1-1-2014

Screening Of Natural Antimicrobial Agents And Antimicrobial-Resistant Bacteria Using A Soleris System

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SCREENING OF NATURAL ANTIMICROBIAL AGENTS AND ANTIMICROBIAL - RESISTANT BACTERIA USING A SOLERIS SYSTEM

by

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THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2014

MAJOR: NUTRITION AND FOOD SCIENCE

Approved by:

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Advisor Date

DEDICATION

I dedicate this thesis and all my hard work behind it to my dear family and friends. Having a family that is always supportive, understanding and loving makes me very grateful and happy no matter what bad situation I met in my life. My friends also have been showing their truthful helps to me during the period of this experience. I would have not been able to go through all the hard times if they were not there around me to give me the amazing support.

ACKNOWLEDGEMENTS

I would like to acknowledge and give my deepest appreciation to my super advisor, Dr. Yifan Zhang, who has taught me the spirit of adventure in regard to research and gave me practical guidance and amazing support. Based on what I have learned not only in the area of food safety and food microbiology, but also valuable experience in my life from Dr. Yifan Zhang, I would graduate from here with calm and confidence to achieve my career goals. In addition, I would also give my sincere gratitude to my committee members, Dr. Kai-Lin Catherine Jen and Dr. Kequan Zhou. They were both very caring and supportive during the whole period of my graduate study. Without their guidance and helps, I would not have the confidence and expertise to finish my best work. Finally, I would also thank my lab mates Vasiana Tomco, Varun Tahlan, and Manisha Polur for their contributions during the research in the lab. Thanks again to all of you.

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CHAPTER 1

INTRODUCTION

1.1 Natural antimicrobial agents

Prevention and control of bacterial contamination in food is an imperative task to ensure food safety. Many antimicrobials derived from animals, plants, and microbial sources have been shown to have antimicrobial activities applied in food industries (1-6). Essential oils are secondary metabolites of plants that are generally recognized as safe (GRAS) as flavoring agents for consumption by animals and humans in the US. They have been shown to be promising alternatives to chemical sanitizers against foodborne bacteria (7-8). Essential oils from clove, basil, lemongrass, and thyme have demonstrated antimicrobial effects on food products (9-15). These natural antimicrobials can be used alone or in combination with other novel preservation methods or compounds to obtain satisfactory result for preservation in food industries (16-18). In particular, plant-derived natural antimicrobials have been newly widely applied in food industries to prevent food spoilage and extend shelf life of food as well (19).

It has been extensively reported that various plant-derived essential oils and their isolates exhibit antimicrobial functions against foodborne pathogens (20-23) They are natural aromatic compounds found in the seeds, bark, stems, roots, flowers, and other parts of plants (1, 21). Essential oils are considered as the blood of plants with antibacterial, anti-inflammatory, and anti-viral characteristics (24-26). Generally, methods used to obtain and produce essential oils include fermentation, expression, extraction and steam distillation (1, 26). Antimicrobial mechanism of essential oils is

involved in several specific targets of microorganism cells. Basically, mechanism of antimicrobial action is associated with hydrophobicity that enables essential oils to penetrate into bacterial cell membrane and mitochondria, further disturbing the membrane structures and rendering them more permeable (27-31). Leakage of ions and other cell contents can subsequently occur. As a severe consequence, extensive loss of cell contents or the exit of critical molecules and ions will lead to death of bacteria. The most effective compounds attributing to antimicrobial abilities of essential oils are believed to be phenolic compounds (30-32).

1.2 Identification of Antimicrobial-Resistant Bacteria

Antimicrobial resistance (AMR) is defined as the resistance of a microbial organism against antimicrobial agents to which it was initially sensitive (33). It is acknowledged that the overuse of antimicrobial agents in animal feed for preventing and/or treating bacterial associated infectious diseases has imposed selective pressure on many foodborne pathogens (34). consequently, such selective pressure promotes acquisition of the antimicrobial-resistant foodborne bacterial pathogens that subsequently transmit to humans as food contaminants (34). Micro-dilution antimicrobial susceptibility testing assay is frequently used to determine the Minimal Inhibitory Concentration (MIC) – defined as the lowest concentration of an antimicrobial substance that inhibits the visible growth of a microorganism *in vitro* after overnight incubation (34-37). It is usually expressed in micrograms per milliliter (ppm) of a specific antimicrobial agent required to inhibit the growth of a specific microorganism (35, 37). MIC is widely used in microbiology laboratories when antimicrobial susceptible testing is performed to screen antimicrobial-resistant or –susceptible microorganisms (33-34). Moreover, MIC takes an

important role in diagnostic laboratories to determine breakpoints of an antimicrobial substance (35-37). Breakpoints refer to MIC of any given antimicrobial agent that can be used to define susceptibility and resistance of bacterial pathogens. The unit is either in concentration (in ug/L or in uL/mL) or diameter (in mm) depending on testing methods (34, 36, 37). Breakpoints are the concentrations of antimicrobial substances at which bacteria are killed successfully. With the increasing prevalence of antimicrobial resistance long time established breakpoints may underestimate antimicrobials dosage levels (34, 37). Thus, new data are needed to obtain the accurate breakpoints of antimicrobial agents (33-34).

As recommended by the Clinical and Laboratory Standards Institute (CLSI), micro-dilution method is a common method to test antimicrobial susceptibility of antimicrobial agents against microorganisms (38-39). Testing is generally performed using a 96 micro-titer plate incorporated with a two-fold serial dilution of individual antimicrobial agent and the specific concentration of bacterial suspension was determined by 0.5 McFarland standard. After overnight incubation, the first clear well is estimated as MIC of the antimicrobial agent (38). However, the main disadvantage of the traditional micro-dilution method is time-consuming and labor-intensive. Limitations also focus on the application of restricted concentrations of antimicrobial agents and inconsistent results when testing fastidious anaerobes due to excessive exposure to oxygen during the preparation procedure (40).

1.3 Soleris Detection Method

A method called Soleris system for rapid automated detection of bacteria was described and developed by Firstenberg-Eden and Shelef (41, 43, 45). The Soleris system is an instrument that is capable of monitoring 32 samples simultaneously at one incubation temperature in the range of 15-60 ℃. The presence of micro-organisms is detected via changes in pH as the micro-organisms grow and produce acid. The unique design of this system is the disposable vials that contain a specific broth in its upper layer and a semi-fluid barrier part at the bottom. Since only small molecules and ions can pass through the semi-fluid barrier, it mirrors the color change in the medium, without the influence of turbidity. Changes in optical units are monitored and recorded to determine the detection time for a specific micro-organism (41-47). Each detection time corresponds with a certain concentration of the microorganism tested within the specific vial. Many studies have reported that using the Soleris system for bacteria detection, such as rapid detection of *E. coli* in ground beef and water, *Listeria* in shell eggs and ready-to-eat meats, *Salmonella* in milk and chicken, etc. (41-47). The Soleris system can be used to screen natural antimicrobials and antimicrobial-resistant bacteria by monitoring the growth of microorganisms under different antimicrobial conditions.

The overall goal of this study was to screen 11 plant-derived essential oils and 2 compounds for their antimicrobial activities against some foodborne pathogens and screen two species of antibiotic-resistant and -susceptible bacteria using the Soleris system. The specific objectives of this study were i) to develop an automated detection technique to test the antimicrobial properties of plant-derived essential oils and compounds against different species of bacteria; (ii) to compare the performance of Soleris system with traditional broth micro-dilution assay for testing MIC (iii) to screen

different strains of methicillin-resistant and -susceptible *Staphylococcus aureus* (*S. aureus*), ß-lactam-resistant and -susceptible *Escherichia coli* (*E. coli*) using Soleris system.

CHAPTER 2

MATERIALS AND METHODS

2.1 Bacterial strains and culture methods

The microorganisms utilized in this study consisted of the following strains: *S. aureus* (NCTC 8325), *E. coli* (ATCC 23631, ATCC 13706, ATCC 25922), methicillin-resistant *S. aureus* (58-2, 276, 47-3, 19-2, 85), methicillin-susceptible *S. aureus* (408, TS40-1, TS 18-3, TS15-1, 925, 83-2, TS20-3, 83-1), ß-lactam-resistant *E. coli* (N39037, N39078, N39872, N39958, N39969, N40530, N40558, N40613). All the ß-lactam-resistant *E. coli* strains were obtained from the National Antimicrobial Resistance Monitoring System (NARMS). The remaining microorganisms were from the Microbiology Laboratory of Wayne State University. The microorganisms were maintained on Tryptic Soy Agar (TSA) at 4℃ and newly sub-cultured on TSA for 18 to 24 h at 37℃ before use.

2.2 Instrument Description

The Soleris system (Ann Arbor, MI) is an automated detection system with an incubator containing 32 vials capacity model that is capable of heating and cooling in the temperature range of 15-60℃. It measures optical changes via color change in pH initiated by microbial growths in the disposable vials. Samples are introduced into a ready-for-use vial that contain specific medium in its upper layer, and at the bottom, a square window containing a soft agar layer which separates liquid broth and the agar barrier. The semi-fluid layer mirrors the color change in the broth without the influence

by sample particles or turbidity. Light from light emitting diodes passes through the bottom potion of the vial and a photodiode measures light transmission at the rate of 10 readings per hour. As soon as the color change expressed as optical units is detected by the optical sensor, the time of such detection is recorded in the computer.

2.3 Preparation of Essential Oil Emulsions

Eleven essential oils including thyme, cinnamon, oregano, clove, bay, rosemary, basil, nutmeg, bergamot, marjoram, sage, lemongrass oils, and two plant-derived compounds consisting of eugenol and carvacrol were purchased from Fisher-scientific Company. Stock solutions (20 µL/mL) were prepared using 600µL of individual essential oil or compound, 300ul Tween 80, and 30ml double-distilled water. A sonic dismembrator model was used to vortex the emulsions thoroughly. All the essential oils stock solutions were stored at 4℃.

2.4 Preparation of Antibiotic Stock Solutions

Cefoxitin, ampicillin and tetracycline stock solutions (2mg/L) were prepared by using 0.002g of individual antibiotic and 50ml deionized water based upon which to guarantee the final concentration of an antibiotic in one specific test vial is 4mg/L. Similarly, 4mg/L, 8 mg/L, 16 mg/L, 32 mg/L, 64 mg/L and 128 mg/L antibiotic stock solutions were prepared by using 0.004g, 0.008g, 0.016g, 0.032g, 0.064g and 0.0128g of individual antibiotic, respectively, and 500ml deionized water for each. All the antibiotic stock solutions were stored at 4℃.

2.5 Generation of calibration curves

In order to compare the reproducibility of the data generated using the Soleris system and traditional culture method, the reproducibility test was performed prior to the rest of the test. The overnight pure culture was serially diluted 10-fold (10^0 - 10^9 cfu/ml) in sterile water. The diluted *S. aureus* (1ml) suspension was aseptically added into vials containing 9ml specific broth. Likewise, diluted *E. coli* suspension (5ml) was transferred into the corresponding test vials containing 5ml test media. The vials were gently inverted several times and loaded into the incubator. An un-inoculated vial was tested as a control of each measurement. Each test was carried out three times. *S. aureus* was tested at 37^0C for 16 h, while *E. coli* was tested at 35^0C for 14 h (41, 42, 47). Colony counts were determined by the traditional culture method.

2.6 Testing procedure using Soleris system

All the tested inoculums were prepared using 18h culture adjusted in reference to the McFarland 0.5 standard and further diluted with Mueller Hinton Broth (MHB) to obtain approximately 10^8cfu/ml. The 9ml specific broth vials for *S. aureus* were inoculated with 500 µl of 10^7 cfu/ml bacterial suspension, while the 5 ml specific medium vials for *E. coli* were inoculated with 278 µl of 10^7cfu/ml bacterial suspension in order to give the final concentration in each vial approximately 5×10^5 cfu/ml. Different concentration of antimicrobial agents or antibiotic stock solutions (500µl) obtained by a two-fold dilution (ranging from 156ppm to 10000ppm) was added into each *S. aureus* vial, while 278µl was added into each *E. coli* vial. One corresponding vial that consists of un-inoculated media was included in each measurement as a control. The ready-for-test vials were inverted 10 times gently prior to their placement into the incubator. Sample information

and parameters were entered into the Soleris system corresponding to the position of each sample in the incubator. *S. aureus* was tested at 37^0C for a maximum of 12.1 h, whereas *E. coli* was tested at 35^0C for a maximum of 10.8 h.

2.7 Determination of minimal inhibitory concentration (MIC)

A broth micro-dilution assay as recommended by NCCLS was used to show the minimal inhibitory concentration (MIC) of antimicrobial properties of the selected plant essential oils and compounds (38-39). All tests for *E. coli* strains were performed with Cation Adjusted Mueller Hinton Broth (CAMHB) alone, while tests for *S. aureus* strains were also tested with 4% Sodium Chloride solution. Initially, a two-fold serial dilution of various essential oils and compounds, ranging from 78ppm to 10000ppm, were incorporated in a 96-well microtiter plate, including one control (CAMHB + sterile distilled water or CAMHB + 4% NaCl + sterile distilled water) at the last row. Bacterial suspensions were standardized to approximately 1×10^8 cfu/ml (using McFarland 0.5 Standard). The specific amount of bacterial suspension (50µl) was subsequently added to each well in order to give a final concentration of approximately 5×10^5 CFU/ml. Plates were incubated overnight at 37 °C for 18 h. Each test was carried out in triplicate. The first clear well was determined as the Minimal Inhibitory Concentration (MIC) of an individual essential oil or compound showing complete inhibition of the tested bacteria.

CHAPTER 3

RESULTS

3.1 Calibration curves of *S. aureus* and *E. coli* standard strains

The *E. coli* (ATCC 23631) and *S. aureus* (NCTC 8325) colony counts obtained by traditional culture method were plotted against the detection times generated by the Soleris system. The regression lines for *E. coli* (ATCC 23631) and *S. aureus* (NCTC 8325) are shown in Fig. 1 and Fig.2, respectively. A total of 30 data points were used to generate the regression lines for *E. coli* resulting in a correlation coefficient of -0.97 and the line equation was log (cfu/mL)= 9.766 – 0.79×DT(Fig. 1). The correlation coefficient for 30 data points obtained for *S. aureus* counts was -0.96 and the line equation was log (cfu/mL)= 9.244 – 0.671×DT (Fig. 2).

3.2 Antimicrobial property expressed by MIC

Antimicrobial activity of 11 different essential oils and 2 compounds obtained from herbs and spices against *E. coli* and *S. aureus* were investigated. The MIC values showed the wide variation in the antimicrobial properties of the tested essential oils and compounds against *S. aureus* and *E. coli* (Table. 1). As shown in Table 1, among 11 essential oils tested, 8 essential oils exhibited antibacterial activity against *S. aureus*. Cinnamon oil (1250ppm), oregano oil (1250ppm), and rosemary oil (1250ppm) turned out to be the top three inhibitory oils examined. However, only 7 essential oils revealed antibacterial properties against *E. coli* in which cinnamon oil (312ppm) was the most effective one examined among the selected antimicrobial agents. By contrast, bergamot oil, marjoram oil, and basil oil failed to inhibit any of the selected strains. Sage oil did not

exhibit antimicrobial activity against *E. coli* in the present study. In addition, carvacrol showed higher antimicrobial property than eugenol against all the tested microorganisms. No obvious difference in susceptibility to the tested antimicrobial agents was found between gram-positive and gram-negative bacteria.

3.3 Antimicrobial activity expressed by Detection Times (DTs)

Antimicrobial activities of various essential oils against *S. aureus* and *E. coli* expressed by Detection Time (DT) are shown in Table 2 and Table 3, respectively. As shown in Table 2 and Table 3, when applying higher concentrations of individual essential oils and compounds in the inoculated media, longer time was required to detect the microorganism in the vial. The *S. aureus* was more sensitive to the oils of thyme, sage and lemongrass than other selected oils. By contrast, the oils of clove, basil, oregano, marjoram, sage, and lemongrass were less active against the tested *E. coli* than other oils examined. In terms of the antimicrobial performance of two plant-derived compounds, carvacrol appeared to possess higher antimicrobial properties than eugenol against all the tested microorganisms.

3.4 Screening MRSA and MSSA

Detection times (DTs) of various cefoxitin concentrations (mg/L) against the selected Methicillin-resistant *S. aureus* and Methicillin-susceptible *S. aureus* strains are shown in Table 4. All the tested MRSA strains were found to demonstrate significant resistance to cefoxitin. Out of 8 different of MSSA strains, 7 of them (TS40-1, TS15-1, TS20-3, 925, 408, 83-2, and 83-1) were sensitive to cefoxitin at a concentration of 4mg/L.

The MSSA strain (TS18-3) indicated higher susceptibility to cefoxitin than the rest of selected MSSA strains.

3.5 Screening cefoxitin-resistant and -susceptible *E. coli*

Detection times (DTs) of various cefoxitin concentrations (mg/L) against the tested ß-lactam-resistant and -susceptible *E. coli* are shown in Table 5. Among 9 different ß-lactam-resistant *E. coli* strains, 6 (N39001, N40602, N39213, N39190, N39200, N40490) were not detected when cefoxitin concentrations were 32mg/L or higher. The rest of the tested strains *(*N39969, N39078, and N39037) exhibited a higher degree of resistance to cefoxitin. By contrast ß-lactam-susceptible *E. coli (*ATCC25922 and ATCC 13706) were less susceptible to cefoxitin than ATCC 23631 did.

3.6 Screening ampicilin-resistant and -susceptible *E. coli*

Detection times (DTs) of different concentration of ampicilin (mg/L) against the selected ß-lactam-resistant and -susceptible *E. coli* strains are shown in Table 6. Only one ß-lactam-resistant *E. coli* strain (N39037) revealed ampicillin resistance in this study. With higher concentration of ampicillin applied, longer time was required to detect the microorganisms in the vials. The ß-lactam-susceptible *E. coli* strains (ATCC13706 and ATCC 23631) showed higher susceptibility to ampicillin, compared with the strain (ATCC 25922).

3.7 Screening tetracycline-resistant and -susceptible *E. coli*

Detection times (DTs) of different concentration of tetracycline (mg/L) against the tested ß-lactam-resistant and susceptible *E. coli* are shown in Table 7. Tetracycline was

active against all the selected ß-lactam-resistant *E. coli* strains (N40602 and N39001) at a concentration of 128mg/L. The ß-lactam-susceptible *E. coli* strain (N39910) failed to exhibit susceptibility to tetracycline at the tested concentration of 2mg/L and 4mg/L. By contrast, the rest of the ß-lactam-susceptible *E. coli* strains of (N39190 and N39872) indicated high susceptibility to tetracycline.

CHAPTER 4

DISCUSSION

In this study, the suitability of the Soleris system for screening natural antimicrobial agents and antimicrobial-resistant bacteria was evaluated. The high correlation coefficients between the Soleris system DTs and traditional culture methods support the application of this system as an alternative method for enumeration of the tested bacteria.

The time required to perform the measurements for 8 different concentrations of individual antimicrobial agent tested by micro-dilution method (media preparation, serial dilution preparation, samples loading, incubation, and results analysis), and the time required by the Soleris system (labeling, serial dilution preparation, vials loading, parameters setting, incubation, data handling) was recorded and compared. The hands-on time (excluding incubation time) of each measurement using the Soleris system and micro-dilution method was 18.5 and 89.5 min, respectively. Moreover, MICs for *E. coli* could be determined within 12.6 h using the Soleris system, while the traditional micro-dilution method required 21 h. Time consumption to test MICs for *S. aureus* using the Soleris system and micro-dilution method was 11.3 h and 21 h, respectively. The ratios of the time required to perform the susceptibility testing using the Soleris system in relation to the traditional micro-dilution assay were 0.6 for *E. coli* and 0.54 for *S. aureus* as shown in Fig. 3. The Soleris system is time-saving and less labor intensive in comparison to the standard micro-dilution assay when performing susceptibility testing.

In this study, the tested antimicrobial agents showed varying antimicrobial activities against the selected bacterial pathogens. Out of 11 essential oils and 2 compounds

examined, oregano, rosemary, thyme, cinnamon oils and carvacrol revealed strong activity against the tested pathogens. Previous studies (9-15, 47-56) have shown that oregano, cinnamon, rosemary, thyme had strong and consistent inhibitory effects against various bacteria. Among all antimicrobial agents investigated in this study, cinnamon oil appeared to be the most effective oil to inhibit the growth of bacterial pathogens. Earlier studies (48, 57) have found better antimicrobial activity for clove and bay oils, however, the present study showed least inhibitory effect of clove and bay oils against the tested *S. aureus*. Moreover, previous studies reported that gram-positive bacteria were more resistant to the antimicrobial agents than gram-negative bacteria (7, 9), however, no obvious difference of susceptibility of the tested antimicrobial agents was found between gram-positive and gram-negative bacteria in this study. The essential oils of nutmeg, bergamot and sage failed to exhibit antimicrobial activity against the selected bacterial pathogens. The differences were probably due to the application of different oil extraction methods, oil preparation methods, testing methods, and discrepant sensitivities of the tested microorganisms.

DT values cannot be compared with literature data since there are no reports on the antimicrobial properties of the tested essential oils and compounds. However, this study showed that the Soleris system is comparable to the standard micro-dilution method with respect to susceptibility testing of antimicrobial agents. The oils of thyme, cinnamon, oregano, bay, rosemary and compound carvacrol revealed higher inhibitory effects than the remaining tested antimicrobial agents. Moreover, one of the advantages of the Soleris system is its ability to determine Minimum Bactericidal Concentration (MBC) in addition to MICs simultaneously. The definition of MBC is concentration at which 99.9% or more

of the initial inoculum is killed (58). The first no detection (ND) was associated with the MBC value of a certain antimicrobial agent. The corresponding concentration of a specific essential oil following the one that showed ND was estimated as MIC value provided by the Soleris system.

Generally, the antimicrobial concentration of the first clear well is estimated as MIC of the tested substance using the standard micro-dilution method (35-36). However, the well that remains clear or less turbid after overnight incubation probably still contains a lower level of viable microorganisms. Another possibility is that all the microbes could have been killed by the antimicrobial agent examined. The above two possibilities cannot be differentiated visually. However, the soleris system can guarantee the absence of microorganism in the test vials that efficiently avoids the inconsistent results due to the limitation of data handling visually. Thus, the Soleris system demonstrated higher efficiency and accuracy than the traditional method when testing MIC values of antimicrobial agents.

Regarding the effectiveness of the Soleris system for screening ß-lactam susceptible *E*. coli, this study showed consistent results with the data measured by broth micro-dilution. In addition, the corresponding MIC values of various antibiotics provided by the Soleris system were also in agreement with earlier literatures (59-63).

CHAPTER 5

CONCLUSION

Conclusions were made based on the results of MIC values and detection time values. First, different essential oils and effective compounds possessed varying antimicrobial abilities against the tested strains. Second, the Soleris system provided us an alternative and time-saving method to test MIC and MBC of different essential oils. Finally, the Soleris system produced comparable data and provided a rapid and cost-efficient alternative method for screening antimicrobial- resistant and -susceptible bacteria. Future research will be designed to test the effective components of essential oils and the exact modes of their antimicrobial activities, and test the system on different bacteria and antimicrobial agents.

Table 1. Antimicrobial activity expressed as the minimum inhibitor concentration (MIC)[(1)] of various essential oils and compounds (ppm) against selected bacteria strains[(2)]

	MIC (ppm)	
Essential Oils	*E. coli*[(2)]	*S. aureus*[(2)]
Thyme	≧1250	≧2500
Cinnamon	≧312	≧1250
Sage	-[(3)]	≧2500
Origanum	≧2500	≧1250
Bergamot	-	-
Eugenol	≧5000	≧5000
Carvacrol	≧312	≧1250
Clove	≧1250	≧5000
Marjoram	-	-
Bay	≧1250	≧5000
Lemongras	≧2500	≧2500
Rosemary	≧2500	≧1250
Basil	-	-

[(1)] Defined as the lowest concentration of essential oil that showed total inhibition after 18 h of incubation at 37°C.

[(2)] All strains in the stationary phase of growth were used at a final concentration of 5×10^5 CFU/mL.

[(3)] Were not detected.

Table 2. Detection time (h) of different concentration of selected essential oils and compounds (ppm) against *S. aureus* (NCTC 8325) by Soleris system.

	10000	5000	2500	1250	615	312	156
Thyme	ND	6.7h	3.9h	3.0h	2.8*h*	2.8h	2.8h
Cinnamon	ND	ND	4.4h	3.2h	3.2*h*	3.1h	3.0h
Oregano	ND	*ND*	6.9h	3.8h	3.6*h*	3.3h	3.1h
Clove	5.8h	4.1*h*	3.8h	3.4h	3.3h	3.3h	3.2h
Bay	5.0h	3.3h	3.1h	2.9h	2.9h	2.9h	2.8h
Rosemary	7.6h	5.6h	3.3h	3.3h	3.1h	3.0h	2.8h
Basil	5.5h	3.5h	3.1h	2.9h	2.9h	2.8h	2.8h
Nutmeg	12.1h	6.3h	4.8h	3.9h	3.6h	3.1h	3.0h
Bergamot	9.8h	5.7h	3.1h	2.9h	2.9h	2.8h	2.8h
Eugenol	10.3h	4.6h	3.9h	3.1h	2.9h	2.9h	2.8h
Majoram	10.6h	4.2h	2.8h	2.8h	2.8h	2.8h	2.8h
Sage	ND	7.8h	4.1h	3.3h	3.1h	3.0h	2.8h
Carvacrol	ND	9.6h	4.1h	3.9h	3.2h	2.9h	2.8h
Lemongrass	ND	4.2h	4.0h	3.2h	3.2h	2.8h	2.8h

ND: No detection time.

Table 3. Detection time (h) of different concentration of selected essential oil

And compounds (ppm) against *E. coli* (ATCC 23631) by Soleris system.

	10000	5000	2500	1250	615	312	156
Thyme	ND	6.4h	3.9h	3.0h	2.8*h*	2.8h	2.8h
Cinnamon	ND	ND	4.4h	3.2h	3.2*h*	3.1h	3.0h
Oregano	8.4h	6.9h	6.0h	3.8h	3.6*h*	3.3h	3.1h
Clove	7.3h	5.8*h*	3.8h	3.4h	3.3h	3.3h	3.2h
Bay	ND	6.2h	3.1h	2.9h	2.9h	2.9h	2.8h
Rosemary	ND	5.6h	3.3h	3.3h	3.1h	3.0h	2.8h
Basil	6.6h	6.1h	4.4h	4.4h	4.2h	4.2h	4.1h
Nutmeg	ND	6.1h	4.1h	3.8h	3.7h	3.6h	3.4h
Bergamot	ND	5.9h	3.9h	3.8h	3.7h	3.6h	3.5h
Eugenol	10.8h	5.9h	5.1h	4.0h	3.7h	3.5h	3.4h
Marjoram	6.9h	6.1h	4.4h	4.3h	3.9h	3.8h	3.8h
Sage	8.2h	6.9h	5.3h	4.4h	4.1h	3.4h	2.8h
Carvacrol	ND	11.7h	4.0h	4.0h	3.7h	3.7h	3.5h
Lemongrass	8.2h	6.6h	5.3h	4.5h	3.6h	3.1h	2.8h

ND: No detection time.

Table 4. Detection time (h) of different concentration of cefoxitin (mg/L) against the selected MRSA[(1)] and MSSA[(2)] by Soleris system.

Strains	**2**	**4**	**8**	**16**	**32**	**64**
85[(1)]				ND		
276[(1)]			7.2	ND		
58-2[(1)]			4.2	ND		
47-3[(1)]				ND		
19-2[(1)]				ND		
TS40-1[(2)]	4.0	ND				
TS18-3[(2)]	ND					
TS15-1[(2)]	2.8	ND				
TS20-3[(2)]	3.9	ND				
925[(2)]	3.9	ND				
408[(2)]	4.0	ND				
83-2[(2)]	10.7	ND				
83-1[(2)]	2.8	ND				

ND: No detection time.

Table 5. Detection time (h) of different concentration of cefoxitin (mg/L) against the selected ß-lactam-resistant[(1)] and -susceptible[(2)] *E. coli* by Soleris system.

Strains	**2**	**4**	**8**	**16**	**32**	**64**
N39001[(1)]				12.4	ND	ND
N40602[(1)]				15.4	ND	ND
N39213[(1)]				9.1	ND	ND
N39190[(1)]				8.7	ND	ND
N39200[(1)]				9.4	ND	ND
N39037[(1)]					8.6	ND
N39969[(1)]					13.1	ND
N39078[(1)]					6.7	ND
N40490[(1)]					ND	
ATCC25922[(2)]	6.1	ND				
ATCC13706[(2)]	10.3	ND				
ATCC23631[(2)]	ND					

ND: No detection time.

Table 6. Detection time (h) of different concentration of ampicilin (mg/L) against the selected ß-lactam-resistant(1) and -susceptible(2) *E. coli* by Soleris system.

Strains	**2**	**4**	**8**	**16**	**32**	**64**	**128**
N39001(1)					6.9	7.0	7.9
N40602(1)					4.6	5.2	5.0
N39213(1)					5.5	6.7	14.7
N39190(1)					6.3	10.8	15.6
N39200(1)					4.5	5.4	16.5
N39037(1)					4.4	5.1	ND
N39969(1)					5.2	8.0	8.2
N39078(1)					5.2	4.5	5.5
ATCC25922(2)	9.7	ND					
ATCC13706(2)	ND						
ATCC23631(2)	ND						

ND: No detection time.

Table 7. Detection time (h) of different concentration of tetracycline (mg/L) against the selected ß-lactam-resistant and -susceptible *E. coli* by Soleris system.

Strains	**2**	**4**	**8**	**16**	**32**	**64**	**128**
N39190		ND					
N39872		ND					
N40602					3.7	14.3	ND
N39001					3.6	8.1	ND
N39910	5.6	6.1					

ND: No detection time.

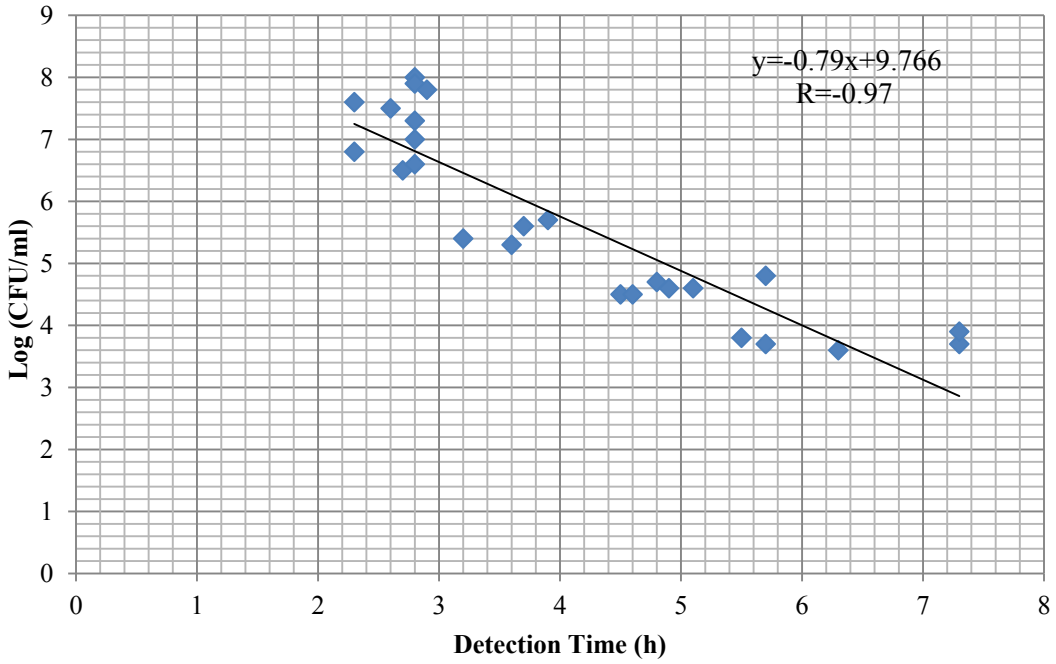

Fig.1. Regression curve for the data from *E. coli* (ATCC 23631) plate culture method plotted against detection time (DT).

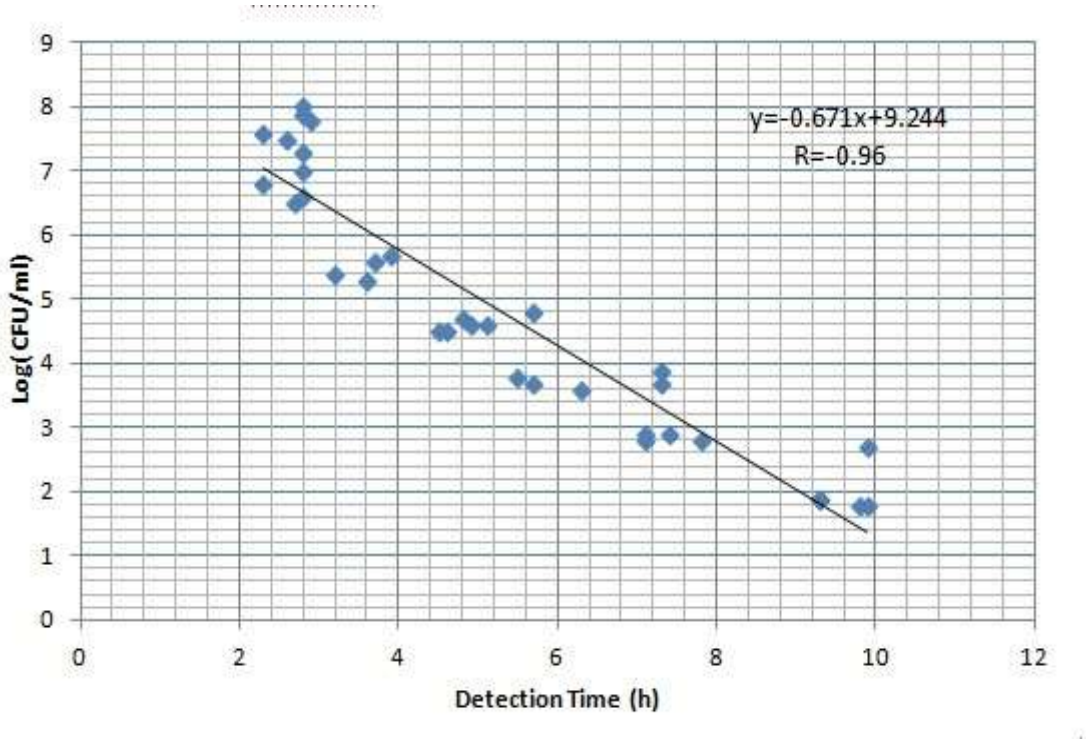

Fig.2. Regression curve for the data from *S. aureus* (NCTC 8325) plate culture method plotted against detection time (DT).

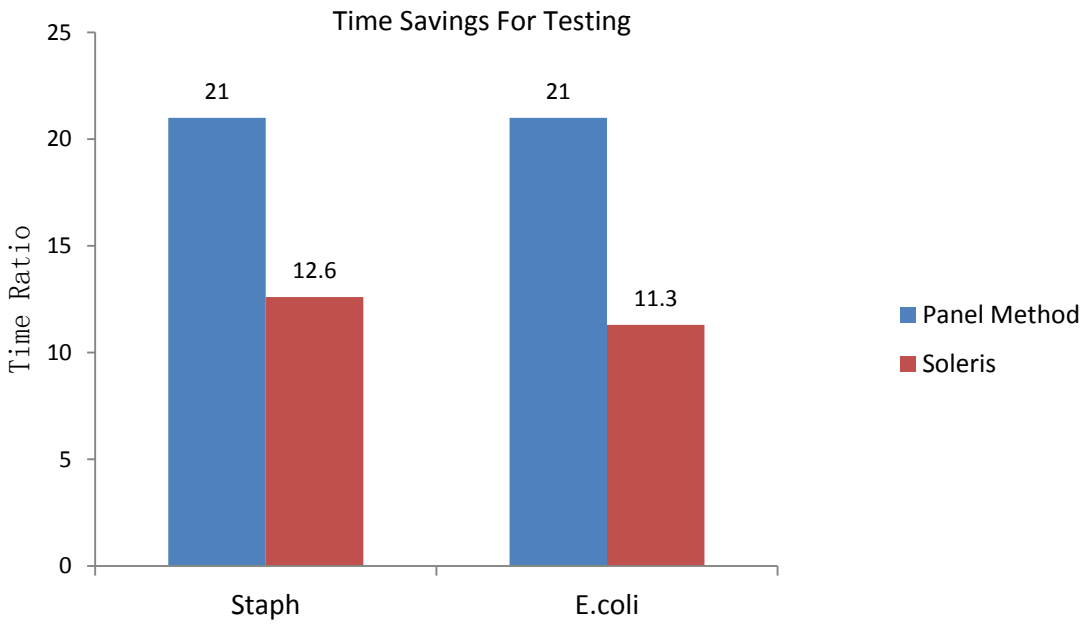

Fig. 3. Time ratio of the Soleris system detection procedures compared to traditional broth micro-dilution method.

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ABSTRACT

SCREENING OF NATURAL ANTIMICROBIAL AGENTS AND ANTIMICROBIAL - RESISTANT BACTERIA USING A SOLERIS SYSTEM

by

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May 2014

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Traditional broth micro-dilution method is a common assay of measuring Minimum Inhibitory Concentration (MIC) to determine the antimicrobial activity of an antimicrobial agent. However, this method is generally time-consuming and labor intensive. Alternatively, an automated optical method using the Soleris system was applied in this study. The system was compared to the traditional broth micro-dilution 96-well assay to test the antimicrobial activity of 11 essential oils and 2 plant-derived compounds against *Escherichia coli* and *Staphylococcus aureus*. We also used the system to differentiate antimicrobial-resistant and –susceptible bacteria based on their antimicrobial resistance phenotypes. MIC values of cefoxitin against 5 strains of methicillin-resistant *S. aureus* (MRSA) and 8 strains of methicillin-susceptible *S. aureus* (MSSA) were tested. MIC values were also determined on cefoxitin, ampicilin and tetracycline against 11 ß-lactam-resistant *E. coli* and 3 susceptible *E. coli*. Most of the selected essential oils revealed strong antimicrobial effects against the tested microorganisms. Cinnamon oil and carvacrol compound were found to be more active

against the test strains than any other selected antimicrobial agents. MICs obtained by Soleris system were comparable to those determined by standard micro-dilution method with respect to susceptibility testing of antimicrobial agents. The advantage of the Soleris system is its time efficiency and ease to perform. It provides a rapid and cost-efficient alternative for screening antimicrobial agents and antimicrobial-resistant bacteria.

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