Environmental Technology & Innovation 36 (2024) 103807

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Evaluation of the biocide activity of tomatine-rich extracts from tomato cannery residues against fungi and bacteria

Alessandro Passera [a], Paola Casati [a], Parisa Abbasi-Parizad [a], Saul Pagnoni [a], Daniele Carullo [b], Stefano Farris [b], Barbara Scaglia [a,*]

[a] *Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of the Study of Milan, Via Celoria 2, Milan 20133, Italy*

[b] *Department of Food, Environmental and Nutritional Sciences, University of the Study of Milan, Via Celoria 2, Milan 20133, Italy*

ARTICLE INFO

Keywords:
critical micellar concentration
gray mold
saponin
plant extract
by-product
pesticides

ABSTRACT

Synthetic pesticides are discouraged for their environmental and health impacts, making research into alternatives essential. Several solutions of vegetal origin are being evaluated. The use of residual biomass from the agri-food system is particularly suitable due to its abundance and often unexplored potential. This study focuses on characterizing and assessing the activity of extracts obtained from wastes of the tomato cannery industry (including green fruit, stems, and leaves), which are rich in steroidal glycoalkaloids such as α-tomatine and tomatidine in different proportion. The antimicrobial activity of these extracts was tested on three bacterial strains belonging to the *Escherichia coli* (EC), *Xanthomonas campestris* (XC), and *Bacillus pumilus* (BP) species, as well as the phytopathogenic fungus *Botrytis cinerea* (BC). In particular, the mechanism of action of the extracts in relation to their surfactant properties was investigated, with the effect of the analytical standard serving as a reference. Both extracts showed strong inhibition of bacterial and fungal growth *in vitro*, with values reaching 100 %.

The inhibitory effect was mainly due to the presence of α-tomatine in the extracts, which reached its aggregated state of micelle at the critical micelle concentration (CMC). Tomatidine, although known for its biocidal properties, did not contribute significantly due to its limited solubility. However, exceptions to this pattern were observed for extract rich in tomatidine, which exhibited efficacy at doses below the CMC. A possible explanation could be the enhanced solubility of tomatidine (which corresponds to enhanced bioactivity) in the presence of surfactant secreted by BP or as a consequence of the interaction between tomatidine and α-tomatine at the pre-micellar state for BC. *In vivo* assays with BC showed a reduction in symptoms comparable to that of a commercial fungicide available for organic agriculture, particularly at low concentrations. The relative content of α-tomatidine and tomatidine in the extracts modulated their bioactivity. An excess of tomatidine relative to α-tomatine led to a decrease in biocidal effect due to the chemical interactions among these species.

Abbreviations: BC, *Botrytis cinerea*; BP, *Bacillus pumilus*; CMC, critical micellar concentration; DM, Dry matter; EC, *Escherichia coli*; GT, Green tomato fruit extract; I%I, infection percentage index; LB, Luria-Bertani broth; OD, Optical density; PDA, Potato dextrose agar; PS, polystyrene; SL, Tomato leaves and stem extract; XC, *Xanthomonas campestris*.

* Correspondence to: Via Celoria 2, Milano 20133, Italy.
E-mail address: barbara.scaglia@unimi.it (B. Scaglia).

https://doi.org/10.1016/j.eti.2024.103807
Received 16 April 2024; Received in revised form 6 August 2024; Accepted 24 August 2024
Available online 26 August 2024

1. Introduction

In recent years, EU policies have discouraged the use of synthetic pesticides, urging research efforts to identify new solutions to overcome the threats posed by pests and pathogens. These policies aim to minimize the environmental impact of pesticides, not only during their application but also throughout their production process (Dhakal and Singh, 2019).

Many non-edible parts of edible plants contain antinutritional or toxic secondary metabolites that can be employed in such approaches (Daraban et al., 2023). Tomatine, in particular, is the primary steroidal glycoalkaloid found in solanaceous plants such as tomatoes, potatoes, and eggplants. It exists in varying concentrations across different plant parts, including stems, leaves, flowers, and fruits. In tomato plants (*Solanum lycopersicum*), tomatine is synthesized in different organs and serves as defense compounds against insects and other pests. Moreover, the accumulation of tomatine is also species- and cultivar-dependent, as clearly demonstrated by seminal studies conducted by Friedman and colleagues (Friedman et al., 1994; Friedman & Levin, 1998; Kozukue et al., 2004). In these studies, α-tomatine ranged from 0.4 to 9.9 mg/100 g of dry weight in ripened commercial varieties, while it reached 225 mg/100 mg dry weight in unripened fruits of the Manteca cultivar parental line. These studies also highlighted how the organ type, the timing and phenological state play a role: for example, green fruits are extremely rich in tomatine, which however undergoes degradation during ripening (Kozukue and Friedman, 2003; Tamasi et al., 2019).

Testing pure standards of the glycosylated (α-tomatine) and aglycated (tomatidine) molecules, biological activities described for tomatine include biocidal effects against bacteria, insects, and fungi (Friedman, 2002). Furthermore, some pharmacological effects such as anti-inflammatory and anti-cancer properties have also been reported (Friedman, 2002; Bailly, 2021).

The biocidal activity of α-tomatine has been attributed to its interaction with the sterols in cell membranes, leading to membrane damage through pore formation, which in turn causes cellular content leakage and ultimately cell lysis (Hoagland, 2009). This effect primarily stems from the surfactant properties of α-tomatine, which comprises hydrophilic glycoside sugar moieties combined with a hydrophobic steroidal portion (i.e., tomatidine) (Lorent et al., 2014). Acting as an anionic surfactant, the effectiveness of α-tomatine is associated with its CMC, marking the formation of micelles (Huang et al., 2020; Böttger et al., 2012). Antibiotic properties of α-tomatines against microbial agents such as *Listeria monocytogenes* (Pingulkar et al., 2001), *Escherichia coli*, *Staphylococcus aureus* (Friedman, 2002), and *Salmonella enterica* (Friedman, 2013) have been demonstrated. Similar effects have been described for saprophytes and pathogens of plant species, except for those specialized for the tomato plant, which are less sensitive to α-tomatine, possibly due to differences in the structure of their membranes or the production of detoxifying enzymes (You and van Kan, 2021).

Tomatidine lacks hydrophilic moieties, thus it does not exhibit surfactant activity. However, it has been described as a biocide against bacteria and fungi. The antifungal effect is believed to result from tomatidine's ability to interfere with the ergosterol biosynthesis pathway, while its antibacterial effect is thought to be related to the inhibition of protein biosynthesis (Bailly, 2021; Lisdiana et al., 2023).

In a recent article, the authors developed a method to obtain tomatine rich-extract from tomato plant residues, typical byproducts of the tomato cannery industry (Abbasi-Parizad et al., 2023). Green tomatoes (GT) and stems and leaves (SL) are the main residues (2–3 % w/w wet weight of incoming tomatoes at the plant) resulting from preliminary sorting and cleaning steps. The composition of the extract varied due to the type of feedstock and seasonal variation in sampling. Nevertheless, all extracts contained both aglycated and glycosylated forms, as well as additional unconventional glycosylated tomatine and non-saponine compounds (Abbasi-Parizad et al., 2023). The potential use of these fractions in the agricultural sector is of growing interest, meeting the need to identify alternative biocide molecules using inexpensive or zero-value materials as feedstock while ensuring performance comparable to currently employed pesticides.

Compared to the pure standards, the vegetal extracts consist of mixtures of molecules whose coexistence may enhance or diminish overall bioactivity (Abbasi-Parizad et al., 2022).

In this context, our study aims to test the antifungal and antibacterial properties of extracts rich in glycoalkaloids obtained from tomato cannery residues, using pure chemical standards as reference. The effectiveness of the extracts was evaluated, and attempts were made to identify their mechanism of action. Special attention was given to studying the surfactant properties of the extracts and their impact on overall bioactivity.

2. Materials and method

2.1. Materials

Tomato residues were collected in a large-scale tomato cannery in Ravarino (Emilia Romagna region, Italy) during the production season of 2021 (Abbasi-Parizad et al., 2023). The daily arrival of tomatoes from different producers and the successive mixing during the production steps make it impossible to identify the specific cultivar of tomato, generally identified as industrial tomato. The biomasses come from the primary sorting step of the industrial transformation, during which rotten red fruits, green fruits (GF) and stems and leaves (SL) were separated and discarded through an optical and manual separation system. The biomasses were and successively brought to the laboratory of the University of Milan for successive characterization.

2.2. Tomatines-rich fraction extraction and chemical characterization

2.2.1. Extraction of the tomatine-rich fraction

The biomasses were dried at 60 °C under vacuum and successively grinded (size < 500 μm). The extraction was carried out as

reported in Abbasi-Parizad et al. (2023). Briefly, 2 g of dry matter (DM) of the residues were extracted with acetic acid (product 1.00062, Merck KGaA, Darmstadt, Germany) (5 % v/v in deionized water) ratio 1:12.5 (w:v) and successively sonicated (T = 30 min), mechanically agitated (t=120 min at 300 rpm), and finally sonicated (T = 30 min) (Taveira et al., 2012). The samples were then centrifuged (T = 15 min, 10,000 rpm) and ammonia (25 % v/v) (product 1.05488, Merck KGaA, Darmstadt, Germany) ratio 10:1 (v/v) added to the surfactant. The reaction was heated at 65 °C for 2 hours and successively stored overnight at 4 °C. The suspension was successively centrifuged (T = 15 min, 8000 rpm) and the fraction of interest (i.e., the precipitated) was washed till pH neutral and successively dried at 40 °C under vacuum (Abbasi-Parizad et al., 2023). This allowed to obtain the two extracts: GT, obtained from the fraction rich in green fruits, and SL, obtained from the fraction rich in stems and leaves.

2.2.2. Analytical characterization

The quali-quantitative content of the tomatines was approached by using HPLC and NMR techniques as reported by Abbasi-Parizad et al. (2023). Briefly, HPLC Agilent 1260 Infinity (Agilent Technologies, Santa Clara, California, United States) was equipped with Luna Omega Polar C18 column (150 × 3.0 mm, 3 μm particle size, Phenomenex, Torrance, USA) employed at temperature of 30°C. The mobile phase was acetonitrile (product 34851, Merck KGaA, Darmstadt, Germany) and trimethylammonium phosphate (product 56778, Merck KGaA, Darmstadt, Germany) at pH = 3 with different ratio, elution flow of 0.4 mL min^{-1}. The identification of the standard glycosylated molecules (α-tomatine and tomatidine) and of the standard aglycated ones (tomatidine and tomatidinedol) were done by using high purity degree standard (DBA, Milan, Italy).

The ^{1}H NMR approach was applied to the SL previously dissolved in deuterated methanol (product 1.06028, Merck KGaA, Darmstadt, Germany) to identify no-standard molecules of the all-tomatine. Both the ^{1}H NMR and Cosy experiments were carried out using Bruker AVANCE NEO 500 MHz equipped with a 5 mm TCI cryoprobe (Abbasi-Parizad et al., 2023).

2.2.3. Contact angle measurement

Contact angle was determined for the analytical standards of α-tomatine and tomatidine and for the extracts (SL and GT). All samples were solubilized in DMSO (10 % v/v) aqueous solution at the concentration of 1 mg mL^{-1}. The stock solution was successively diluted to obtain solution (1 % v/v DMSO) in the concentration range from 0 μg mL^{-1} to 1000 μg mL^{-1}.

Contact angle analyses were performed using an optical contact angle apparatus (OCA 15 Plus – Data Physics Instruments GmbH, Filderstadt, Germany) equipped with a video measuring system with a high-resolution CCD camera and a high performance-digitizing adapter. The software SCA 20 (Data Physics Instruments GmbH, Filderstadt, Germany) was used to capture the droplet images and to determine the contact angle by image processing. To emphasize the influence of the surfactant concentration on the surface tension of the liquid, a low surface energy (SE) material (5 × 2 cm^2 polystyrene – PS – sheets, 120 μm thick, SE = 32 ± 1.2 mN/m) was used. PS surface was first cleaned using ethanol to avoid any influence possibly arising from impurities and/or dust. Then, PS specimens were fixed and kept flat by means of a special sample holder with parallel clamping jaws. The contact angle (θ, deg) of SL and GT solutions in air was measured by the sessile drop method, by gently dropping a droplet of 4 ± 0.5 μL onto the PS surface, according to the so-called pick-up procedure (Boyaci et al., 2019). To minimize the effect of evaporation, analyses were performed at 23 ± 1 °C and 100 % relative humidity using a laboratory-made climatic cabinet.

A minimum of 10 droplets were examined for all samples on both the left and right sides and the resulting mean values were then plotted against the surfactant concentration for the calculation of the CMC according to the analytical method proposed by Alkawareek et al. (2018).

2.3. In vitro tests of antibiotic and antifungal activity

The antibiotic and antifungal effect of the analytical standards and extracts were assayed through *in vitro* tests. The following microorganisms were used in the tests: *Botrytis cinerea* strains 2N15, 2Z15, and 3G1, isolated from different weeds present in a vineyard in northern Italy (Toffolatti et al., 2020); *Escherichia coli* strain DH5α (EC), *Xanthomonas campestris* isolated from *Brassica napus* plants by our group (XC), and a *Bacillus pumilus* isolated from *Zea mays* plants by our group (BP). For the assays against *B. cinerea* the extracts, the analytical standards of α-tomatine and tomatidine, and different mixtures of the two standards were used (Supporting information, Table S1); for the bacteria, different concentrations of SL and GT have been tested (Supporting information, Table S2). All tests were carried out in a total volume of 200 μL containing 80 μL of growth medium Potato Dextrose Broth (PDB) for fungi, Luria-Bertani (LB) broth for bacteria), 20 μL of microbial inoculum, and 100 μL of treatment solution, with a final concentration of 1 % DMSO.

For *B. cinerea* strains, microbial inoculum was a suspension of conidia in water (10^5 conidia mL^{-1}) harvested from Potato Dextrose Agar (PDA) plates in which the fungus grew for ten days, while for bacterial isolates the microbial inoculum was an aliquot of an overnight culture in LB broth.

The treatments were compared to the results obtained in the negative controls, in which the 100 μL of treatment solution were replaced either by sterile distilled water or sterile distilled water with 1 % final concentration of DMSO, and in the positive controls, in which the 100 μL of treatment solution were replaced with either a fungicide for *B. cinerea* strain, or with the kanamycin antibiotic (50 μg mL^{-1}) (Sigma-Aldrich, Germany). Three different fungicides effective against *Botrytis cinerea* have been used as positive controls: one containing boscalid and pyraclostrobin (Signum; BASF, Germany), one containing cyprodinil and fludioxonil (Switch; Syngenta, Switzerland), and the last containing tribasic copper sulfate (King; Diachem, Italy). Each fungicide was used at the concentrations suggested by the manufacturer, as reported in Table S1.

Each treatment or control was incubated in a well of an optical 96-well plate and replicated 8 times. *B. cinerea* conidia were incubated for 6 days at 24 °C, while bacterial isolates were incubated for 24 hours at 37 °C for EC and at 24 °C for XC and BP.

Microbial growth was evaluated by measuring turbidity as an increase in optical density (OD) measured at 450 nm for *B. cinerea* and at 600 nm for the bacteria with a Magellan plate reader (Tecan Trading AG, Switzerland). To account for the different initial turbidity of some solutions, OD values were first normalized by calculating a ΔOD value by subtracting the OD values at the start of incubation from the OD values measured at the end of incubation. These ΔOD values were compared among treatments with the following formula to calculate the growth inhibition percentage (GIP):

$$(1 - (\Delta OD_T / \Delta OD_C)) * 100$$

Where ΔOD_T is the average ΔOD value in the treatment and ΔOD_c is the average ΔOD value in the negative controls.

No difference was observed in the growth of the microorganisms between the two negative controls (with or without 1 % DMSO), therefore the ΔOD_c values are calculated considering both negative controls together.

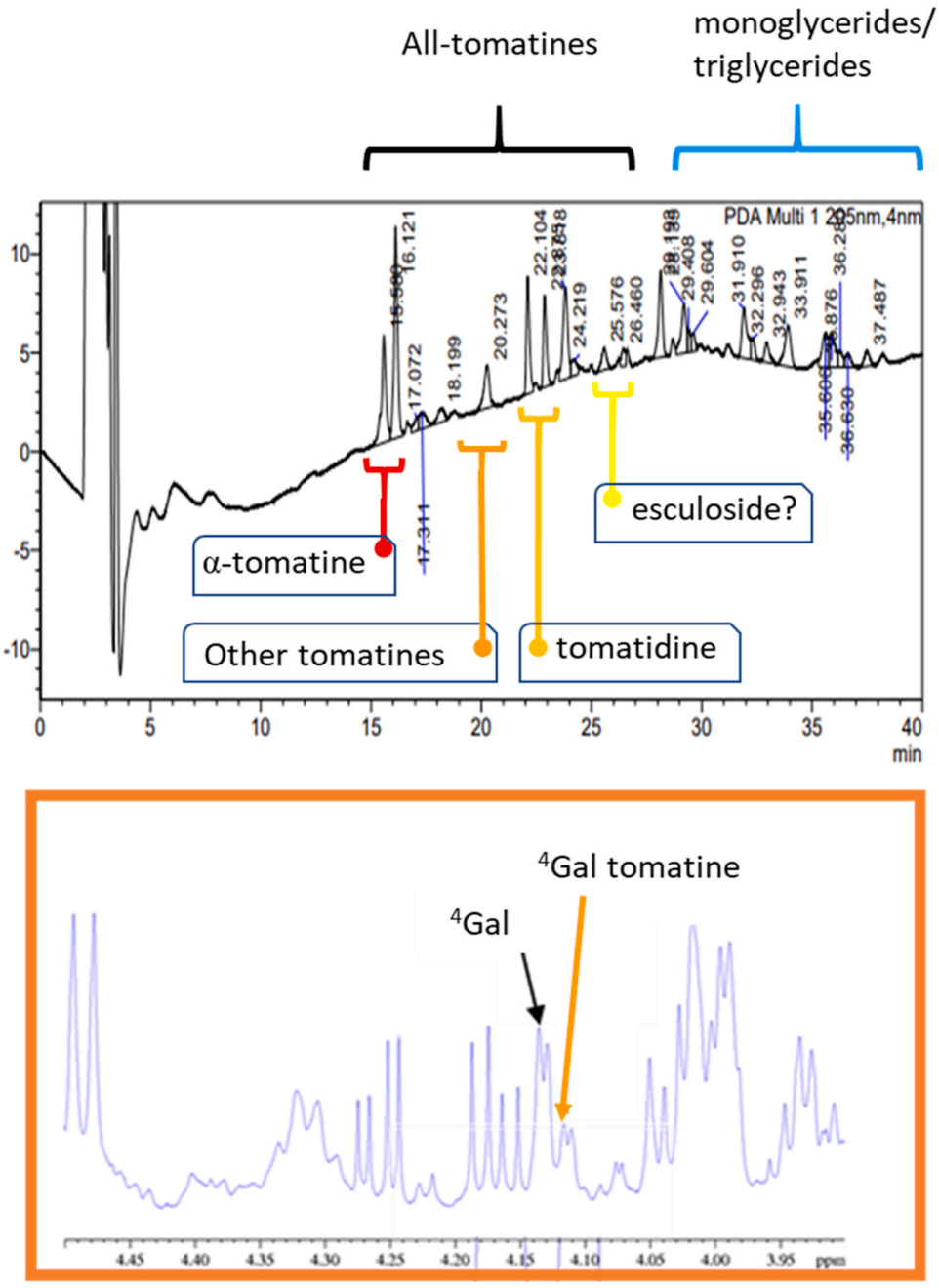


Fig. 1. Spectra HPLC and ^{1}H NMR of the sample SL (Adapted from Abbasi-Parizad et al., 2023).

No statistically significant differences were detected between the results obtained on the three isolates of *B. cinerea*, therefore the results presented for *B. cinerea* consider the three isolates as a single entity. Indicated as BC.

2.4. In vivo test of antifungal activity

The antifungal effect of the SL and GT extracts against BC was assayed through an *in vivo* test, compared to that of commercial fungicides. For this assay, healthy leaves from tomato plants were inoculated and placed in a Petri dish containing sterile 1 % agar: water medium, used to maintain humidity of the leaf. Each dish contained three tomato leaves, and each leaf was inoculated near the middle of the leaf with a 20 μL droplet of either sterile ringer solution (healthy control), BC conidia solution (10^5 conidia mL^{-1}) in sterile ringer solution (negative control), BC conidia solution with different concentrations of GT extract (30, 63, 125, or 500 μg mL^{-1}) in sterile ringer solution, BC conidia solution with different concentrations of SL extract (63, 125, 250, or 500 μg mL^{-1}) in sterile ringer solution, BC conidia solution with three different commercial fungicides at the concentrations suggested by the manufacturers (Switch 700 μg mL^{-1}; Signum 1500 μg mL^{-1}; King 3 μg mL^{-1}) (positive controls).

After 5 days from inoculation, with the plates stored at 20 °C, the damage caused on each leaf was assessed visually, determining a percentage of damage on the leaf, converting it to a symptom class from 0 to 6 based on the following scale (0 = 0 %; 1 = from 1 % to 10 %; 2 = from 11 % to 25 %; 3 = from 26 % to 50 %; 4= from 51 % to 75 %; 5 = above 75 %; 6 = 100 % and with visible sporulation) (Vercesi et al., 2014), and ultimately calculating an infection percentage index (I%I) based on the formula by Townsend and Heuberger (1943), as follows:

$$I\%I = \frac{\sum(ni \times vi)}{N \times V}$$

Where *ni* is the number of leaves in any one symptom class, *vi* is the number associated with that class, N is the total number of observed leaves and V is the number associated with the highest symptom class (in our case 6).

2.5. Statistical elaboration

The results of growth inhibition for all the microorganisms and of I%I caused by BC were compared between treatments by a non-parametric Kruskal-Wallis test, since the datasets did not satisfy the requirements for a parametric test. The statistical elaboration was carried out using the SPSS software version 29 (IBM, New York, NY, USA).

3. Results and Discussion

3.1. Chemical composition and detection of the surfactant properties of the extracts

A multidisciplinary analytical approach was used to identify the composition of the extracts (Fig. 1) (Abbasi-Parizad et al., 2023). Preliminary detection by HPLC analysis of the pure standard of α-tomatine and tomatidine identified the retention time at min 13–14 and 21 respectively (Abbasi-Parizad et al., 2023). Additional confirmation of the identification of the glycosylated and aglycated forms was obtained by acid hydrolysis of the pure α-tomatine standard. After 1 hour of the process, the pick of tomatidine appeared instead of that of α-tomatine. Finally, with a longer treatment time (time = 2 h), the tomatidine also disappeared and a new pick, probably a degraded form of tomatine such as esculoside, was observed (time = 25 min) (Fig. 1).

Table 1
Composition and chemical characterization of the tomato cannery residues and tomatines rich-extracts.

Residues	GT	SL
Composition[a]	Green fruits (80 %); Red fruits (20 %)	Leaves + stems (78.35 %); Green fruits (5.53 %); Red fruits (16.12 %)
Extract quantity[b]	12.4±4	10.8±0.4
All-tomatine[c]	661	864
α-tomatine[d]	530±70	310±40
tri/tetrasaccharides-tomatine[e]	101	124
tomatidine[f]	30±8	430±10
no-tomatine[g]	339	136

[a] : Composition expressed as w/w wet weight basis;
[b] : quantity of obtained extract is expressed as mg g^{-1} DM of original biomass;
[c] : quantity, expressed as μg mg^{-1} DM of the extract, of α-tomatine+Dehydro-tomatine, tri/tretrasaccharides-tomatine, and tomadine+tomatidinedol identified by HPLC and using pure standard as reference;
[d] : quantity, expressed as μg mg^{-1} DM of the extract, of α-tomatine+dehydro-tomatine identified by HPLC and using pure standard as reference.
[e] : quantity, expressed as μg mg^{-1} DM of the extract, of tri/tetrasaccharides-tomatine identified by NMR approach and using the pure standard of α-tomatine and HPLC for quantification;
[f] : quantity, expressed as μg mg^{-1} DM of the extract, of tomadine+tomatidinedol identified by HPLC and using pure standard as reference;
[g] : quantity, expressed as μg mg^{-1} DM of the extract, of no tomatine compound calculates as: 1000- tomatine;

The use of the ^{1}H NMR approach allowed a more complete characterization of the molecules of the extract SL with HPLC (Fig. 1).

The NMR characterization confirmed the presence of several compounds generally identifiable as tomatine (all-tomatine) and aliphatic molecules tentatively identified as mono/triglycerides (Fig. 1). In addition, the elaboration of the analytical data coming from ^{1}H NMR and HSQC identify a pick attributable to galactose sugar identified as a component of the glycosylated portion of alternative tomatine (Fig. 1) (Abbasi-Parizad et al., 2023).

The deeper analyses of this aspect by HSQC have been extensively explained in a previous work of the Authors (Abbasi-Parizad et al., 2023), who consider the sugars as part of trisaccharide-tomatine (sugar: Gal-Glu-Glu or Gal-Glu plus Glu characterized by some chemical modifications) or tetrasaccharide-tomatine (sugar: Gal-probably Glu-anomeric β-glucose form).

The integration of all previous data allows the complete description of the extracts in terms of α-tomatine, tomatidine, other tomatine and no-tomatine molecules (Table 1). The yield of the extractions from tomato industry residues were comparable to those previously found considering the different tomato plant fractions (Taveira et al., 2012). The residues showed a very different composition that affected not only the amount but also the quality composition in tomatine fraction.

The SL (rich in leaves and stems) had a significant concentration of tomatidine (49.79 % of the tomatine), while in GT the α-tomatine content was 80.2 % tomatine.

The additional forms of glycosylated tomatine, which sugars were tentatively identified as trisaccharide or tetrasaccharide portions, that correspondent to the 14.3 % and 15.28 % of the all-tomatine for the SL and GT, respectively (Abbasi-Parizad et al., 2023).

Surfactant properties of the extracts were investigated through contact angle tests. The theoretical background behind this method is that increasing the concentration of a surfactant in a polar solvent (e.g., water) will decrease the contact angle of the same liquid on a hydrophobic surface, due to a decrease of the surface tension of the liquid. This is because the surfactant molecules will displace at the liquid/air and at the liquid-solid interfaces decreasing the interfacial tension, thus promoting the spreading of the liquid on the hydrophobic surface (Fig. 2a–c). The contact angle decrease is observed until the surfactant molecules can move at the liquid/air interface, which occurs upon reaching the critical micellar concentration (CMC). At the CMC, the liquid-air and the liquid-solid interfaces are saturated, that is, there is no additional free space available for the surfactant molecules to occupy that interface. Consequently, any addition of surfactant above the CMC will result in the self-assembly of the surfactant molecules to form micelles (Fig. 2d–e).

Accordingly, plotting the 'contact angle versus surfactant concentration' will result in a first descending part (contact angle decrease) followed by a steady part (the contact angle does not decrease longer after the CMC). The CMC of the surfactant is represented by the intersection point of the two straight lines, which can be analytically determined by solving the system of equations given by the fitting lines of the two straight parts of the plot obtained by linear regression.

This test was performed first for the α-tomatine standard (Fig. 3 A) to obtain reference data. The plot shows a typical trend for surfactants, with a straight reduction at lower concentrations followed by a steady portion. The point of intersection between the two lines corresponds to the CMC. Accordingly, the CMC calculated for α-tomatine was 75.9 μg mL^{-1}, which is in the typical range of non-ionic surfactant (Huang et al., 2020). This value is different from a previous reference that indicated a CMC = 99 μg mL^{-1} (Yamanaka et al., 2008), but several factors such as temperature, ionic strength of the medium, and solvent can influence the aggregation behavior of α-tomatine (Lorent et al., 2014; Samal et al., 2017). The non-surfactant nature of tomatidine was confirmed by the test carried out on the analytical standard; the plot (Fig. 3A) did not show any significant trend, and highlighted the very limited solubility of the molecule in the solvent (water:DMSO 90:10 v/v) due to its highly hydrophobic nature.

Both SL and GT showed the same contact angle trend as α-tomatine, confirming the surfactant nature of the extracts (Fig. 3 B-C). The CMC was equal to 102.5 μg mL^{-1} and 165.1 μg mL^{-1} for the SL and GT, respectively. The higher CMC values for SL and GT compared to pure α-tomatine indicate the lower surfactant capacity extracts. Considering the heterogeneous composition of extracts, this result was expected: as the overall surfactant activity is given by the presence and concentration of both surfactant and non-

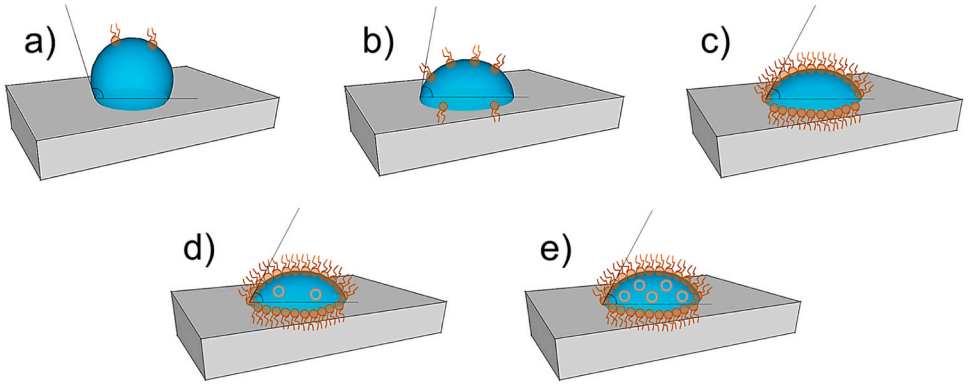


Fig. 2. Schematic drawing of the effect of a surfactant on the wettability of a polar liquid on a hydrophobic surface. Increasing the surfactant concentration (from panel a through panel e), the contact angle decreases until the CMC of the surfactant is reached because of the reduction of the interfacial tension at both liquid-gas and liquid-solid interfaces (a–c). After the CMC, micelle formation occurs, and the contact angle does not change longer (d–e) (adapted from Alkawareek et al., 2018).

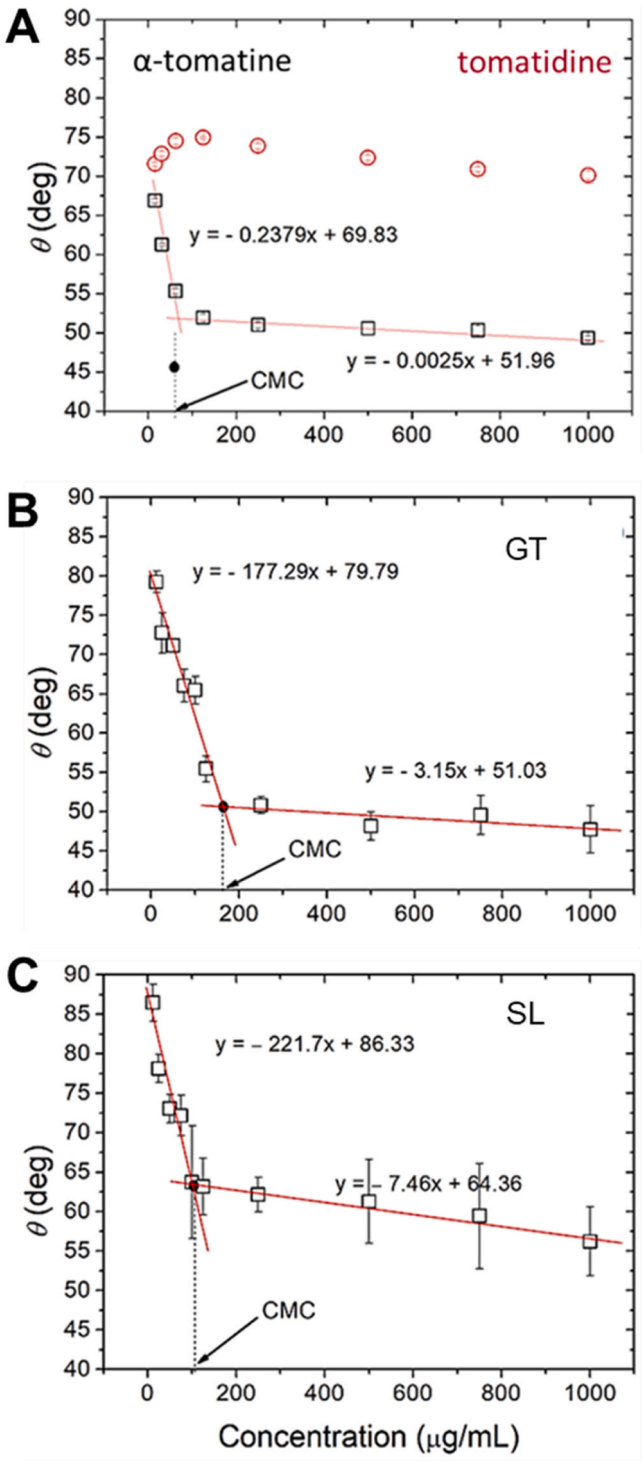


Fig. 3. Contact angle versus concentration plots for A) the α-tomatine and tomatidine standard solutions and, for the extracts B) GT and C) SL. Equations obtained from linear regression (red line) of experimental data (empty squares) are displayed for both descending and steady parts of the plot. Error bars indicate the standard deviation for each experimental data (mean value).

surfactant components (Scaglia et al., 2016), the extracts undergo a dilution effect compared to pure α-tomatine. Taking into consideration the CMC of the α-tomatine standard and its content in both extracts, a theoretical CMC $_{\text{α-tomatine due}}$ of 143.2 μg mL^{-1} and 244.8 μg mL^{-1} were calculated for GT and SL, respectively. In the case of GT, the value was very similar to that obtained

experimentally, suggesting that the surfactant behavior is primarily ascribed to the α-tomatine. Despite the expected data, the experimental CMC of SL was 2.4 times lower than CMC of α-tomatine alone, suggesting that additional molecules must contribute to the overall surfactant property.

Other surfactant molecules are present in the extracts: the tri/tetrasaccharide-tomatines are known to have surfactant properties depending by the number and type of sugars in the glycosylated portion (Arneson and Durbin, 1968; Yamanaka et al., 2008). Previous studies on these molecules highlighted a lower surfactant attribute than α-tomatine, which further decreased at the reduction of the number of sugars. The non-tomatine fraction characterized as lipid (Abbasi-Parizad et al., 2023) can contribute or hinder the surfactant capacity of the extract in the case of monoglyceride or triglyceride nature, respectively (Table 1).

In the extracts, both alternative-glycosylated tomatines and monoglycerides are suitable to be involved in the formation of mixed-micelles with α-tomatine, whose composition affected physical (i.e., size and form) and chemical (i.e., surfactant property) characteristics compared to α-tomatine-only micelles.

3.2. Tests of antibiotic and antifungal activity of the extracts

3.2.1. Biocide activity against bacteria

The assays to evaluate the bactericidal effect of the extracts showed a clear dose effect, the trends were characterized by a progressive increase of the antibiotic activity until a plateau was reached that corresponded to the complete inhibition of the growth for EC and BP, while it only partially inhibited the growth of XC (Fig. 4). The efficacy of both SL and GT are comparable to those of the antibiotic kanamycin, used as a reference standard (Fig. 4). In the case of GT, the maximum of the inhibition was reached at the same concentration for all bacteria tested, while SL was more effective against Gram-positive BP than Gram-negative bacteria EC and XC, for which significant reduction of the growth occurred at concentration 10- to 20-fold higher.

The expression of the extracts concentration in terms of CMC units, rather than absolute concentration, highlighted differences among the extracts: in the case of GT, the maximum inhibition of all three bacteria occurred around 1 CMC; for SL, similar results were obtained at concentration that correspondent to 0.1 CMC for BP, or to 2.5 CMC for EC and XC (Fig. 4 A, C).

The main mode of action at the basis of the effectiveness of GT seems to be its surfactant properties, caused by the α-tomatine content while other compounds seemed to have a dilution affect. In particular, this surfactanct property is caused by the presence of the lycotetraose residue with polar features, while the aglycone part is nonpolar, conferring the molecule an amphiphilic behavior in a pH range of 5–8 (Friedman, 2002).

In agreement with literature the change of membrane permeability and disruption is a mechanism effective against all bacteria, both Gram-positive and Gram-negative (Pingulkar et al., 2001; Friedman, 2002 Friedman, 2013; Lorent et al., 2014).

Typically, the cholesterol-binding activity of α-tomatine, and consequently its biocidal effect, has an optimum around pH 7.8–8 when the amino group (-NH) present in the F ring of the glycoalkaloid is in its unprotonated form. Lowering the pH to more acidic conditions (e.g., pH 4) promotes the protonation of the amino group, generating a partial charge on the aglycon portion, which in turn impairs the amphiphilic features of the molecule (Bailly, 2021; Friedman, 2002).

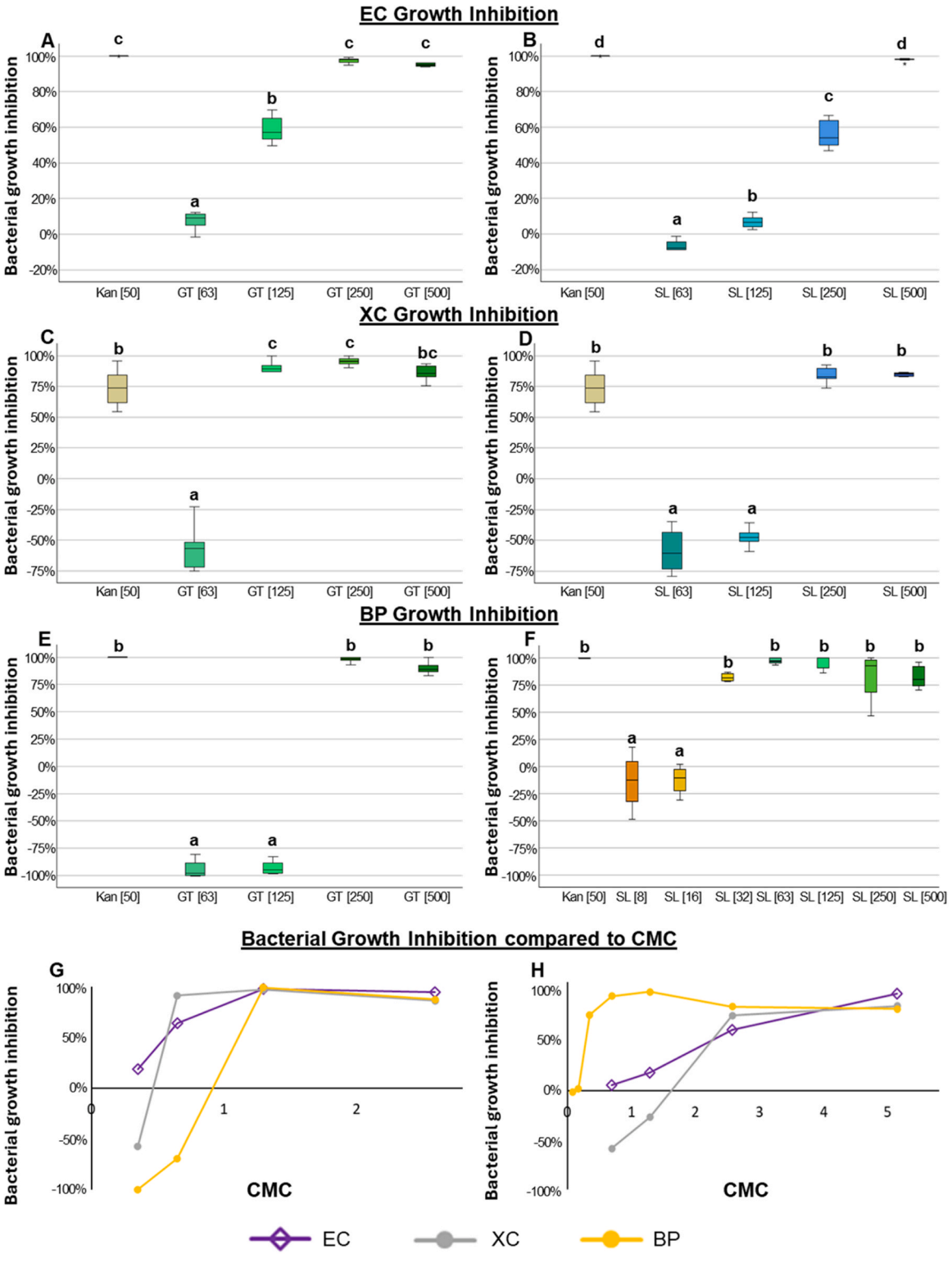
Firstly, aglycon portion form reversable bonds with sterols, then, when a high density of tomatine molecules has bound to sterols, their glycosidic residues can interact with one another forming a matrix of irreversible α-tomatine-cholesterol bonds that bend the membrane, eventually causing the budding off of small vesicles that undermine the integrity of the membrane and cause leakage and ultimately cell death (Milner et al., 2011).

Instead, on a first look, the bioactivity of SL did not seem to be directly linked to the surfactant properties. Still, as previously mentioned, the CMC of the α-tomatine portion of SL is 2.4 times higher than that of the whole extract. When taking this into consideration, and the fact that the effect against EC and XC was seen at a concentration of 2.5 CMC units, it is reasonable to suggest that the effect against these bacteria is associated to the surfactant properties of the α-tomatine contained in the SL.

Completely different was the case of SL against BP, where the inhibition remains very strong at concentrations significantly lower than CMC.

Surfactant molecules are usually described as being in a single-molecule state at concentrations below their CMC, nevertheless the existent capability to reduce the surface tension (Fig. 2) was more recently attributed to the presence of molecules-cluster or pre-micellar aggregation (LeBard et al., 2012; Sen et al., 2016). This partial reduction of surface tension might be sufficient to partially solubilize the tomatidine fraction of the SL extract, which accounts for approximately 50 % of the extract.

Tomatidine is known to have bacteriostatic and bactericidal properties against *Staphylococcus aureus* and *Bacillus* spp. small-colony variants, by interfering with protein biosynthesis and ATP-synthase activity (Guay et al., 2018; Lamontagne Boulet et al., 2018; Langlois et al., 2024; Mitchell et al., 2011). This latter mode of action has been linked to the integrity of the spiroaminoketal moiety of tomatidine and specific features of the C3 carbon; in fact, extensive modification of the 3β-hydroxyl group of the molecule is deleterious for its bactericidal effect (Lamontagne Boulet et al., 2018). In addition, tomatidine has been shown to enhance the toxicity of other molecules (i.e. gentamycin), thereby lowering their effective concentration (Chagnon et al., 2014). However, tomatidine is strongly hydrophobic and its reduced availability in solution impairs all these toxicity mechanisms. On its own, the molecule minimizes the contact surface with water through weak hydrogen bonds that produce insoluble aggregates (Maibaum et al., 2004; Bogunia and Makowski, 2020). Moreover, its solubility may improve after interaction with surfactant that limited tomatidine-water repulsion thank to the formation of bound with the hydrophobic moieties of the surfactant (Jeffrey et al., 2010). In both the extracts similar interactions are possible among α-tomatine and tomatidine in particular in the SL that has a more proportionate amount of the molecules. However, no or very limited improvement of the bioavailability and biocide effect was recorded. On the contrary, this item may be the explanation of the high toxicity highlighted in the trial with *Bacillus* species known to secrete large amount of surfactant



(caption on next page)

Fig. 4. Graphs reporting the inhibitory effect of bacterial growth of the GT and SL extracts. In each graph, the Y-axis reports the percentage of growth inhibition compared to the growth measured in the non-treated control. For graphs A to F, the X-axis reports the treatment, either GT or SL extract, or the control with the antibiotic Kanamycin (Kan) and the concentration of the treatment, expressed as μg mL^{-1}, between squared brackets. For graphs G and H, the X-axis reports the concentration of GT and SL extracts, respectively, expressed as CMC units. Graphs A, C, E and G report the results obtained with the GT extracts, while B, D, F, and H report the results of SL extracts. Graphs A and B report the results obtained with EC, graphs C and D the results with XC, graphs E and F the results with BP, while graphs G and H report the results with all three bacteria as lines with different colours, as reported in the legend. Different letters (a, b, c, d) over a bar indicate statistically different results according to a Kruskal-Wallis non-parametric test ($P < 0.05$).

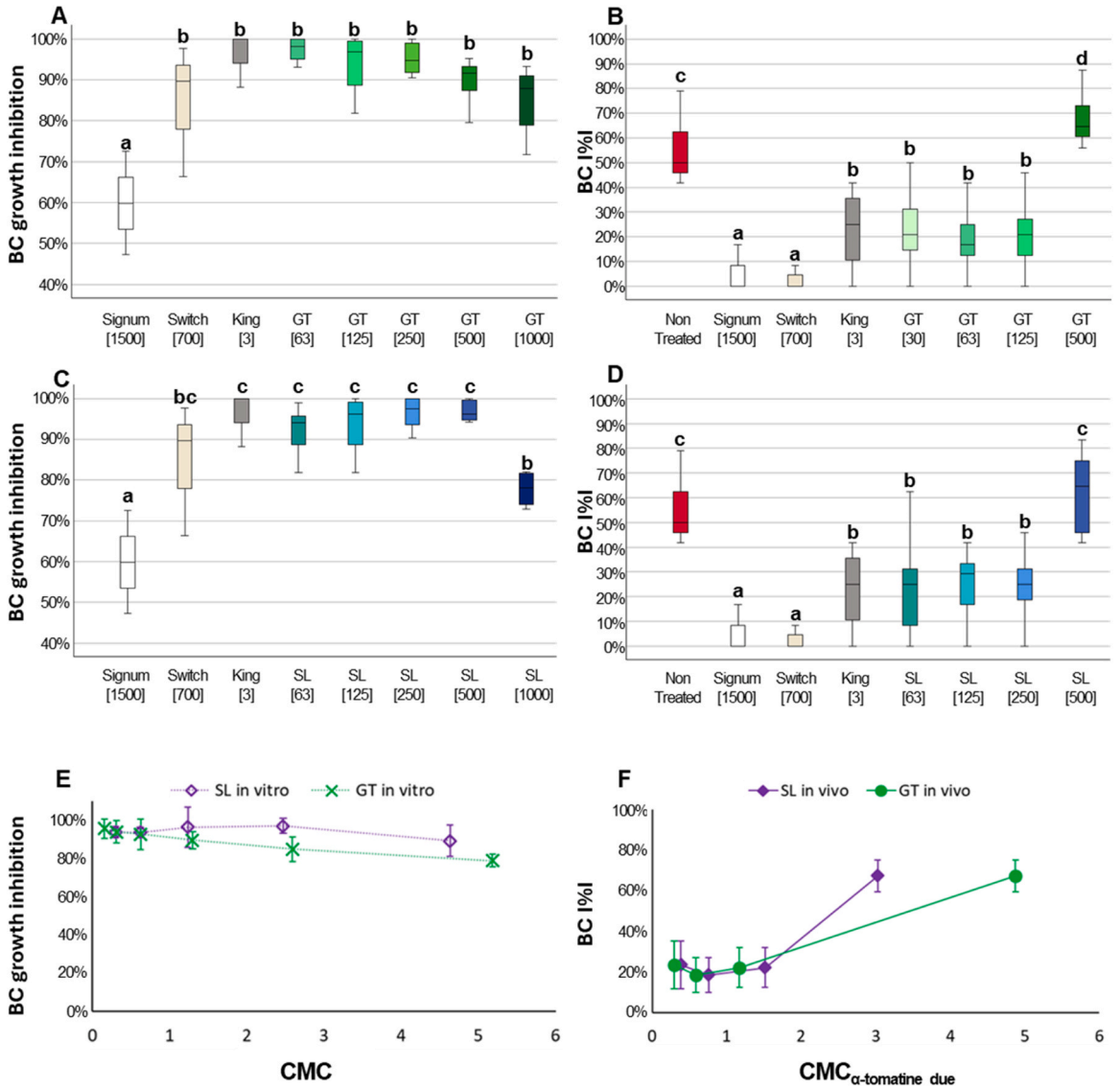


Fig. 5. Graphs reporting the inhibitory effect on BC growth in vitro and on BC infection on tomato leaves of the GT and SL extracts. In graphs A, C, and E, the Y-axis reports the inhibition of BC growth, while in graphs B, D, and F it reports the percentage of BC infection (I%I). In graphs A, B, C, and D the X-axis reports the treatment (either GT or SL extract, or the control with the fungicides Signum, Switch, or King) and the concentration of the treatment, expressed as μg mL^{-1}, between squared brackets. For graph E, the X-axis reports the concentration of GT and SL extracts expressed as CMC units. For graph F, the X-axis reports the concentration of GT and SL extracts expressed as CMC units calculated on the base of α-tomatine concentration only. For the in vitro results, concentrations ranging from 63 to 1000 μg mL^{-1} were used, while the in vivo experiments used concentrations between 30 and 500 μg mL^{-1} as reported in Supplementary Table 1. Different letters (a, b, c, d) over a bar indicate statistically different results according to a Kruskal-Wallis non-parametric test ($P < 0.05$).

called surfactins (Théatre et al., 2021).

Surfactins are cyclic lipopeptides with strong surfactant properties (Ongena and Philippe, 2008) composed of a hydrophilic protein head and a fatty acid chain which length ranges from C12 to C15. The length of the fatty acid influences the size of the aggregates, CMC, and number of molecules involved in the micelle formation (Bochynek et al., 2023). The protein part of surfactins often shows an apolar domain with aliphatic amino acids, mainly leucine (Théatre et al., 2021), an amino acid with which tomatidine is known to have strong interactions (Lisdiana et al., 2023) making the bonding between surfactin and tomatidine very likely. Since the surfactin micelle is characterised by a large surface and small internal volume, it is reasonable to suppose an almost outward position of the tomatidine in the micelle allowing its chemical reactivity as well as biocide effect (Long et al., 2015).

3.2.2. *Biocide activity against Botrytis cinerea*

The *in vitro* tests showed the capability of the extracts to reduce the growth of BC reaching levels of inhibition comparable or higher than those obtained by the fungicides (Fig. 5 A,C). The maximum effect was reached at very low concentration and maintained at plateau for some successive doses before, in the case of SL, decreasing at the highest concentrations tested.

The expression of dose in CMC unit highlighted that the maximum effect for doses below or equal to CMC $_{\alpha\text{-tomatine due}}$ (Fig. 5) as described before against BP. Then the reduction occurred with different degree after those concentrations. Similar trend has been described for fungicide with colloidal nature that at higher concentration are subjected to precipitation phenomenon (Owen et al.,

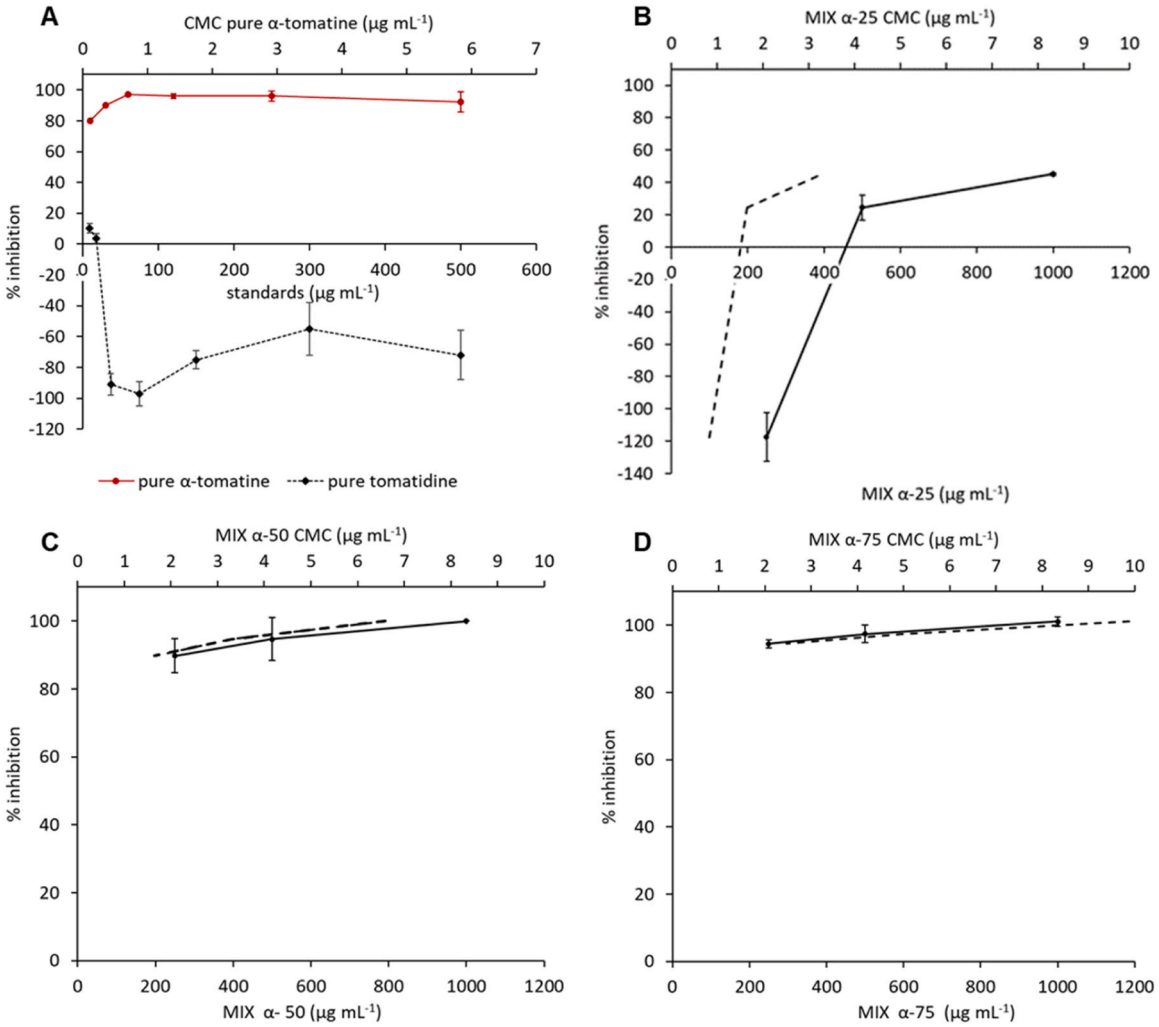


Fig. 6. Graphs reporting the inhibitory effect on BC growth *in vitro* of A) pure analytical standards of α-tomatine and tomatidine and three mixes of the two standards: B) MIX α-25, C) MIX α-50, and D) MIX α-75. The Y-axis reports the percentage of growth inhibition compared to a non-treated control. The lower X-axis reports the concentration of the standards or mixtures used as µg mL^{-1}, while the higher X-axis reports the same concentrations as α-tomatine CMC. In graph A, the red solid line indicates α-tomatine standards, while the black dotted line indicates tomatidine standards. For graphs B, C, and D the solid lines report the effects of the treatment related to the lower X-axis, absolute concentration, while the dotted lines report the effects of the treatment related to the top X-axis, α-tomatine CMC.

2014). The α-tomatine micelles can form super-micelles or micelles aggregates thus destined to precipitate. In this case, however, the toxic effect of α-tomatine may not be due exclusively to its surfactant nature. It is reported in literature that, on top of the previously mentioned effects of α-tomatine due to pore formation, other toxicity mechanisms of α-tomatine include accumulation of reactive oxygen species, activation of phosphotyrosine kinase and monomeric G protein signaling pathways leading to increased Ca^{2+}, and induction of apoptosis (Sandrock and Vanetten, 1998; Lorent et al., 2014; Tam et al., 2021).

The *in vivo* assay against BC gave the following results: without any inoculation with the pathogen, the leaves showed no development of symptoms, while the leaves inoculated with the pathogen and without any treatment showed high levels of rot, with an average of I%I around 50 % but reaching peaks of 80 % in some plates; the treatment with commercial fungicides greatly reduced the infection. In particular, the synthetic fungicides Switch and Signum entirely negated the infection in most cases, rarely allowing the development of the pathogen and, even then, just to very low degree. The King fungicide, which is available in organic agriculture, still managed to greatly reduce the development of symptoms to around half of that registered in the non-treated controls.

Most of the treatments made with the SL and GT extracts had the same efficacy as the King fungicide, being in the same range of efficacy and validated by statistical analysis (Fig. 5 B,D). The only exception is the highest concentration tested for both extracts (500 μg mL^{-1}) which showed infection values comparable or even higher than the non-treated control. This is in line with the results obtained from the *in vitro* assays, in which the lowest concentration tested had higher efficacy in inhibiting BC growth and higher concentrations eventually lost efficacy.

It is important to note that, with the exception of these last high-concentration conditions, all treatments managed to lower – at least in some cases – the infection index to 0 %, an important factor when determining the actual applicability in field conditions since only a complete halt to the growth of the pathogen can not only protect the plant organ, but also reduce the overall damage by preventing secondary infections that can happen throughout the season, in particular from leaf to fruit.

To achieve a deeper evaluation of the interaction, *in vitro* tests were performed with the pure standards and their mix (Fig. 6). The pure α-tomatine standard gave a significant reduction of BC growth, achieving the almost complete inhibition of the growth of the fungi at the lowest concentration of 30 μg mL^{-1} and maintaining the efficacy at higher concentrations. Instead, tomatidine had a limited inhibition effect at very low concentrations (9–18 μg mL^{-1}) and no inhibitory effect was seen at higher concentrations. On the contrary, the presence of high tomatidine concentration (37–500 μg mL^{-1}) seemed to promote the growth of the fungus. This increase in growth rate was registered at concentrations at which precipitation phenomenon was recorded (Fig. 3 A) avoiding the BC-tomatidine interaction. The calculation of the theoretical CMC of the MIX based on the CMC of the α-tomatine and α-tomatine/tomatidine proportion gave values of CMC equal to 101.2 μg mL^{-1}, 151.6 μg mL^{-1}, and 303.6 μg mL^{-1} for MIX $_{\alpha\text{-}75}$, $_{\alpha\text{-}50}$, $_{\alpha\text{-}25}$ respectively. Expressed as CMC the doses of the MIX $_{\alpha\text{-}75}$, $_{\alpha\text{-}50}$ were very similar or higher than CMC thus confirming the association effect and surfactant activity.

Despite this, the MIX $_{\alpha\text{-}25}$ was less effective in inhibiting fungal growth than the other MIX even at concentrations higher than CMC = 1 (Fig. 6 B). This reduced bioactivity may be due to a sequestering of available α-tomatine by the surplus of tomatidine: part of the α-tomatine will be involved in hydrophobic bonds with the tomatidine and therefore be unavailable to act against the fungus.

The other mix were tested at value > 1 CMC therefore no considerations may be done in this sense. However, the very good correspondence between the inhibition vs. concentration or CMC data highlighted almost well correspondence (Fig. 6 C-D).

The SL has a proportion glycated-aglycated saponine not yet tested with the MIX, but although it is borderline, no depressive effect on bioactivity against BC occurred. A possible explanation considered the involvement of other glycosylated tomatidine instead of α-tomatine in the interaction with tomatine to mitigate its negative effect. In fact, summing this fraction to the α-tomatine content (Table 1), the SL become a MIX $_{\text{glycosylated-50}}$ ratio typical safe from the depressive effect of tomatidine.

4. Conclusion

This study confirms the strong antimicrobial potential of tomatines and contributes to expanding knowledge on the use of extracts from tomato residues, as opposed to highly purified analytical standard molecules. Particularly noteworthy is the observation the GT extract, rich in α-tomatine and lacking tomatidine, exhibited an exceptionally high antifungal effect even at very low concentrations, while the antibacterial effect was significant only at concentrations higher than 1 CMC. This suggests that such natural extracts could serve as relatively selective molecules capable of inhibiting the growth of fungal pathogens while preserving bacterial diversity and the presence of mutualist bacteria. Further trials are necessary to gather more data on the effects of these extracts on non-target organisms. Nevertheless, the current results imply that tomatine-rich extracts might be an effective product for plant protection obtained from a circular economy approach.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data Availability

Data will be made available on request. All data produced as part of this study is reported in the present manuscript.

Acknowledgements

This work was supported by the Cariplo Foundation, Call "Circular Economy – Promoting research for a sustainable future – 2022" Project: "Development of biocide tomatine-biochar product for agriculture using the tomato industry waste residues as feedstock and a cascade production approach. BECOME -BiopEstiCide from tOMato rEsidues" Grant Number 0675-2022). The authors are grateful to Demetrio Marcianò from University of Milan for valuable help in reviewing literature for the writing of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.103807.

References

Abbasi-Parizad, P., De Nisi, P., Pepé Sciarria, T., Scarafoni, A., Squillace, P., Adani, F., Scaglia, B., 2022. Polyphenol bioactivity evolution during the spontaneous fermentation of vegetal by-products. Food Chem. *374*, 131791 https://doi.org/10.1016/j.foodchem.2021.131791.

Abbasi-Parizad, P., Salvino, R.A., Passera, A., Follador, A., Cosentino, C., Jucker, C., Savoldelli, S., Bacenetti, J., Casati, P., Scaglia, B., 2023. Development of a cascade production system finalized to the extraction of all-tomatine-rich fraction using the tomato cannery waste as feedstock. J. Clean. Prod. 401, 136743 https://doi.org/10.1016/j.jclepro.2023.136743.

Alkawareek, M.Y., Akkelah, B.M., Mansour, S.M., Amro, H.M., Abulateefeh, S.R., Alkilany, A.M., 2018. Simple experiment to determine surfactant critical micelle concentrations using contact-angle measurements. J. Chem. Educ. 95, 2227–2232. https://doi.org/10.1021/acs.jchemed.8b00276.

Arneson, P., Durbin, P.D., 1968. Studies on the mode of action of tomatine as a fungitoxic agent. Plant Phys. 43, 683–686. https://doi.org/10.1104/pp.43.5.683.

Bailly, C., 2021. The steroidal alkaloids α-tomatine and tomatidine: panorama of their mode of action and pharmacological properties. Steroids 176, 108933. https://doi.org/10.1016/j.steroids.2021.108933.

Bochynek, M., Lewińska, A., Witwicki, M., Dębczak, A., Łukaszewicz, M., 2023. Formation and structural features of micelles formed by surfactin homologues. Front. Bioeng. Biotechnol. 11, 1211319. https://doi.org/10.3389/fbioe.2023.1211319.

Bogunia, M., Makowski, M., 2020. Influence of Ionic Strength on Hydrophobic Interactions in Water: Dependence on Solute Size and Shape. J. Phys. Chem. B 124, 10326–10336. https://doi.org/10.1021/acs.jpcb.0c06399.

Böttger, S., Hofmann, K., Melzig, M.F., 2012. Saponin can perturb biological menbranes and reduce the surface tension of aqueous solution: A correlation? Bioorg. Med. Chem. 20, 2822–2828. https://doi.org/10.1016/j.bmc.2012.03.032.

Boyaci, D., Iorio, G., Sozbilen, G.S., Alkan, D., Trabattoni, S., Pucillo, F., Farris, S., Yemenicioğlu, A., 2019. Development of flexible antimicrobial zein coatings with essential oils for the inhibition of critical pathogens on the surface of whole fruits: Test of coatings on inoculated melons. Food Packag. Shelf Life 20, 100316, 1016/j.fpsl.2019.100316.

Chagnon, F., Guay, I., Bonin, M.A., Mitchell, G., Bourab, K., Malouin, F., Marsault, E., 2014. Unveiling the structure-activity relationship of tomatidine, a steroid alkaloid with unique antibiotic properties against persistent forms of *Staphylococcus aureus*. Eur. J. Med. Chem. 80, 605–620. https://doi.org/10.1016/j.ejmech.2013.11.019.

Daraban, G.M., Hlihor, R.M., Suteu, D., 2023. Pesticides vs. Biopesticides: from pest management to toxicity and impacts on the environment and human health. Toxics 11, 983. https://doi.org/10.3390/toxics11120983.

Dhakal, R., Singh, D.N., 2019. Biopestidiced: a key to sustainable agriculture. Int. J. Pure Appl. Biosci. 7, 391–396. https://doi.org/10.18782/2320-7051.7034.

Friedman, M., 2002. Tomato glycoalkaloids: role in the plant and in the diet. J. Agr. Food Chem. 50, 5751–5780. https://doi.org/10.1021/jf020560c.

Friedman, M., 2013. Anticarcinogenic, cardioprotective, and other health benefits of tomato compounds lycopene, α-tomatine, and tomatidine in pure form and in fresh and processed tomatoes. J. Agr. Food Chem. 61, 9534–9550. https://doi.org/10.1021/jf402654e.

Friedman, M., Levin, C.E., 1998. Dehydrotomatine content in tomatoes. Journal of Agricultural and Food Chemistry 46 (11), 4571–4576. https://doi.org/10.1021/jf9804589.

Friedman, M., Levin, C.E., McDonald, G.M., 1994. α-Tomatine Determination in Tomatoes by HPLC Using Pulsed Amperometric Detection. Journal of Agricultural and Food Chemistry 42 (9), 1959–1964. https://doi.org/10.1021/jf00045a024.

Guay, I., Boulanger, S., Isabelle, C., Brouillette, E., Chagnon, F., Bouarab, K., Marsault, E., Malouin, F., 2018. Tomatidine and analog FC04-100 possess bactericidal activities against Listeria, Bacillus and Staphylococcus spp. BMC Pharmacology and Toxicology 19 (1), 1–12. https://doi.org/10.1186/s40360-018-0197-2.

Hoagland, R.E., 2009. Toxicity of tomatine and tomatidine on weeds, crops and phytopathogenic fungi. Allelopath. J. 23, 425–436.

Huang, Z., Yao, Y., Ayele, B.A., Chen, Q., Hue, Q., 2020. Solubilization mechanism of diesel in saponin micelle for contaminated montmorillonite washing. J. Environ. Chem. Eng. 8 (5), 104203 https://doi.org/10.1016/j.jece.2020.104203.

Jeffrey, L., Woodhead, J.L., Hall, C.H., 2010. Simulation of micelle formation in the presence of solutes. Langmuir 26 (19), 15135–15141. https://doi.org/10.1021/la1024444.

Kozukue, N., Friedman, M., 2003. Tomatine, chlorophyll, B-carotene and lycopene content in tomatoes during growth and maturation. J. Sci. Food Agric. 83, 195–199. https://doi.org/10.1002/jsfa.1292.

Kozukue, N., Han, J.S., Lee, K.R., Friedman, M., 2004. Dehydrotomatine and α-Tomatine Content in Tomato Fruits and Vegetative Plant Tissues. Journal of Agricultural and Food Chemistry 52 (7), 2079–2083. https://doi.org/10.1021/jf0306845.

Lamontagne Boulet, M., Isabelle, C., Guay, I., Brouillette, E., Langlois, J., Jacques, P., Rodrigue, S., Brzezinski, R., Beauregard, P.B., Bouarab, K., Boyapelly, K., Boudreault, P., Marsault, É., Malouin, F., 2018. Tomatidine Is a Lead Antibiotic Molecule That Targets Staphylococcus aureus ATP Synthase Subunit C. Antimicrob Agents Chemother 62, 1–18. https://doi.org/10.1128/aac.02197-17.

Langlois, J.-P., Larose, A., Brouillette, E., Delbrouck, J.A., Boudreault, P.-L., Malouin, F., 2024. Mode of Antibacterial Action of Tomatidine C3-Diastereoisomers. Molecules 29, 1–19. https://doi.org/10.3390/molecules29020343.

LeBard, D.N., Levine, B.G., DeVane, R., Shinoda, W., Klein, M.L., 2012. Premicelles and monomer exchange in aqueous surfactant solutions above and below the critical micelle concentration. Chem. Phys. Lett. 522, 38–42. https://doi.org/10.1016/j.cplett.2011.11.075.

Lisdiana, L., Iswari, S.R., Christijanti, W., Harjono, H., Sasi, F.A., Dafip, M., 2023. Tomatidine Extraction from Various Solvents and Cancer Inhibition Mechanism Through In Silico Analysis. Trend Sci. 20 (10), 195–199. https://doi.org/10.48048/tis.2023.5738.

Long, J.A., Rankin, B.M., Amotz, D.B., 2015. Micelle Structure and Hydrophobic Hydration. J. Am. Chem. Soc. 137 (33), 10809–10815. https://doi.org/10.1021/jacs.5b06655.

Lorent, J.H., Quentin-Leclercq, J., Mingeot-Leclercq, M.P., 2014. The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. Org. Biomol. Chem. 12, 8803–8822. https://doi.org/10.1039/c4ob01652a.

Maibaum, L., Dinner, A.R., Chandler, D., 2004. Micelle Formation and the Hydrophobic *Effect*. J. Phys. Chem. 108, 6778–6781. https://doi.org/10.1021/jp037487t.

Milner, S.E., Brunton, N.P., Jones, P.W., O'Brien, N.M., Collins, S.G., Maguire, A.R., 2011. Bioactivities of glycoalkaloids and their aglycones from *Solanum* species. J. Agric. Food Chem. 59, 3454–3484. https://doi.org/10.1021/jf200439q.

Mitchell, G., Gattuso, M., Grondin, G., Marsault, E., Bourab, K., Malouin, F., 2011. Tomatidine inhibits replication of *Staphylococcus aureus* Small-Colony Variants in Cystic Fibrosis airway epithelial cells. Antimicr. Ag. Chemother. 55, 1937–1945. https://doi.org/10.1128/AAC.01468-10.

Ongena, M., Philippe, J., 2008. Bacillus lipopeptides: versatile weapons for plant disease biocontrol. Tr. Microbiol. 16, 115–125. https://doi.org/10.1016/j.tim.2007.12.009.

Owen, S.C., Doak, A.K., Ganesh, A.N., Nedyalkvoa, L., McLaughlin, C.K., Shoichet, B.K., Shoichet, M.S., 2014. Colloidal drug formulations can explain "bell-shaped" concentration-response curves. ACS Chem. Biol. 9, 777–784. https://doi.org/10.1021/cb4007584.

Pingulkar, K., Kamat, A., Bongirwar, D., 2001. Microbiological quality of fresh leafy vegetables, salad components and ready-to-eat salads: an evidence of inhibition of *Listeria monocytogenes* in tomatoes. Int. J. Food Sc. Nutr. 52, 15–23. https://doi.org/10.1080/09637480020027219.

Samal, K., Das, C., Mohanty, K., 2017. Eco-friendly biosurfactant saponin for the solubilization of cationic and anionic dyes in aqueous system. Dyes Pigm 140, 100–108. https://doi.org/10.1016/j.dyepig.2017.01.031.

Sandrock, R.W., Van Etten, H.D., 1998. Fungal sensitivity to and enzymatic degradation of the phytoanticipin α-tomatine. Phytopathology 88, 137–143. https://doi.org/10.1094/PHYTO.1998.88.2.137.

Scaglia, B., Baglieri, A., Tambone, F., Gennari, M., Adani, F., 2016. Chlorpyrifos-methyl solubilisation by humic acids used as bio-surfactants extracted from lignocelluloses and kitchen wastes, 208e213 Chemosphere 159. https://doi.org/10.1016/j.chemosphere.2016.06.008.

Sen, P.K., Mukherjee, P., Pal, B., 2016. Effects of pre-micelles of anionic surfactant SDS on the electron transfer reaction between methylene blue and ascorbic acid. J. Mol. Liq. 224, 472–479. https://doi.org/10.1016/j.molliq.2016.10.034.

Tam, C.C., Nguyen, K., Nguyen, D., Hamada, S., Kwon, O., Kuang, I., Gong, S., Escobar, S., Liu, M., Kim, J., Hou, T., Tam, J., Cheng, L.W., Kim, J.H., Land, K.M., Friedman, M., 2021. Antimicrobial properties of tomato leaves, stems, and fruit and their relationship to chemical composition. BMC Compl. Med. Ther. 21, 1–9. https://doi.org/10.1186/s12906-021-03391-2.

Tamasi, G., Pardini, A., Bonechi, C., Donati, A., Pessina, F., Marcolongo, P., 2019. Characterization of nutraceutical components in tomato pulp, skin and locular gel. Eur. Food Res. Techn. 245, 907–918. https://doi.org/10.1007/s00217-019-03235-x.

Taveira, M., Ferreres, F., Gil-Izquierdo, A., Oliveira, L., Valentao, P., Andrade, P.B., 2012. Fast determination of bioactive compounds from Lycopersicon esculentum Mill. Leaves. Food Chem. 135, 748–755. https://doi.org/10.1016/j.foodchem.2012.05.016.

Théatre, A., Cano-Prieto, C., Bartolini, M., Laurin, Y., Deleu, M., Niehren, J., Fida, T., Gerbinet, S., Alanjary, M., Medema, M.H., Léonard, A., Lins, L., Arabolaza, A., Gramajo, H., Gross, H., Jacques, P., 2021. The surfactin-like lipopeptides from *Bacillus* spp.: natural biodiversity and synthetic biology for a broader application range. Front. Bioeng. Biotech. 9, 623701 https://doi.org/10.3389/fbioe.2021.623701.

Toffolatti, S.L., Maddalena, G., Marcianò, D., Passera, A., Quaglino, F., 2020. A molecular epidemiology study reveals the presence of identical genotypes on grapevines and ground cover weeds and the existence of separate genetic groups in a *Botrytis cinerea* population. Plant Path 69, 1695–1707. https://doi.org/10.1111/ppa.13257.

Townsend, G.K., Heuberger, J.W., 1943. Methods for estimating losses caused by disease in fungicide experiments. Plant Dis. 27, 340–343.

Vercesi, A., Toffolatti, S.L., Venturini, G., Campia, P., Scagnelli, S., 2014. Characterization of Botrytis cinerea populations associated with treated and untreated cv. Moscato vineyards. Phytopath. Medit. 53, 108–123. https://doi.org/10.14601/Phytopathol_Mediterr-12367.

Yamanaka, T., Vincken, J.P., De Waard, P., Sanders, M., Takada, N., Gruppen, H., 2008. Isolation, characterization, and surfactant properties of the major triterpenoid glycosides from unripe tomato fruits. J. Agr. Food Chem. 56, 11432–11440. https://doi.org/10.1021/jf802351c.

You, Y., van Kan, J.A.L., 2021. Bitter and sweet make tomato hard to (b)eat. N. Phytol. (1), 90–100. https://doi.org/10.1111/nph.17104.