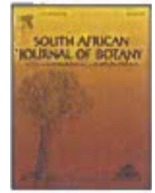




ELSEVIER

## South African Journal of Botany

journal homepage: [www.elsevier.com/locate/sajb](http://www.elsevier.com/locate/sajb)

# Enhancing the antimicrobial efficacy of common herbs and spices through an optimized polyherbal approach

T. Mapeka<sup>a,b</sup>, M. Sandasia<sup>b</sup>, E. Ncube<sup>a</sup>, A. Viljoena<sup>b</sup>, S. van Vuuren<sup>1,\*</sup><sup>a</sup> Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Preroria 0001, South Africa<sup>b</sup> SAMRC Herbal Drugs Research Unit, Faculty of Science, Tshwane University of Technology, Private Bag X680, Preroria 0001, South Africa<sup>1</sup> Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of Witwatersrand, 7 York Road, Parktown 2193, South Africa

## ARTICLE INFO

## Article history:

Received 23 August 2023

Revised 3 November 2023

Accepted 20 November 2023

Available online xxx

Edited by: Dr. M Vambe

## Keywords:

Anti-bacterial

Minimum inhibitory concentration

Sum of fractional inhibitory concentration index

Design of experiments

## ABSTRACT

Herbs and spices are used globally by most population groups across the world. In sub-Saharan Africa, they have medicinal significance in addition to their culinary uses. Although herbs and spices are often used in combination, there are few studies that report on their interactive antimicrobial effect. This study screened 17 culinary herbs and spices (crude extracts and essential oils) for antimicrobial activity, individually, followed by 1:1 combinations of the active extracts, to determine the outcome of combining these for antibacterial activity. The minimum inhibitory concentrations (MIC) were determined against six common foodborne pathogens using the broth microdilution assay. The design of experiments (DOE) approach was subsequently employed in MODDE 9.1<sup>®</sup> software, to optimise the experimental runs so as to identify the best interaction that would produce the best possible antimicrobial effect. Phytochemical profiling of the most active extracts was achieved using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) analysis. The results demonstrated that combining *Rosmarinus officinalis* with either *Syzygium aromaticum* or *S. officinalis* methanol extracts produced synergistic antimicrobial effects towards *B. cereus* with IFIC = 0.25 mg/ml and 0.31 mg/ml respectively. The DOE predicted that a combination of higher ratios of *R. officinalis* (59.5:1), higher ratios of *S. officinalis* (40:1), and lower ratios of *S. aromaticum* (0.5:1) would produce the best antimicrobial effect with MIC = 0.17 mg/ml. This was experimentally confirmed and there was a strong correlation ( $r$ -value 0.73) between the predicted and experimental MIC values, leading to the identification of an optimal antimicrobial combination. The combination of *R. officinalis* (56:1) and *S. officinalis* (44:1) produced the antimicrobial effect (MIC = 0.19 mg/ml) which can be recommended for future studies.

© 2023 The Authors. Published by Elsevier B.V. on behalf of SAAB. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

## 1. Introduction

The use of herbs and spices is practiced daily by most population groups globally. In sub-Saharan Africa, herbs and spices not only form part of the culinary repertoire, but they also have medicinal, as well as cultural significance (Djiazet et al., 2022). The importance of herbs and spices in South Africa is highlighted by an ethnobotanical study undertaken in the Eastern Cape province where 58 plant species were reported for use by the Xhosa communities (Asowata-Ayodele et al., 2016). In this survey, the herbs and spices with high therapeutic relevance were identified as *Uppia javanica* Spreng. (Verbenaceae), *Mentha aquatica* L (Lamiaceae), *Mentha longifolia* (L) L (Lamiaceae), *Mentha spicata* L (Lamiaceae) and *Capsicum annum* L (Solanaceae).

The inherent antimicrobial nature of most herbs and spices contributes to their use for food preservation, combating pathogenic spoilage bacteria which cause foodborne diseases which are a major threat to public health. Bacteria such as *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella sonnei*, *Shigella flexneri*, and *Shigella dysenteriae* are often associated with foodborne illnesses and gastrointestinal tract infections (Biorisis, 2017). Due to the increasing resistance to different classes of antibiotics, infections caused by foodborne pathogens are reported to prolong hospitalization, as well as increase morbidity and mortality (Rozman et al., 2021). As a result, studies have focused on identifying plant-derived antimicrobial alternatives from culinary spices and herbs, among other natural products. Plant extracts and essential oils have antimicrobial activity owing to the presence of bioactive compounds such as phenolic acids, flavonoids, quinones and tannins. There are numerous studies reporting on the antibacterial activities of spice and herb extracts, as well as essential oils, towards different types of micro-organisms, including foodborne pathogens (Sethi

\* Corresponding author.

E-mail address: [sandy.vanvuuren@wits.ac.za](mailto:sandy.vanvuuren@wits.ac.za) (S. van Vuuren)

et al., 2013; Mith et al., 2014; Sah et al., 2020; Yadav et al., 2022). Spices such as *Allium sativum* L (garlic), *Cinnamomum zeylanicum* Blume (cinnamon), *S. aromaticum* (L) Merr. & LM Perry (clove), *Thymus vulgaris* L (thyme), and *S. officinalis* L (sage) to name a few, have been reported to possess antibacterial activity against a range of Gram-positive and Gram-negative bacteria, including pathogens of the gastrointestinal tract.

In traditional medicine practices such as Ayurveda, African traditional medicine (ATM) and Traditional Chinese Medicine (TCM), medicinal plants are usually co-administered as combinations, where these polyherbal formulations are believed to work in synergy to produce enhanced biological effects compared to using single herbs (Parasuraman et al., 2014; Ramamoorthy et al., 2019; Mussarat et al., 2021). This also allows for lower concentrations of individual plants to be used, which has the potential to reduce toxicity (Parasuraman et al., 2014; Ramamoorthy et al., 2019). Although herbs and spices are commonly used in combination, few studies have specifically reported on their interactive effects as antimicrobial agents (Kon and Rai, 2012; Bag and Chattopadhyay, 2015; Garcia-Diez et al. 2017; Maharjan et al., 2019).

To determine the optimum herb combinations which produce the best antimicrobial effect in a laboratory setting is practically challenging, time consuming and uneconomical, as various extract combinations are randomly assigned, and many experiments are run with the hope of a favourable outcome. Furthermore, when experiments are based on trial and error, without proper experimental design, there is a possibility of low reproducibility, reliability, and versatility (Politis et al. 2017; Das and Dewanjee, 2018). One strategy to overcome this is to use the design of experiments (DOE) in order to perform experiments in a systematic way with a limited number of runs, thus saving time and resources (Lamberti et al., 2022). In the present study, the DoE was used to develop a polyherbal formulation comprising herbs and spices, at optimum concentrations to produce an enhanced antimicrobial effect.

## 2. Materials and methods

### 2.1. Sourcing and preparation of study macen/a/s

Seventeen commonly used culinary herbs and spices with documented antibacterial activities were selected for this study (Table 1). Culinary herbs and spices were purchased from Warren Chemical Specialties (Pty) Ltd Uohannesburg, South Africa). The identification was based on the supplier product labelling, as the products were obtained in powder form, and were accompanied by the certificate of analysis. The plant materials were stored in airtight containers under room temperature, and away from direct light. Phytochemical analysis using ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) analysis and fingerprinting were performed in-house. All plant materials were checked for contamination before experiments were carried out.

Extracts were prepared for each of the herbs and spices by macerating the powders in solvents of varying polarities (water, methanol and dichloromethane) at a 1:10 solvent ratio, followed by shaking in the dark for 24 h. at room temperature using a mechanical shaker. The mixtures were filtered through Whatman No.1 filter paper and the filtrates were evaporated to dryness using a rotavapor (H50- 500, Magna Analytical, Labtech, South Africa). The 17 essential oils used in this study were purchased from Prana Monde CC (Belgium). All commercial essential oils were accompanied by a certificate of analysis. The chromatographic profiles were also obtained in-house using gas chromatography- mass spectrometry (GC-MS) analysis and the marker compounds identified for each species.

The stock solutions of the extracts (1 mg/ml) were prepared by dissolving 1 mg of the sample in 1 ml of either methanol, water or dichloromethane. The solutions were stored at 4 °C until further use.

The plants used in the study have been extensively phytochemically profiled in many previous studies. Therefore, the analytical method and chemical fingerprinting for *R. officinalis*, *S. officinalis* and *S. aromaticum* is only presented as Supplementary material, St.

### 2.2. Micro-organisms and growth conditions

Six pathogens associated with foodborne diseases and gastrointestinal tract infections were used in the study to evaluate the antimicrobial effects of the selected herbs and spices. The strains included *S. aureus* ATCC 25923, *B. cereus* ATCC 11778, *S. enterica* serovar *Typhimurium*. ATCC 1403, *S. sonnei* ATCC 9290, *E. coli* ATCC 8739 and *Enterobacter faecalis* ATCC 29212 ( Davies Diagnostics, South Africa). Stock cultures were kept at -20 °C, then subcultured onto Tryptone Soya agar (TSA) and subsequently in Tryptone Soya broth (TSB) and incubated at 37 °C for 24 h.

### 2.3. Minimum inhibitory concentration (MIC) determination

The broth micro-dilution assay was performed to determine the MICs of extracts and oils against the test organisms (Eloff, 1998; Clinical and Laboratory Standards Institute, 2020). Although the herb and spice selection has been well studied in the past, baseline MIC values were obtained for all samples before interactive studies, as variability may exist between present samples in the study and those documented in the literature. Extracts and oils were diluted to a concentration of 32 mg/ml using acetone as a diluent. A volume of 100 µl of sterile broth was plated out in triplicate in each of the wells of a 96-well microtiter plate. Extracts and oils were added at a volume of 100 µl, followed by serial dilutions to yield concentrations of 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06 mg/ml. Microbial cultures were diluted using sterile TSB at a 1:100 dilution to achieve an approximate concentration of  $1 \times 10^6$  colony-forming units (CFU)/ml. Test cultures were then added (100 µl) at 0.5 MacFarland's turbidity standard to all the wells of the microtiter plates. The plates were sealed with a sterile adhesive sealing film to prevent loss of volatile components and then incubated at 37 °C for 24 h. After incubation, 40 µl of p-iodonitrotriazolium chloride (INT) at a concentration of 0.40 mg/ml was added to each well. Viable micro-organisms interact with INT resulting in a colour change from clear to red/purple. The lowest dilution with no colour change was considered the MIC for that extract or essential oil. The assays were performed in triplicate and the mean values recorded. Ciprofloxacin (positive control) and acetone (negative control) were included.

### 2.4. Sum of fractional inhibitory concentration index (IFIC) determination

The sum of the fractional inhibitory concentration index (IFIC) was used to measure interactions of various 1:1 combination of herbs, spices, and essential oils with promising antibacterial effects based on the MIC results. Samples of two extracts/oils (50 µl each) were combined in an Eppendorf tube (1:1), mixed and added in each of the wells of a microtiter plate. The same procedure for determining the MIC was followed where the cultures were added, the plates incubated and INT added for colour change. The IFIC for each of the combinations was calculated using Eq. (1).

$$\text{IFIC} = \text{FIC}(i) + \text{FIC}(ii) \quad (1)$$

$$\text{FIC}(i) = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}}$$

$$\text{FIC}(ii) = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) independently}}$$

**Table 1**

Selected common culinary herbs and spices with documented antimicrobial activities used in this study.

Spice/herb	Extract type	Justification for inclusion in the study	Refs.
<i>Allium salivum</i>	Methanol, ethanol, aqueous	Inhibited the growth of <i>E. coli</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>S. enterica</i> serovar Typhimurium, and <i>Shigella</i> species (MIC ranging from 0.03 to 20.00 mg/ml)	Nedorostova et al. (2009), Petropoulos et al. (2018)
	Essential oil	Inhibited the growth of <i>S. aureus</i> and <i>E. coli</i> at MIC values of 0.01 and 0.53 µL/ml, respectively	
<i>Jneithum graveolens</i>	Aqueous, acetone	Demonstrated antibacterial activity against <i>E. faecalis</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>S. enterica</i> serovar Typhimurium, at MIC values ranging from 5.00 to 25.00 mg/ml	Isopencuand Ferde (2012), Sharopov et al. (2011), Peerkam et al. (2014)
	Essential oil	Inhibited the growth of <i>S. aureus</i> and <i>E. coli</i> at MIC values of 0.006 and 0.06 mg/ml, respectively	
<i>Apium graveolens</i>	Methanol, ethanol, hexane, aqueous	Showed antibacterial activity towards <i>E. coli</i> , <i>S. enterica</i> serovar Typhimurium, and <i>S. aureus</i> (MIC ranging from 0.12 to 1.17 µg/ml) and MIC values ranging from 0.01 to 1.25 mg/ml	Baananou et al. (2013), Udona et al. (2015)
	Essential oil	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>E. faecalis</i> , and <i>S. aureus</i> at MIC values ranging from 5.00 to 40.00 µg/ml	
Cinnamomum Zeylonicum	Ethanol, methanol	Inhibited the growth of <i>E. coli</i> , <i>S. aureus</i> , and <i>B. cereus</i> at MICs ranging from 0.63 to 6.25 mg/ml	Nanasombal and Wimunigool (2011), Madha et al. (2017), Salmactat (2019)
	Essential oil	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>S. enterica</i> serovar Typhimurium, and <i>E. faecalis</i> (MIC ranging from 0.10 to 4.80 mg/ml)	
Coriandrum sativum	Methanol	Inhibited the growth of <i>B. subtilis</i> , <i>E. coli</i> , <i>S. aureus</i> , and <i>S. enterica</i> serovar Typhimurium, (MIC ranging from 50.00 to 500.00 µg/ml)	Casetti et al. (2012), Jamnani et al. (2014)
	Essential oil	Showed antibacterial activity against <i>S. aureus</i> at MIC values ranging from 0.04 to 0.25X v/v	
<i>Cymbopogon citratus</i>	Methanol, chloroform, ethanol	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>S. aureus</i> , and <i>S. enterica</i> serovar Typhimurium, (MIC values ranging from 0.04 to 2.50 mg/ml)	Ilasse et al. (2011), Danlami et al. (2011), Zulfarhan et al. (2016)
	Essential oil	Inhibited the growth of <i>E. coli</i> , <i>E. faecalis</i> , <i>S. aureus</i> , <i>S. enterica</i> serovar Typhimurium, <i>S. dysenteriae</i> , and <i>S. enterica</i> at MIC values ranging from 1.00 to 2.50 µg/ml	
taurus nobilis	Ethanol, aqueous	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , and <i>S. aureus</i> at MIC values ranging from 0.78 to 103.12 mg/ml	Ciputo et al. (2017), Tomar et al. (2020)
	Essential oil	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>S. faecalis</i> at MIC values ranging from 0.04 to 1.00 mg/ml	
<i>Melissa officinalis</i>	Aqueous, ethanol	Inhibited the growth of <i>B. cereus</i> and <i>S. aureus</i> at MIC values of 0.78–3.12 mg/ml, respectively.	Cryhan et al. (2012), Abdellatif et al. (2014)
	Essential oil	Inhibited the growth of <i>E. coli</i> , <i>S. aureus</i> , and <i>B. cereus</i> at MIC values of 0.48–3.00 µg/ml	
<i>Mentha x piperita</i>	Methanol	Inhibited the growth of <i>S. aureus</i> , <i>E. coli</i> , and <i>S. enterica</i> serovar Typhimurium, (MIC values ranging from 0.01 to 0.03 mg/ml)	Abdellaur et al. (2014), Smgh et al. (2015)
	Essential oil	Inhibited the growth of <i>S. aureus</i> and <i>E. coli</i> at MIC of 0.5% and 0.71 v/v, respectively	
Ocimum basilicum	Methanol, ethyl acetate, chloroform	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>B. subtilis</i> , <i>S. aureus</i> , and <i>S. enterica</i> serovar Typhimurium, with MIC values ranging from 0.06 to 0.25 mg/ml	Hossamet et al. (2010), Beatovic et al. (2015)
	Essential oil	Showed antibacterial activity with MIC values ranging from 0.009 to 23.48 µg/ml for <i>B. cereus</i> , <i>E. coli</i> , <i>E. faecalis</i> , <i>S. enterica</i> serovar Typhimurium, and <i>L. monocytogenes</i>	
<i>Origanum majorana</i>	Methanol, aqueous, dichloromethane	Showed antibacterial activity with MIC values ranging from 0.05 to 1.00 mg/ml for <i>E. coli</i> and <i>S. aureus</i>	Hajdoui et al. (2016), Al-Falim, (2018)
	Essential oil	Inhibited the growth of <i>B. cereus</i> , <i>S. aureus</i> , <i>E. faecalis</i> , <i>E. coli</i> and <i>S. enterica</i> serovar Typhimurium, (MIC values ranging from 0.08 to 1.56 mg/ml)	
<i>Petroselinum crispum</i>	Methanol	Inhibited the growth of <i>S. aureus</i> at MIC value of 37 µg/ml	Alsaqali et al. (2016), Linde et al. (2016)
	Essential oil	Inhibited the growth of <i>B. cereus</i> , <i>S. aureus</i> and <i>E. coli</i> (MIC ranging from 0.04 to 1.00 mg/ml)	
<i>Rosmarinus officinalis</i>	Aqueous, methanol, ethanol, hexane	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>E. faecalis</i> , <i>S. aureus</i> and <i>S. enterica</i> serovar Typhimurium, with MIC ranging from 0.78 to 3.12 mg/ml	Weerakkody et al. (2010), Ceyhan et al. (2012)
	Essential oil	Inhibited the growth of <i>S. aureus</i> and <i>E. coli</i> at MIC values ranging from 0.03 to 0.06 v/v	
<i>Salvia officinalis</i>	Essential oil	Showed antibacterial activities with MIC values in the range of 0.01–0.30 mg/ml against <i>E. coli</i> and <i>S. aureus</i>	Bouaziz et al. (2009), Generali et al. (2012)
	Ethanol	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> and <i>S. aureus</i> with MICs in the range of 0.02–0.99 mg/ml.	
<i>Syzygium aromaticum</i>	Aqueous, methanol	Showed antibacterial activities with MIC ranging from 0.31 to 2.31 mg/ml against <i>S. aureus</i> and <i>E. coli</i>	Pandey et al. (2011), Thiemann et al. (2019)
	Essential oil	Showed antibacterial activities with MIC values in the range of 0.40–0.8 mg/ml against <i>B. cereus</i> , <i>E. coli</i> , <i>S. aureus</i> , and <i>S. enterica</i> serovar Typhimurium.	
Thymus vulgaris	Essential oil	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>S. aureus</i> , and <i>S. enterica</i> serovar Typhimurium, at MIC values ranging from 0.40 to 0.801 µg/ml	Al Maqart et al. (2011), Ahakbarlu and Shamel, (2013)
	Methanol	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , <i>S. aureus</i> , and <i>S. sonnei</i> at MIC values ranging from 0.07 to 1.10 mg/ml	
<i>Zingiber officinale</i>	Methanol, ethanol, aqueous	Inhibited the growth of <i>B. cereus</i> , <i>E. coli</i> , and <i>S. enterica</i> serovar Typhimurium, at MIC values ranging from 0.02 to 20.00 µg/ml	Li et al. (2017), Wang et al. (2020)
	Essential oil	Inhibited the growth of <i>S. aureus</i> , <i>S. enterica</i> serovar Typhimurium, and <i>S. Sonnei</i> and <i>B. cereus</i> (MIC values ranging from 0.25 to 1.00 mg/ml)	

Where (a) is the MIC of the first extract or essential oil in the combination and (b) is the MIC of the second extract or essential oil. The IFIC for each combination was interpreted as; synergistic if IFIC is  $\leq$  0.5; additive if IFIC is  $> 0.5$  but  $\leq$  1.0; indifference if IFIC is  $> 1.0$  but  $\leq 4.0$ , and antagonism if IFIC is  $> 4.0$  (van Vuuren and Viljoen 2011). For the antimicrobial assays, both the positive (ciprofloxacin) and negative (acetone) controls were included. Assays were performed in triplicate and the mean values recorded.

### 2.5. Design of experiments (DOE) modeling to determine synergistic antibacterial combinations

The MODDE software version 9.0 (Umetrics AB, Umea, Sweden) was used to determine input parameters that would result in the desired outcomes. The three factors (plant extracts) were input as fraction units each ranging from 0 to 1. The response (MIC) was set to range from 0.00025 to 8 mg/ml with a target MIC value of 0.5 mg/ml. A full factorial design was applied and the models were fitted with partial least squares (PLS) analysis and adjusted by removing non-significant terms. A total of 12 experimental runs with varying combinations were obtained from the design; three runs with individual extracts, another three runs with two extracts in combination and six runs with three extracts in combination and three center points. A mixture design worksheet for the extracts was produced and the modeling of responses was generated to confirm the best model fit. A prediction contour plot was generated, which showed the average prediction (point estimate prediction) for every possible combination of the tested extracts. The predicted combinations with low MIC values were selected and the reliability of the model in predicting those combinations was confirmed experimentally through determination of MIC values against the selected pathogens.

## 3. Results

### 3.1. Antibacterial activity of crude extracts and essential oils

The antibacterial activity results of crude extracts and essential oils are presented in Table 2. For the extracts, good antibacterial activity is considered as MIC  $< 0.1$  mg/ml, moderate activity is  $0.1 < \text{MIC} < 0.625$  mg/ml and weak (activity MIC values  $> 0.625$  mg/ml) (Kuethe, 2010). All the extracts tested exhibited moderate to weak antimicrobial activity towards the tested bacterial pathogens. Generally, six plants namely, *A. sativum*, *C. zeylanicum*, *O. marjorana*, *R. officinalis*, *S. officinalis* and *S. aromaticum* displayed moderate antimicrobial activity against the various pathogens tested as indicated in bold in Table 2. All the water extracts displayed poor activity towards all the tested pathogens (MIC values  $> 1$  mg/ml). Compared to all the moderately active plants, *S. aromaticum* methanol extracts displayed a broader range of activity inhibiting the growth of four pathogens (*B. cereus*, *E. coli*, *E. faecalis* and *S. aureus*) out of the six, with MIC values ranging from 0.42 to 0.67 mg/ml. The dichloromethane extract of the same plant was only able to inhibit the growth of *E. faecalis* and *S. aureus* with MICs of 0.46 mg/ml and 0.62 mg/ml, respectively. *Rosmarinus officinalis* and *S. officinalis* methanol extracts were active towards *B. cereus* and *E. coli*, while the dichloromethane extracts inhibited *S. aureus* and *S. sonnei*. In total, each of the two plants displayed activity against four pathogens, irrespective of the type of solvent extract tested. Cinnamomum zeylanicum showed activity against two pathogens (*B. cereus* and *E. faecalis*) where the methanol extract was active towards *E. faecalis* (MIC = 0.42 mg/ml) and the dichloromethane extract showed activity towards *B. cereus* (MIC = 0.50 mg/ml). The last two moderately active plants were *A. sativum* and *O. marjorana*, where the dichloromethane extracts were each active against only *E. faecalis* with MIC values of 0.46 mg/ml and 0.50 mg/ml, respectively.

With reference to essential oils, good antibacterial activity is interpreted as MICs of 0.10-0.50 mg/ml, whilst the oils with MIC values of 0.50-1.00 mg/ml are reported to exhibit moderate activity and  $> 1.00$  mg/ml show weak activity (Freires et al., 2015). Seven out of the 17 essential oils displayed strong inhibition towards the growth of only one pathogen (*B. cereus*) out of the six tested pathogens. These active oils included *C. zeylanicum*, *C. dravastis*, *L. nobilis*, *M. officinalis*, *R. officinalis*, *S. aromaticum*, and *Z. officinale* with MIC values ranging from 0.25 to 0.50 mg/ml. Moderately active essential oils (MIC values of 0.50-1.00 mg/ml) towards at least one pathogen include *A. sativum*, *A. graveolens*, *C. sativum*, *M. piperita*, *O. marjorana*, *P. crispum*, *S. o. l. idna/ls* and *T. vulgaris*. Two other essential oils (*A. graveolens* and *O. basilicum*) exhibited weaker activity (MIC values  $> 1.00$  mg/ml) against the tested pathogens. The ciprofloxacin control values for the pathogens were in accordance with the breakpoint values of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) (EUCAST, 2020; CLSI, 2020).

These results provided the basis for herb/spice selection to use in the subsequent studies. Extracts that displayed moderate to good antimicrobial activity when tested individually were selected, and these included *A. sativum*, *C. zeylanicum*, *O. marjorana*, *R. officinalis*, *S. officinalis* and *S. aromaticum*. Either the methanol or dichloromethane extract was subsequently tested on a specific pathogen depending on the activity observed for that particular extract. Selection of the essential oils was also based on the set criteria mentioned and seven essential oils were included in the interactive studies namely, *C. zeylanicum*, *C. citratus*, *L. nobilis*, *M. officinalis*, *R. officinalis*, *S. aromaticum* and *Z. officinale*. A total of 14 extract combinations (1:1) and 21 essential oils combinations (1:1) were evaluated for antimicrobial interaction and the results are presented in Table 3.

### 3.2. Interactive antibacterial studies

The combinations were classified as either synergistic (IFIC is  $\leq$  0.5), antagonistic (IFIC is  $> 4.0$ ), additive (IFIC is  $> 0.5$  but  $\leq 1.0$ ) or indifferent (IFIC is  $> 1.0$  but  $\leq 4.0$ ) (van Vuuren and Viljoen, 2011). Out of the 14 extract combinations tested, two displayed synergistic interactions (I.R.C value of 0.25 and 0.31) and two combinations were additive (IFIC value of 0.88 and 1.00). The synergistic interactions were observed when *R. officinalis* was combined with either *S. aromaticum* (IFIC value of 0.25) or *S. officinalis* (I.R.C value of 0.31) and tested against *B. cereus*. The same combination of *R. officinalis* and *S. aromaticum* displayed an additive effect when tested against *E. coli* (I.F.C value of 1.00), whilst the *R. officinalis* and *S. officinalis* had an additive effect against *S. sonnei* (IFIC value of 0.88) when dichloromethane extracts were combined. The remaining extract combinations were either indifferent or antagonistic, and these two outcomes were also observed for all the essential oil combinations tested. Based on these results, the plants extracts that showed a synergistic interaction in a 1:1 combination namely, *R. officinalis*, *S. aromaticum*, and *S. officinalis*, were selected for further analysis in the DoE, to determine the optimum proportions that would produce the best possible antimicrobial effect.

### 3.3. Design of experiments (DOE) optimisation of herbal combinations

The three plants namely, *R. officinalis*, *S. aromaticum*, and *S. officinalis* were combined in various proportions in the MODDE software and a PIS model was constructed with an R<sup>2</sup> value of 0.72 which signified a high variation in the response (MIC), and a strong fit between the data and the model. The Q<sup>2</sup> value of 0.18 demonstrated a significant predictive power of the model (Q<sub>i</sub>  $> 0.10$ ) and the high model validity (0.84), showed a good model fit (Bhatia et al., 2016). The value obtained for model reproducibility (0.52) exceeded the requisite value of 0.5, indicating good experimental control and low error.

Table 2

Antibacterial activity of 17 culinary herbs and spices expressed as MICs (mg/ml).

Spice/hero	Extract	<i>B. cereus</i> ATCC 11778	<i>E. coli</i> ATCC 8739	<i>E. faecalis</i> ATCC 29212	<i>S. aureus</i> ATCC 25923	<i>S. sonnei</i> ATCC 9220	<i>S. enterica</i> serovar Typhimurium. ATCC 1403
Allium <i>sotivum</i> L	Methanol	6.67	5.33	4.00	>8.00	3.00	>8.00
	Water	4.00	6.67	4.00	<b>8.00</b>	4.00	3.00
	Dichloromethane	2.00	4.00	0.46	3.33	3.33	1.27
Anethum <i>graveolens</i> L	Essential oil	2.00	4.00	1.00	4.00	4.00	2.00
	Methanol	2.00	<b>&gt;8.00</b>	<b>4.00</b>	4.00	2.67	2.67
	Water	4.67	<b>4.00</b>	6.00	4.00	4.00	8.00
	Dichloromethane	2.00	–8.00	2.67	3.33	2.67	2.67
Apium <i>graveolens</i> L	Essential oil	2.00	2.67	2.00	4.00	2.00	2.00
	Methanol	2.67	2.67	2.67	4.00	<b>4.00</b>	2.00
	Water	2.67	6.00	2.67	<b>8.00</b>	4.00	4.00
Cinnamomum <i>zeylanicum</i> Blume	Dichloromethane	5.33	<b>8.00</b>	<b>5.33</b>	2.67	2.00	2.00
	Essential oil	0.67	2.00	4.00	2.67	4.00	2.00
	Methanol	2.67	4.00	0.42	2.67	4.00	>8.00
Coriandrum <i>sativum</i> (hort. ex DC) Stapf	Water	2.67	4.00	2.67	>8.00	4.00	2.00
	Dichloromethane	0.50	0.67	1.00	1.00	1.67	0.67
	Essential oil	0.25	1.00	0.67	1.00	1.00	1.00
	Methanol	6.67	<b>8.00</b>	8.00	8.00	>8.00	2.00
Cymbopogon <i>citratus</i> L	Water	2.67	4.00	2.67	8.00	>8.00	2.67
	Dichloromethane	2.00	2.67	2.67	2.00	2.67	2.00
	Essential oil	0.67	1.00	0.83	2.00	2.00	2.00
	Methanol	4.00	–8.00	>8.00	5.33	>8.00	2.67
Laurus <i>nobilis</i> L	Water	5.33	5.33	2.00	8.00	>8.00	8.00
	Dichloromethane	1.33	2.00	>8.00	1.00	2.00	2.67
	Essential oil	0.25	1.00	1.00	>8.00	2.00	2.00
	Methanol	>8.00	5.33	4.00	<b>8.00</b>	8.00	>8.00
Melissa <i>officinalis</i> L	Water	5.33	8.00	2.67	5.33	2.67	2.67
	Dichloromethane	1.33	5.33	2.67	2.67	2.67	2.00
	Essential oil	0.50	2.00	2.67	4.00	2.67	4.00
	Methanol	2.00	4.00	2.67	5.33	>8.00	2.67
Mentha <i>piperita</i> L	Water	1.67	2.67	1.67	2.67	2.67	2.67
	Dichloromethane	2.00	8.00	2.00	6.67	4.00	8.00
	Essential oil	<b>8.00</b>	2.00	1.33	>8.00	2.00	2.00
	Methanol	4.00	2.00	<b>2.00</b>	8.00	2.33	>8.00
Ocimum <i>basilicum</i> L	Water	2.00	<b>4.00</b>	6.67	>8.00	4.00	2.67
	Dichloromethane	2.67	<b>8.00</b>	2.00	2.67	2.67	2.67
	Essential oil	1.00	2.00	4.00	2.00	4.00	2.00
	Methanol	2.67	4.00	– <b>8.00</b>	6.00	2.67	2.00
Oenothera <i>majorana</i> L	Water	2.67	6.00	6.67	>8.00	4.00	4.00
	Dichloromethane	2.00	8.00	6.00	2.67	2.00	2.00
	Essential oil	1.17	2.00	–8.00	4.00	<b>4.00</b>	2.00
	Methanol	2.00	<b>8.00</b>	1.33	4.00	8.00	2.67
Petroselinum <i>crispum</i> L	Water	2.67	5.33	2.67	4.00	<b>8.00</b>	2.67
	Dichloromethane	6.00	6.67	0.50	2.67	2.00	4.00
	Essential oil	0.67	1.33	4.00	4.00	3.33	>8.00
	Methanol	2.00	4.00	2.00	4.00	5.33	>8.00
Rosmarinus <i>officinalis</i> L	Water	4.00	5.33	4.00	4.00	4.00	>8.00
	Dichloromethane	2.00	8.00	2.67	2.67	4.00	2.00
	Essential oil	0.83	2.00	3.33	4.00	<b>4.00</b>	2.00
	Methanol	0.50	0.50	0.80	1.33	1.00	2.00
Salvia <i>officinalis</i> L	Water	2.00	4.00	>8.00	4.00	>8.00	5.33
	Dichloromethane	2.67	1.33	1.00	0.50	0.33	4.00
	Essential oil	0.50	2.00	3.33	<b>8.00</b>	>8.00	2.00
	Methanol	0.25	0.42	0.80	2.00	2.67	2.00
Syzygium <i>aromaticum</i> (L) Merr. & LM Perry	Water	6.00	4.00	4.00	2.00	>8.00	2.00
	Dichloromethane	1.00	2.67	1.00	0.50	0.25	1.67
	Essential oil	100	2.00	2.00	4.00	3.33	2.00
	Methanol	0.67	0.50	0.42	0.62	6.67	2.00
Thymus <i>vulgans</i> L	Water	5.33	1.00	1.67	2.00	2.00	2.67
	Dichloromethane	2.67	1.33	0.46	0.62	2.00	2.00
	Essential oil	0.42	1.00	1.00	2.00	2.00	1.00
	Methanol	2.00	>8.00	2.00	2.67	2.67	2.67
Zingiber <i>officinale</i> Roscoe	Water	5.33	5.33	>8.00	4.00	>8.00	2.67
	Dichloromethane	2.00	8.00	1.67	2.00	2.00	2.67
	Essential oil	1.00	1.00	3.33	2.00	4.00	2.00
	Methanol	2.00	4.00	2.00	2.00	2.67	4.00
Positive control	Water	2.67	4.00	2.00	8.00	4.00	2.00
	Dichloromethane	1.33	2.00	1.33	2.67	2.00	1.67
	Essential oil	0.42	1.00	1.00	4.00	2.00	1.00
Negative control	Ciprofloxacin (1 µg/ml)	0.62	0.31	0.62	0.16	0.62	0.62
	Acetone in water (mg/ml)	<b>&gt;8.00</b>	>8.00	<b>8.00</b>	<b>8.00</b>	>8.00	>8.00

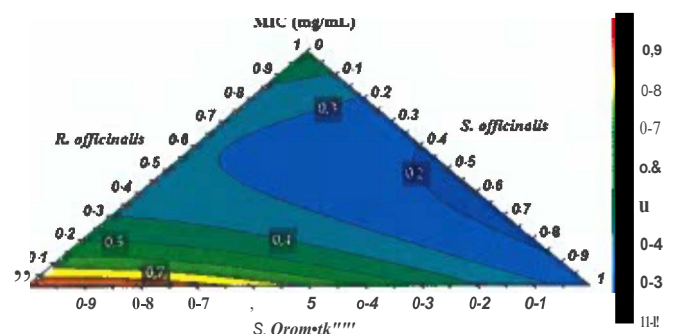
Values represent mean MICs for experiments performed in triplicate (n = 3). Values in bold indicate extracts with moderate activity and essential oils with good antibacterial activity.

**Table3**  
Antibacterial interactions or 1:1 combinations or extracts and essential oils against selected pathogens.

Pathogen	I:1 herb combinations	Type of sample	:£AC	Interpretation
<i>B. cereus</i> ATCC 11778	<i>R. officinalis</i> •	Methanol	0.25	Synergy
	<i>S. aromaticum</i> <i>R. officinalis</i> +	Methanol	0.31	Synergy
	<i>S. officinalis</i> <i>S. aromolicum</i> +	Methanol	1.31	Indifferent
<i>E. coli</i> ATCC 8739	<i>S. ojjicinalis</i> <i>R. ojjicinalis</i> +	Methanol	1.00	Additive
	<i>S. aromalicum</i> <i>R. officinalis</i> +	Methanol	1.83	Indifferent
	<i>S. officinalis</i> <i>S. aromaricum</i> •	Methanol	2.19	Indifferent
<i>E. fatcalis</i> ATCC 29212	<i>S. ojjicinalis</i> • <i>S. aromalicum</i>	Methanol	2.50	Indifferent
	<i>Asolivum</i> + <i>O. majorana</i>	dichloromerh-	4.00	Antagonism
	<i>Asalivum</i> + <i>S. aromalicum</i>	dichloromerhane	2.00	Indifferent
<i>S. a11t11s</i> ATC(25923)	<i>O. majoro11a</i> + <i>S. aromalicum</i>	dichloromethane	4.00	Antagonism
	<i>R. officinalis</i> + <i>S. ojjicinalis</i>	dichloromethane	3.49	Indifferent
	<i>R. officinalis</i> + <i>S. aromalicum</i>	dichloromethane	1.81	Indifferent
<i>S. sonnei</i> ATCC 9290	<i>S. officinalis</i> + <i>S. aromaricum</i>	dichloromethane	2.40	Indifferent
	<i>R. officinalis</i> + <i>S. officinalis</i>	dichloromethane	0.88	Additive
	<i>C. citratus</i> + <i>R. ojjicinalis</i>	essential oils	6.00	Antagonism
<i>B. cereus</i> ATCC 11778	<i>C. cimitus</i> • <i>M. officinalis</i>	essential oils	6.00	Antagonism
	<i>C. dtralus</i> + <i>Lnobilis</i>	essential oils	6.00	Antagonism
	<i>C. dtrolus</i> + <i>Z. officinale</i>	essential oils	6.38	Antagonism
<i>B. cereus</i> ATCC 11778	<i>[. citralls</i> + <i>C zeylanicum</i>	essential oils	4.00	Indifferent
	<i>Ceitrus</i> + <i>S. aromaricum</i>	essential oils	6.38	Antagonism
	<i>R. officinalis</i> + <i>M. ojjicinalis</i>	essential oils	5.33	Antagonism
<i>B. cereus</i> ATCC 11778	<i>R. officinalis</i> + <i>Lnobilis</i>	essential oils	4.00	Indifferent
	<i>R. ojjicinalis</i> + <i>Z. officina</i>	essential oils	8.76	Antagonism
	<i>R. officinalis</i> • <i>C. zeylanicum</i>	essential oils	6.00	Antagonism
<i>B. cereus</i> ATCC 11778	<i>R. officinalis</i> • <i>S. aromaricum</i>	essential oils	4.38	Antagonism
	<i>M. officinalis</i> + <i>L nobiUs</i>	essential oils	4.00	Indifferent
	<i>M. ojjicinalis</i> + <i>Z. officinale</i>	essential oils	4.38	Antagonism
<i>B. cereus</i> ATCC 11778	<i>M. officinalis</i> • <i>C zeylanicum</i>	essential oils	3.00	Indifferent
	<i>M. ojjicinalis</i> + <i>S. aromaricum</i>	essential oils	3.98	Indifferent
	<i>Lnobilis</i> + <i>Z. ojjicinalis</i>	essential oils	6.43	Antagonism
<i>B. cereus</i> ATCC 11778	<i>Lnobilis</i> + <i>C. zeylanicum</i>	essential oils	3.00	Indifferent
	<i>L nobilis</i> + <i>S. aromaricum</i>	essential oils	4.38	Antagonism
	<i>Z. officinale</i> + <i>C. zeylanicum</i>	essential oils	4.25	Antagonism
<i>B. cereus</i> ATCC 11778	<i>Z. officinale</i> + <i>S. aromaricum</i>	essential oils	7.94	Antagonism
	<i>C zeylanicum</i> • <i>S. aromaricum</i>	essential oils	3.19	Indifferent

**Table4**  
The 12 plant combinations setup generated by the PLS model in DOE and the corresponding MIC values determined experimentally.

Experiment No	Predicted ratios for methanol extract proportions in combination			Actual MIC (mg/ml) values from combination
	<i>R. officinalis</i>	<i>S. aromaticum</i>	<i>S. officinalis</i>	
1		0	0	0.50
2	0	1	0	1.00
3	0	0	1	0.25
4	0.67	0.17	0.17	0.37
5	0.17	0.67	0.17	0.31
6	0.17	0.17	0.67	0.25
7	0	0.50	0.50	0.75
8	0.50	0	0.50	0.12
9	0.50	0.50	0	0.25
10	0.33	0.33	0.33	0.50
11	0.33	0.33	0.33	0.50
12	0.33	0.33	0.33	0.25



**Fig. 1.** Response contour plot showing the extract combinations and predicted responses (MIC values).

Table 4 indicates the DOE output of twelve experimental runs with the different plant combinations that were tested against *B. cereus* and the resulting MIC values are also indicated for the combinations. All the 12 combinations showed noteworthy antimicrobial activity (MIC < 1 mg/ml) indicating that selecting any combinations of the three plants would give a formulation with good antimicrobial activity. However, to pursue the objective of identifying the optimum proportions for the best antimicrobial activity, a response contour plot was constructed based on the data provided in Table 4 and this is indicated in Fig. 1. According to the key, plant combinations with coordinates in the blue region of the plot are predicted to give the lowest MIC values (0.3 mg/ml) which translates to the best antimicrobial activity.

The predicted MIC values in the blue region were therefore based on the combinations with a high proportion of *R. officinalis*, low *S. aromaticum* and a varying amount of *S. officinalis*. The optimizer function assisted in determining the best ratio at which the extracts should be combined for optimum synergistic antibacterial effects. Five combinations in the blue region of the response contour plot were selected and the MIC assay was performed experimentally (Table 5), to confirm the predictions of the PLS model. All five combinations displayed noteworthy antimicrobial activity as observed in Tables. The highest antibacterial activity (Experimental MIC value of 0.19 mg/ml) was achieved with combination 3 comprising of *R. officinalis* (59.5%), *S. officinalis* (40%) and *S. aromaticum* (0.5%) as well as combination 2, comprising only of *R. officinalis* (56%) and *S. officinalis* (44%). The model contributions plot indicated that the presence of *S. aromaticum* had no significant antimicrobial effect for this combination. Although experimentally, combination 2 displayed the same MIC value as combination 3, the PLS model predicts that trace amounts of *S. aromaticum* in the mixture is beneficial (predicted

**TmltS**Experimental antimicrobial confirmation against *B. cereus*

Combinauon	<i>R. officinalis</i>	<i>S. aromaticum</i>	<i>S. officinalis</i>	Predicted MIC (mg/ml)	Experimental MIC (mg/ml)
1	0.78	0.01	0.21	0.30	0.38
2	0.56	0.00	0.44	0.19	0.19
3	0.595	0.005	0.40	0.17	0.19
4	0.23	0.23	0.54	0.29	0.25
S	0.04	0.03	0.92	0.25	0.38

MIC = 0.17 mg/ml compared to no *S. aromaticum* at all (predicted MIC = 0.19 mg/ml). The results of these experiments confirmed the reliability and fitness of the model because the correlation coefficient ( $r = 0.73$ ), calculated using Microsoft Excel showed a strong correlation between the experimental and the predicted MIC values for all the combinations (Table 5).

#### 4. Discussion

The findings of this study generally show that the methanol and dichloromethane extracts of *R. officinalis*, *S. officinalis*, and *S. aromaticum* were the more effective antibacterial agents towards all the tested pathogens except *S. enterica serovar Typhimurium*. A previous study has reported on the antibacterial activity of the alcoholic extract of *S. officinalis* against *B. cereus* at an MIC value of 0.23 mg/ml (Generalic et al., 2012), which agrees with the current study. In addition, some studies also reported on the activity of *S. aromaticum* methanol extract against *S. aureus* (Pandey et al., 2011; Thielmann et al., 2019). These results are congruent with the findings from this study. Generally, the water extracts displayed weaker or no antibacterial activity which is a similar observation to previous studies by Weerakkody et al. (2010) and Witkowska et al. (2014).

The antibacterial activity of *C. zeylanicum* essential oil against *B. cereus* was reported by other researchers (Unlu et al., 2010; Nanasombat and Wimuttigol, 2011). Their findings are congruent with the current study where an MIC value of 0.56 mg/ml was also obtained. Linde et al. (2016) reported an MIC value of 1 mg/ml for *P. crispum* oil against *B. cereus*, which relates to the findings in this study. Tomar et al. (2020) reported the activity of *L. nobilis* oil against *B. cereus* at an MIC value of 0.75 mg/ml and the results are consistent with the current findings. The inhibitory effect of *M. officinalis* and *O. marjorana* essential oils was also reported by other researchers (Abdellatif et al., 2014; Hajlaoui et al., 2016). Although the 17 extracts tested in the current study were selected based on documented antimicrobial activity, it is clear that many more screening studies must be conducted in order to confirm the effectiveness of these herbs and spices as antimicrobial agents. It is important to challenge these on a range of pathogenic strains under different laboratory conditions so that the highly potent candidates can be identified and investigated further. This study also confirms high variability in antimicrobial activity of many spices and herbs which depends on the type of plant, extraction solvent, test media and pathogens tested, an important consideration which discourages the use of herbs and spices as a primary preservative method (Al-Wabel and Farhi, 2012).

Overall the results of the interactive studies demonstrate that selected crude extracts of culinary herbs and spices can be combined to achieve either synergistic, additive, indifferent or antagonistic interactions. This information is particularly important as the use of herb combinations is common in many traditional medicine practices around the globe, with the primary aim of achieving an enhanced biological effect (synergy) while minimizing undesired effects (Che et al., 2013). The findings of this study, however, clearly demonstrate that only a few (three) of the spice extracts were able to achieve synergistic interactions out of the 17 tested. The *R. officinalis* combined

with *S. aromaticum* or *S. officinalis* methanol extracts and tested against *B. cereus* were synergistic. One previous study reported an additive effect when the alcoholic extract of *Origanum vulgare* (oregano) was combined with either *R. officinalis* or *S. officinalis* and tested against *S. aureus* (Witkowska et al., 2014), which partially supports the effectiveness of the two plants when used in combination. In the current study, although a synergistic effect was observed with the 1:1 combination of the three plants, the FIC values for both combinations were still > 0.1 mg/ml which is the maximum value for an extract to be regarded as displaying good antimicrobial activity (Kueer (2010). The 1:1 combinations can therefore be regarded as moderately active. Whether combining these extracts achieves a significant effect when compared to the individual extracts is an important factor to consider when aiming to produce a highly effective polyherbal formulation. The optimization procedure using DoE is therefore important to ensure that optimum proportions of the extracts are combined for the best possible antimicrobial outcome. The current study illustrates the importance of DoE as the best antimicrobial effect was observed when the three plants were used in a mixture in varying proportions compared to the 1:1 combination. The most effective combination comprised of methanol extracts of *R. officinalis* (59.5%), *S. officinalis* (40%), and *S. aromaticum* (0.5%), yielding an MIC = 0.19 mg/ml, which was the lowest recorded in this study. Combining *R. officinalis* (56%), *S. officinalis* (44%) alone was able to achieve the same effect (MIC = 0.19 mg/ml), therefore we can infer that the later combination is more economical and recommend this for further studies.

It is also important to highlight that in the current study, none of the tested essential oil combinations exhibited synergy or additive effects. However, previous studies have reported an additive activity when *S. aromaticum* and *R. officinalis* oils were combined and tested against *E. coli* and *S. aureus* (Fu et al., 2007). Bag and Chattopadhyay (2015) reported synergy when combining *C. sativum* and *Cuminum cyminum* L (cumin) essential oils against *B. cereus*, *E. coli*, and *S. aureus*, with IFIC values of 0.25 and 0.50, respectively. Bassole and colleagues, also reported synergism from combining *M. piperita* and *O. basilicum* essential oils against *S. aureus*, *E. faecalis*, *L. monocytogenes*, *E. coli*, and *Shigella dysenteriae* with IFIC values of 0.36, 0.37, 0.27, 0.29, and 0.35, respectively, whilst the combination was additive against *S. enterica serovar Typhimurium* with an IFIC value of 0.69. Combining *O. vulgare* with either *O. marjorana*, *R. officinalis*, *T. vulgaris* or *O. basilicum* oil yielded additive effects against *B. cereus* and *E. coli* (Bassole and Juhani, 2012). Kon and Rai (2012) evaluated the antibacterial activity of *T. vulgaris* oil in combination with *C. zeylanicum*, *M. piperita*, and *M. officinalis* oils against *E. coli* and *S. aureus* and synergistic interactions were observed when *T. vulgaris* and *C. zeylanicum* oils were combined and tested against *S. aureus* (IFIC value of 0.26), and when *T. vulgaris* and *M. officinalis* oils were combined and tested against *E. coli* (IFIC value of 0.34). Gyorgy (2010) reported synergism from the combination of *S. officinalis* and *T. vulgaris* oils against *S. aureus*, *B. subtilis*, and *L. monocytogenes*. It was also reported that synergy from the combination of *O. vulgare* and *O. basilicum* essential oils against *S. aureus* was evident (Lv et al., 2011). Additive effects were observed when *T. vulgaris* and *M. piperita* oils were combined and tested against *E. coli* and *S. aureus* with IFIC values of 0.55.

The findings of this study may contribute to the successful application of combined herbs and spices as natural antimicrobials which can be successfully applied in food preservation.

## 5. Conclusion

It is evident that the use of selected spice extracts in combination increases the antibacterial activity due to synergistic interactions. Synergy was observed by combining *R. officinalis* and *S. aromacicum* methanol extracts (IFIC value of 0.25) and from combination of *R. officinalis* and *S. officinalis* methanol extracts (IFIC value of 0.31) against *S. cereus*. Instead of combining extracts randomly, the DOE study provided an interesting and promising cost-effective approach whereby spices and herbs extracts can be combined at optimum proportions for the best biological effect. The DOE therefore created an innovative opportunity for the future development of the most effective antimicrobial agents for the pharmaceutical and food industries.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors are grateful for the financial support provided by the National Research Foundation, South African Medical Research Council, and Faculty of Research Committee Individual Research Grant, WITS University. The University of Witwatersrand, Department of Pharmacy and Pharmacology is also thanked for providing the facilities and resources to carry out the experimental work.

## Supplementary materials

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.sajb.2023.11.030.

## References

- Abdellatif, F., Boudjelal, H., Zouin, A., Hassani, A., 2014. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinalis* L. *EXCLI J.* 13 (7), 772–781.
- Al-fatm, M., 2018. Volatile constituents, antimicrobial and antioxidant activities of the aerial parts of *Origanum majorana* L from Yemen. *J. Pharm., Res.* 13 (4) 1–10.
- Al Maqtari, M.A., Alghabbi, S.M., Alhamzy, E.H., 2011. Chemical composition and antimicrobial activity of essential oil of *Thymus vulgaris* from Yemen. *Turk. J. Biochem.* 36 (4), 342–349.
- Aliakbarlu, J., Shamel, F., 2013. *In vitro* antioxidant and antibacterial properties and total phenolic contents of essential oils from *Thymus vulgaris*, *T. korschyanus*, *Ziziphora tenuifolia* and *Z. clinopodioides*. *Turk. J. Biochem.* 38 (4), 425–431.
- Alsaqah, M., El-Shibiny, A.A., Adel, M., Abdel-Samir, M.A.S., Ghoneim, S., 2016. Use of some essential oils as antimicrobial agents to control pathogenic bacteria in beef burger. *World J. Dairy Food Sci.* 11 (1), 109–120.
- Al Wabel, N.A., Fatima, S.M., 2012. Antimicrobial activities of spices and herbs. In: Proceedings of the II International Conference on Antimicrobial Research - ICAR2012, pp. 21–23.
- Asowala-Ayodele, A.M., Afolayan, A.J., Otunola, G.A., 2016. Ethnobotanical survey of culinary herbs and spices used in the traditional medicinal system of Nkonkobe Municipality, Eastern Cape, South Africa. *S. Afr. J. Bot.* 104, 69–75.
- Baananou, S., Bouficira, I., Mahmoud, A., Boufek, K., Marongiu, B., Boughattas, N.A., 2013. Antitumor and antibacterial activities of *Apium graveolens* essential oil and extract. *Nat. Prod. Res.* 27 (12) 1075–1083.
- Bag, A., Chactopadhyay, R.R., 2015. Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. *PLoS One* 10 (7), e0131321.
- Bassole, J.H.N., Lamiem-Mtda, A., Bayala, B., Obame, L.C., Ilboudo, A.J., Franz, C., Novak, N., Neb, R.C., Dicko, M.H., 2011. Chemical composition and antimicrobial activity of *Cymbopogon dravans* and *Cymbopogon giganteus* essential oils alone and in combination. *Phytomed.* 18 (12), 1070–1074.
- Bassole, J.H.N., Juhana, H.R., 2012. Essential oils in combination and their antimicrobial properties. *Molecules* 17 (4), 3989–4006.
- Beatovic, O., Krstic-Milovic, D., Trifunovic, S., Siljegovic, J., Glamoclija, J., Rislit, M., Jelacic, S., 2015. Chemical composition, antioxidant and antimicrobial activities of the essential oils of twelve *Oidium basilicum* L cultivars grown in Serbia. *Rec. Nat. Prod.* 9 (1), 62–75.
- Bhatia, H., Read, E., Agarabi, C., Brorson, K., Lule, S., Yoon, S., 2016. A design space exploration for control of critical quality attributes of mAb. *Int. J. Pharm.* 512 (1), 242–252.
- Binisis, T., 2017. Foodborne pathogens. *AIMS Microbiol.* 3 (3), 529–563.
- Bouaziz, M., Yangui, T., Sayadi, S., Dhoub, A., 2009. Disinfectant properties of essential oils from *Salvia officinalis* L cultivated in Tunisia. *Food Chem. Toxicol.* 47 (11), 2755–2760.
- Caputo, L., Nazzaro, F., Souza, L.F., Aliberti, L., De Martino, L., Fratianni, F., Coppola, R., De Feo, V., 2017. *Lau111S nobilis*: composition of essential oil and its biological activities. *Molecules* 22 (6), 930.
- Casati, F., Bartelke, S., Biehler, K., Augustin, M., Schempp, C.M., Frank, U., 2012. Antimicrobial activity against bacteria with dermatological relevance and skin flora of the essential oil from *Coriandrum sativum* L fruits. *Phytother. Res.* 26 (3), 420–424.
- Ceyhan, N., Keskin, D., Ugur, A., 2012. Antimicrobial activities of different extracts of eight plant species from four different family against some pathogenic microorganisms. *J. Food Agric. Environ.* 10 (1), 193–197.
- Che, C.T., Wang, Z.J., Chow, M.S., Lam, C.W., 2013. Herb-herb combination for therapeutic enhancement and advancement: theory, practice and future perspectives. *Molecules* 18 (5), 5125–5141.
- Clinical and Laboratory Standards Institute (CLSI), 2020. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S11. Clinical and Laboratory Standards Institute, Wayne, USA.
- Olanmi, U., Rebecca, A., Machan, O.S., Asuquo, T.S., 2011. Comparative study on the antimicrobial activities of the ethanolic extracts of lemon grass and *Polyalthia longifolia*. *J. Appl. Pharm. Sci.* 01 (09), 174–176.
- Das, A.K., Dewanjee, S., 2018. Optimization of extraction using mathematical models and computation. Sarker D. S., Nahar, L. *Computational Phytochemistry*. Elsevier, United Kingdom.
- Ojiazet, S., Kenfack, L.B.M., Ngangoum, E.S., Nzali, H.G., Tchigang, C., 2022. Indigenous spices consumed in the food habits of the populations living in some countries of Sub-Saharan Africa: utilization value, nutritional and health potentials for the development of functional foods and drugs: a review. *Food Res. Int.* 157, 111280.
- Eloft, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Plant. Med.* 64 (8), 711–713.
- EUCAST, The European Committee on Antimicrobial Susceptibility Testing., 2020. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 10.0. <https://www.eucast.org/>. (Accessed 3/10/2023)
- Fu, I.A., Denny, C., Benso, B., Alencar, S.M.D., Rosalen, P.L., 2015. Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: a systematic review. *Molecules* 20 (4), 7329–7358.
- Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., Eitner, T., 2007. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *J. Appl. Pharm. Sci.* 21 (10), 989–994.
- Garcia-Otez, J., Alheiro, J., Pinto, A.L., Falco, V., Fraqueza, M.J., Patarata, L., 2017. Synergistic activity of essential oils from herbs and spices used on meat products against foodborne pathogens. *Nat. Prod. Commun.* 12 (2), 281–286.
- Gener, I., Skroza, O., Sutjak, J., Motina, S.S., Ljubenkovic, I., Kujahnic, A., Simac, V., Kalinic, V., 2012. Seasonal variations of phenolic compounds and biological properties of sage (*Salvia officinalis* L). *Chem. Biodivers.* 9 (2), 441–457.
- Gyorgy, E., 2010. Study of the antimicrobial activity and synergistic effect of some plant extracts and essential oils. *Rev. Romana Med. Lab.* 18 (1/4), 49–56.
- Hajlaoui, H., Mighri, H., Aouni, M., Gharallah, N., Kadri, A., 2016. Chemical composition and *in vitro* evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana* L essential oil. *Microb. Pathog.* 95, 86–94.
- Hossain, M.A., Kabir, M.J., Salehuddin, S.M., Rahman, S.M.M., Das, A.K., Singha, S.J., Alam, K.M.D., Rahman, A., 2010. Antibacterial properties of essential oils and methanol extracts of sweet basil *Oidium basilicum* occurring in Bangladesh. *Pharm. Biol.* 48 (5), 504–511.
- Ispencu, G., Ferde, M., 2012. The effect of *Anerthum graveolens* upon the growth of *E. coli*. *UPB Sci. Bull. Ser. B* 74 (3), 85–92.
- Jastaniah, S.D., 2014. The antimicrobial activity of some plant extracts, commonly used by Saudi people, against multidrug resistant bacteria. *Life Sci.* 11 (8), 78–84.
- Kon, K., Rai, M., 2012. Antibacterial activity of *Thymus vulgaris* essential oil alone and in combination with other essential oils. *Nus. ant. Biosci.* 4 (2), 2087–2094.
- Kucek, V., 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Med.* 76 (14), 1479–1491.
- Lambert, F., Mazzariol, C., Spolaore, F., Ceccato, R., Salmaso, L., Gross, S., 2022. Design of experiment: a rational and still unexplored approach to inorganic materials' synthesis. *Sustam. Chem.* 3 (1), 114–130.
- Linde, G., Gazim, Z., Cardoso, B., Jorge, L., TeSevic, V., Glamoclija, J., Sokovic, M., Colauto, N., 2016. Antifungal and antibacterial activities of *Perroselinum crispum* essential oil. *Genet. Mol. Res.* 15 (3), 1–11.
- Lopez, E.J.C., Balcazar, M.F.H., Mendoza, J.M.R., Ortiz, A.D.R., Melo, M.T.O., Parrales, R.S., Delgado, T.H., 2017. Antimicrobial activity of essential oil of *Zingiber officinale* Roscoe (Zingiberaceae). *Am. J. Plant Sci.* 8 (7), 1511–1524.
- Lv, F., Liang, H., Yuan, Q., Li, C., 2011. *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Res. Int.* 44 (9), 3057–3064.
- Madiha, I.Y., Rukayadi, Y., Norhayat, H., 2017. Effects of extraction conditions on yield, total phenolic contents and antibacterial activity of methanolic *Cinnamomum zeylanicum* Blume leaves extract. *Ind. Food J.* 24 (2), 779–786.

- Malik, R., Thapa, S., Acharya, A., 2019. Evaluation of antimicrobial activity and synergistic effect of spices against few selected pathogens. *IntJM* 6 (1), 10-18.
- Mith, H., Dure, R., Delcensene, V., Zhiri, A., Odube, G., Clinquart, A., Oaube, A., 2014. Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. *Food Sci, Nutr* 2 (4), 403-416.
- Mussarat, S., Adnan, M., Begum, S., Rehman, S.U., Hashem, A., Abd-Allah, E.F., 2021. Antimicrobial screening of polyherbal formulations traditionally used against gastrointestinal diseases. *Saudi J. Biol. Sci.* 28 (12), 6829-6843.
- Nanisombat, S., Wiumtiggosol, P., 2011. Antimicrobial and antioxidant activity of essential oils. *Food Sci. Biotechnol.* 20 (1), 45-53.
- Nedorostova, L., Kloucek, P., Kokoska, L., Stolcova, M., Pulkrabek, J., 2009. Antimicrobial properties of selected essential oils in vapour phase against food borne bacteria. *Food Control* 20 (2), 157-160.
- Pandey, A.K., Smgh, P., Tripathi, N.N., 2011. Antibacterial activity of *Syzygium aromaticum* (clove) with metal effect against food borne pathogen *Asan*. *J. Plndt Sn Res.* 11 (2), 69-80.
- Parduraman, S., Thing, G.S., Dhanaraj, S.A., 2014. Polyherbal formulation. concept of Ayurveda. *Pharmacogn. Rev.* 8, 73-80.
- Peerakam, N., Wattanathorn, J., Punjaisee, S., Buamongkol, S., Srisa-ard, P., Chansakaow, S., 2014. Chemical profiling of essential oil composition and biological evaluation of *Anethum graveolens* L. (seed) grown in Thailand. *J. Nat. Sci. Res.* 4 (16), 34-41.
- Petropoulos, S., Fernandes, A., Barros, L., Cmc, A., Sokovic, M., Ferre, I., 2018. Antimicrobial and antioxidant properties of various Greek garlic genotypes. *Food Chem.* 245, 7-12.
- Pohl, N., Stavros Colombo, P., Colombo, G.M., Rekkas, D., 2017. Design of experiments (DoE) in pharmaceutical development. *Drug Dev. Ind. Pharm.* 43 (6), 889-901.
- Ramamoorthy, R., Muthalagu, M., Andra, S., Rajesh, B., Narayanasamy, M., 2019. Investigation on antimicrobial, antioxidant and cytotoxicity properties of tangle bark extract formulated using traditional medicinal plants. *NApp. Sci.* 1, 1-7.
- Roman, U., Pusk, M., Kmetec, S., Duh, S.D., Sosur Tutk, S., 2021. Reduced susceptibility and increased resistance of bacteria against disinfectants: a systematic review. *Microorganisms* 9 (12), 2550.
- Singh, S.N., Khanal, H., Achary, D.R., 2020. Antibacterial activity of common spices extracts on bacterial isolates found in Kachhila, a Newari cuisine. *IntJM* 7, 8-18.
- Salma, U., Saha, S.K., Sultana, S., Ahmed, S.M., Haque, S.O., Mostaqim, S., 2019. The antibacterial activity of ethanolic extract of cinnamon (*Cinnamomum zeylanicum*) against two food borne pathogens: *Staphylococcus aureus* and *Escherichia coli*. *Mymensingh Med. J.* 28 (4), 767-772.
- Sethi, S., Dutta, A., Gupta, R.L., Gupta, S., 2013. Antimicrobial activity of spices against isolated food borne pathogens. *Int. J. Pharm. Pharm. Sci.* 5 (1), 260-262.
- Sharopov, F.S., Wink, M., Gulmurodov, S.I., Isupov, J.S., Zhang, H., Setzer, W.N., 2013. Composition and bioactivity of the essential oil of *Anethum graveolens* L. from Tajikistan. *Int. J. Med. Aromar. Pl. Inrs* 3 (2), 125-130.
- Singh, R., Shushni, M.A., Belkheir, A., 2015. Antibacterial and antioxidant activities of *Memha piperita* L. *Arab. J. Chem.* 8 (3), 322-328.
- Thielmann, J., Muranyi, P., Kazman, P., 2019. Screening essential oils for their antimicrobial activities against the foodborne pathogenic bacterium *Escherichia coli* and *Staphylococcus aureus*. *Helvion* 5 (6), e01860.
- Tomar, D., Ajeera, G., Giik, V., Ramadan, M.F., 2020. Composition and antibacterial effects of Laurel (*Laurus nobilis* L.) leaves essential oil. *J. Essent. Oil Bear. Plants* 2 (2), 414-421.
- Uddan, Z., Shad, A.A., Bakht, J., Ullah, I., Jan, S.J., 2015. In vitro antimicrobial, antioxidant and phytochemical screening of *Apium graveolens*. *P. J. Pharm. Sci.* 28 (5), 1699-1704.
- Unlu, M., Ergene, E., Unlu, G.V., Zeytinoglu, H.S., Vural, N., 2010. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae). *Food Chem. Toxicol.* 48 (11), 3274-3280.
- van Vuuren, S., Viljoen, A., 2011. Plant-based antimicrobial studies: methods and approaches to study the interaction between natural products. *Planta Med.* 77 (1), 1168-1182.
- Wang, X., Shen, Y., Thakur, K., Han, J., Zhang, J.C., Hu, F., Wei, Z.J., 2020. Antibacterial activity and mechanism of ginger essential oil against *Escherichia coli* and *Staphylococcus aureus*. *Molecules* 25 (17), 3955. <https://doi.org/10.3390/molecules25173955>.
- Weerakkody, N.S., Callin, N., Turner, M.S., Dykes, G.A., 2010. In vitro antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. *Food Control* 21 (10), 1408-1414.
- Witkowska, A.M., Hickey, D.K., Wilkinson, M.G., 2014. Effect of variation in food components and composition on the antimicrobial activity of oregano and clove essential oils in broth and in a reformulated reduced salt vegetable soup product. *J. Food Res.* 3 (6), 92-106.
- Yadav, R., Yadav, K.S., Soni, S., Mili, S.B., 2022. Antibacterial activity of some common kitchen spices against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. *Int. J. Pharm. Sci. Res.* 13 (3), 1125-1134.
- Zulfa, Z., Chit, C.T., Rukayadi, Y., 2016. In vitro antimicrobial activity of *Cymbopogon distachyoides* (lemongrass) extracts against selected foodborne pathogens. *Int. Food Res. J.* 23 (2), 1262-1267.

