Green Plum (*Prunus mume*) Concentrate: Antimicrobial and Fumigant Insecticidal Bioactivity Against Food-Relevant Bacteria and Stored Product Beetles

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Abstract
Green plum (*Prunus mume* Siebold & Zucc.) is a fruit-bearing tree of the family Rosaceae, deeply rooted in East Asian culinary and medicinal traditions, particularly in China, Japan, and Korea. Green plum concentrate is a thick yellow brown to dark brown paste with a characteristic aroma, obtained by solvent extraction of the fruit. Its complex composition of bioactive compounds has attracted increasing scientific interest for natural antimicrobial and insecticidal applications. The aim of this study was to evaluate the antimicrobial activity of green plum 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 showed inhibition zones ranging from 7.67 to 13.33 mm, with the strongest activity observed against *L. innocua* (13.33 mm) and *S. epidermidis* (12.33 mm). MIC_{50} values ranged from 1.435 mg/mL (*S. epidermidis*) to 4.661 mg/mL (*C. koseri*), while MIC_{90} values ranged from 2.382 mg/mL (*S. epidermidis*) to 5.862 mg/mL (*C. koseri*). At the highest concentration (100 %), insecticidal activity reached 96.67 % mortality for *C. maculatus* and 92.00 % for *M. dorsalis*. Green plum concentrate showed moderate antimicrobial activity, with the most pronounced effects against Gram-positive bacteria, alongside strong insecticidal efficacy against both tested beetle species. These findings highlight its potential as a plant-derived bioactive extract for food protection applications.

Keywords: *Prunus mume*; plant extract; antibacterial efficacy; stored-grain insects; botanical insecticide; food preservation

1. Introduction

Prunus mume Siebold & Zucc., known in Chinese as *mei* and commonly referred to as Japanese apricot, Chinese plum, or green plum, belongs to the subfamily Amygdaloideae of the family Rosaceae. The species is native to China and has been cultivated across East Asia for more than two millennia, with China remaining the primary producer and consumer. It is also widely cultivated in Japan, where it is known as *ume*, and in Korea, where it is called *maesil*. The annual global production of *P. mume* fruit is estimated to reach several hundred thousand tonnes, with Chinese production predominating [1]. The fruit plays a dual role in East Asian culture: it is both a widely consumed food product—processed into pickled plums, vinegar, wine, juice, and dried

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preparations and a traditional medicinal material, historically used in decoctions recorded in classical Chinese pharmacopoeias for antidiarrhoeal, antitussive, antiparasitic, and digestive purposes.

The phytochemical composition of *P. mume* fruit is highly complex. Comprehensive analyses have identified approximately 192 compounds across different plant parts, with the fruit pulp containing the highest concentration of bioactive constituents [1]. The dominant chemical classes include organic acids particularly citric, malic, succinic, and oxalic acids which account for a substantial proportion of the fruit's dry weight and contribute to its characteristic sour taste. Phenolic compounds represent the second major group, including phenolic acids such as chlorogenic, caffeic, and protocatechuic acids; flavonoids such as quercetin, rutin, and kaempferol; and hydrolysable tannins. Triterpenoids, including oleanolic and ursolic acids, have been identified in fruit, leaves, and seeds. In addition, mumefural a thermally derived compound formed from citric acid in the presence of glucose is considered a characteristic marker of processed *P. mume* products [2].

The antimicrobial potential of *Prunus mume* has been documented in several studies. Seneviratne et al. [3] demonstrated that *P. mume* extracts inhibited a range of oral pathogenic bacteria and proposed their application in natural oral care formulations. Mitani et al. [4] identified phenolic compounds, particularly chlorogenic acid and its derivatives, as key contributors to antimicrobial activity. More recently, Li et al. [5] reported that *P. mume* hydrosol obtained by steam distillation, with benzaldehyde as the dominant volatile component, exhibited significant antibacterial activity against multiple strains, including *Listeria monocytogenes* and *Bacillus subtilis*, with stronger effects against Gram-positive bacteria. Similarly, Kim et al. [6] confirmed broad-spectrum antibacterial activity of *P. mume* extracts against foodborne pathogens. Despite these findings, no study to date has investigated the antimicrobial and insecticidal properties of green plum concentrate, a full-spectrum aromatic extract obtained by non-polar solvent extraction under controlled laboratory conditions.

The two insect species investigated in this study represent major threats to stored legume commodities. *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae, Bruchinae) is one of the most destructive primary pests of stored pulses worldwide, capable of causing losses exceeding 90% of stored grain within a few months under favourable conditions [7]. Its life cycle occurs largely within the seed, where larvae feed internally on the cotyledon, rendering the grain unsuitable for consumption or planting. *Megabruchidius dorsalis* (Fahraeus), originally native to East Asia, has expanded its distribution to Europe, North Africa, and North America, primarily infesting seeds of *Gleditsia triacanthos* and other legumes. Plant-derived fumigants, particularly volatile aromatic extracts, represent an environmentally compatible alternative for controlling these pests in enclosed storage systems, avoiding the drawbacks associated with synthetic insecticides such as residue accumulation and resistance development [8].

Based on this background, the objective of the present study was to evaluate, for the first time, the antimicrobial and fumigant insecticidal activities of green plum (*Prunus mume*) concentrate against six bacterial strains relevant to food safety and two species of stored-product bruchid beetles, and to interpret the results in relation to the known phytochemical composition of *P. mume*.

2. Materials and methods

2.1 Plant material

Green plum concentrate derived from the fruit of *Prunus mume* Siebold & Zucc. was obtained from GuiLin Four Seasons Sunshine Co., Ltd. (Guilin, Guangxi, China). The product was prepared by extraction of green plum fruit with aliphatic hydrocarbon solvents, followed by complete solvent removal under reduced pressure. The resulting extract was a dense yellow brown to dark brown aromatic paste with a characteristic sour–sweet fruity aroma. Upon receipt, the concentrate was transferred into sealed amber glass vials and stored at 4 °C in the dark to minimise oxidative degradation and loss of volatile constituents.

2.2 Bacterial strains

Six bacterial strains 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.

Prior to each experiment, strains were activated by two successive subcultures in Mueller–Hinton broth (MHB; Oxoid, Basingstoke, UK) at 37 °C for 24 h. Bacterial suspensions were prepared by adjusting turbidity to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL).

2.3 Disc diffusion antimicrobial assay

Preliminary antimicrobial activity was evaluated using the agar disc diffusion method according to Kačániová et al. [9]. Mueller–Hinton agar (MHA; Oxoid) plates were inoculated by uniform swabbing in three directions with bacterial suspensions standardised to 0.5 McFarland turbidity.

Sterile filter paper discs (6 mm diameter; Whatman) were impregnated with 10 µL of green plum concentrate solution prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich) to ensure adequate solubilisation 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 discs containing DMSO alone 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 determination

MIC_{50} and MIC_{90} values were determined using the broth microdilution method in sterile 96-well microplates (Nunc), following Kačániová et al. [10]. Green plum 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 bacteria) were included on each plate. Plates were sealed with adhesive film and incubated at 37 °C for 24 h.

Optical density at 600 nm was measured using a GloMax® microplate reader (Promega), and percentage growth inhibition relative to the control was calculated. MIC_{50} and MIC_{90} values were derived from concentration–response curves 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 green plum concentrate vapours was evaluated against adult *C. maculatus* and *M. dorsalis*. Insects were reared on mung bean 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 filter paper disc (3 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 ensure exposure exclusively to the vapour phase.

After 24 h of exposure at 27 ± 1 °C, insects were examined under a stereomicroscope, and individuals not responding to gentle stimulation were recorded as dead. Mortality (%) was calculated for each replicate, with three independent replicates performed per concentration.

2.6 Statistical analysis

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

3. Results and discussion

3.1 Disc diffusion antimicrobial activity

The disc diffusion results for green plum concentrate are presented in Table 1. Inhibition zones were recorded for all six tested bacterial

strains, ranging from 7.67 mm (*C. koseri*) to 13.33 mm (*L. innocua*). The most susceptible strains were the Gram-positive organisms *L. innocua* (13.33 mm) and *S. epidermidis* (12.33 mm), whereas Gram-negative bacteria showed generally lower susceptibility. Among the Gram-negative strains, *K. pneumoniae* produced an inhibition zone of 10.67 mm, *Enterobacter aerogenes* 8.33 mm, and *C. koseri* 7.67 mm the lowest value observed. *B. cereus*, despite its Gram-positive cell wall structure, showed a comparatively lower inhibition zone (11.67 mm), which may be partially associated with its spore-forming capability.

Table 1. Disc diffusion antimicrobial activity of green plum (*Prunus mume* Siebold & Zucc.) concentrate against selected bacterial strains.

Bacterial strain	Inhibition zone (mm) Mean ± SD	ABT (mm) Mean ± SD
Bacillus cereus	11.67 ± 0.58^{bc}	30.33 ± 0.58^{ab}
Enterobacter aerogenes	8.33 ± 0.58^{d}	28.67 ± 0.58^{c}
Klebsiella pneumoniae	10.67 ± 0.58^{c}	30.67 ± 0.58^{ab}
Listeria innocua	13.33 ± 0.58^{a}	31.67 ± 0.58^{a}
Staphylococcus epidermidis	12.33 ± 0.58^{ab}	29.67 ± 0.58^{bc}
Citrobacter koseri	7.67 ± 0.58^{d}	28.33 ± 0.58^{c}

These findings are consistent with previous studies on *Prunus mume*, which have reported greater susceptibility of Gram-positive bacteria compared to Gram-negative species. Li et al. [5] demonstrated that *P. mume* hydrosol exhibited stronger activity against Gram-positive bacteria such as *Listeria monocytogenes* and *Bacillus subtilis*, while Gram-negative species showed lower sensitivity. This pattern reflects the absence of an outer membrane in Gram-positive bacteria, allowing direct interaction of hydrophobic bioactive compounds with the cytoplasmic membrane. In contrast, Gram-negative bacteria possess a lipopolysaccharide-rich outer membrane that acts as a permeability barrier and limits the uptake of lipophilic antimicrobial agents [11,12]. The reduced susceptibility of *K. pneumoniae* and *E. aerogenes* observed in this study is therefore consistent with their known intrinsic resistance mechanisms, including outer membrane impermeability and active efflux systems.

In comparison with other extracts evaluated in this study series, the disc diffusion activity of green plum concentrate (7.67-13.33 mm) was comparable to that of longan concentrate and slightly lower than that of jasmine absolute, indicating a moderate antimicrobial profile consistent with polyphenol-rich plant extracts.

3.2 Minimum inhibitory concentration (MIC_{50} and MIC_{90})

MIC_{50} and MIC_{90} values determined by broth microdilution and probit analysis are presented in Table 2. In contrast to the disc diffusion results, which identified *L. innocua* as the most susceptible strain, the microdilution assay indicated *Staphylococcus epidermidis* as the most sensitive organism, with MIC_{50} of 1.435 mg/mL and MIC_{90} of 2.382 mg/mL. This discrepancy between diffusion-based and broth-based methods is well documented for hydrophobic plant extracts, as limited diffusion of high-molecular-weight or poorly soluble constituents in agar may underestimate their true inhibitory activity in liquid systems [13].

L. innocua exhibited MIC_{50} and MIC_{90} values of 1.582 mg/mL and 2.735 mg/mL, respectively, closely comparable to *S. epidermidis*, while *B. cereus* showed intermediate susceptibility (MIC_{50} 1.459 mg/mL; MIC_{90} = 2.694 mg/mL). All Gram-positive strains required MIC_{50} values within the range of 1.4-1.6 mg/mL, significantly lower than those observed for Gram-negative bacteria.

Among Gram-negative strains, *E. aerogenes* required MIC_{50} of 3.516 mg/mL and MIC_{90} of 4.563 mg/mL, while *K. pneumoniae* exhibited similar values (MIC_{50} = 3.699 mg/mL; MIC_{90} =

4.581 mg/mL). The most resistant organism was *C. koseri*, with MIC_{50} of 4.661 mg/mL and MIC_{90} of 5.862 mg/mL. The very low standard deviation for MIC_{90} suggests a highly consistent and steep concentration–response relationship.

Table 2. Minimum inhibitory concentrations (MIC_{50} and MIC_{90}) of green plum (*Prunus mume* Siebold & Zucc.) concentrate against selected bacterial strains.

Bacterial strain	MIC50 (mg/mL) Mean ± SD	MIC90 (mg/mL) Mean ± SD
Bacillus cereus	1.459 ± 0.187^{b}	2.694 ± 0.215^{c}
Enterobacter aerogenes	3.516 ± 0.277^{a}	4.563 ± 0.248^{b}
Klebsiella pneumoniae	3.699 ± 0.217^{a}	4.581 ± 0.319^{b}
Listeria innocua	1.582 ± 0.223^{bc}	2.735 ± 0.123^{c}
Staphylococcus epidermidis	1.435 ± 0.211^{c}	2.382 ± 0.186^{c}
Citrobacter koseri	4.661 ± 0.289^{a}	5.862 ± 0.025^{a}

Compared to longan concentrate and jasmine absolute, green plum concentrate showed higher MIC values across all strains, indicating lower inhibitory potency at the level of bacterial growth suppression. This observation is consistent with the known mechanisms of action of *P. mume* constituents. Phenolic acids such as chlorogenic acid derivatives primarily act through membrane destabilisation, protein binding, and interference with bacterial adhesion processes, rather than through strong membrane-disruptive effects typical of terpenoid compounds [1,4].

3.3 Fumigant insecticidal activity

The fumigant insecticidal activity of green plum concentrate against *Callosobruchus maculatus* and *Megabruchidius dorsalis* is presented in Table 3. Both species exhibited a clear concentration-dependent increase in mortality across the tested range. For *C. maculatus*, mortality reached 96.67 % at 100 % concentration, indicating near-complete lethality. Mortality decreased progressively with dilution, reaching 75.00 % at 50%, 54.33 % at 25 %, 32.33 % at 12.5 %, 21.67 % at 6.25 %, and 9.33 at 3.125 %. The relatively low variability at intermediate concentrations indicates a consistent biological response.

A similar trend was observed for *M. dorsalis*, with mortality of 92.00 % at 100 %, followed by 75.00 % at 50 %, 56.67 % at 25 %, 33.00 % at 12.5 %, 22.33 % at 6.25 %, and 9.67 % at 3.125 %. Slightly higher variability at the highest concentration may reflect inter-individual differences in sensitivity to volatile compounds.

The strong fumigant activity observed is consistent with previous studies on plant-derived volatile compounds. Li et al. [5] identified benzaldehyde as a major volatile component of *P. mume* hydrosol, and benzaldehyde has been reported to exhibit insecticidal activity against stored-product pests [14,15]. According to Isman [16], such compounds may act through inhibition of mitochondrial respiration and disruption of neurotransmission, including interference with GABA-regulated pathways [17–19]. Additional aromatic compounds, including esters and phenolic volatiles, may contribute synergistically to the observed toxicity.

Probit regression analysis yielded LC50 values of 17.49 % and 18.69 % for *C. maculatus* and *M. dorsalis*, respectively. The LC90 value for *C. maculatus* was 78.46 %. For *M. dorsalis*, the LC90 value of 100.86 % slightly exceeded the upper boundary of the tested concentration range, suggesting that complete control of this species would require concentrations at or beyond the maximum tested level.

Table 3. Insecticidal activity of Green Plum (*Prunus mume*) concentrate against *C. maculatus* and *M. dorsalis.*

Concentration (%)	*Callosobruchus maculatus*	*Megabruchidius dorsalis*
	Mean ± SD (%)	Mean ± SD (%)
100	96.67 ± 2.52[a]	92.00 ± 6.08[a]
50	75.00 ± 3.61[b]	75.00 ± 2.65[b]
25	54.33 ± 1.53[c]	56.67 ± 1.15[c]
12.5	32.33 ± 2.08[d]	33.00 ± 1.73[d]
6.25	21.67 ± 4.16[e]	22.33 ± 1.15[e]
3.125	9.33 ± 2.08[f]	9.67 ± 3.21[f]
LC_{50} (%)	17.49	18.69
LC_{90} (%)	78.46	100.86

3.4 Integrated discussion

Overall, green plum concentrate demonstrated moderate antibacterial activity combined with strong fumigant insecticidal efficacy. Compared with other extracts evaluated in this study series, its antimicrobial activity was comparable to longan concentrate but lower than that of jasmine absolute, whereas its insecticidal activity ranked among the highest.

This profile is consistent with the chemical composition of *Prunus mume*, which is dominated by organic acids and phenolic compounds rather than highly lipophilic terpenoids [20,21]. While these compounds may provide moderate antimicrobial activity, their volatility and bioactivity contribute significantly to fumigant toxicity [17]. The results therefore suggest that green plum concentrate may be particularly suitable for applications targeting insect control in stored products, with additional antimicrobial benefits [22].

Future studies should focus on detailed chemical characterisation of the extract using GC-MS and LC-MS, identification of key bioactive compounds, and evaluation of sublethal effects on insect reproduction and development.

4. Conclusion

This study provides the first evaluation of the antimicrobial and fumigant insecticidal activity of green plum (*Prunus mume*) concentrate. The results demonstrate moderate antibacterial activity, with Gram-positive bacteria showing higher susceptibility than Gram-negative strains, and strong concentration-dependent fumigant toxicity against both *Callosobruchus maculatus* and *Megabruchidius dorsalis*. These findings support the potential of green plum concentrate as a natural bioactive agent for applications in food preservation and stored-product protection.

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