a major global challenge, particularly in tropical and subtropical regions with limited storage infrastructure. *C. maculatus* (Fabricius), the cowpea weevil, is capable of destroying more than 90 % of stored grain within a few months under favourable conditions [13]. *Megabruchidius dorsalis* (Fahraeus), originally native to East Asia, has expanded its distribution to Europe and North America and infests seeds of *Gleditsia triacanthos* and other legumes [14,15]. The extensive use of synthetic fumigants such as phosphine and methyl bromide has led to increasing resistance, environmental concerns, and regulatory restrictions, highlighting the need for alternative plant-based solutions [16]. Furfural, a key component of tamarind-derived products, has been reported as an effective fumigant against stored-product insects, including bruchids, *Tribolium castaneum*, and *Sitophilus* spp., acting primarily through inhibition of respiratory enzymes and disruption of nervous system function [17].

Based on this background, the aim of the present study was to evaluate the antimicrobial and fumigant insecticidal activity of tamarind (*Tamarindus indica*) concentrate against six bacterial strains relevant to food safety and two species of stored-product bruchid beetles. Particular attention was given to the relationship between the semi-solid physicochemical nature of tamarind concentrate and its biologically active volatile fraction responsible for fumigant activity, as well as to its positioning within a broader comparative series of plant-derived aromatic extracts.

2. Materials and methods

2.1 Plant material

Tamarind concentrate derived from the fruit of *Tamarindus indica* L. was supplied by GuiLin Four Seasons Sunshine Co., Ltd. (Guilin, Guangxi, China). The product was obtained by

hydrocarbon solvent extraction of tamarind fruit material, followed by complete removal of the solvent under reduced pressure. The resulting extract was a dense, tan to yellow–brown paste with a characteristic sweet–sour aroma and markedly higher viscosity compared to other concentrates evaluated in this study series. This high viscosity reflects the substantial content of co-extracted fatty acid esters and waxes alongside the volatile aromatic fraction. The concentrate was stored in sealed amber glass vials at 4 °C in the dark until use.

2.2 Bacterial strains

Six bacterial strains obtained from the Czech Collection of Microorganisms (CCM, Brno, Czech Republic) were used: *Bacillus cereus* CCM 2010, *Enterobacter aerogenes* CCM 2531, *Klebsiella pneumoniae* CCM 2318, *Listeria innocua* CCM 4030, *Staphylococcus epidermidis* CCM 4418, and *Citrobacter koseri* CCM 2535.

Working cultures were prepared by two successive subcultures in Mueller–Hinton broth (MHB; Oxoid, Basingstoke, UK) at 37 °C for 24 h. Bacterial suspensions were standardised to the 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL) using a digital densitometer prior to all experiments [18].

2.3 Disc diffusion assay

Preliminary antimicrobial activity was evaluated using the agar disc diffusion method according to Kačániová et al. [19]. Mueller–Hinton agar (MHA; Oxoid) plates were uniformly inoculated with bacterial suspensions using triple-direction swabbing.

Sterile filter paper discs (6 mm diameter; Whatman) were impregnated with 10 µL of tamarind concentrate solution prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and placed on the agar surface. After a 10 min pre-diffusion period at room temperature, plates were incubated aerobically at 37 °C for 24 h.

Inhibition zone diameters (including the disc) were measured to the nearest 0.5 mm using a digital calliper. Antibiotic discs (ABT; Oxoid) were used as positive controls, while DMSO-loaded discs served as negative controls. Each strain was tested in triplicate, and results are expressed as mean ± standard deviation (SD).

2.4 Broth microdilution and MIC_{50}/MIC_{90} determination

Minimum inhibitory concentrations were determined using the broth microdilution method in sterile 96-well flat-bottom microplates (Nunc), following Kačániová et al. [20]. Tamarind concentrate was dissolved in DMSO and serially diluted two-fold in Mueller–Hinton broth to obtain concentrations ranging from 10 mg/mL to 0.0098 mg/mL.

Each well contained 50 µL of diluted extract and 50 µL of bacterial suspension adjusted to the 0.5 McFarland standard, resulting in a total volume of 100 µL. Growth controls (inoculated broth without extract) and sterility controls (broth with extract but without inoculum) were included on each plate. Plates were sealed with adhesive film and incubated at 37 °C for 24 h.

Optical density was measured at 600 nm using a GloMax® microplate reader (Promega, Madison, WI, USA), and percentage growth inhibition relative to the control was calculated. MIC_{50} and MIC_{90} values were derived from concentration–response data using probit regression analysis in JMP Student Edition 18.0 (SAS Institute, Cary, NC, USA). All assays were performed in triplicate.

2.5 Fumigant insecticidal bioassay

Fumigant insecticidal activity of tamarind concentrate vapours was evaluated against adult *Callosobruchus maculatus* and *Megabruchidius dorsalis* using a closed vapour-phase bioassay. Both species were reared on mung bean (*Vigna radiata*) seeds at 27 ± 1 °C and 65 ± 5 % relative humidity, and adults aged 1-7 days post-emergence were used for testing.

Six concentrations (100 %, 50 %, 25 %, 12.5 %, 6.25 %, and 3.125 %; v/v in acetone) were prepared. A 10 µL aliquot of each dilution was applied to a circular filter paper disc (9 cm diameter; Whatman No. 1) attached to the inner side of a Petri dish lid. Ten adult insects were placed in the base of each 9 cm Petri dish, which was immediately sealed with the treated lid and Parafilm to prevent vapour leakage.

Insects were exposed exclusively to the vapour phase for 24 h at 27 ± 1 °C. After exposure, individuals were examined under a stereomicroscope, and those not responding to gentle stimulation were recorded as dead. Mortality (%) was calculated for each replicate,

with three independent replicates per concentration. Acetone control assays confirmed the absence of solvent-induced mortality.

2.6 Statistical analysis

All data are presented as mean ± standard deviation (SD) of three independent replicates (n = 3). Statistical differences among treatments were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test at a significance level of $p \leq 0.05$, performed in IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). MIC_{50} and MIC_{90} values were calculated by probit regression analysis assuming a log-normal concentration–response relationship.

3. Results and discussion

3.1 Antimicrobial activity - disc diffusion assay

The disc diffusion results for tamarind concentrate are presented in Table 1. Inhibition zones were detected against all six tested bacterial strains, ranging from 9.33 mm (*E. aerogenes*) to 14.33 mm (*B. cereus*). Notably, tamarind concentrate showed the strongest disc diffusion activity against *B. cereus* of all four concentrates and absolutes evaluated in this study series, matching the values recorded for jasmine absolute against the same strain. *L. innocua* was the second most susceptible organism with an inhibition zone of 13.67 mm, followed by *S. epidermidis* (13.33 mm). All three Gram-positive strains produced inhibition zones exceeding 13 mm, placing tamarind concentrate among the more potent extracts against this bacterial group.

Table 1. Disc diffusion antimicrobial activity of tamarind (*Tamarindus indica*) concentrate against selected bacterial strains (Mean ± SD, n = 3).

Bacterial strain	Inhibition zone (mm) Mean ± SD	ABT (mm) Mean ± SD
Bacillus cereus	14.33 ± 0.58^{a}	30.33 ± 0.58^{ab}
Enterobacter aerogenes	9.33 ± 0.58^{b}	28.67 ± 0.58^{c}
Klebsiella pneumoniae	10.67 ± 0.58^{b}	30.67 ± 0.58^{ab}
Listeria innocua	13.67 ± 0.58^{a}	31.67 ± 0.58^{a}
Staphylococcus epidermidis	13.33 ± 0.58^{a}	29.67 ± 0.58^{bc}
Citrobacter koseri	9.67 ± 0.58^{b}	28.33 ± 0.58^{c}

Among Gram-negative organisms, *K. pneumoniae* yielded 10.67 mm, *C. koseri* showed 9.67 mm, and *E. aerogenes* was the least susceptible at 9.33 mm. These values are consistent with the outer membrane barrier characteristic of Gram-negative bacteria, which restricts penetration of hydrophobic aromatic compounds including fatty acid esters and furan derivatives present in tamarind concentrate [21]. Ghaly et al. [11] similarly reported lower antibacterial activity of *T. indica* ethanolic extract against Gram-negative organisms including *Pseudomonas aeruginosa* compared to Gram-positive species, confirming the preferential Gram-positive susceptibility pattern observed here.

The strong activity against *B. cereus* (14.33 mm) may reflect synergistic interactions between multiple bioactive constituents of tamarind concentrate particularly 5-hydroxymethylfurfural, furfural, and amphiphilic fatty acid esters which together may more effectively compromise the cytoplasmic membrane than any individual component alone [22]. It is important to note that tamarind concentrate, despite its markedly dense, paste-like physical consistency, demonstrated disc diffusion activity comparable to or exceeding that of less viscous preparations. This indicates that the volatile bioactive fraction is sufficiently released under assay conditions to produce meaningful inhibition.

3.2 Antimicrobial activity - minimum inhibitory concentrations (MIC_{50} and MIC_{90})

MIC_{50} and MIC_{90} values for tamarind concentrate are presented in Table 2. Unlike the disc diffusion results, where *B. cereus* showed the largest inhibition zone, the broth microdilution assay identified *S. epidermidis* as the most susceptible

strain, with MIC_{50} of 0.770 mg/mL and MIC_{90} of 1.618 mg/mL. This divergence between disc diffusion and broth microdilution rankings is consistent across all four extracts in this study series and reflects the known limitation of the disc diffusion method for hydrophobic and viscous aromatic products, whose higher molecular weight constituents diffuse poorly through agar yet exert stronger inhibitory effects in liquid systems [23].

Table 2. Minimum inhibitory concentration (MIC_{50} and MIC_{90}) of tamarind (*Tamarindus indica*) concentrate against selected bacterial strains (Mean ± SD, n = 3).

Bacterial strain	MIC_{50} (mg/mL) Mean ± SD	MIC_{90} (mg/mL) Mean ± SD
Bacillus cereus	1.455 ± 0.172^{b}	2.533 ± 0.414^{b}
Enterobacter aerogenes	2.625 ± 0.267^{a}	3.757 ± 0.090^{a}
Klebsiella pneumoniae	2.588 ± 0.259^{a}	3.596 ± 0.474^{a}
Listeria innocua	1.266 ± 0.191^{bc}	0.779 ± 0.121^{d}
Staphylococcus epidermidis	0.770 ± 0.125^{c}	1.618 ± 0.164^{c}
Citrobacter koseri	2.625 ± 0.241^{a}	3.728 ± 0.231^{a}

L. innocua showed MIC_{50} of 1.266 mg/mL and MIC_{90} of 0.779 mg/mL. This inversion is atypical and may reflect a steep concentration–response relationship, where a narrow concentration window separates partial and near-complete inhibition. Such behaviour has been observed for plant extracts rich in phenolic or amphiphilic compounds [12]. *B. cereus* showed MIC_{50} of 1.455 ± 0.172 mg/mL and MIC_{90} of 2.533 mg/mL, with the highest variability observed among the tested strains.

Among Gram-negative strains, *Enterobacter aerogenes* and *C. koseri* both showed MIC_{50} values of 2.625 mg/mL respectively, while their MIC_{90} values were 3.757 mg/mL and 3.728 mg/mL. *K. pneumoniae* required MIC_{50} of 2.588 mg/mL and MIC_{90} of 3.596 mg/mL. The similar MIC values across these Gram-negative species suggest a common resistance mechanism related to the outer membrane permeability barrier. The antimicrobial activity of *T. indica* extracts has been attributed to disruption of membrane integrity leading to bacterial lysis, as confirmed by scanning electron microscopy [11].

3.3 Fumigant insecticidal activity

The fumigant insecticidal activity of tamarind concentrate against *Callosobruchus maculatus* and *Megabruchidius dorsalis* across six concentrations is presented in Table 3. Both species showed a clear concentration-dependent increase in mortality. For *C. maculatus*, mortality at 100 % was 92.67 %, followed by 75.67 % at 50 %, 53.33 % at 25 %, 30.67 % at 12.5 %, 22.33 % at 6.25 %, and 8.33 % at 3.125 %. Although slightly lower than values observed for other extracts in this study series, this still represents a high level of fumigant activity. For *M. dorsalis*, mortality at 100 % was 88.67 %, followed by 72.67 % at 50 %, 52.00 % at 25 %, 33.00 % at 12.5 %, 23.33 % at 6.25 %, and 10.33 % at 3.125 %. At several intermediate concentrations, mortality values were comparable to those observed for *C. maculatus*. The slightly higher variability at some concentrations may reflect inter-individual differences in sensitivity.

The observed fumigant activity confirms that even highly viscous concentrate preparations release sufficient volatile compounds to exert biological effects. Tamarind concentrate contains furan derivatives such as furfural and 5-hydroxymethylfurfural, which have documented fumigant insecticidal activity. Furfural has been shown to inhibit mitochondrial respiration and interfere with neural signalling pathways in insects [24, 25], contributing to the observed mortality.

Probit regression analysis yielded LC_{50} values of 19.31 % and 20.26 % for *C. maculatus* and *M. dorsalis*, respectively, and an LC_{90} value of 98.10 % for *C. maculatus*. For *M. dorsalis*, the LC_{90} value of 128.38 % exceeded the tested concentration range, indicating that complete control of this species would require

concentrations beyond those evaluated in the present study.

Table 3. Insecticidal activity of Tamarind (*Tamarindus indica*) concentrate against *C. maculatus* and *M. dorsalis*.

Concentration (%)	*C. maculatus* Mean ± SD (%)	*M. dorsalis* Mean ± SD (%)
100	92.67 ± 3.21[a]	88.67 ± 5.51[a]
50	75.67 ± 2.31[b]	72.67 ± 1.53[b]
25	53.33 ± 4.51[c]	52.00 ± 4.58[c]
12.5	30.67 ± 2.31[d]	33.00 ± 4.58[d]
6.25	22.33 ± 2.52[e]	23.33 ± 6.51[d]
3.125	8.33 ± 0.58[f]	10.33 ± 2.08[e]
LC_{50} (%)	19.21	20.09
LC_{90} (%)	98.10	123.36

3.4 Integrated discussion

Overall, tamarind concentrate demonstrated notable antimicrobial activity combined with strong fumigant insecticidal efficacy. Among the four aromatic extracts evaluated in this study series, tamarind concentrate showed the strongest disc diffusion activity against *Bacillus cereus* (14.33 mm), while its activity against Gram-negative bacteria remained moderate, consistent with the outer membrane permeability barrier characteristic of this bacterial group [13].

The antimicrobial profile of tamarind concentrate is consistent with its complex chemical composition, which includes furan derivatives, amphiphilic fatty acid esters, and phenolic compounds [1,4]. These constituent classes act through complementary mechanisms, including membrane disruption, interference with enzymatic activity, and pH reduction mediated by organic acids, which together may account for the broader antimicrobial spectrum observed [2,6].

From the entomological perspective, tamarind concentrate exhibited strong concentration-dependent fumigant toxicity against both *Callosobruchus maculatus* and *Megabruchidius dorsalis*, despite its markedly dense physical consistency. This confirms that the volatile fraction of the extract is sufficiently released under enclosed vapour-phase conditions to exert significant biological effects. Plant-derived fumigants represent an increasingly important research direction in sustainable postharvest protection, given the growing restrictions on synthetic fumigants and the emergence of resistance [8]. Furfural and 5-hydroxymethylfurfural, identified as key volatile constituents of tamarind concentrate, are known to act through inhibition of respiratory enzymes and disruption of neural signalling in stored-product insects [9,14].

Overall, the combination of antibacterial and insecticidal properties in a single plant-derived preparation is particularly relevant for food protection systems where multiple biological hazards coexist [7]. Further studies should address full chemical characterisation of tamarind concentrate by GC-MS and LC-MS, validation of efficacy in food matrix models, and evaluation of sublethal effects on insect reproduction and development.

4. Conclusions

This study provides the first systematic evaluation of the antimicrobial and fumigant insecticidal properties of tamarind (*Tamarindus indica*) concentrate. Tamarind concentrate demonstrated notable disc diffusion activity (9.33-14.33 mm), particularly against *Bacillus cereus*, and measurable MIC_{50} values ranging from 0.770 mg/mL (*S. epidermidis*) to 2.625 mg/mL (*E. aerogenes* and *C. koseri*). Fumigant insecticidal activity reached 92.67 % for *C. maculatus* and 88.67 % for *M. dorsalis* at 100% concentration. These results confirm that the volatile fraction of tamarind concentrate retains significant biological activity despite its dense physical consistency and support its further investigation as a natural agent for food preservation and stored-product protection.

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