Lysis of *Microcystis aeruginosa* with Extracts from Chinese Medicinal Herbs

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**Abstract:** Boiling water extracts of 66 selected Chinese medicinal herbs were screened for their anticyanobacterial activity against *Microcystis aeruginosa* by the soft-agar overlayer (SAO) method. Results indicated that extracts from 16 materials could inhibit the growth of this bacterial species. Among these anticyanobacterial samples, eight extracts showed low minimum inhibitory concentrations (MIC), including four extracts with MICs between 1 and 6 mg/mL, and four extracts with MICs < 1 mg/mL which could be considered useful to prevent the outbreak of cyanobacteria before the appearance of cyanobacterial blooms. Further study showed that three extracts with MIC values < 1 mg/mL induced intensive chlorophyll-a lysis within 7 days at the MIC. The results suggested that highly efficient anticyanobacterial compounds must be involved in the inhibitory activities. The final results indicated these three extracts (from *Malaphis chinensis*, *Cynips gallae-tinctoriae* and *Fructus mume*) had the potential to be developed as algicides due to their remarkably anticyanobacterial activities.  

**Keywords:** Chinese medicinal herbs; *Microcystis aeruginosa*; cyanobacteria; lysis
1. Introduction

Cyanobacteria (blue-green algae) are photoautotrophic Gram-negative bacteria which commonly evoke occurrence of blooms and scums in lakes, reservoirs, slow-flowing rivers [1]. Due to their musty odor and production of potent toxins, cyanobacteria populations are a great concern in reservoir supplies and recreational water systems [2]. Microcystis aeruginosa, found globally in fresh waters, is a cyanobacterium which can produce toxins threatening public health [3], so M. aeruginosa has been the subject of increasing research over the last decades. To minimize the threat, methods of prevention and control for bloom problems have been adopted, such as chemical treatments with algaecides [4] and biological control [5]. Algicidal compounds are widely used in industrial waters, for the sanitation of swimming pools and the like. Copper sulfate, chelated copper compounds, and diuron (3-[3,4-dichlorophenyl]-1,1-dimethylurea) are the only compounds currently approved by the U.S. Environmental Protection Agency for use as algicides in catfish production ponds. Unfortunately, these compounds have the following undesirable characteristics: (i) broad-spectrum toxicity towards phytoplankton that can result in the death of the entire phytoplankton community and subsequent water quality deterioration that may stress or kill catfish; (ii) lengthy persistence in the environment that creates concerns about environmental safety; and (iii) the public’s negative perception of the use of synthetic herbicides in food fish production ponds [6]. Ideally, in the concentration employed, they should be harmless for man and animals.

Thousands of plants worldwide are used in traditional medicine as treatments for bacterial infections and especially traditional Chinese medicinal herbs which contain abundant potential antimicrobial agents have been used for the treatment of a wide variety of diseases for thousands of years [7]. In recent years, screening of plant resources for antimicrobial compounds has being intensively carried on worldwide, but there has been no screening of Chinese herbs extracts against M. aeruginosa.

In this study, we aimed to investigate potential useful plant compounds or extracts from Chinese herbs which should ideally display lysis activity against cyanobacteria. We expect that this study will contribute to the control of pollution by M. aeruginosa and the following identification of active compounds for emergencies caused by this species.

2. Results and Discussion

2.1. Results

2.1.1. Anticyanobacterial Screening

By the SAO method, diverse levels of anticyanobacterial activities were observed in the plates containing different tested extracts. The final screening results are listed in Table 1. Generally, it was found that extracts from 16 materials showed inhibitory activity towards M. aeruginosa. Among the 16 extracts, Herba Patriniae, Forsythia suspensa (Thunb.) Vahl, Rubia cordifolia, Polygala tenuifolia, Acorus tatarinowii, Sophora flavescens, Rhizoma Chuanxiong, Rhizoma Corydalis and Ranunculus ternatus extracts showed low inhibition levels to M. aeruginosa with DIZ (diameter of inhibition zone)
values ≥ 10 mm; Fraxinus rhynchophylla, Crataegus pinnatifida, and Euphorbia humifusa extracts showed moderate levels of activity, with DIZ values ≥ 20 mm; Cornus officinalis Sieb. et Zucc, Malaphis chinensis, Cynips gallae-tinctoriae and Fructus mume extracts showed high activity levels, with DIZ values ≥ 30 mm.

Table 1. Anticyanobacterial activity of various extracts with boiling water by the disc diffusion method.

| Botanical name                      | English name                      | Part tested | M. aeruginosa |
|-------------------------------------|-----------------------------------|-------------|---------------|
| Control                             |                                   | N           | −             |
| P                                   |                                   | ++          |               |
| Cornus officinalis Sieb. et Zucc    | Medical Dogwood Fruit             | Fruit       | +++           |
| Malaphis chinensis                  | Gallnut                           | Gallae      | +++           |
| Fructus mume                        | Smoked Plum                       | Fruit       | +++           |
| Cynips gallae-tinctoriae Olivier    | Nutgall                           | Gallae      | +++           |
| Euphorbia humifusa Wild             | Humifuse Spurge                   | Whole plant | ++            |
| Fraxinus rhynchophylla Hance        | Ash Bark                          | Peel        | ++            |
| Crataegus pinnatifida               | Hawthorn                          | Fruit       | ++            |
| Rubia cordifolia Linn.              | India Madder Root                 | Whole plant | +             |
| Polygala tenuifolia Wild            | Thinline Milkwort Root            | Whole plant | +             |
| Acerus tatarinowii Schott           | Rhizoma Acori Tatarinowii         | Whole plant | +             |
| Sophora flavescens Alt              | Lightyellow Sophora Root          | Rhizome     | +             |
| Rhizoma Chuanxiong                  | Ligusticum Chuanxiong Hort        | Rhizome     | +             |
| Rhizoma Corydalis                   | Yanhusuo                          | Whole plant | +             |
| Forsythia suspensa (Thunb.) Vahl    | Weeping Forsythia                 | Whole plant | +             |
| Herba Patriniae                     | White flower Patrinia Herb        | Whole plant | +             |
| Ranunculus ternatus Thunb           | Radix Ranunculi Ternati           | Whole plant | +             |
| Eichhornia crassipes (Mart.) Solms  | Weter Hyacinth                    | Branch      | −             |
| Suaeda glauca Bge                   | Common Seepweed Herb              | Whole plant | −             |
| Houttuynia cordata Thub             | Herba Houttuyniae                 | Whole plant | −             |
| Isatis tinctoria L                  | Isatis Root                       | Rhizome     | −             |
| Herba Taraxaci                      | Dandelion                         | Whole plant | −             |
| Pulsatilla chinensis (Bunge) Regel  | Anemone                           | Whole plant | −             |
| Coptis chinensis Franch             | Coptis Chinensis                  | Whole plant | −             |
| Folium Isatidis                     | Folium Isatidis                   | Leaf        | −             |
| Prunella vulgaris                   | Spica Prunellae                   | Whole plant | −             |
| Punica granatum                     | Pomegranate                       | Peel        | −             |
| Terminalia chebula Retz             | Medicine Terminalia Fruit         | Fruit       | −             |
| Gardenia: jasminoides Ellis         | Cape Jasmine                      | Fruit       | −             |
| Violia philippica ssp. munda W. Beck| Purple flower Violet              | Leaf        | −             |
| Anemarrhena asphodeloides Bunge     | Common Rhizoma Anemarrhena        | Fruit       | −             |
| Rhizoma Cyperi                      | Nutgrass Galingale Rhizom         | Fruit       | −             |
| Lithospermum erythrorhizon Sieb.et Zucc | Gromwell Root                | Whole plant | −             |
| Herba Artemisiae Annuae             | Sweet Wormwood Herb               | Whole plant | −             |
| Zanthoxylum bungeanum               | Pricklyash Peel                   | Fruit       | −             |
| Aucklandia lappa Decne              | Radix Aucklandia                  | Rhizome     | −             |
Table 1. Cont.

| Herb Name                                      | Origin/Part                  | Form    | Activity  |
|-----------------------------------------------|------------------------------|---------|-----------|
| Glycyrrhiza uralensis Fisch                   | Licorice Roots, Northwest   | Whole plant | −         |
| Rhizoma Smilacis Glabrae                      | Glabrous Greenbrier Rhizome | Rhizome | −         |
| Sanguisorba officinalis Linn                  | Garden Burnet                | Rhizome | −         |
| Pericarpium Citri Reticulatae                 | Dried Tangerine peel        | Peel    | −         |
| Herba Senecionis Scandentis                   | Climbing Groundsel Herb     | Whole plant | −         |
| Schisandra chinensis (Turcz.) Baill           | Chinese Magnolivine Fruit   | Fruit   | −         |
| Clematis chinensis Osbeck                     | Radix Clematidis            | Whole plant | −         |
| Drynaria fortunei (Kunze) J. SM               | Rhizome                     | −        |
| Benincasa hispide Thunb                      | White gourd                 | Peel    | −         |
| Astragalus membranaceus (Fisch.) Bunge        | Astragali                   | Rhizome | −         |
| Bupleurum chinense DC                         | Bupleuri                    | Rhizome | −         |
| Geranium wilfordii Maxim                      | Herba erodii                | Whole plant | −         |
| Subgen. Tsutsusi (G. Don) Pojarkova           | Loquat                      | Leaf    | −         |
| Cortex Cinnamomi Cassiae                      | Cinnamon                    | Rhizome | −         |
| Andrographis paniculata (Burn.f.) Nees        | Common Andrographis Herb    | Whole plant | −         |
| Lonicera japonica Thunb                      | Flos Lonicerae              | Flower  | −         |
| Tradescantia albiflora                        | Whole plant                 | −        |
| Portulaca oleracea Linn                       | Purslane                    | Whole plant | −         |
| Plantago asiatica L                           | Plantain                    | Whole plant | −         |
| Trachelospermum jasminoides (Lindl.) Lem      | Caulis Trachelospermii      | Whole plant | −         |
| Semen Cassiae                                 | Cassia Seed                 | Seed    | −         |
| Pogostemon cablin (Blanco) Benth              | Agastache rugosa            | Whole plant | −         |
| Folium illicis Latifoliae                     | Broadleaf Holly leaf        | Leaf    | −         |
| Cyrtomium fortunei J. Sm                     | Cyrtomii Rhizoma            | Rhizome | −         |
| Herba Menthae Heplocalycis                    | Wild Mint Herb              | Leaf    | −         |
| Syzygium aromaticum (L.) Merr. Et Perry       | Flos Caryophyllata          | Leaf    | −         |
| Platycladus orientalis (Linn.) Franco         | Arborvitae                  | Leaf    | −         |
| Magnolia liliiflora Desr                      | Flos Magnoliae              | Fruit   | −         |
| Areca catechu Linn                            | Betel nut                   | Peel    | −         |
| Dryobalanops aromatica Gaertn. f.             | Borneol                     | Resin   | −         |
| Sterculia lychnophera Hance                   | Boat-fruited Sterculia Seed | Fruit   | −         |
| Gymnostemma pentaphyllium Thunb. Makino       | Fiveleaf Gymnostemma Herb   | Leaf    | −         |
| Reynoutria japonica Houtt                     | Rhizoma Polygoni Cuspidati  | Leaf    | −         |

Abbreviations: N, negative control (distilled water); P, positive control (CuSO4 20 μg/mL); Grading of results: ++++, complete inhibition (DIZ: 30~40 mm); ++, moderate inhibition (DIZ: 20~30 mm); +, partial inhibition (DIZ: 10~20 mm); −, no inhibition (DIZ: 8 mm); The outside diameter of oxford cup on the soft-agar overlayer is 8 mm and the diameter of inhibition zone (DIZ) of negative control is also 8 mm. If the DIZ value is 8 mm, that means the extract has no inhibitory activity against *M. aeruginosa*.

2.1.2. Determination of *M. aeruginosa*-Inhibiting Abilities of 16 Selected Chinese Herbs Extracts

The inhibitory abilities of selected Chinese herbs extracts were confirmed by determining the MIC towards *M. aeruginosa*. All the results were listed in Table 2. Extracts of *C. gallae-tinctoriae*,
M. chinensis, F. mume, Herba Patriniae exhibited the lowest MIC values (<1 mg/mL); The MIC values of C. pinnatifida, C. officinalis Sieb. Et, F. rhynchophylla, E. humifusa are all at 3.125 mg/mL. MIC values of the remaining eight extracts were all higher than 6 mg/mL, which meant their M. aeruginosa-inhibiting abilities were very low and so they were excluded in the subsequent study.

### Table 2. MIC values of several Chinese herbs against the growth of Microcystis aeruginosa.

| Botanical name                      | English name                  | Part tested     | MIC (mg/mL) |
|------------------------------------|-------------------------------|-----------------|-------------|
| Cynips gallae-tinctoriae Olivier   | Nutgall                       | Gallae          | 0.39        |
| Malaphis chinensis                 | Gallae                        | Gallae          | 0.39        |
| Herba Patriniae                    | White flower Patrinia Herb    | Whole plant     | 0.78        |
| Fructus mume                       | Smoked Plum                   | Fruit           | 0.78        |
| Crataegus pinnatifida              | Nippon Hawthorn Fruit         | Fruit           | 3.125       |
| Cornus officinalis Sieb. et Zucc   | Medical Dogwood               | Fruit           | 3.125       |
| Fraxinus rhynchophylla Hance       | Ash Bark                      | Peel            | 3.125       |
| Euphorbia humifusa Wildl           | Humifuse Spurge               | Whole plant     | 3.125       |
| Forsythia suspensa (Thunb.) Vahl   | Weeping Forsythia             | Whole plant     | 6.25        |
| Polygala tenuifolia Wildl          | Thinleaf Milkwort Root        | Whole plant     | 6.25        |
| Rubia cordifolia Linn.             | India Madder Root             | Whole plant     | 6.25        |
| Ranunculus ternatus Thunb          | Radix Ranunculi Ternati       | Whole plant     | 12.5        |
| Acorus tatarinowii Schott          | Rhizoma Acori Tatarinowii     | Whole plant     | 12.5        |
| Rhizoma Chuanxiong                 | Ligusticum Chuanxiong Hort    | Rhizome         | 12.5        |
| Sophora flavescens Alt             | Lightyellow Sophora Root      | Rhizome         | 25          |
| Rhizoma Corydalis                  | Yanhusuo                      | Whole plant     | 25          |

2.1.3. Dynamic Analysis of Chlorophyll-a in M. aeruginosa with Remained 8 Chinese Herbs Extracts

Changes of the chlorophyll a (Chl-a) contents of the M. aeruginosa in a sterile BG11 culture solution for seven days were recorded as a time course curve (Figure1). Effects of the eight finally selected Chinese herbs extracts on the M. aeruginosa growth could be observed from corresponding curve. Generally speaking, each extract finally caused the Chl-a lysis of M. aeruginosa, but their lysing actions differed with regards to the inception time and efficacy. From Figure 1, it is seen that except for treatment with P. mume extract, the Chl-a of all treated M. aeruginosa started lysing two days after the inoculation of tested extracts. C. gallae-tinctoriae, M. chinensis, C.officinalis Sieb. Et and F. mume extracts almost induced Chl-a complete lysis in seven days (Figures 1 a-d). However, F. rhynchophylla, E. humifusa, C. pinnatifida and H. Patriniae extracts only induced partial lysis of Chl-a in seven days (Figures 1 e-h).
Figure 1. Effects of different Chinese herbs extracts on the contents of chlorophyll a at MIC value. Control: M. aeruginosa (■); Treat: Chinese herbs extracts (●) [a: C. gallae-tictoriae (0.39 mg/mL); b: M. chinensis (0.39 mg/mL); c: C. officinalis Sieb. Et (3.125 mg/mL); d: F. mume (0.78 mg/mL); e: F. rhynchophylla (3.125 mg/mL); f: E. humifusa (3.125 mg/mL); g: C. pinnatifida (3.125 mg/mL); h: H. Patrinae (0.78 mg/mL)]. Each point represents mean ±SE of three replications.
2.2. Discussion

China has a rich flora that is widely distributed throughout the country. Chinese medicinal herbs have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods. In fact, many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as sources of antimicrobial agents against foodborne pathogens [8]. In this study, it was indicated that many extracts from selected Chinese medicinal herbs possessed ideal inhibition activity against cyanobacteria.

The SAO method was employed for screening the anticyanobacterial Chinese herbs. Anticyanobacterial activity was evaluated by measuring the DIZ of the tested cyanobacterium. In such tests, extracts containing active insolvable compounds would escape detection, but considering the potential applications, it was preferred to focus on the extracts containing active soluble compounds. Solubility and diffusion of active components in agar media could play a major role in the formation of inhibition zone, which suggested that DIZ might not be absolutely equivalent to the anticyanobacterial capability, hence the MIC values of all positive extracts screened by SAO were determined to evaluate their anticyanobacterial capability. Extracts of C. gallae-tinctoriae, M. chinensis, F. mume, and H. Patriniae exhibited low MIC values (<1 mg/mL), which indicated their potential to be timely applied to prevent the outbreak of cyanobacteria before the occurrence of cyanobacterial bloom.

The M. aeruginosa-lytic activity of the selected extracts was evaluated for their potential as algicides. It was found that extracts of C. gallae-tinctoriae, M. chinensis, F. mume and C. officinalis Sieb. Et showed obvious lytic activity towards M. aeruginosa, while extract of H. Patriniae showed too weak lytic activity to be a useful algicide. Considering the far higher MIC and average M. aeruginosa-lytic activity, the extract of C. officinalis Sieb Et was not recommend for development as an algicide. It should be noted that the initial time for lytic actions of these extracts were different at their MICs, and F. mume extract exhibited fast acting lysis activity against Chl-a of M. aeruginosa which was different from other extracts. These results suggested that P. mume extract probably contained some special active compounds involved in the lysis of M. aeruginosa, and such extract could be considered as acute algaecide applied in some emergencies caused by M. aeruginosa.

Many volatile organic compounds have been found to have lytic activity against cyanobacteria. It was confirmed that volatile terpenoid compounds produced by plants had lytic activity [9]. According to Cowan [10], aqueous plant extracts mainly contain anthocyanins, starches, tannins, saponins, terpenoids, polypeptides, lectins, etc, so Chinese herbs extracts could be expected to have universal anticyanobacteria activity, but this was not really the case. Many extracts did not display any anticyanobacteria activity. Therefore, it was implied that the content of the active ingredients was different in different Chinese medicinal herbs. For instance, the aqueous extracts of F. mume mainly contain organic acids, terpenoids, sterols, flavonoids, carbohydrates and amino acids. Especially, the concentration of organic acids was very high (up to 40.5%) [11]. Malic acid and citric acid were the major organic acid constituents, while ethanedioic acid, glycolic acid, lactic acid, succinic acid, formic acid, acetic acid, propionic acid, etc were also present. Lots of these organic acids have been reported to have inhibition activity towards some microbe species [12-14], so it was very likely that these organic acids induce the lysis of cyanobacteria. Of course, terpenoids (ursolic acid) and flavonoids may show synergistic effect on the growth of cyanobacteria. In addition, some amino acids (e.g., lysine,
histidine, alanine) may also accelerate the lysis of cyanobacteria [15]. *M. chinensis* is a traditional Chinese herb distributed widely in southern China. It is the gallae that is produced by some parasitic aphids (family Pemphigidae) on Rhus leaves of the family Anacardiaceae (mainly *Rhus chinensis* Mill, *Rhus potaninii* Maxim, and *Rhus punjabensis* var. sinica (Diels) Rehd. et Wils) [16]. The surface feature of *C. gallae-tinctoriae* is very similar to *M. chinensis*. Both of them are gallae, and the active compounds from their aqueous extracts should be also similar, containing a large amount of gallotannin, a typical hydrolysable tannin, with the content of up to 70% of its weight [17]. The content of gallic acid in the extract is about 2%-4%. It was reported that gallotannin and gallic acid could inhibit the growth of intestinal bacteria [18], so it was presumed that gallotannin and gallic acid were at least partially responsible for the observed lysis of cyanobacteria by the extracts from *M. chinensis* or *C. gallae-tinctoriae*. Even the active extracts showed diverse levels in efficiency and inception time. The results indicated that multiple and diverse active compounds with anticyanobacteria activity existed in the extracts. It would be of interest to purify and identify the responsible bioactive components from these extracts.

3. Experimental Section

3.1. Cyanobacteria Culture and Chinese Medicinal Herbs

*M. aeruginosa* (FACHB 905) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), located in China. The strain was grown in BG-11 medium [19] at 25 °C with illumination at 3,000 lx under a 16 L/8 D cycle. Sixty-six Chinese medicinal herbs were obtained from traditional medicine stores in Nanjing. The different parts of the plant used were the leaves, the branches, the fruits, the rhizomes, the peels, the gallaes shown in Tables 1 and 2.

3.2. Preparation of the Extracts

Stock solutions were prepared with the traditional process currently used in Chinese clinics and scientific studies by boiling 100 g of raw material with 1,000 mL of distilled water for 1 h. The material was centrifuged and filtered through filter paper. The residue remaining on the filter paper was reboiled with 1,000 mL distilled water, centrifuged, and refiltered. The resulting two batches of the solution were mixed and then boiled again until 100 mL remained. This solution was regarded as a concentration of 100% (100 mL of HC solution made from 100 g of raw material). After being autoclaved, the stock solution was stored at 4 °C until used.

3.3. Measurement of Anticyanobacterial Activity

The anticynobacterial activity was determined using the soft-agar overlayer (SAO) method [20]. Approximately 5 mL of cyanobacterial cells (2 × 10⁶ cells/mL) were mixed with warmed 5 mL of 0.8% (w/v) soft agar and over-layered on a 10 mL of 1.2% (w/v) agar layer solidified in a plate. After the cyanobacterium containing layer was solidified, Sterilized Oxford cups (8 mm in external diameter) were put up on the cyanobacterium-containing layer regularly and 200 μL extract solutions
were respectively deposited on the disc, and the concentration of the test herb extracts were diluted to 200 mg/mL. 20 µg/mL CuSO₄ and distilled water were used as positive and negative controls, respectively. The plates were incubated at 25 °C for 2 days. Anticyanobacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested cyanobacterium. DIZ was expressed in millimeters. All tests were performed in triplicate.

3.4. Determination of Minimum Inhibitory Concentration (MIC)

Several anticynobacterial extracts were screened from the material extracts by disc diffusion method. A broth microdilution method was used to determine the MIC [21,22]. An aliquot of 0.1 mL of a serially diluted Chinese herb extracts in BG11 medium was added to a 96-well microplate and 0.1 mL of cyanobacteria cultured broth (2 × 10⁶ cells/mL) was added to each well. Then the resulting solution was incubated at 25 °C under 3,000 lx continuous illumination for a week. The MIC is defined as the lowest concentration of the extracts at which the cyanobacteria does not demonstrate visible growth. Each test was performed in triplicate.

3.5. Extraction and Measurement of Cyanobacterial Chlorophyll a

A solution of 90% methanol was used for extraction of chlorophyll a (chl-a) from cyanobacteria. Measurement of chl-a was according to the method of Parsons and Strickland [23]. Cyanobacteria collected by centrifugation (12,000 r.p.m., 15 min, 4 °C) were subjected to extraction with 90% methanol in a water bath (60 °C) for 10 min. After extraction, the solid suspension was removed by centrifugation (12,000 r.p.m., 15 min, 4 °C). Then, the absorbance of extracts at 665, 645 and 635 nm was measured using a spectrophotometer (Perkin Lambda 25). The concentration of chl-a was calculated using the following equation [15]: Chl-a (µg/mL) = 11.6A₆₆₅-1.31A₆₄₅-0.14A₆₃₅.

4. Conclusions

In this study, it was indicated that many Chinese herbs possess anticyanobacteria activity. Our results showed that C. gallae-tinctoriae, M. chinensis, and F. mume have potential as algicides due to their remarkable anticyanobacterial effects.

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References and Notes

1. Chow, C.W.K.; House, J.; Velzeboer, R.M.A.; Drikas, M.; Burch, M.D.; Steffensen, D.A. The effect of ferric chloride flocculation on cyanobacterial cells. Water Res. 1998, 32, 808-814.
2. Codd, G.A.; Bell, S.G.; Kaya, K.; Ward, C.J.; Beattie, K.A.; Metcalf, J.S. Cyanobacterial toxins, exposure routes and human health. *Eur. J. Phycol.* **1999**, *34*, 405-415.

3. Reynolds, C.S.; Walsby, A.E. Water blooms. *Biol. Res.* **1975**, *50*, 437-481.

4. Hrudey, S.; Burch, S.; Burch, M.; Drikas, M.; Greorgy, R. Toxic cyanobacteria in water, a guide to their public health consequences, monitoring and management. In *Remedial Measures*; Chorus, I., Bartram, J., Eds.; Routledge: London, UK, 1999; pp. 275-312.

5. Choi, H.J.; Kim, B.H.; Kim, J.D.; Han, M.S. Streptomyces neyagawaensis as a control for the hazardous biomass of *Microcystis aeruginosa* (Cyanobacteria) in eutrophic freshwaters. *Biol. Control.* **2005**, *33*, 335-343.

6. Schrader, K.K.; Nanayakkara, N.P.D.; Tucker, C.S.; Rimando, A.M.; Ganzera, M.; Schaneberg, B.T. Novel derivatives of 9,10-anthraquinone are selective algicides against the musty-odor cyanobacterium *Oscillatoria perornata*. *Appl. Environ. Microbiol.* **2003**, *69*, 5319-5327.

7. Bensky, D.; Gamble, A. Chinese herbal medicine. In *Materia Medica*, Rev. ed.; Eastland Press, Inc.: Seattle, WA, USA, 1993; pp. 13-17.

8. Deans, S.G.; Ritchie, G.A. Antimicrobial properties of plant essential oils. *Int. J. Food Microbiol.* **1987**, *5*, 165-180.

9. Ozaki, K.; Ohta, A.; Iwata, C.; Horikawa, A.; Tsuji, K.; Ito, E.; Ikai, Y.; Harada, K.I. Lysis of cyanobacteria with volatile organic compounds. *Chemosphere* **2008**, *7*, 1531-1538.

10. Cowan, M.M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **1999**, *12*, 564-582.

11. Shen, H.M.; Qiao, C.Z.; Su, Z.W. Quantitative dynamic analysis of the main component of organic acid in the *Fructus mume*. *Chin. Pharmaceut. J.* **1995**, *30*, 133-135.

12. Nikolaus, B.E.; Wayman, B.E.; Encinas, E. The bactericidal effect of citric acid and sodium hypochlorite on anaerobic bacteria. *J. Endod.* **1988**, *14*, 31-34.

13. Georgopoulou, M.; Kontakiotis, E.; Nakou, M. Evaluation of the antimicrobial effectiveness of citric acid and sodium hypochlorite on the anaerobic flora of the infected root canal. *Int. Endod. J.* **1994**, *27*, 139-143.

14. Greer, G.G.; Dillts, B.G. Lactic acid inhibition of the growth of spoilage bacteria and cold tolerant pathogens on pork. *Int. J. Food Microbiol.* **1995**, *25*, 141-151.

15. Takamura, Y.; Yamada, T.; Kimoto, A.; Kanahama, N.; Tanaka, T.; Nakadaira, S.; Yagi, O. Growth inhibition of microcystis cyanobacteria by L-lysine and disappearance of natural microcystis blooms with spraying. *Microb. Environ.* **2004**, *19*, 31-39.

16. Tian, F.; Li, B.; Ji, B.P.; Yang, J.H.; Zhang, G.Z.; Chen, Y.; Luo, Y.C. Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: The polarity affects the bioactivities. *Food Chem.* **2009**, *113*, 173-179.

17. Sun, D.W. *Chemistry of Vegetable Tannins (in Chinese)*; Chinese Forest Press: Beijing, China, 1992; Chapter 1, pp. 257-260.

18. Ahn, Y.J.; Lee, C.O.; Kweon, J.H.; Ahn, J.W.; Park, J.H. Growth-inhibitory effects of *Galla Rhois*-derived tannins on intestinal bacteria. *J. Appl. Microbiol.* **1998**, *84*, 439-443.

19. Stanier, R.; Kunisawa, R.; Mandel, M.; Cohen-Bazire, G. Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol. Rev.* **1971**, *35*, 171-205.
20. Uchida, H.; Kouchiwa, T.; Watanabe, K.; Kawasaki, A.; Hodoki, Y.; Otani, I.; Yamamoto, Y.; Suzuki, M.; Harada, K.I. A coupled assay system for the lysis of cyanobacteria. *Jpn. J. Water Treat. Biol.* **1998**, *34*, 67-75.

21. National Committee for Clinical Laboratory Standards (NCCLS). *Performance Standards for Antimicrobial Susceptibility Test, Ninth International Supplement. M100-S9*; NCCLS: Wayne, PA, USA, 1999.

22. Bassole, I.H.N.; Quattara, A.S.; Nebie, R.; Quattara, C.A.T.; Kabore, Z.I.; Traore, S.A. Chemical composition and antibacterial activities of the essential oils of Lippia chevalieri and Lippia multiflora from Burkina Faso. *Phytochemistry* **2003**, *62*, 209-212.

23. Parsons, T.R.; Strickland, J.D.H. Discussion of spectrophotometric determination of marine-plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. *J. Mar. Res.* **1963**, *21*, 155-163.

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