Large-Scale Screening of 239 Traditional Chinese Medicinal Plant Extracts for Their Antibacterial Activities against Multidrug-Resistant Staphylococcus aureus and Cytotoxic Activities

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Abstract: Novel alternative antibacterial compounds have been persistently explored from plants as natural sources to overcome antibiotic resistance leading to serious foodborne bacterial illnesses. In this study, the ethanolic extracts from 239 traditional Chinese medicinal plants (TCMP)’ materials were screened to discover promising candidates that have strong antibacterial properties against multidrug-resistant Staphylococcus (S.) aureus and low cytotoxicity. The results revealed that 74 extracts exhibited good antibacterial activities (diameter of inhibition zone (DIZ) ≥ 15 mm). Furthermore, 18 extracts (DIZ ≥ 20 mm) were determined their minimum inhibitory concentrations (MIC) and minimum bactericide concentrations (MBC), ranging from 0.1 to 12.5 mg/mL and 0.78 to 25 mg/mL, respectively. In addition, most of the 18 extracts showed relatively low cytotoxicity (a median lethal concentration (LC50) >100 µg/mL). The 18 extracts were further determined to estimate possible correlation of their phenolic contents with antibacterial activity, and the results did not show any significant correlation. In conclusion, this study selected out some promising antibacterial TCMP extracts with low cytotoxicity, including Rhus chinensis Mill., Ilex rotunda Thunb., Leontice kiangnanensis P.L.Chiu, Oroxylum indicum Vent., Isatis tinctorial L., Terminalia chebula Retz., Acacia catechu (L.f.) Wild., Spatholobus suberectus Dunn, Rabdosia rubescens (Hems.) H.Hara, Salvia miltiorrhiza Bunge, Fraxinus fallax Lingelsh, Coptis chinensis Franch., Agrimonia Pilosa Ledeb., and Phellodendron chinense C.K.Schneid.

Keywords: medicinal plant; antimicrobial activity; drug resistance; foodborne pathogens; cytotoxicity; polyphenols
1. Introduction

Foodborne diseases are mainly transmitted by bacterial-contaminated food and pose serious public health problems that cause significant morbidity and mortality worldwide [1]. According to the World Health Organization (WHO) report, approximately 2.2 million people annually die from foodborne diseases caused by pathogens [2]. *Staphylococcus* (*S.* *aureus*), one of the major foodborne pathogens, leads to a wide range of severe foodborne diseases due to its invasiveness, multidrug resistance, and virulence [3,4]. The indiscriminate use of antibiotics in animal husbandry and hospitals have accelerated the emergence of *S. aureus* to acquire resistance to various antibacterial agents [5–8]. The rapid development of multidrug resistance has led to a decrease in the effectiveness of commercial antibiotics and has resulted in untreatable infections [9]. Moreover, a challenging complication is presented by the slow pace of research and development of new antibiotics that may add to the existing arsenal of antimicrobials to circumvent the outbreak and transmission of drug resistant bacteria [10]. As such, to overcome the outbreak and proliferation of multidrug-resistant bacterial infections, novel antibacterial compounds of plant, animal, bacterial, algal, and fungal origin have been persistently explored as an alternative to conventional antibiotics. In recent years, plants have particularly played a pivotal role as viable sources for the discovery of strong antibacterial agents applied in the pharmaceutical, food, and animal feed industries.

Plants produce various secondary metabolites that have antimicrobial properties such as phenolics, alkaloids, flavonoids, tannins, terpenoids, polyacetylenes, and quinones [6,11–13]. These phytochemical compounds, namely phytoalexins, are naturally occurring antimicrobials produced by plant as active defense mechanisms against phytopathogens such as bacteria, fungi, and viruses in the environment [14]. In addition, these biomolecules possess a wide spectrum of chemical properties including unique chemical scaffolds and complex structures, providing potentiating inhibitory effects associated with possible distinctive mechanisms of action against microorganisms [15,16]. There have been several previous studies reporting a significant advantage of these phytochemicals to impede the development of drug-resistant bacteria [17,18]. Some of these phytochemicals and plant extracts are designated by the United States Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS), and have low toxicity and good acceptability to food and medical applications [18]. All these features in combination support that plants are a promising source of natural antimicrobials.

There has been a growing interest in discovering new and effective antimicrobial substances from traditional Chinese medicinal plants (TCMP). In China, 11,118 species of plants have been used as medicinal materials for centuries [19]. By virtue of diverse bioactive compounds synthesized from the plants, their therapeutic applications have been reported in many ethnopharmacological studies including anticancer, hepatoprotection, anti-inflammatory, antidiarrheal antioxidant, antiviral, and antimicrobial activities [20,21]. Numerous studies have been demonstrated on the antimicrobial activities of TCMP extracts against different types of microbes, but there still remain challenges to discover novel antimicrobial molecules from TCMP. Therefore, 239 TCMP materials (89 families, 206 genera, and 233 species) were selected from the Chinese literature that have antibacterial effects in this present study. It was aimed to take a large-scale screening of TCMP for the assessment of potentially strong antibacterial activity against multidrug-resistant *S. aureus* to evaluate the provision of promising baseline information for the potential use of TCMP as novel antimicrobial agents in pharmaceutical, food and animal feed industries.

2. Results and Discussion

2.1. Screening of Antibacterial TCMP Extracts against Antibiotic-Resistant *S. aureus*

The 239 ethanolic TCMP extracts were used to screen for their potential antibacterial activity against *S. aureus* including one reference strain ATCC 25923 and one antibiotic-resistant strain SJTUF 20827 based on the agar diffusion method (Table S1 in Supplementary Material and Figure 1). Antibacterial activities of TCMP extracts, evaluated by the measurement of diameter of inhibition zone (DIZ), were
classified as very strong (DIZ ≥ 20 mm), strong (20 > DIZ ≥ 15 mm), moderate (15 > DIZ ≥ 10 mm), and weak (DIZ < 10 mm) [22]. Fifty-two out of 239 extracts showed strong antibacterial activities against S. aureus ATCC 25923, whereas 41 extracts had strong antibacterial activities against S. aureus SJTUF 20827 (DIZ ≥ 15 mm) that had similar values of DIZ (12.9–34.6 mm) with conventional antibiotics such as oxacillin (32 µg/mL) and ampicillin (4 µg/mL). Of the extracts, Speranskia tuberculata Baill. exhibited the best inhibitory effects on the growth of both reference and antibiotic-resistant S. aureus strains with DIZ values 26.8 and 25.2 mm, respectively. In this study, antibacterial properties of TCMP against S. aureus may be attributed to the simplicity of its cell membrane structure and composition in Gram-positive bacteria, which can be easily accessible to permeation of hydrophobic active compounds in TCMP [13]. Therefore, antibacterial molecules can directly access the target sites both on the cell wall and in the cytoplasm, resulting in pore formation, intercellular constituent leakages, structural or functional abnormalities of the bacterial membrane phospholipid bilayer, and inhibition of its biosynthesis [23–26]. In addition, these active compounds are commonly targeted at multiple sites rather than one specific site of antibacterial action [27,28]. For example, Ebelle et al. [29] reported that the methanolic extract of Enantia chlorantha had several modes of antibacterial action including the extension of the bacterial latency period, the deactivation of H+ - ATPases activity in cell membrane, the loss of the salt tolerance of the S. aureus, and inhibition of the biofilm formation. Since TCMP extracts contain diverse chemical substances, they probably influence the bacteria cell constituents or molecular targets by various mechanisms.

![S. aureus ATCC25923 and S. aureus SJTUF20827](image)

**Figure 1.** Screening of 239 TCMP extracts for antibacterial activities against S. aureus ATCC 25923 (reference strain) and SJTUF 20827 (antibiotic-resistant strain). Number in parentheses indicates the number of TCMP extracts, which exhibited DIZ value in four different ranges of DIZ value; DIZ < 10 mm, 10 ≤ DIZ < 15 mm, 15 ≤ DIZ < 20 mm and DIZ ≥ 20 mm.

The results also revealed that the reference S. aureus was more susceptible to TCMP extracts compared with the antibiotic-resistant strain. These differences may be attributed to the fact that the antibiotic-resistant bacteria have acquired complex resistance mechanisms to survive in the presence of the antimicrobial molecules, rendering the antibacterial agents inactive. Indeed, bacterial pathogens have developed strategies for antibacterial resistance by chemical alteration and destruction of antibacterial molecules, decrease in their penetration, extrusion of the antibacterial compound, and interference in their access to target sites [30,31]. In addition, they are able to synthesize a protective polysaccharide layer on their surface, known as a bacterial biofilm [32]. These resistant factors might influence the activity of TCMP extracts against antibiotic-resistant S. aureus in this study. Further studies are needed to determine the mechanisms of antibacterial action of the extracts and their specific targets in antibiotic-resistant S. aureus strains.
2.2. Selected TCMP Extracts with a Wide Range of Antibacterial Activities against Multidrug-Resistant Bacteria

Based on initial screening results in which both the reference and antibiotic-resistant *S. aureus* model strains were found to be susceptible to TCMP extracts, we selected 74 strong TCMP extracts (DIZ ≥ 15 mm) and proceeded to the assessment of the wide range of antibacterial activities against multidrug-resistant *S. aureus* strains SJTUF 20745, 20746, 20758, 20978, and 20991 (Table 1). In this study, results showed that the selected TCMP extracts exhibited different antibacterial effects against multidrug-resistant pathogens; however, most extracts revealed strong and extensive antibacterial activities. As shown in Figure 2, 51.35%, 62.16%, 64.86%, 95.94%, and 77.97% of TCMP extracts possessed strong inhibitory effects (DIZ ≥ 15 mm) against *S. aureus* SJTUF 20745, 20746, 20758, 20978, and 20991, respectively.

However, certain TCMP extracts such as *Cnidium monnieri* Cusson and *Nervilia fordii* Schltr. showed poor inhibitory effects against most multidrug-resistant *S. aureus* strains (Table 1). In total, TCMP extracts of *S. tuberculata*, *Acacia catechu* (L.f.) Willd., *Coptis chinensis* Franch., *Quercus infectoria* Oliv., *Leontice kiangnanensis* P.L. Chiu, *Rhus chinensis* Mill., *Rabdosia rubescens* (Hems.) H. Hará, and *Dalbergia odorifera* T.C. Chen had the best antibacterial activities against antibiotic-resistant bacteria with DIZ values ranging from 18.2 to 31.9 mm compared with tested conventional antibiotics (DIZ = 11.9–21.7 mm). The strong antibacterial activities of these TCMP have been reported in many studies, which are correlated well with the results obtained in this study. For example, previous study showed that the methanolic extract of *A. catechu* at a concentration of 100 mg/mL had a strong antibacterial activity against *S. aureus*, with DIZ value 20 mm [33]. Feng and Xu [34] demonstrated that *R. rubescens* extract had broad-spectrum inhibitory effects on *S. aureus*, *Symphoricarpos albus* and *Bacillus subtilis* strains with DIZ values in range of 17.5–22.8 mm, 18.2–22.3 mm, and 17.3–25.6 mm, respectively. The antibacterial effects of *Q. infectoria* gall extract against multidrug-resistant bacteria has also been studied [35]. The ethanolic extracts of *Q. infectoria* (1 mg/disc) exhibited inhibitory effects against methicillin-resistant *S. aureus* (MRSA, DIZ = 13.3–15.3 mm), methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS, DIZ = 19.3 mm), and multidrug-resistant *Acinetobacter* spp. (DIZ = 12.7 mm).

![Figure 2](image-url)
Table 1. Antibacterial activities of 74 selected TCMP extracts against multidrug-resistant S. aureus strains based on diameter of inhibition zone (DIZ).

| No. | Family                  | Scientific Name                      | Common Name                  | Extracted Plant Part | DIZ (mm) against S. aureus |
|-----|-------------------------|--------------------------------------|------------------------------|----------------------|---------------------------|
| 3   | Anacardiaceae           | Rhus chinensis Mill.                 | Nutgall tree                 | gall                 | SJTUF 20745               |
| 8   | Apiaceae                | Crinum molleri Curson                | Mernier’s snowpawsley        | fruit                | 21 ± 1                    |
| 22  | Aquifoliaceae           | Ilex rotunda Thunb.                  | Kurogane holly               | bark                 | 17.2 ± 0.5                |
| 26  | Araceae                 | Alpinia officinarum L.               | Gaultheria lutea             | root                 | 18.3 ± 0.4                |
| 31  | Aristolochiaceae        | Aristolochia fimbriata L.            | Aristolochia fimbriata L.    | aerial part           | 6.1 ± 0.4                 |
| 36  | Asphodelaceae           | Helichrysum italicus                 | Asteraceae                   | aerial part           | 12.3 ± 0.5                |
| 46  | Asteraceae              | Hemistepta lyrate Bunge              | Asteraceae                   | aerial part           | 12.4 ± 0.3                |
| 51  | Asparagaceae            | Inula japonica Thunb.                | Inula japonica Thunb.        | flower               | 16.4 ± 0.4                |
| 58  | Berberidaceae           | Leonotis leonurus L.                 | Leonotis leonurus L.         | flower               | 19.1 ± 0.7                |
| 61  | Bignoniaceae            | Oroxylum indicum Vent.               | Indian trumpet flower        | seed                 | 18.5 ± 0.3                |
| 62  | Boraginaceae            | Lithospermum erythrorhizon Siebold & Zucc. | Boraginaceae               | leaf                 | 11.8 ± 0.2                |
| 65  | Brassicaceae            | Isatis tinctoria L.                  | Purple gromwell              | leaf                 | 21 ± 1                    |
| 81  | Combretaceae            | Terminalia chebula Retz.            | Dyer’s wood                  | leaf                 | 21 ± 1                    |
| 87  | Dioscoraceae            | Dioscorea bulbifera L.              | Meconopsis VK               | twig                 | 18.2 ± 0.5                |
| 90  | Ebenaceae               | Diospyros kaki Thunb.               | Chinese persimmon            | calyx                | 16.7 ± 0.4                |
| 92  | Ericaceae               | Pyrola calliantha Andres             | Chinese pyrole               | aerial part           | 13.7 ± 0.1                |
| 95  | Euphorbiaceae           | Euphorbia humifusa Willd.           | Herba euphorbiae             | aerial part           | 13.8 ± 0.2                |
| 96  | Euphorbiaceae           | Sparrskaia tuberculata Baill.       | Herba sparrskae             | tuberculata          | 24.1 ± 0.4                |
| 97  | Fabaceae                | Acacia catechu (L.) Willd.          | Catechu                      | branch               | 23 ± 0.8                  |
| 101 | Cassia occidentalis L.  | Pueraria tuberosa L.                | Cassia occidentalis L.       | seed                 | 14.9 ± 0.1                |
| 102 | Cassia tora L.          | Dipsacus sylvestris (L.)            | Sickle Cassia               | seed                 | 14.0 ± 0.4                |
| 103 | Dalbergia odorifera T. C. Chen | Fragrant rosewood                | Indian trumpet flower        | trunk                | 18.2 ± 0.5                |
| 107 | Glechoma hederacea L.   | Chinese hoven locust                | Chinese hoven locust        | branch               | 14.0 ± 0.4                |
| 110 | Glycyrrhiza uralensis L. | Licorice                            | Chinese hoven locust        | branch               | 12.8 ± 0.1                |
| 112 | Lablab purpureus (L.)   | Lablab Bean                         | Lablab Bean                  | seed                 | 14.6 ± 0.4                |
| 114 | Lablab purpureus (L.)   | Aleppoa oak                         | Callistephus sp.             | gall                 | 22.4 ± 0.5                |
| 115 | Sophora tonkensis Gagnepain | Vietnamese sophora                 | Vietnamese sophora           | thymol               | 14.3 ± 0.4                |
| 117 | Spastymbos suberocostus Dunn | Cauious spastymbos              | Cauious spastymbos           | aerial part           | 17 ± 0.1                  |
| 122 | Hypericaceae            | Hypericum androsaicum Thunb.        | Mattet St. John’s-wort       | aerial part           | 17.8 ± 0.4                |
| 126 | Lamiaceae               | Isodon serra Kudo                   | Ruprechtia                  | aerial part           | 17.8 ± 0.4                |
| 132 | Raddia radicans (Hemsl.) H. Harra | Herba radicosia                 | Herba radicosia             | aerial part           | 15.8 ± 0.2                |
| 133 | Salvia miltiorrhiza Bunge | Vietnamese sophora                | Vietnamese sophora           | thymol               | 17.3 ± 0.5                |
| 139 | Lardizabalaceae         | Sargentodoxa cunninge Redher & E. H. Wilson | Sargentodoxa cunninge      | stem                 | 14.5 ± 0.1                |
| 140 | Laurusaceae             | Cinnamomum cassia (L.) Presl       | Sargentodoxa cunninge       | stem                 | 15.2 ± 0.1                |
| 148 | Lecythidaceae           | Diphasiastrum complanatum (L.) Holub | Groundsel                   | aerial part           | 20.8 ± 0.2                |
| 151 | Magnoliaceae            | Magnolia denudata Oess.             | Lilypity                     | bud of flower         | 15.2 ± 0.1                |
| 153 | Malvaceae               | Bombax malabaricum DC.             | Bombax malabaricum DC.      | root bark            | 10.3 ± 0.2                |
| 154 | Helicteres angustifolia L. | Narrowleaf screeetree              | Helicteres angustifolia L.   | root                 | 17.7 ± 0.1                |
| 155 | Pterospermum heterophyllum Hance | Helicteres angustifolia L.   | Pterospermum heterophyllum Hance | root                 | 15.7 ± 0.2                |

SJTUF 20745
SJTUF 20746
SJTUF 20758
SJTUF 20797
SJTUF 20991

Note: The values in the table represent the diameter of inhibition zone (DIZ) in millimeters (mm). The values are rounded to one decimal place. The table includes the number of strains based on diameter of inhibition zone (DIZ).
Table 1. Cont.

| No. | Family          | Scientific Name                  | Common Name                             | Extracted Plant Part | DIZ (mm) against *S. aureus* |
|-----|-----------------|----------------------------------|-----------------------------------------|-----------------------|------------------------------|
| 159 | Meliaceae       | *Melia azedarach* L.             | Chinaberry tree                         | bark & root bark      | 18.4 ± 0.3                   |
| 168 | Oleaceae        | *Fraxinus fujian Lingelsh.*       | Largeleaf Chinese ash                   | bark                  | 13.4 ± 0.5                   |
| 169 |                   | *jasminum nudiflorum* Lind.      | Winter jasmine                          | bud of flower         | 11.3                         |
| 172 | Orchidaceae     | *Nervilia fordii Schlr.*         | Ford nervilla                           | rhizome & leaf        | NIZ                          |
| 173 | Pholiota chinensis Lindl. | *Pholiota chinensis* Lindl. | Chinese photinia herb                 | stem                  | 14.9 ± 0.3                   |
| 177 | Orobanchaceae   | *Strya asiatica* (L.) Kunzite    | Asiatic witchweed                      | aerial part            | 11.9                         |
| 178 | Paoniaeae       | *Paonia lactiflora* Pall.        | Chinese peony                           | root                  | 15.6 ± 0.4                   |
| 179 | Paoniaeae       | *Paonia suffruticosa* Andrews    | root bark                               | 14.1 ± 0.1            |
| 180 | Paoniaeae       | *Paonia veitchii* Lynch          | root                                    | 14.5 ± 0.1            |
| 182 | Phyllanthaceae  | *Phyllanthus emblica* L.         | Emblica                                 | fruit                 | 16.6 ± 0.5                   |
| 183 | Pinaceae        | *Pseudolarix amabilis* Rebder    | Chinese golden larch                    | root bark             | 17.4 ± 0.5                   |
| 185 | Poaceae         | *Bambusa tuludoides* Munro       | Puntungpole bamboe                      | stem                  | 9.4                          |
| 186 |                   | *Chrysopogon aciculatus* Trin.   | Mackie’s pest                           | aerial part            | 11.4 ± 0.2                   |
| 192 | Polygonaceae    | *Polygonum bistorta* L.          | Meadow bistort                          | rhizome               | 15.3 ± 0.2                   |
| 193 | Polygonaceae    | *Polygonum chinense* L.          | Chinese knotweed                        | aerial part            | 14.2 ± 0.1                   |
| 194 | Polygonaceae    | *Polygonum multiflorum* Thunb.   | Tuberc fleecflower                      | stem                  | 16.6 ± 0.3                   |
| 195 | Polygonaceae    | *Polygonum multiflorum* Thunb.   | Tuberc fleecflower                      | tuberous root         | 14 ± 1                       |
| 196 | Rumex obtusifolius L. | *Rumex obtusifolius* L. | Bitter dock                             | root                  | 12.8                         |
| 197 | Primulaceae     | *Ardisia japonica* Blume         | Marlberry                               | aerial part            | 16.5 ± 0.6                   |
| 198 | Ranunculaceae   | *Lysimachia christinae* Hancke   | Herb westlake                           | 14.3 ± 0.3            |
| 200 | Ranunculaceae   | *Coptis chinensis* Franch.       | Chinese goldthread                     | rhizome               | 23 ± 1                       |
| 201 | Thalictrum aquilegfolium L. | *Thalictrum aquilegfolium* L. | French meadow-rue                       | rhizome & root        | 14.4                         |
| 202 | Rosaceae        | *Agrimonia pilosa* Ledeb.        | Herba agrimoniae                       | aerial part            | 16.5 ± 0.2                   |
| 203 | Rosaceae        | *Duchesnea indica* (Andr.) Focke | Indian strawberry                       | aerial part            | 11.5                         |
| 205 | Rosaceae        | *Graum aleppicum* Jacq.          | Aleppo avens                           | aerial part            | 15.0 ± 0.5                   |
| 206 | Rubiaceae       | *Punica mume* Siebold & Zucc.    | Japanese apricot                       | fruit                 | 12.1 ± 0.1                   |
| 210 | Rubiaceae       | *Serissa serissoides* (DC.) Druce | Snowrose                                | aerial part            | 16.1 ± 0.3                   |
| 217 | Rutaceae        | *Phellodendron chinense* C. K. Schneid. | Chinese corktree                     | bark                  | 20 ± 1                       |
| 218 | Zingiberaceae   | *Zanthoxylum nitidum* DC.        | Shiny-leaf prickly-ash                  | root                  | 15.5 ± 0.4                   |
| 222 | Saxifragaceae   | *Saxifraga stolonifera* Meerb.   | Creeping saxifrage                      | aerial part            | 11.5                         |
| 227 | Solanaceae      | *Lycium chinense* Mill.          | Chinese boxthorn                        | bark                  | 19 ± 0.2                     |
| 230 | Tamariaceae     | *Tamarix chinensis* Lour.        | China tamarisk                          | branch & leaf         | 13.6 ± 0.2                   |
| 231 | Thymelaeaceae   | *Daphne genkwa* Siebold & Zucc.  | Chinese daphne                          | bud of flower          | 22.1 ± 0.1                   |
| 239 | Zingiberaceae   | *Curcuma phaeocaulis* Valetton   | Rhizoma zedoariae                      | rhizome               | 14.1 ± 0.4                   |
|     | Ampicillin      |                                  |                                        |                       | 21.7 ± 0.3                   |
|     | Oxacinil        |                                  |                                        |                       | 15.5 ± 0.6                   |
|     | DMSO            |                                  |                                        |                       | NIZ                          |

1 74 TCMP extracts (100 mg/mL) with strong inhibitory effects on the growth of reference *S. aureus* strain ATCC 25923 and antibiotic-resistant *S. aureus* strain SJTU 20827 (DIZ ≥ 15 mm, Table S1 in Supplementary Material), were selected to investigate the wide range of antibacterial activities against multidrug-resistant *S. aureus* strains SJTU 20745, 20746, 20758, 20978, and 29991. Diameter of inhibition zone (DIZ) was determined by the agar diffusion method in triplicate and value of DIZ was expressed as mean ± standard deviation (SD). DIZ values less than 8.0 mm was defined as “no inhibition zone (NIZ)”. Ampicillin (32 μg/mL) and oxacillin (4 μg/mL) were used as positive controls, while DMSO was used as a negative control.
2.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of TCMP Extracts against Multidrug-Resistant *S. aureus*

Next, 18 selected TCMP extracts with the highest DIZ values were subjected to investigations of their MIC and MBC against multidrug-resistant *S. aureus* strains (Table 2). The MIC values for the extracts ranged from 0.1 to 12.5 mg/mL and the MBC values ranged from 0.78 to 25 mg/mL. Of the TCMP extracts evaluated, multidrug-resistant *S. aureus* strains were impacted with a high degree of susceptibilities, expressed as MIC, to *R. chinensis* (0.1–0.195 mg/mL), followed by *Q. infectoria* (0.195 mg/mL), *C. chinensis* (0.195–0.39 mg/mL), *D. odorifera* (0.39 mg/mL), *Agrimonia pilosa* Lede. (0.1–0.78 mg/mL), *A. catechu* (0.195–0.78 mg/mL), *Phellodendron chinense* C.K. Schneid. (0.195–0.78 mg/mL), *Terminalia chebula* Retz. (0.39–0.78 mg/mL), *Oroxylum indicum* (0.39–1.56 mg/mL), and *Spatholobus suberectus* Dunn. (0.39–1.56 mg/mL). Conversely, *Isatis tinctoria* L. showed relatively poor antibacterial activity with the high MIC values (3.125–12.5 mg/mL) and MBC values (12.5–25 mg/mL). Accordant results were previously reported from other studies. In one example, Tian et al. [36] reported that gall extract of *R. chinensis* was more effective against *S. aureus* (MIC = 0.25 mg/mL) among tested microorganisms. Moirangthem et al. [37] found that the bark extract from *O. indicum* exhibited strong antibacterial effects against *S. aureus* (MIC = 62.5 µg/disc), and also showed a broad antibacterial spectrum against Gram-positive and Gram-negative bacteria with the MIC values ranging from 62.5 to 250 µg/disc. Similar to the results obtained in this study, Tayel et al. [38] reported that the extract of *Q. infectoria* exhibited strong inhibitory effects against *S. aureus* with the MIC value for 0.313 mg/mL. Wan et al. [35] also determined the antibacterial activities of *Q. infectoria* that MIC values of the ethanolic extract against multidrug-resistant bacteria were in the range of 0.03 to 0.63 mg/mL, indicating their strong antibacterial activities. Other TCMP extracts with good antibacterial activities against other bacterial pathogens reported in other studies [39–41], are in agreement with the results in Table 2. The observed slight differences in the susceptibility of test bacteria were due to different solvents, extraction methods, antibacterial test methods, harvesting time, and the variation in the proportion of bioactive compounds.

**Table 2.** Minimal inhibitory concentration (MIC, mg/mL) and minimal bactericidal concentration (MBC, mg/mL) of selected TCMP extracts against multidrug-resistant *S. aureus*.

| No. | Scientific Name          | ATCC 25923 | SJTF 20745 | SJTF 20746 | SJTF 20758 | SJTF 20758 | SJTF 20827 | SJTF 20978 | SJTF 20979 | SJTF 20991 |
|-----|--------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 3   | *Rhus chinensis* Mill.   | 0.1        | 3.125      | 0.195      | 1.56       | 0.78       | 0.195      | 0.125      | 0.195      | 0.195      |
| 22  | *Ilex rotunda* Thunb.    | 0.78       | 1.56       | 0.78       | 1.56       | 0.78       | 1.56       | 0.78       | 1.56       | 0.78       |
| 51  | *Isula japonica* Thunb.  | 3.125      | 25         | 0.195      | 1.56       | 0.78       | 0.195      | 0.125      | 0.195      | 0.195      |
| 58  | *Lemtice kriottanensis* PL.Chiu | 0.78     | 0.156     | 0.78       | 0.156      | 0.78       | 0.156      | 0.78       | 0.156      | 0.78       |
| 61  | *Oroxylum indicum* Vent. | 0.39       | 0.156     | 0.39       | 0.156      | 0.39       | 0.156      | 0.39       | 0.156      | 0.39       |
| 85  | *Isatis tinctoria* L.    | 3.125      | 25         | 3.125      | 25         | 3.125      | 25         | 3.125      | 25         | 3.125      |
| 81  | *Terminalia chebula* Retz.| 0.195      | 0.25       | 0.195      | 0.25       | 0.195      | 0.25       | 0.195      | 0.25       | 0.195      |
| 96  | *Speranta tuberculata* Bail. | 0.78     | 0.78       | 0.78       | 0.78       | 0.78       | 0.78       | 0.78       | 0.78       | 0.78       |
| 97  | *Acacia catechu* (L.f.) Will.d. | 0.195    | 0.3125     | 0.625      | 0.195      | 0.625      | 0.195      | 0.625      | 0.195      | 0.625      |
| 103 | *Delbergia odorata* T.C.Chen | 0.39     | 0.3125     | 0.39       | 0.625      | 0.39       | 0.625      | 0.39       | 0.625      | 0.39       |
| 112 | *Quercus indica* Oliv.   | 0.1        | 0.125      | 0.195      | 0.25       | 0.195      | 0.125      | 0.195      | 0.195      | 0.195      |
| 115 | *Spatholobus suberectus* Dunn | 0.195   | 0.195      | 0.195      | 0.195      | 0.195      | 0.195      | 0.195      | 0.195      | 0.195      |
| 123 | *Rhabdosia rubescens* (Hems.l.) H.Hara | 1.56  | 12.5      | 15.6       | 12.5       | 12.5       | 12.5       | 12.5       | 12.5       | 12.5       |
| 133 | *Salvia miltiorrhiza* Bunge | 0.39     | 0.3125     | 0.39       | 0.625      | 0.39       | 0.625      | 0.39       | 0.625      | 0.39       |
| 168 | *Fraxinus falax* Lingdan. | 1.56      | 0.25       | 0.195      | 0.25       | 0.195      | 0.25       | 0.195      | 0.25       | 0.195      |
| 200 | *Capia chinensis* Franch. | 0.39       | 0.195      | 0.39       | 0.195      | 0.39       | 0.195      | 0.39       | 0.195      | 0.39       |
| 202 | *Agrimonia pilosa* Lede.  | 0.1        | 0.156      | 0.156      | 0.156      | 0.156      | 0.156      | 0.156      | 0.156      | 0.156      |
| 217 | *Phellodendron chinense* C.K. Schneid. | 0.78    | 0.3125    | 0.3125     | 0.3125     | 0.3125     | 0.3125     | 0.3125     | 0.3125     | 0.3125     |
| 310 | *Amicinillin* (µg/mL) | 0.05       | 0.25       | 0.25       | 0.25       | 0.25       | 0.25       | 0.25       | 0.25       | 0.25       |

18 selected TCMP extracts with the highest DIZ values (DIZ ≥ 20 mm) were subjected to investigations of their MIC and MBC against one reference (ATCC 25923), one erythromycin-resistant (SJTUF 20827) and five multidrug-resistant (SJTUF 20745, SJTF 20746, SJTF 20758, SJTF 20978 and SJTF 20991) *S. aureus* strains (n = 3). Ampicillin and Oxacillin were used as positive controls. 1 NA, not applicable.
In general, this study selected out some TCMP with strong and extensive growth inhibitory effects on multidrug-resistant \textit{S. aureus}, including \textit{R. chinensis}, \textit{Q. infectoria}, \textit{C. chinensis}, \textit{D. odorifera}, \textit{A. pilosa}, \textit{A. catechu}, \textit{P. chinense}, \textit{T. chebula}, \textit{O. indicum}, \textit{S. suberectus}, \textit{S. tuberculate} \textit{Inula japonica} Thunb., \textit{R. rubescens}, and \textit{Salvia miltiorrhiza} Bunge. Additionally, to the best of our knowledge, our study is the first to report the promising antimicrobials of plant extracts, including \textit{I. rotunda}, \textit{I. japonica}, \textit{L. kiangnanensis}, \textit{S. tuberculate} and \textit{F. fallax}, all of which had strong and broad-range of antibacterial activities against multidrug-resistant \textit{S. aureus}.

2.4. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of TCMP Extracts

Plant species naturally produce various secondary metabolites leading to possession of valuable biological activities including antibacterial activity [20]. Selecting a potential antibacterial agent from plant-derived compounds might be an appropriate strategy since these substances are produced by the outcome of plant defense responses against biotic stress from bacterial, fungi, viruses, and abiotic stress, and commonly have antibacterial activities [18]. Major plant-derived compounds that are responsible for antibacterial activity include phenolics, phenolic acids, flavonoids, quinones, tannins, coumarins, terpenoids, and alkaloids [16]. The structural diversity and various chemical compositions of antibacterial compounds result in strong and broad-range of antibacterial activities with diverse mechanisms of antibacterial action [15].

Multiple studies have reported that phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins) presented in TCMP contribute to good antibacterial properties against bacterial pathogens [42–44]. Polyphenolic compounds such as flavan-3-ols, flavonols, tannins, and phenolic acids are known to exhibit wide spectra and strong antimicrobial activity compared with other polyphenols [45]. The phenolic compounds are also able to suppress microbial virulence factors such as quorum sensing, bacterial biofilms, bacterial motility, bacterial toxins, and bacterial surfactant [16]. For example, Wang et al. [46] reported that vesticarpan derived from \textit{D. odorifera} as the phenolic compound showed antibacterial activity against \textit{Ralstonia solanacearum}. Four flavonoids isolated from \textit{D. odorifera}, including sativanone, (3R)-vestitone, liquiritigenin and isoliquiritinigenin, exhibited strong antibacterial activity against \textit{R. solanacearum} with the DIZ values ranging from 11.2 to 16.6 mm [47]. Sithisarn et al. [48] demonstrated that flavones such as baicalein, baicalin, and chrysin in \textit{O. indicum} are active compounds for antibacterial activities against \textit{Staphylococcus intermedius}, \textit{Streptococcus suis}, \textit{Pseudomonas aeruginosa} and extended-spectrum \(\beta\)-lactamase (ESBL)-producing \textit{Escherichia coli}. Cho et al. [49] also found active flavonoids like 7-hydroxy-6-methoxy-flavanone and formononetin isolated from \textit{S. suberectus} that inhibited \textit{S. aureus} derived sortase A, which is responsible for anchoring surface protein virulence factors.

Therefore, we further determined the phenolic content in 18 TCMP extracts with strong antibacterial effect to demonstrate whether their major antibacterial compounds are attributed to polyphenols. Total phenolic content (TPC) and total flavonoid content (TFC) were determined by the Folin-Ciocalteu method and AlCl3-based colorimetric method, respectively. The TPC in the extracts were in the range of 31.4 to 646 mg gallic acid equivalent (GAE)/g dry weight (DW), whereas TFC were in the range of 5.27 to 377 mg catechin equivalent (CE)/g DW (Table 3). Consistent with previous studies, strong antibacterial TCMP extracts such as \textit{R. chinensis}, \textit{Q. infectoria}, \textit{A. Pilosa}, \textit{A. catechu} and \textit{S. suberectus} contained high amounts of polyphenols, with TPC 632, 646, 371, 545, and 489 mg GAE/g DW, respectively, leading to a suspicion that polyphenols might be the actual active compounds responsible for antibacterial activity against \textit{S. aureus}. A case in point being the gall extract of \textit{Q. infectoria} which presented the highest polyphenol level (646 mg GAE/g DW), but it presented a relatively low flavonoids level (38 mg CE/g DW), indicating that major phenolic components in \textit{Q. infectoria} might be consisted of non-flavonoids such as phenolic acids, stilbenes and lignans. Arina and Harisun [50] found that \textit{Q. infectoria} gall mainly contained polyphenols with a high concentration of tannic acid (2233 mg/g). Tannins, well-known as antibacterial agents, have been shown to inhibit the growth of various pathogens by destroying their bacterial plasma membrane and forming hydrogen bonds between tannins and the proteins.
in bacterial cells, resulting in the protein denaturation and their coagulation [51]. Conversely, it was also found that some other strong antibacterial TCMP extracts like *C. chinensis*, *S. tuberculate*, and *S. miltiorrhiza* included relatively low TPC (95, 31.4 and 56 mg GAE/g DW, respectively) and TFC (23.1, 13.4 and 34.5 mg CE/g DW, respectively), suggesting that their major antibacterial substances might be nonphenolic antibacterial compounds.

Table 3. Total phenolic content (TPC) and the total flavonoid content (TFC) in selected TCMP extracts.

| No. | Scientific Name                      | TPC (mg GAE/g DW) | TFC (mg CE/g DW) |
|-----|--------------------------------------|-------------------|------------------|
| 3   | *Rhus chinensis* Mill.               | 632 ± 4           | 36.8 ± 0.9       |
| 22  | *Ilex rotunda* Thunb.                | 143 ± 3           | 16.9 ± 0.2       |
| 51  | *Iruia japonica* Thunb.              | 92 ± 6            | 62 ± 3           |
| 58  | *Leontice kiangnanensis* P. L. Chiu  | 33 ± 1            | 5.27 ± 0.08      |
| 61  | *Oroxylum indicum* Vent.             | 158 ± 3           | 18.1 ± 0.6       |
| 65  | *Isatis tinctoria* L.                | 60 ± 3            | 8.35 ± 0.2       |
| 81  | *Terminalia chebula* Retz.           | 553 ± 4           | 27 ± 0.8         |
| 96  | *Spermska tuberculata* Baill.        | 31.4 ± 0.6        | 13.4 ± 0.5       |
| 97  | *Acacia catechu* (L.f.) Wild.        | 545 ± 2           | 377 ± 3          |
| 103 | *Dalbergia odorifer* T. C. Chen      | 215 ± 6           | 62 ± 1           |
| 112 | *Quercus infectoria* Oliv.           | 646 ± 3           | 38 ± 3           |
| 115 | *Spatholobus suberectus* Dunn        | 489 ± 5           | 214 ± 11         |
| 132 | *Rhabdosia rubescens* (Hems.) H. Har| 135 ± 4           | 41 ± 1           |
| 133 | *Salvia miltiorrhiza* Burge          | 56 ± 5            | 34.5 ± 0.9       |
| 168 | *Fraxinus fallax* Lingelsh.          | 363 ± 15          | 106 ± 5          |
| 200 | *Coptis chinensis* Franch.           | 95 ± 3            | 23.1 ± 0.3       |
| 202 | *Agrimonia pilosa* Ledeb.            | 371 ± 11          | 154 ± 10         |
| 217 | *Phellodendron chinense* C. K. Schnei| 123 ± 2           | 35 ± 2           |

The experiments were performed in triplicate and the results were expressed as mean ± SD. One–way analysis of variance (ANOVA) plus post hoc Tukey test was performed and different superscript lowercase letters (a–l) indicated statistically significant difference (*p* < 0.05). GAE, gallic acid equivalent; CE, catechin equivalent; DW, dry weight.

Although the spectrophotometric methods such as Folin-Ciocalteu method and AlCl<sub>3</sub>-based colorimetric method are commonly used to quantify total phenolics in plant extracts [52–57], these analyses are not accurate measurements. The Folin-Ciocalteu assay can contribute to the overestimation of TPC due to the presence of reducing compounds such as ascorbic acid, reducing sugars, and aromatic amino acids (tyrosine and tryptophan), leading to the disruption of phenolic oxidation reaction [58–61]. Similar to the limitation for TPC, the AlCl<sub>3</sub>-based method for TFC has a constraint on the measurement of all classes of flavonoids in the extracts and the considerable contents of total flavonoid can be attributed to the presence of phenolic acids in the extract during the absorbance measurement at 510 nm [62]. Therefore, more accurate and precise analyses such as chromatographic methods would be necessary to conduct for qualification of total phenolic compounds in the TCMP extracts. In this present study, these spectrophotometric methods were used for simple comparison among the selected TCMP extracts. However, further research would be required using more accurate and reliable analytical methods such as chromatographic assays in order to investigate phytochemical constituents and to identify major antimicrobial compounds in each extract.

2.5. Cytotoxicity and Safety of the TCMP Extracts

In order to utilize the crude TCMP extracts as antimicrobial agents in pharmaceuticals, food, and animal feed industries, it is essential to ensure their safety. The 18 TCMP extracts with good antibacterial effect were evaluated for their cytotoxicity in normal human foreskin fibroblast (HFF) cells. Cell viability varies with the extracts when the cells were exposed at the concentration of 100 µg/mL for 24 h (Figure 3). *S. tuberculate* extract had a considerably low level of cell viability with 4.83%, followed by *D. odorifera* (7.14%), *I. japonica* (24.9%), *R. chinensis* (34.5%), and *Q. infectoria* (47.5%). Additionally, in order to investigate the cytotoxic effects of the TCMP extracts, dose-response
experiments were conducted (Figure S1 in Supplementary Material) and the median lethal concentration (LC₅₀) values were calculated (Table 4). The cytotoxicity of plant extracts with LC₅₀ value ≤ 20 µg/mL was regarded as a possible cytotoxic plant extract [63]. The results indicated that most plant extracts had low cytotoxicities with considerably high LC₅₀ value ≥ 100 µg/mL, excluding S. tuberculate (LC₅₀ = 25.9 µg/mL), D. odorifera (LC₅₀ = 44.1 µg/mL), I. japonica (LC₅₀ = 54.1 µg/mL), R. chinensis (LC₅₀ = 77.6 µg/mL), and Q. infectoria (LC₅₀ = 91.6 µg/mL), supporting by previous studies that also found a weak cytotoxicity of T. chebula [64,65], S. suberectus [66], Q. infectoria [67,68] and O. indicum [37]. However, it is necessary to test the toxicity by in vivo studies, since in vitro cellular toxicity might provoke different consequences in animals associated with gut interactions and bioavailability of the extracts [18].

![Figure 3](image)

**Figure 3.** The viability of HFF cells exposed to selected TCMP extracts (100 µg/mL) for 24 h assessed by the colorimetric assay using MTT. RC = R. chinensis; IR = I. rotunda; IJ = I. japonica; LK = L. kiangnanensis; OI = O. indicum; IT = I. tinctoria; TCR = T. chebula; STB = S. tuberculate; AC = A. catechu; DO = D. odorifera; QI = Q. infectoria; SS = S. suberectus; RR = R. rubescens; SM = S. miltiorrhiza; FF = F. fallax; CCF = C. chinensis; AP = A. Pilosa; PC = P. chinense; Control = 0.1% DMSO. Results are expressed as mean ± standard deviation (SD) and the experiments were conducted in triplicate (p < 0.05).

**Table 4.** Cytotoxicity (LC₅₀) on the human foreskin fibroblast (HFF) cells and selectivity index (SI) of selected TCMP extracts. SI value greater than 1 indicate that the extract is more toxic to the pathogen than to human cells, suggesting possible safety.

| Scientific Name                      | Cytotoxicity (LC₅₀, µg/mL) | Selectivity Index (SI = LC₅₀/MIC) |
|--------------------------------------|-----------------------------|----------------------------------|
| 3                                    | Rhus chinensis Mill.        | 77.6                             | 0.77                             |
| 22                                   | Ilex rotunda Thunb.         | >100                             | 554                              |
| 51                                   | Inula japonica Thunb.       | 54.1                             | 0.02                             |
| 58                                   | Leontice kiangnanensis P. L. Chiu | >100                  | 2687                             |
| 61                                   | Oroxylum tinctorium Vent.   | >100                             | NA                               |
| 65                                   | Isatis tinctoria L.         | >100                             | 76.0                             |
| 81                                   | Terminalia chebula Retz.    | >100                             | 2.20                             |
| 96                                   | Speranokia tuberculata Baill. | 25.9                    | 0.03                             |
| 97                                   | Acacia catechu (L.f.) Willd. | NA ³                           | NA                               |
| 103                                  | Dalbergia odorifera T. C. Chen | 44.1                     | 0.11                             |
| 112                                  | Quercus infectoria Oliv.    | 91.6                             | 0.47                             |
| 115                                  | Spatholobus suberectus Dunn | >100                             | 138                              |
| 132                                  | Rabdosia rubescens (Hemsl.) H. Har. | >100                  | 158                              |
| 133                                  | Salvia miltiorrhiza Bunge    | NA                               | NA                               |
| 168                                  | Fraxinus fallax Lingelsh.   | >100                             | 5.55                             |
| 200                                  | Coptis chinensis Franch.     | >100                             | 86.1                             |
| 202                                  | Agrimonia pilosa Ledeb.      | >100                             | 204                              |
| 217                                  | Phellodendron chinense C. K. Schneid. | >100                  | 1.85                              |

¹ NA, not applicable.
To verify the safety of plant extracts, selectivity index (SI) of plant extracts was also determined by LC50 dividing by MIC value (Table 4). The SI value greater than 1 indicates that a plant extract is more toxic to the pathogen than the host cell [63]. In other words, the plant is safe and possible to be developed as antibacterial agents [13]. I. japonica extract had the lowest SI value of 0.02 against S. aureus, followed by S. tuberculate (0.03), D. odorifera (0.11), Q. infectoria (0.47), and R. chinensis (0.77). Besides the five extracts, most other plant extracts had strong inhibitory effects on S. aureus, but were less cytotoxic to human cells, suggesting that these plants could be developed as herbal medicine, food additive or preservative in the future.

2.6. Correlations Analysis among Polyphenolic Content, Antibacterial Effect, and Cytotoxicity of TCMP Extracts

The presence of polyphenolic compounds in the TCMP extracts can be related to strong antibacterial activity against multidrug-resistant S. aureus. In previous study, Shan et al. [42] demonstrated good linear correlations of phenolic content with the antibacterial activity of medicinal herbs against foodborne bacteria including S. aureus ($r^2 = 0.93$). Pavić et al. [52] also reported that TPC in a medicinal plant, namely Ruta graveolens L., was strongly correlated to the MIC values of E. coli, B. subtilis, and S. aureus ($r = 0.973, p < 0.050$). These results suggest that polyphenols might contribute to antibacterial properties of plant extracts. The antibacterial activity of polyphenols can involve various mechanisms of action such as disrupting the cell integrity, destroying membrane proteins, increasing permeability of cell membrane, inhibiting biofilm formation, inactivating microbial enzymes, up/down-regulating proteins involved in DNA and RNA synthesis, and deprivation of metal iron by their chelating ability [69,70]. For instance, some phenolics such as chlorogenic acid, tannic acid, and caffeic acid inhibited on the bacterial growths due to hyperacidification at the plasma membrane interface, resulting in disrupting H⁺-ATPase pump and thereby causing cell death [53].

In order to understand the interrelationships between the antibacterial activity of TCMP extracts and polyphenolic contents, 18 TCMP extracts with good effects were used in an analysis of correlations among MIC, TPC, and TFC values (Figure 4). The results with high $p$ values ($p > 0.05$) are interpreted as without statistical significance that might be confounded by the small sample size ($n = 18$) in this study because of $p$ value’s dependence on the sample size [71,72]. Unlike $p$ value, the effect size is independent of sample size [71]. In this study, Pearson’s correlation coefficient ($r$) was introduced as an effect size index to quantify difference among variables and to understand the correlations of antibacterial activity with phenolic contents. A moderate negative correlation was obtained between TPC and MIC with Pearson’s correlation coefficient $r = -0.400 (p = 0.100)$, and a very weak correlation was also shown between TFC and MIC with $r = -0.226 (p = 0.366)$, in agreement of the results obtained by a previous study [54], showing moderate correlations of antibacterial activity with phenolic contents in spice extracts ($r = 0.541, p < 0.001$).

The $r$ values were low and moderate based on the standards of effect size, which are classified as small ($r = \pm 0.2$), medium ($r = \pm 0.3$), and large ($r = \pm 0.5$) [71]. The effect size can be influenced by factors such as amount of variability, linear relationship among two variables, presence of outlier and measurement errors [73]. The relationships between phenolic contents and antibacterial activities showed the inclination to nonlinearity, leading to the low values of Pearson’s correlation (Figure 4). In addition, the narrow range of MIC values (0.01–12.5 mg/mL) compared to TPC (31.4–646 mg GAE/g DW) and TFC (5.27–214 mg CE/g DW) might be attributed to low $r$ values. This tendency for the weak correlations between phenolic contents and antibacterial activity might be suspected to the high levels of TPC, resulting from overestimated TPC values due to the presence of reducing compounds in the TCMP extracts [58–61]. Therefore, the phytochemical profiles of TCMP extracts need to be investigated by accurate and precise analyses in the future to reliably comprehend the correlations of antibacterial activity with phenolic compounds in the extracts. In other respects, the results may be also suggested that antibacterial activity of TCMP extracts is attributed to nonphenolic substances, especially the extracts of C. chinensis, S. tuberculate, and S. miltiorrhiza, which had relatively low polyphenols contents.
(Table 3). Indeed, Lee et al. [74] discovered that a terpenoid compound, namely dihydrotanshinone I, isolated from the roots of *S. miltiorrhiza* was the strong antibacterial compound against the broad range of gram-positive bacteria such as *B. subtilis* with a low MIC value of 3.1 µg/mL.

![Figure 4](image_url)

**Figure 4.** Pearson correlation analysis. (A) Correlation of total phenolic content (TPC) with minimum inhibitory concentration (MIC). (B) Correlation of TPC with cell viability (%) at 100 µg/mL. (C) Correlation of total flavonoid content (TFC) with MIC. (D) Correlation of TFC with cell viability (%). (E) Correlation of TPC with TFC. (F) Correlation of MIC with cell viability (%).

For the purpose of evaluating possible antibacterial compounds in TCMP extracts with their safety for use, we also analyzed the correlations of antibacterial activity with cell viability (%), showing the weak positive correlation with *r* = 0.257 (*p* = 0.303) (Figure 4). The results indicate that cytotoxic compounds might be discordant with antibacterial compounds [13,63]. Indeed, Dzoyem et al. [75] found
that antibacterial compounds such as ursolic acid, quercitrin, and entadanin from *Entada abyssinica* were relatively low cytotoxic on Vero monkey kidney cells. Two flavonoids isolated from *Pappea capensis*, quercetin-3-O-rhamnoside and epicatechin, exhibited a wide range of antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli* and *Klebsiella pneumoniae* and showed considerably low cytotoxic on Vero monkey kidney cells with LC$_{50}$ values $> 200$ µg/mL [76]. Therefore, there still remain the possibilities to identify strong antibacterial compound(s) from *S. tuberculate*, *I. japonica*, *D. odorifera* and *Q. infectoria* that were relatively more toxic to the pathogen than the host cells (Table 4).

3. Materials and Methods

3.1. Chemicals and Reagents

Dimethyl sulfoxide (DMSO) and 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Beyotime (Shanghai, China). Ethanol was purchased from Titan Chem. (Shanghai, China). Luria Bertani (LB) broth, agar bacteriological, and Mueller–Hinton broth were from Oxiod (Basingstoke, UK). Ampicillin and oxacillin were purchased from Meilune (Dalian, China). Sodium chloride, sodium nitrite, sodium hydroxide, potassium chloride, disodium phosphate and monopotassium phosphate were purchased from General-Reagent® (Shanghai, China). Aluminum chloride was purchased from Sinopharm Chemical Reagent (Shanghai, China). Sodium carbonate was from J&K Scientific (Beijing, China). Resazurin was purchased from Adamas (Shanghai, China). Gallic acid was from 87 Energy Chemical (Shanghai, China). Folin-Ciocalteu reagent was from Macklin (Shanghai, China). Deionized water was used in all experiments.

3.2. Collection of Plant Samples

The 239 dried TCMP (89 families, 206 genera, and 233 species) were collected from the markets in Shanghai, China. The TCMP samples presented in Table 1 and Table S1 in the Supplementary Material were sorted in term of family, scientific name, and common name identified from GBIF (http://www.gbif.org/) and Tropicos® (http://www.tropicos.org/).

3.3. Preparation of Plant Extracts

The dried TCMP samples were pulverized by a lab-scale miller (S025, IKA, Staufen, Germany). In this study, ethanol was used as an extraction solvent due to its safety and good extraction activity to obtain the desired antimicrobial components from the plant materials [77]. 4.0 g dried powder of each TCMP was extracted two times with 40 mL of 80% ($v$/$v$) ethanol using an ultrasound-assisted extraction method (1 h, 40 °C, and 480 W) to improve extraction efficiency and the yield of antibacterial compounds in the plants [55,78,79]. Each extract was centrifuged at room temperature (900× g, 15 min), and the supernatants were collected, combined, and concentrated using a rotary evaporator (RE-52AA, Shanghai Ya Rong Co., Ltd, Shanghai, China) at 40 °C under vacuum. The concentrated extract was dried by a vacuum freeze-dryer (SJIA-5FE, Ningbo Shuang Jia instrument Co., Ltd, Ningbo, China). The freeze-dried extract was dissolved in dimethyl sulfoxide (DMSO) at 100 mg/mL and was stored at $-20$ °C for further use.

3.4. Microorganisms and Culture Samples

One reference strain of *S. aureus* ATCC 25923 and six antibiotic-resistant *S. aureus* strains were used in this study. The list of six *S. aureus* consisted of five multidrug-resistant strains (SJTUF 20745, SJTUF 20746, SJTUF 20758, SJTUF 20978, and SJTUF 20991), and one erythromycin-resistant strain (SJTUF 20827), verified in our previous study [54], is shown in Table 5. *S. aureus* ATCC 25923 was used as a reference strain. Single colonies of the bacteria growing on Luria Bertani (LB) agar plate were inoculated in LB culture medium and grown overnight in a shaking incubator at 37 °C and 250 rpm. The bacterial suspension was used at a concentration of $1 \times 10^6$ colony-forming units (CFU)/mL for the following antibacterial experiments.
Table 5. List of antibiotic-resistant *S. aureus* strains used in this study and their antibiotic resistance profiles [54].

| *S. aureus* Strain Name | Antibiotic Resistance Profile |
|-------------------------|------------------------------|
| SJTUF 20745             | Streptomycin, ciprofloxacin, clindamycin, erythromycin |
| SJTUF 20746             | Gentamicin, ciprofloxacin, clindamycin, erythromycin |
| SJTUF 20758             | Penicillin, streptomycin, clindamycin, erythromycin |
| SJTUF 20827             | Erythromycin |
| SJTUF 20978             | Ciprofloxacin, erythromycin, sulfisoxazole |
| SJTUF 20991             | Ciprofloxacin, clindamycin, erythromycin, tetracycline |

3.5. Determination of Antibacterial Activity

3.5.1. Measurement of Diameter of Inhibition Zone (DIZ)

The antibacterial activity of 239 TCMP extracts (100 mg/mL) against *S. aureus* ATCC 25923 and SJTUF 20827 was evaluated based on the DIZ determined by agar diffusion methods as described by Chan et al. [22] with some modifications. Ampicillin (32 µg/mL) and oxacillin (4 µg/mL) were used as the positive controls, and DMSO (60 µL/cup) were used as the negative controls. The DIZ values less than or equal to 8.0 mm was regarded as no antibacterial activity. In order to test the wide-spectrum antibacterial effects on multidrug-resistant *S. aureus*, 74 TCMP extracts with DIZ ≥ 15 mm from the screening results were further investigated their DIZ against another five multidrug-resistant *S. aureus* strains (SJTUF 20745, SJTUF 20746, SJTUF 20758, SJTUF 20978, and SJTUF 20991) as the procedure above. The assay was conducted in triplicate in two independent experiments.

3.5.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC)

TCMP samples with DIZ ≥ 20 mm were used to determine their MIC and MBC against *S. aureus* strains in triplicate. The MIC and MBC were determined by a serial dilution microplate method and Mueller–Hinton (MH) agar counting, respectively [22,80]. Ampicillin and oxacillin were used as the positive controls. This assay was performed in triplicate in three independent experiments.

3.6. Determination of the Total Phenolic and Flavonoid Content

Total phenolic content (TPC) was determined using a Folin-Ciocalteu method, as previously described by Blainski et al. [81]. To quantify TPC, a linear calibration curve of gallic acid was established as a standard (y = 0.0511 + 9.014x, r² = 0.9916). TPC was calculated using the linear equation and expressed as mg gallic acid equivalent (mg GAE)/g dry weight (DW) of the sample. Total flavonoids content (TFC) was quantified using an AlCl₃-based colorimetric method [56]. Catechin was used as a standard to establish a linear calibration curve with function (y = 0.0229 + 0.0033x, r² = 0.999). The assay was performed in triplicate in two independent experiments. TFC was calculated using the linear equation and expressed as mg catechin equivalent (mg CE)/g DW of sample.

3.7. In vitro Cytotoxicity Assay

The cytotoxicity of 18 TCMP ethanolic extracts on human foreskin fibroblast (HFF) cells, which are human normal cells, was determined by the colorimetric assay using 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described by Senthilraja and Kathiresan [82] with slight modification. Briefly, 2 × 10⁴ cells/mL were cultured with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum into each well of 96-well microplates and incubated at 37 °C in a humidified 5% CO₂ incubator overnight. The cells were treated with various concentrations (ranging from 1.56 to 100 µg/mL) of TCMP extracts and incubated at 37 °C for 24 h, whereas the untreated cells were used as control. Next, cells in each well were treated with 100 µL MTT (5 mg/mL, in PBS) and were maintained for 3 h at 37 °C in the dark. After removal of MTT solution, 100 µL
DMSO was added to dissolve insoluble formazan crystal. The absorbance was measured at 570 nm by a microplate reader (SpectraMax iD3, Molecular Devices, Silicon Valley, NC, USA). This assay was conducted in triplicate in four independent experiments. The percentage of cell viability was calculated based on the following equation:

\[
\text{Cell viability (\%)} = \left( \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right) \times 100, \tag{1}
\]

The median lethal concentration (LC\(_{50}\)) was calculated according to the log dose-response curve of cytotoxicity against concentration and was defined as a 50\% reduction of cell viability compared with the control. The selectivity index (SI) value was calculated based on the equation below [63]:

\[
\text{Selectivity index (SI)} = \frac{\text{LC}_{50} (\text{mg/mL})}{\text{MIC (mg/mL)}}, \tag{2}
\]

### 3.8. Statistical Analysis

All assays were conducted in triplicate and all experimental results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Microsoft Excel 2018 (Microsoft, Seattle, WA, USA) and SPSS 22.0 (IBM SPSS Statistics, IBM Corp, Somers, NY, USA). The cytotoxicity data (cell viability and LC\(_{50}\)) were analyzed by GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Statistical significance was defined at \(p\)-value less than 0.05.

### 4. Conclusions

This study conducted the large-scale screening of 239 TCMP extracts to discover strong antibacterial activities against multidrug-resistant \textit{S. aureus}. This study selected out several TCMP extracts with promising antibacterial activity as well as low cytotoxicity, including \textit{R. chinensis}, \textit{I. rotunda}, \textit{L. kiangranensis}, \textit{O. indicum}, \textit{I. tinctoria}, \textit{T. chebula}, \textit{S. suberectus}, \textit{R. rubescens}, \textit{S. miltiorrhiza}, \textit{F. fallax}, \textit{C. chinensis}, \textit{A. pilosa}, and \textit{P. chinense}. The results of plant extracts, \textit{I. rotunda}, \textit{I. japonica}, \textit{L. kiangranensis}, \textit{S. tuberculate} and \textit{F. fallax}, were first reported about their antibacterial effects on \textit{S. aureus} in the present study. Further study would be required to investigate phytochemical profiles in TCMP extracts by more sensitive analytical methods. It would be necessary to identify their antibacterial active compounds, potential mechanisms of antibacterial action, and in vivo toxicity prior to applying them as new antibacterial agents in pharmaceutical, food, and animal feed industries.

### Supplementary Materials

The following are available online at http://www.mdpi.com/2076-0817/9/3/185/s1, Table S1: Screening 239 TCMP extracts for antibacterial activities against reference \textit{S. aureus} ATCC 25923 and antibiotic-resistant \textit{S. aureus} SJTUF 20827 based on diameter inhibition zone (DIZ, mm), Figure S1: Dose-response curves for the viability of HFF cells exposed to selected TCMP extracts.

### Author Contributions:

Conceptualization, R.-Y.G. and H.C.; methodology, G.K. and R.-Y.G.; validation, G.K.; formal analysis, G.K.; investigation, G.K., D.Z., and A.K.F.; data curation, G.K.; writing—original draft preparation, G.K. and R.-Y.G.; writing—review and editing, R.-Y.G., O.H., VM., H.-B.L., X.-H.W., and H.C.; supervision, R.-Y.G. and H.C. All authors have read and agree to the published version of the manuscript.

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### Conflicts of Interest:

The authors declare no conflict of interest.

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