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# Inhibitory parameters of the essential oil and various extracts of *Metasequoia glyptostroboides* Miki ex Hu to reduce food spoilage and food-borne pathogens

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**Abstract**

The aim of this work was to examine the chemical composition of the essential oil and various solvent extracts isolated from the floral cone of *Metasequoia glyptostroboides* Miki ex Hu and to test their efficacy against a diverse range of organisms comprising food spoilage and food-borne pathogenic bacteria. The chemical composition of essential oil isolated by hydrodistillation was analysed by GC-MS. It was determined that 59 compounds, which represented 97.06% of total oil, were present in the oil. The oil contains mainly α-pinene (29.54%), totarol (9.37%), α-thujene (8.63%), bornylene (8.63%), β-caryophyllene (4.40%), totarol acetate (3.98%), δ-3-carene (3.19%) and 2-β-pinene (2.25%). The oil was found containing mainly the oxygenated mono- and sesquiterpenes and their respective hydrocarbons. Antibacterial activity of essential oil, methanol extract and various organic sub-fractions of methanol extract of *M. glyptostroboides* was determined in vitro using agar diffusion method and MIC determination test against eleven (four Gram-positive, seven Gram-negative) bacterial strains including food spoilage and food-borne pathogens. The essential oil (5 μl/ml, corresponding to 1000 ppm/disc), methanol extract and various organic sub-fractions (7.5 μl/ml, corresponding to 1500 ppm/disc) of *M. glyptostroboides* exhibited great potential of antibacterial activity against four Gram-positive bacteria such as *Bacillus subtilis* (ATCC 6633), *Listeria monocytogenes* (ATCC 19166), *Staphylococcus aureus* (KCTC 1916), *S. aureus* (ATCC 6538) and one Gram-negative bacterium, *Pseudomonas aeruginosa* (KCTC 2004). The zones of inhibition of different concentrations of essential oil, methanol extract and its derived various organic sub-fractions against the tested bacteria were found in the range of 10 ~ 20 mm and the MIC values were recorded between 125 and 1000 μg/ml. This study shows that *M. glyptostroboides* mediated essential oil and extracts can be applied in food industries as a natural preservatives or flavoring additives to control food spoilage and food-borne pathogenic bacteria causing severe destruction in food.


*Keywords:* *Metasequoia glyptostroboides*; Essential oil composition; α-Pinene; Sabinene; α-Thujene; Food spoilage and food-borne pathogens; Antibacterial activity

## 1. Introduction

Food-borne illnesses associated with *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella enteritidis* and *Listeria monocytogenes,* present a major public health concern throughout the world (Hall, 1997). A number of methods have been employed to control or prevent the growth of these pathogens in food, including the use of synthetic and natural antimicrobial agents (Branen & Davudson, 2000; Payne, Davidson, Oliver, & Christen, 1990). There is a wealth of literature on antimicrobial compounds and the use of these compounds in food systems to eliminate or control the growth of pathogenic micro-organisms.

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The increasing incidence of food-borne diseases, coupled with the 'resultant social and economic implications, means there is a constant striving to produce safer food and to develop new natural antimicrobial agents. There is, therefore, still a need for new methods of reducing or eliminating food spoilage and food-borne pathogens, possibly in combination with existing methods.

Most plants produce antimicrobial secondary metabolites, either as part of their normal programme of growth and development or in response to pathogen attack or stress. A well known novel way to reduce the proliferation of micro-organisms is the use of plant-based essential oils or organic plant extracts, due to their less chance of being toxic. The oils are natural products extracted from plant materials which, because of their antibacterial, antifungal, antioxidant and anticarcinogenic properties, can be used as natural additives in many foods (Aureli, Costantini, & Zolea, 1992; Lambert, Skandamis, Coote, & Nychas, 2001). Essential oils have been proven to be inhibitory against a wide range of food-spoiling microbes, depend upon their concentration, method of testing, and active constituents present (Aureli et al., 1992; Lis-Balchin, Ochoka, Deans, Asztemborska, & Hart, 1999).

In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin & Deans, 1997; Reynolds, 1996). Some of the oils used on the basis of their reputed antimicrobial properties have well documented in vitro activity (Deans & Ritchie, 1987; Marino, Bersani, & Comi, 2001). Mono- and sesquiterpenoids, the main volatile constituents of the essential oils, have historically been used in pharmaceutical, food and perfume industries because of their antibacterial properties, culinary and fragrance, respectively. Some studies have concentrated exclusively on one oil or one micro-organism. While these data are useful, the reports are not directly comparable, due to methodological differences, such as choice of plant extract(s), test micro-organism(s) and antimicrobial test method (Janssen, Scheffer, & Svendsen, 1987).

Various micro-organisms cause food spoilage and constitute the most important matter of concern to the food industry. Many different chemical and synthetic compounds have been used as antimicrobials to inhibit bacteria in foods. But, due to concerns regarding the synthetic antimicrobial agents, there has been an increase in consumer demand for naturally-derived compounds, such as essential oil and plant extracts, as antimicrobial substances for food industries (Negi, Chauhan, Sadia, Rohinishree, & Ramteke, 2005).

*M. glyptostroboides* is a deciduous conifer of the redwood family of Cupressaceae. It is the only living species in the genus *Metasequoia* propagated and distributed in many parts of Eastern Asia and North America, as well as in Europe. We sought to develop a set of general measuring equations to estimate the potentiality of *M. glyptostroboides* as a potent antibacterial agent. However, there is no report available in the literature on the analyses of essential oil of *M. glyptostroboides*, in general, or its antibacterial properties in particular. Hence, efforts have been made here, specifically to investigate the role of essential oil and the various solvent extracts of *M. glyptostroboides* as a potent antimicrobial agent against food spoilage and food-borne pathogenic bacteria.

Therefore, the aims of the present study were (a) to examine the chemical compositions of essential oil, methanol extract and various sub-fractions of methanol extract, isolated from the floral cones of *M. glyptostroboides* and (b) to test their efficacy against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria.

## 2. Materials and methods

### 2.1. Plant material

The floral cones of *M. glyptostroboides* were collected from the Pusan area of South Korea, in September, 2005, and initially identified by morphological features and the data base present in the library, and a voucher specimen has been deposited in the herbarium of the Department of Biotechnology, Daegu University, South Korea.

### 2.2. Isolation of the essential oil

The air-dried plant material (200 g) was subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The oil was dried over anhydrous $Na_2SO_4$ and preserved in a sealed vial at 4 °C prior to further analysis.

### 2.3. Preparation of crude extract and various fractions

The air-dried floral cones of *M. glyptostroboides* were pulverized into powdered form. The dried powder (50 g) was extracted three times with 70% MeOH (200 ml × 3) at room temperature and the solvents from the combined extracts were evaporated by vacuum rotary evaporator (EYELA N1000, Japan). The methanol extract (5.42 g) was suspended in water and extracted, successively, with hexane, chloroform and ethyl acetate at room temperature to give hexane (2.31 g), chloroform (0.75 g), ethyl acetate (0.78 g) and residual methanol (0.92 g) sub-fractions, respectively. Solvents (analytical grade) for extraction were purchased from Sigma–Aldrich Co., USA.

### 2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

The GC–MS analysis of the essential oil was performed using a Shimadzu GC–MS (GC-17A) equipped with a ZB-1 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow

rate of 1 ml/min. Injector and MS transfer line temperature were set at 220 °C and 290 °C, respectively. The oven temperature was programmed from 50 °C to 150 °C at 3 °C/min, then held isothermal for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1.0 μl were injected manually in the splitless mode. The relative percentage of the oil constituents was expressed as percentages by peak area normalization.

Identification of components of the essential oil was based on GC retention time on a ZB-1 capillary column relative to computer matching of mass spectra with those of standards (Wiley 6.0 data of GC–MS system) and, whenever possible, by co-injection with authentic compounds.

### 2.5. Micro-organisms

In all, eleven bacterial strains including food spoiling, such as *Bacillus subtilis* ATCC 6633 and *Pseudomonas aeruginosa* KCTC 2004, and food-borne pathogens, namely *Enterobacter aerogenes* KCTC 2190, *Salmonella typhimurium* KCTC 2515, *E. coli* ATCC 8739, *E. coli* O157:H7 ATCC 43888, *E. coli* O157:H7 (human), *L. monocytogenes* ATCC 19166, *S. enteritidis* KCCM 12021, *S. aureus* ATCC 6538 and *S. aureus* KCTC 1916, were obtained from the Korea Food and Drug Administration (KFDA), Daegu, South Korea. Cultures of each bacterial strain were maintained on Luria broth (LB) agar medium (Acumedia Manufacturers, Inc. Lansing, Michigan, USA) at 4 °C.

### 2.6. Antibacterial activity assay

The agar diffusion method (Murray, Baron, Pfaller, Tenover, & Yolke, 1995) was used for antibacterial assay. Petri plates were prepared by pouring 20 ml of LB medium and allowed to solidify. Plates were dried and 1 ml of standardized inoculum suspension was poured and uniformly spread. The excess inoculum was drained away and the inoculum was allowed to dry for 5 min. A Whatman No. 1 sterile filter paper disc (6 mm diameter) was impregnated with 5 μl/ml of essential oil, corresponding to 1000 ppm/disc, and 7.5 μl/ml, corresponding to 1500 ppm/disc, of MeOH extract and its derived sub-fractions. Negative controls were prepared using the same solvent employed to dissolve the samples. Standard reference antibiotics, streptomycin and tetracycline (10 μg/disc, each from Sigma–Aldrich Co., USA), were used as positive controls for the tested bacteria. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria. Each assay in this experiment was replicated three times.

### 2.7. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) of essential oil, methanol extract and methanol-derived sub-fractions of hexane, chloroform and ethyl acetate, were tested by a twofold serial dilution method (Chandrasekaran & Venkatesalu, 2004). The tests of oil, methanol extract and its derived sub-fractions were incorporated into Luria-Broth medium to get a concentration of 1000 μg/ml and serially diluted to achieve 500, 250 125 and 75 μg/ml, respectively. Ten micro-litres of standardized suspension of each tested organism ($10^8$ cfu/ml) were transferred to each tube. The control tubes, containing only bacterial suspension, were incubated at 37 °C for 24 h. The lowest concentrations of the test samples, which did not show any growth of tested organism after macroscopic evaluation, were determined as MICs.

## 3. Results

### 3.1. Chemical composition of the essential oil

The hydrodistillation of the floral cone of *M. glyptostroboides* Miki ex Hu gave a dark-yellowish oil. GC–MS analyses of the oil led to the identification of 59 different components, representing 97.1% of total oil. The identified compounds are listed in Table 1 according to their elution order on a ZB-1 capillary column. The oil was a complex mixture, consisting mainly of oxygenated mono- and sesquiterpenes and their respective hydrocarbons. The leading and major components in the oil detected were α-pinene (29.5%), α-thujene (8.63%), δ-3-carene (3.19%), bornylene (8.63%), totarol (MW: 300; 9.37%), totarol (MW: 286; 5.28%), 2-β-pinene (2.25%), β-caryophyllene (4.40%) and totarol acetate (3.98%). Aliphatic hydrocarbons and acids were characteristic constituents of the oil of *M. glyptostroboides*. γ-Caryophyllene (0.11%), α-terpineol (0.30%) and limonene (0.06%) were found to be the minor components of *M. glyptostroboides* oil.

### 3.2. In vitro antibacterial activity

The in vitro antibacterial activities of essential oil, methanol extract and methanol-derived sub-fractions of *M. glyptostroboides*, against the employed bacteria, were qualitatively and quantitatively assessed by the presence or absence of inhibition zones. According to the results given in Table 2, in all, eleven food spoilage and food-borne bacterial strains, including four Gram-positive and seven Gram-negative bacteria, were tested. The oil exhibited antibacterial activity against all four Gram-positive and one Gram-negative bacterium at the concentration of 5 μl/ml (1000 ppm/disc). The oil exhibited potent inhibitory effect against *S. aureus* KCTC 1916, *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, *L. monocytogenes* ATCC 19166 and *P. aeruginosa* KCTC 2004, with diameter zones of inhibition of 16, 14, 13 and 14 mm, respectively, as shown in Table 2. Methanol extract of *M. glyptostroboides* and its derived sub-fractions also revealed considerable antibacterial activity against all four Gram-positive bacteria and one of the Gram-negative bacteria, namely *P. aeruginosa*, at the concentration of 7.5 μl/ml, corresponding to

Table 1
Chemical composition of volatile oil isolated by hydrodistillation from *Metasequoia glyptostroboides* Miki ex Hu

| No. | Rt[a] (min) | Compound | Percentage (%) | Identification method |
|---|---|---|---|---|
| 1 | 7.344 | α-Pinene | **29.5** | MS |
| 2 | 7.416 | α-Thujene | **8.63** | MS |
| 3 | 7.447 | δ-3-Carene | **3.19** | MS |
| 4 | 7.516 | Sabinene | **1.87** | MS |
| 5 | 7.893 | 2-Cyanoaziridine | 0.22 | MS |
| 6 | 8.049 | 2-β-Pinene | **2.25** | MS |
| 7 | 8.200 | 1-β-Pinene | 0.06 | MS |
| 8 | 8.433 | Linalyl acetate | **3.95** | MS |
| 9 | 8.618 | β-Myrcene | 0.05 | MS |
| 10 | 9.427 | Bornylene | **8.63** | MS |
| 11 | 9.828 | Limonene | 0.06 | MS |
| 12 | 9.910 | 2-Cyanoaziridine | 0.03 | MS |
| 13 | 10.013 | (R)-Cuparene | 0.05 | MS |
| 14 | 10.328 | 5-Ethyl-2(5H)-furanone | 0.10 | MS |
| 15 | 10.401 | α-Chamigrene | 0.04 | MS |
| 16 | 10.469 | Tricyclene | 0.23 | MS |
| 17 | 10.534 | α-Terpitine | 0.07 | MS |
| 18 | 10.848 | Cyclofenchene | 0.06 | MS |
| 19 | 10.972 | γ-Terpitine | 0.16 | MS |
| 20 | 12.135 | Terpineol-4 | 0.65 | MS |
| 21 | 12.267 | *cis*-Sabinenehydrate | 0.03 | MS |
| 22 | 12.370 | Solanone | 0.66 | MS |
| 23 | 12.506 | α-Terpineol | 0.30 | MS |
| 24 | 12.596 | Linalyl propionate | 0.22 | MS |
| 25 | 14.135 | Endo bornyl acetate | **2.22** | MS |
| 26 | 14.237 | Bornoil formate | 0.13 | MS |
| 27 | 14.328 | Exo bornyl acetate | 0.04 | MS |
| 28 | 15.058 | Dihydrocarvyl acetate | 0.40 | MS |
| 29 | 15.944 | Geranyl acetate | 0.04 | MS |
| 30 | 16.563 | β-Caryophyllene | **4.40** | MS |
| 31 | 16.754 | γ-Caryophyllene | 0.11 | MS |
| 32 | 16.921 | α-Humulene | **1.18** | MS |
| 33 | 17.048 | β-Selinene | 0.04 | MS |
| 34 | 17.507 | 2,3,3-Trimethyl tricycle heptane | 0.03 | MS |
| 35 | 18.691 | Caryophyllene oxide | **4.49** | MS |
| 36 | 18.748 | (Z)-3-Heptadecen-5-yne | 0.06 | MS |
| 37 | 18.896 | 13-Heptadecyn-1-ol | 0.66 | MS |
| 38 | 19.175 | Citronellyl acetate | 0.36 | MS |
| 39 | 19.401 | *cis*-Carane | 0.12 | MS |
| 40 | 19.582 | Methylol pinene | 0.49 | MS |
| 41 | 20.169 | Myrtenol | 0.03 | MS |
| 42 | 21.104 | *cis*-Farnesol | 0.04 | MS |
| 43 | 22.518 | β-Bisabolene | 0.03 | MS |
| 44 | 23.195 | Longipinenepoxide | 0.12 | MS |
| 45 | 23.412 | $C_{12}H_{10}N_4O_3$ (MW: 258) | 0.08 | MS |
| 46 | 23.580 | Hexaenoic acid | 0.05 | MS |
| 47 | 24.245 | Homomyrtenol | 0.11 | MS |
| 48 | 24.414 | Methyl ester | 0.48 | MS |
| 49 | 24.554 | Perylene | 0.04 | MS |
| 50 | 25.205 | Nopyl acetate | 0.04 | MS |
| 51 | 26.526 | Carnosol | 0.27 | MS |
| 52 | 26.610 | Ferruginol | 0.15 | MS |
| 53 | 27.312 | Totarol (MW: 286) | **5.28** | MS |
| 54 | 27.885 | $C_{20}H_{30}O_2$ (MW: 302) | 0.95 | MS |
| 55 | 28.167 | Isopropyl acetate | 0.07 | MS |
| 56 | 29.546 | Totarol (MW: 300) | **9.37** | MS |
| 57 | 29.915 | Sugiol | 0.12 | MS |
| 58 | 30.923 | Ruginol | 0.19 | MS |
| 59 | 32.675 | Totarol acetate | **3.98** | MS |

[a] Retention time of authentic compounds on ZB-1 capillary column; MS, mass spectra identified according to computer matching of mass spectra with those of standards Wiley 6.0 data of GC–MS system.

1500 ppm/disc (Table 2). Methanol extract showed the strongest antibacterial effect against both the strains of *S. aureus* (KCTC 1916 and ATCC 6538) with their respective diameter zones of inhibition of 20 and 18 mm, as compared to the standard drug streptomycin. Hexane, chloroform and ethyl acetate fractions showed antibacterial effects with diameter zones of inhibition in the range 10 ~ 18 mm. In this study, in some cases, the oil, methanol extract and organic sub-fractions exhibited higher antibacterial activity than that of streptomycin, while tetracycline showed higher activity, in some other cases, than those of the essential oil and solvent fractions. However, the residual methanol sub-fraction did not show any activity against any of the bacterial strains tested (data not shown). The blind control does not inhibit the growth of the bacteria tested. Methanol extract of *M. glyptostroboides* and its derived sub-fractions (hexane, chloroform and ethyl acetate) exhibited moderate inhibitory effect against *B. subtilis* (ATCC 6633), with diameter zones of inhibition in the range 10 ~ 12 mm.

### 3.3. Minimum inhibitory concentration (MIC)

As shown in Table 3, the MIC values for the oil showed more susceptibility of *S. aureus* KCTC 1916, *P. aeruginosa* and *S. aereus* ATCC 6538 (500 μg/ml) than of *B. subtilis* and *L. monocytogenes* (1000 μg/ml). MIC values of the methanol extract and its derived sub-fractions (hexane, chloroform and ethyl acetate) against the tested bacteria were in the range 250 ~ 500 μg/ml (Table 3). Ethyl acetate and chloroform fractions showed higher antibacterial activity by minimum inhibitory concentrations than did the hexane fraction. As shown in the results, Gram-positive bacteria were found to be more susceptible to the essential oil and various solvent extractions than were Gram-negative bacteria.

## 4. Discussion

The traditional use of plants, provides the basis for indicating the type of essential oils and plant extracts useful for specific food purposes. Historically, many plant oils and extracts have been reported to have antimicrobial properties (Hoffman, 1987). Also, the renewal of interest in the food industry, and increasing consumer demand for effective, safe, natural products, means that quantitative data on plant oils and extracts are required.

Various publications have documented the antimicrobial activity of essential oil constituents and plant extracts (Morris, Khettry, & Seitz, 1979). In recent years, several researchers have also reported mono- and sesquiterpenoids as the major components of essential oils, which are phenolic in nature. It seems reasonable to assume that their antimicrobial mode of action might be related to the phenolic compounds present (Cakir, Kordali, Zengin, Izumi, & Hirata, 2004). Most of the studies on the mechanism of phenolic compounds have focussed on their effects on cellular

Table 2
Anti-bacterial activity of essential oil, MeOH extract and sub-fractions of *Metasequoia glyptostroboides* Milki ex Hu against food spoilage and food-borne pathogens

| Micro-organisms | Zones of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Essential oil[a] | MeOH extract[b] | Sub-fractions of MeOH exract[c] | | | Antibiotics[d] | |
| | | | Hexane | $CHCl_3$ | EtOAc | SM | TC |
| *S. aureur* (KCTC 1916) | 16.0 ± 2.1 | 20.0 ± 1.6 | 18.0 ± 1.4 | 16.0 ± 2.1 | 16.0 ± 1.3 | 14 ± 0.9 | 18 ± 0.5 |
| *B. subtils* (ATCC 6633) | 13.0 ± 0.7 | 12.0 ± 1.2 | 10.0 ± 0.5 | 10.0 ± 0.5 | 12.0 ± 1.1 | 14 ± 0.2 | 18 ± 0.5 |
| *S. aureus* (ATCC 6538) | 14.0 ± 0.9 | 18.0 ± 1.9 | 15.0 ± 1.1 | 15.0 ± 1.9 | 18.0 ± 1.8 | 14 ± 0.6 | 19 ± 0.6 |
| *P. aeruginose* (KCTC 2004) | 14.0 ± 2.1 | 15.0 ± 1.2 | 14.0 ± 0.9 | 14.0 ± 0.7 | 13.0 ± 1.2 | 19 ± 0.4 | 20 ± 1.0 |
| *L. monocytogenes* (ACTC 19166) | 14.0 ± 1.7 | 15.0 ± 0.7 | 14.0 ± 0.7 | 15.0 ± 1.2 | 14.0 ± 0.9 | 14 ± 0.6 | 19 ± 0.5 |
| *E. coli* 0157:H7 (ACTC43888) | nd[e] | nd | nd | nd | nd | 15 ± 0.9 | 20 ± 0.5 |
| *E. coli* (ACTC 8739) | nd | nd | nd | nd | nd | 24 ± 0.7 | 17 ± 1.1 |
| *E. coli* 0157 (Human) | nd | nd | nd | nd | nd | 15 ± 0.7 | 19 ± 1.2 |
| *E. aetogenes* (KCTC 2190) | nd | nd | nd | nd | nd | 13 ± 0.2 | 20 ± 0.6 |
| *A. typhimurium* (KCTC 2515) | nd | nd | nd | nd | nd | 13 ± 0.2 | 21 ± 0.6 |
| *S. enteridis* (KCTC 12021) | nd | nd | nd | nd | nd | 14 ± 0.6 | 21 ± 1.0 |

[a] Diameter od inhibition zones of essential oil including diameter of disc 6 mm (tested at a volume of 1000 ppms/disc).
[b] MeOH extract (1500 ppm/disc).
[c] Sub-fractions of MeOH extract (1500 ppm/disc).
[d] Standard antibiotic SM, streptomycin and TC, tetracycline (10 μg/disc).
[e] nd, not detected. Values are given as mean ± S.D of triplicate experiment.

Table 3
Minimum inhibition concentration of essential oil, MeOH extract and sub-fractions of *Metasequoia glyptostroboides* Miki ex Hu against the growth of food spoilage and food-borne pathogens

| Micro-organisms | Minimum inhibitory concentration (MIC)[a] | | | | |
|---|---|---|---|---|---|
| | Essential oil[b] | MeOH extract[c] | Sub-fractions of MeOH exract[d] | | |
| | | | Hexane | $CHCl_3$ | EtOAc |
| *S. aureur* (KCTC 1916) | 500 | 250 | 250 | 250 | 250 |
| *B. subtils* (ATCC 6633) | 1000 | 500 | 500 | 250 | 500 |
| *S. aureus* (ATCC 6538) | 500 | 250 | 250 | 250 | 250 |
| *P. aeruginose* (KCTC 2004) | 500 | 250 | 500 | 250 | 250 |
| *L. monocytogenes* (ACTC 19166) | 1000 | 500 | 500 | 250 | 250 |
| *E. coli* 0157:H7 (ACTC43888) | nd[e] | nd | nd | nd | nd |
| *E. coli* (ACTC 8739) | nd | nd | nd | nd | nd |
| *E. coli* 0157 (Human) | nd | nd | nd | nd | nd |
| *E. aetogenes* (KCTC 2190) | nd | nd | nd | nd | nd |
| *A. typhimurium* (KCTC 2515) | nd | nd | nd | nd | nd |
| *S. enteridis* (KCTC 12021) | nd | nd | nd | nd | nd |

[a] Minimum inhibitory concentration (MIC).
[b] Essential oil.
[c] MeOH extract.
[d] Sub-fractions of MeOH extract (values in μg/ml).
[e] nd, not detected.

membranes. Actually, phenolics not only attack cell walls and cell membranes, thereby affecting their permeability and release of intracellular constituents (e.g. ribose, Na glutamate) but they also interfere with membrane function (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Thus, active phenolic compounds might have several invasive targets which could lead to the inhibition of bacteria.

Also, the results of the antibacterial screening showed that essential oil, methanol extract and its derived sub-fractions (hexane, chloroform and ethyl acetate) have potential activity against some bacterial strains, such as *S. aureus* KCTC 1916, *L. monocytogenes*, *S. aureus* ATCC 6538, *B. subtilis* and *P. aeruginosa*. This might be the result of the α-pinene (29.5%), totarol (9.37%) and totarol acetate (3.98%) present in the essential oil of *M. glyptostroboides* as these findings are strongly supported by earlier reports (Gary, Evans, & Richard, 2000; Gary, Richard, Michael, Gregory, & Scoot, 1999).

Essential oils, which are odorous and volatile products of plant secondary metabolism, have a wide application in the food flavouring and preservation industries (Smith-Palmer, Stewart, & Fyfe, 2001). *M. glyptostroboides*-mediated oil also contained high percentages of α-thujene, bornylene and δ-3-carene and, as earlier reported, the major components of the various essential oils, have enormous potential against food-borne pathogenic bacteria (Maria-Rose et al., 2004). These findings were confirmed

in the present investigation. In addition, it is also possible that the minor components, such as γ-caryophyllene, α-terpineol, α-humulene, along with methyl esters, might be involved in some type of antibacterial synergism with other active components of essential oil, as was also evidenced by the work of Marino et al. (2001).

When comparing the data obtained in different studies, most publications provide generalization about whether or not a plant oil or extract possesses activity against Gram-positive and Gram-negative bacteria. However, few provide details about the extent or spectrum of this activity. Deans, Noble, Hiltunen, Wuryani, and Penzes (1995) observed that the susceptibility of Gram-positive and Gram-negative bacteria to plant volatile oils had little influence on growth inhibition (Deans et al., 1995; Deans & Ritchie, 1987). However, some oils appeared more specific, exerting a greater inhibitory activity against Gram-positive bacteria. It is often reported that Gram-negative bacteria are more resistant to the plant-based essential oils (Reynolds, 1996). The hydrophilic cell wall structure of Gram-negative bacteria is constituted essentially of a lipo-polysaccharide (LPS) that blocks the penetration of hydrophobic oil and avoids the accumulation of essential oils in target cell membrane (Bezic, Skocibusic, Dinkic, & Radonic, 2003). This is the reason that Gram-positive bacteria were found to be more sensitive to the essential oil, methanol extract and various methanol-derived sub-fractions of *M. glyptostroboides* than were Gram-negative bacteria.

In this research, we found that essential oil, methanol extract and various methanol-derived sub-fractions of *M. glyptostroboides* severely inhibited the growth of food spoilage and food-borne pathogens. Therefore, essential oils and plant extracts are being considered as potential alternatives to synthetic bactericides or as a lead compounds for new classes of natural bactericides.

The results of this study suggest that *M. glyptostroboides* may act as an alternative to synthetic bactericides for use in the food industries, where bacterial pathogens cause severe destruction. However, if plant oils and extracts are to be used for food preservation or medicinal purposes, issues of safety and toxicity will always need to be addressed.

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