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Essential oils from 9 exotic and endemic medicinal plants from Mauritius shows in vitro antibacterial and antibiotic potentiating activities

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ABSTRACT

Essential oils (EOs) extracted from botanical resources have greatly been appraised as antimicrobials against a broad spectrum of microorganisms. A panoply of experimental studies have pointed out the immense potency of EOs as natural antimicrobial agents at relatively low doses; largely attributed to the synergistic interactions of various bioactive components. In the present study, 10 EOs prepared from two endemic (Pit-tosporum senacia Putterl. subsp. Senacia and Syzygium coriaceum J. Bosser & J. Gueho) and seven exotic (Cin-namomum camphora (L.) Nees & Eberm, Citrus aurantium L., Curcuma longa L, Morinda citrifolia L., Petroselinum crispum (Mill.) Fuss, Plectranthus amboinicus (Lour.) Sprengel and Syzygium samarangense (Blume) Merr. & L. M. Perry) aromatic medicinal plants from Mauritius, were screened for their growth inhib-itory activities against eight bacteria (ATCC strains and clinical isolates) using broth microdilution techniques. The EOs were found to possess varying degree of antibacterial potency. The most active EOs were found to have minimum inhibitory concentration (MIC) of 0.25 4 mg/mL and minimum bactericidal concentration (MBC) of 0.25 16 mg/mL. In particular, at its MIC values, P. amboinicus EO showed bactericidal effects against four strains. However, all tested bacteria were insensitive to P. senecia and C. aurantium fruit peel EOs. Bacillus spizizenii was found to be the most susceptible strain to the active EOs. Additionally, P. amboinicus and the two Syzygium spp. EOs showed antibiotic potentiating activities. Mostly synergistic and partial synergistic actions of the EOs in combination with the conventional antibiotics (streptomycin, chloramphenicol and cip-rofloxacin) at a ratio of 1:1 were obtained against the tested bacteria. Thus, results from this study highlighted the antibacterial potential and the efficacy of the most active EOs as antibiotic potentiating agents that could potentially be used in combinational therapies along with conventional antibiotics for a synergistic approach in the treatment/management of clinically relevant bacterial infections.

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1. Introduction

The global emergence of resistant microbial strains is increasingly restricting the potency of currently available drugs leading to failure in the treatment and management of infections ([Tanwar et al., 2014](#page1)). Consequently, the effectiveness of prevalent antibiotics has been found to reduce steadily, leading the way to a 'post-antibiotic' era ([Reardon, 2014](#page1)). In addition, it has been estimated that if there is a persistent rise in antibiotic resistance, it would lead to 10 million deaths annually by 2050 ([O’neill, 2014](#page1); [Mendelson, 2015](#page1)). Concur-rently, the misuse and overuse of antibiotics are even worsening the situation. Therefore, there is a pressing need for the exploration of

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novel drugs and strategies to combat drug-resistant pathogenic microbes. Since multidrug resistance of microorganisms is a major concern and represents a global threat to public health, the screening of natural products in the quest for new antimicrobial agents has become the need of the hour ([Zgoda et al., 2001](#page1)).

In fact, in recent times, plants and their secondary metabolites have attracted much attention of the scientific community with regard to their therapeutic potentials ([Saleem et al., 2016](#page1), [2019](#page1); [Llor-ent-Martinez et al., 2020](#page1)), thus making them important candidates in the search for novel antidotes. Indeed, a huge array of plants used in traditional medicine for curing different ailments, have been evi-denced to be more effective, less expensive compared with conven-tional drugs, and also showing less lethal side effects ([Aziz et al.,](#page1) [2014](#page1); [Nisar et al., 2017](#page1)). Besides, the antimicrobial properties of plants have been particularly appraised and found to offer promising avenues against a range of microbial infections and in the combat of microbial resistance ([Gupta and Birdi, 2017](#page1)). Moreover, while some antibiotics at even low doses, have been reported to induce

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nephrotoxicity and renal impairment in patients ([Cosgrove et al.,](#page1) [2009](#page1); [Ditchfield et al., 2018](#page1)), plant antimicrobials have shown to mit-igate many of the side effects that are often associated with synthetic antimicrobials ([Iwu et al., 1999](#page1)). Indeed, an essential part of the chemical diversity produced by plants is considered to protect them against pathogens. In this context, a number of plant extracts and their derived phytocompounds have been reported to possess signifi-cant antimicrobial potency both in vivo and in vitro ([Gibbons, 2004](#page1)).

In particular, essential oils (EOs) which are concentrated natural bioactive compounds with strong smell ([Nazzaro et al., 2013](#page1)), have been greatly appraised for same. Furthermore, aromatherapeutic lit-erature has identified a number of EOs used against dermatological infections, thus highlighting the efficacy of EOs against pathogens responsible for infections ([Orchard and Van Vuuren, 2017](#page1)). More-over, due to the presence of a wide range of compounds, the antimi-crobial activity of EOs can be attributed to multiple mechanisms rather than a single one. For instance, involving different biochemical and structural mechanisms at various sites on the cell surface as well as within the cell ([Carson et al., 2002](#page1); [Nazzaro et al., 2013](#page1)). Interest-ingly, several investigations have also confirmed the effectiveness of EOs against multidrug resistant bacteria. For instance, some EOs can induce irreversible membrane damage and inhibit quorum sensing, characterized by reduced production of bioluminescence in multi-drug resistant E. coli ([Yap et al., 2014](#page1)a, [2014b](#page1), [2015](#page1)). Furthermore, other EOs have been found to affect the systems employed by bacte-ria to synchronize gene expression in relation to the microbial popu-lation density, including the production of virulence factors ([Hussain](#page1) [et al., 2015](#page1)). Besides, EOs have been comprehensively investigated for their antimicrobial synergistic effects with conventional drugs ([Hemaiswarya et al., 2008](#page1); [Rodrigues et al., 2009](#page1); [Aleksic et al.,](#page1) [2014](#page1)). Thus, EOs and their various preparations have found their applications as natural antimicrobial agents in pharmaceuticals, food

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preservation, and cosmetics ([Burt, 2004](#page1); [Ozdemir et al., 2018](#page1)). Indeed, Mauritius is composed of a rich flora with an array of

indigenous, endemic and native plants commonly used in folk medi-cine for treating several ailments ([Chintamunnee and Mahomoodally,](#page1) [2012](#page1)). However, only few medicinal plant species have been scientif-ically assessed for their possible medicinal attributes and thus, there is still a dearth of scientific information supporting traditional claims of these species in Mauritius. Moreover, in an endeavor to valorize local bioresources in view to explore their potential industrial appli-cations as natural ingredients, this present work was undertaken. For this purpose, the in vitro antibacterial potential and antibiotic poten-tiating effect of 10 EOs prepared from nine aromatic medicinal plants were evaluated against key bacterial strains of clinical relevance, some of which are considered as the major causative agents of noco-somial infections ([De Billerbeck, 2007](#page1); [Pendleton et al., 2013](#page1)). Of these nine plants, were 2 endemic species, namely Syzygium coria-ceum J. Bosser & J. Gueho and Pittosporum senacia Putterl. subsp. Sena-cia, as well as 7 exotic species Cinnamomum camphora (L.) Nees & Eberm, Citrus aurantium L., Curcuma longa L, Morinda citrifolia L., Pet-roselinum crispum (Mill.) Fuss, Plectranthus amboinicus (Lour.) Spren-gel, and Syzygium samarangense (Blume) Merr. & L. M. Perry.

2. Materials and methods

2.1. Chemicals and bacterial culture used

Mueller hinton agar (MHA), Mueller hinton broth (MHB), refer-ence strains microorganisms from the American Type Culture Collec-tion (ATCC), namely Escherichia coli (ATCC 29194), Staphylococcus epidermidis (ATCC 12228), Staphylococcus aureus (ATCC 25923), Methillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeru-ginosa (ATCC 27853), and Bacillus spizizenii (ATCC 6633). The clinical isolates of Enterococcus faecalis and Klebsiella pneumoniae were obtained from Central Laboratory, Victoria Hospital, Candos,

Mauritius. Iodonitrotetrazolium chloride (INT) and the antibiotics chloramphenicol (CHL), ciprofloxacin (CIP), and streptomycin (STR) were purchased from Sigma-Aldrich Co. (Germany).

2.2. Plant materials

Plants were selected on the basis of their traditional uses as natu-ral remedies among the local population, in the treatment and man-agement of various disease conditions in Mauritius (Gurib-[Fakim,](#page1) [1990](#page1); [Gurib-Fakim et al., 1996](#page1); [Gurib-Fakim, 2006](#page1); [Seebaluck and](#page1) [Mahomoodally, 2013](#page1)). All plant specimens were identified and authenticated by a local botanist. Leaves of S. coriaceum (MAU 0027510) and C. camphora (MAU 0027508) were collected from Mon-vert nature park in October 2018, while the leaves of P. amboinicus (MAU 0027507), the fruits of P. senacia (MAU 0027512) and M. citrifo-lia (MAU 0027506), and both the fruits and leaves of C. aurantium

(MAU 0027511) were collected from University farm, Reduit in the month of May to September 2018. On the other hand, leaves of S. samarangense (MAU 0027509) and rhizomes of C. longa (MAU 0027514) were harvested from the southern regions of Mauritius (March to May 2018). Finally, P. crispum (MAU 0027505) (aerial parts) was obtained from the local market.

2.3. Extraction of EOs

Fresh plant materials, cut into small pieces, were subjected to the process of hydrodistillation using a clevenger-type apparatus for 3 h. The EO distillates once yielded, were dried over anhydrous magne-sium sulfate, filtered, and then stored in dark vials at 4 °C until fur-ther analysis ([Aumeeruddy-Elalfi et al., 2015](#page1)).

2.4. Chemical composition of EOs

Analysis of EOs’ chemical compounds was carried out using gas chromatography-flame ionization detector (GC-FID) and gas chroma-tography-mass spectrophotometry (GC MS) techniques. GC MS analysis was conducted by an Agilent 5975 GC-MSD system coupled to an Agilent 7890A GC (Agilent Technologies Inc., Santa Clara, CA). HP-Innowax FSC column (60 m £ 0.25 mm, 0.25 mm film thickness) was used with helium (purity 99.99%) as carrier gas (1.2 mL/min). The GC oven temperature was programmed as previously described by [Zengin et al. (2016)](#page1). GC-FID analysis was carried out by simulta-neous auto-injection using Agilent 7693A series autosampler; 1 mL injections were inserted. To obtain the same elution order with GC MS, simultaneous injections were made in triplicates using simi-lar column and operational conditions. FID temperature was 300 °C. The EO components were then subjected to identification based on retention index (RI) determined by co-injection with reference to a homologous series of n-alkanes (C8-C30), under the same experi-mental conditions. Further identifications were achieved by compari-son of their mass spectra with those from NIST 05 and Wiley 8th version, including their RIs with literature values ([Zengin et al., 2016](#page1); [Mahomoodally et al., 2018](#page1)).

2.5. Antimicrobial assay

2.5.1. Microdilution broth susceptibility assay

A two-fold serial microdilution technique was employed to assess the minimum inhibitory concentration (MIC) of the EOs as performed by [Seebaluck-Sandoram et al. (2018)](#page1) with minor modifications. Each EO (100 mL) was serially diluted two-fold in triplicates with MHB in 96-well microtitre plates. Fresh bacterial inoculums were then pre-pared and adjusted to 0.5 McFarland standard which were further diluted at a ratio of 1:100 with fresh broth in order to yield starting inoculums of approximately 106 CFU/mL. Next, 100 mL of bacterial culture was added to each well of the plates. The antibiotics, CIP

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(10 mg/mL) and CHL and STR (100 mg/mL) were used as positive con-trols, while broth was used as negative control. The bacterial plates were incubated for 24 h at 37 °C. Following incubation, 40 mL of INT (0.2 mg/mL) was added to each well and the plates were incubated for another 20 min. Bacterial growth was denoted by red/pink colour-ation. The well containing the lowest concentration in which no pink-ish red colouration was observed was regarded to be the MIC. All tests were carried out in triplicate.

2.5.2. Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) for the EOs was determined according to [Aumeeruddy-Elalfi et al. (2015)](#page1). Briefly, 10 mL of broth from the uncolored wells (whereby no growth were observed in the earlier MIC assay), corresponding to the MIC value, MIC£2 (one dilution higher than MIC), and MIC£4 (one dilution higher than MICx2) were inoculated on MHA and incubated at 37 °C for 24 h. The MBC was defined as the lowest recorded EO concentration of the MIC wells in which bacteria failed to grow on the MHA. Alternatively, if growth was observed following inoculation on MHA, the concentra-tion of the corresponding well used for inoculation (MIC, MIC£2, and MIC£4) was referred as the bacteriostatic (BS) concentration. Both negative and positive controls were used for comparison.

2.5.3. Antibiotic potentiating assay

The antibiotic potentiating activity of the EOs was assessed according to [Seebaluck-Sandoram et al. (2018)](#page1) with slight modifica-tions. The EOs that were observed to be the most active in the anti-bacterial assays and for which the lowest MIC values were reported against most of the tested bacteria were investigated in combination with commercial antibiotics namely CIP, CHL, and STR to determine any possible synergistic activity. A ratio of 1:1 of EO:antibiotic were prepared by mixing known volume of stock solution of the EO with CIP, CHL and STR respectively. The assay was then carried out by using microdilution method as described in the previous section. One hundred microlitres of each EO:antibiotic (1:1) combination were serially diluted two-fold with MHB, in triplicate in a 96-well micro-plate for each of the ATCC bacterial strains and clinical isolates. The different antibiotics (CIP, CHL, and STR) were used alone as positive controls and MHB as negative control. Inoculum (100 mL) was added to each well and the plates were allowed to incubate at 37 °C over-night. After incubation, 40 ml of INT (0.2 mg/mL) was added to each well and the plates were further incubated for 20 min at 37 °C. The MICs were recorded and the results of the combined effects of the antibiotics and the EOs were calculated and expressed in terms of a fractional inhibitory concentration index (FICI) which is denoted by the following formula: FICI = FIC(EO)+FIC(Antibiotic); whereby FIC (EO), the fractional inhibitory concentration of the EO, is calculated as the MIC of EO in combination/MIC of EO alone and FIC(Antibiotic) which is the fractional inhibitory concentration of the antibiotic used is calculated as the MIC of antibiotic in combination/MIC of antibiotic alone.

3. Results and discussion

In this study, ten essential oils (EOs) obtained from nine medicinal plants by the process of hydrodistillation were evaluated for their growth inhibitory activities against eight bacterial strains. Broth micro-dilution techniques were used to determine the MIC values. In addi-tion, the MBC, defined as the concentration at which 99.9% or more of the initial bacterial inoculums are killed; most commonly used to esti-mate bactericidal activity, was determined ([Canillac and Mourey,](#page1) [2001](#page1)) for the active EOs. Moreover, EOs demonstrating the most potent antibacterial activity ( 2 mg/mL) in this present investigation were further considered for assessment of their antibiotic potentiating effects with three conventional antibiotics. Analysis of the EOs’ anti-bacterial properties was carried out in light of their chemical

Table 1

Major components of EOs identified by GC-FID and GC MS.

|  |  |
| --- | --- |
| EOs | Major components identified |
|  |  |
| CAF | Limonene (84.3%), 9-octadecanoic acid (3.9%), germacrene D (2.5%), |
|  | myrcene (2.3%) |
| CAL | Sabinene (38.1%), citronellal (13.7%), (E)-b-ocimene (11.6%), citro- |
|  | nellyl acetate (5.2%), terpinen-4-ol (5.1%), g-terpinene (4.0%), |
|  | b-pinene (3.0%), myrcene (3.2%), limonene (2.6%), a-terpinene |
|  | (2.3%), a-pinene (2.0%) |

1. 1,8-cineole (54.0%), sabinene (14.6%), a-terpineol (9.8%), a-pinene (4.8%), terpinen-4-ol (3.4%), b-pinene (3.5%)

CL Turmerone (31.4%), ar-turmerone (16.1%), turmerol (14.6%), terpino-

lene (11.0%), a-zingiberene (5.2%), b-sesquiphellandrene (4.8%), b-caryophyllene (3.5%)

MC Octanoic acid (78.9%), hexanoic acid (11.3%), [octanoic acid, methyl

ester] (5.4%)

PA Carvacrol (17.9%), d 3-carene (15.2%), camphor (12.9%), p-cymene

(9.9%), g-terpinene (6.6%), b-caryophyllene (6.1%), b-selinene

(4.2%), a-terpinene (4.1%), trans-b-bergamotene (4.0%)

PC Myristicin (40.3%),1,3,8-p-dimenthatriene (17.9%), b phellandrene

(15.0%), myrcene (4.2%), a, p-dimethylstyrene (3.7%), terpinolene

(2.6%), limonene (2.5%)

PS Myrcene (62.2%), germacrene D (7.8%), limonene (3.4%), 9-octadeca-

noic acid (3.1%), b phellandrene (2.9%), d-cadinene (2.9%)

SC (E)-b-ocimene (24.4%), (Z)-b-ocimene (10.7%), a-guaiene (12.6%),

b-selinene (9.7%), myrcene (7.8%), d-guaiene (7.2%), selin-11-en-4 a-ol (3.8%), a-selinene (3.1%)

* 1. b-pinene (21.3%), a-pinene (8.9%), g-terpinene (7.9%), limonene (7.7%), p-cymene (5.9%), b-selinene (3.8%), selin-11-en-4 a-ol

(3.6%), b-caryophyllene (3.5%), a-selinene (3.4%), d-cadinene (2.9%), 1‑epi‑cubenol (2.2%), terpinolene (2.1%), a-terpineol (2.1%)

1. Cinnamomum camphora; CAL: Citrus aurantium leaf; CAF: Citrus aurantium fruit (peel), CL: Curcuma longa; MC: Morinda citrifolia; PC: Petroselinum crispum; PS: Pit-tosporum senacia; PA: Plectranthus amboinicus; SC: Syzygium coriaceum; SS: Syzy-gium samarangense.

compounds that were identified by GC MS/GC-FID, given that the antimicrobial activities of EOs have been strictly associated with their chemical composition ([Teneva et al., 2019](#page1)).

3.1. Chemical composition of EOs

The results from the GC MS and GC-FID analyses are summarized in [Table 1](#page1). Briefly, C. aurantium fruit peel EO was found to be domi-nated by the monoterpene hydrocarbon, limonene (84.3%), while its leaf EO contained sabinene (38.1%), citronellal (13.7%), and (E)-b-oci-mene (11.6%) in the highest amounts. On the other hand, C. camphora leaf EO was found to contain 1,8-cineole (54.0%) predominantly, fol-lowed by other components such as sabinene (14.6%), a-terpineol (9.8%), and a-pinene (4.8%). In C. longa rhizome EO, turmerone (31.4%), ar-turmerone (16.1%), and turmerol (14.6%) were identified as the three most dominant compounds. Conversely, M. citrifolia fruit EO was found to contain appreciable amounts of alkanoic acids, for instance 78.9% octanoic and 11.3% hexanoic acids. In P. amboinicus leaf EO, carvacrol (17.9%), d 3-carene (15.2%), and camphor (12.9%) were obtained as the principal components, while in P. crispum EO, the phenylpropanoid, myristicin (40.3%) was detected to be the most abundant compound present. Additionally, S. samarangense leaf EO contained b-pinene (21.3%), a-pinene (8.9%), g-terpinene (7.9%), lim-onene (7.7%), and p-cymene (5.9%) as major components, whereas myrcene (62.2%) and (E)-b-ocimene (24.4%) were observed to be the main component in P. senacia and S. coriaceum, respectively.

3.2. Antibacterial activity

Based on the results, modest to relatively good antibacterial activ-ity was exhibited by the EOs, while P. senacia and C. aurantium fruit peel EOs were found to be completely inactive at the tested concen-trations. In particular, P. amboinicus, M. citrifolia, C. longa, S.

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Table 2

Antibacterial activities of EOs and antibiotics against ATCC bacterial strains and clinical isolates.

MIC/MBC (mg/mL)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Bacteria tested | |  |  |  |  |  |  | EOs |  |  |  |  |  |  | Antibiotics |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  | CAF | | CAL | CC | CL | MC | PA | PC | PS | SC | SS |  | STR | CHL | CIP | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Gram-negative | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | E. coli[a](#page1) | | - | | - | (32) | (32) | (8) | (2) | (32) | - | (16) | (16) |  | (3.13 × 10−3) | (3.13 × 10−3) | (1.95 × 10−5) | |
|  |  |  |  |  |  | BS | BS | BS | BC | BC |  | BS | BS |  | BS | BS | BS | |
|  |  |  |  |  |  | [ND] | [ND] | [16] |  |  |  | [ND] | [32] |  | [ND] | [ND] | [ND] | |
| K. pneumoniae[b](#page1) | | | - | | (32) | (32) | (32) | (8) | (2) | (32) | - | (8) | (32) |  | (1.25 × 10−2) | (1.25 × 10−2) | (7.81 × 10−5) | |
|  |  |  |  |  | BS | BS | BS | BC | BC | BS |  | BS | BS |  | BS | BS | BS | |
|  |  |  |  |  | [ND] | [ND] | [ND] |  |  | [ND] |  | [32] | [ND] |  | [ND] | [ND] | [ND] | |
| P. aeruginosa[a](#page1) | | | - | | (8) | - | (16) | (8) | (32) | - | - | (2) | (16) |  | (1.25 × 10−2) | + | (3.13 × 10−4) | |
|  |  |  |  |  | BS |  | BS | BS | BS |  |  | BS | BS |  | BS |  | BS | |
|  |  |  |  |  | [16] |  | [ND] | [16] | [ND] |  |  | [ND] | [ND] |  | [ND] |  | [ND] | |
|  | Gram-positive | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | B. spizizeniia |  | - | | (16) | (32) | (4) | (4) | (0.5) | (16) | - | (0.5) | (0.25) |  | (1.56 × 10−3) | (1.56 × 10−3) | (3.91 × 10−5) | |
|  |  |  |  |  | BS | BC | BS | BC | BC | BC |  | BS | BC |  | BS | BS | BS | |
|  |  |  |  |  | [32] |  | [16] |  |  |  |  | [2] |  |  | [3.13 × 10−3] | [ND] | [7.81 × 10−5] | |
| E. faecalis[b](#page1) | | | - | | (16) | - | (16) | (8) | - | (16) | - | (32) | (16) |  | (1.56 × 10−3) | (3.13 × 10−3) | (4.88 × 10−6) | |
|  |  |  |  |  | BS |  | BS | BS |  | BS |  | BS | BS |  | BS | BS | BS | |
|  |  |  |  |  | [ND] |  | [ND] | [16] |  | [ND] |  | [ND] | [ND] |  | [ND] | [ND] | [ND] | |
| MRSA [a](#page1) | | | - | | (32) | (32) | (8) | (4) | (2) | (16) | - | (1) | (4) |  | (6.25 × 10−3) | (6.25 × 10−3) | (6.25 × 10−4) | |
|  |  |  |  |  | BS | BS | BS | BS | BC | BS |  | BS | BS |  | BS | BS | BS | |
|  |  |  |  |  | [ND] | [ND] | [16] | [8] |  | [ND] |  | [4] | [8] |  | [ND] | [ND] | [ND] | |
| S. aureus[a](#page1) | | | - | | (32) | (32) | (4) | (4) | (2) | (16) | - | (2) | (8) |  | (6.25 × 10−3) | (6.25 × 10−3) | (3.13 × 10−4) | |
|  |  |  |  |  | BS | BS | BS | BS | BS | BS |  | BS | BS |  | BS | BS | BS | |
|  |  |  |  |  | [ND] | [ND] | [16] | [8] | [4] | [ND] |  | [ND] | [ND] |  | [ND] | [ND] | [ND] | |
| S. epidermidis[a](#page1) | | | - | | (32) | (32) | (8) | (4) | (1) | (4) | - | (4) | (8) | + | | (3.13 × 10−3) | (1.56 × 10−4) | |
|  |  |  |  |  | BS | BS | BS | BS | BS | BS |  | BS | BS |  |  | BS | BS | |
|  |  |  |  |  | [ND] | [ND] | [16] | [8] | [2] | [ND] |  | [8] | [16] |  |  | [ND] | [ND] | |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; CC: Cinnamomum camphora; CAL: Citrus aurantium (leaf); CAF: Citrus

aurantium (fruit peel); CL: Curcuma longa; MC: Morinda citrifolia; PC: Petroselinum crispum; PS: Pittosporum senacia; PA: Plectranthus amboinicus; SC: Syzygium

coriaceum; SS: Syzygium samarangense;-: not active; +: growth at tested concentration; STR: streptomycin; CHL: chloramphenicol; CIP: ciprofloxacin; (): MIC;

BS: bacteriostatic at MIC; BC: bactericidal at MIC; []: new MBC in case not bactericidal at MIC; [ND]: not determined if MBC>MICx4.

1. : ATCC strains
2. : clinical isolates

samarangense, and S. coriaceum EOs could be identified as the most active in comparison with the other EOs ([Table 2](#page1)).

It is noteworthy to emphasize that, the present study is the first endeavor to extract and evaluate the antibacterial properties of the EOs from two endemic plants of Mauritius (P. senacia and S. cori-ceum). However, unlike P. senacia, S. coriaceum EO showed interesting antibacterial activity, especially against the Gram-positive ATCC bac-terial strains (MIC: 0.5 4 mg/mL).

The antibacterial potential of the methanolic and ethylacetate extracts of the S. coriaceum has also been investigated. For instance, MIC values of 3.13 12.50 mg/mL and 1.56 6.25 mg/mL were obtained against S. aureus, S. epidemidis B. cereus, E. coli, and P. aerugi-nosa for methanolic and ethylacetate extracts, respectively. In addi-tion, S. coriaceum extracts were found to potentiate the antibacterial effects of conventional antibiotics, ampicillin and streptomycin ([Mahomoodally et al., 2020](#page1)). Similarly, aqueous and methanolic extracts of leaves and twigs of P. senacia have been studied for their antimicrobial effects. Out of 10 strains of microorganisms that were tested, the leaf extract was active against only 4 strains, namely E. coli, S. aureus, B. subtilis, and B. cereus, while the twig extract against B. cereus and E. coli (MIC: 2 12 mg/mL) ([Mahomoodally et al., 2010](#page1)). Besides, P. senacia leaf extract was subjected to another investigation by [Mahomoodally et al. (2019)](#page1) whereby it was found to display anti-bacterial effects against a range of pathogens yielding MIC of 31.25 250 mg/mL and MBC of 31.25 1000 mg/mL.

Among the studied EOs, P. amboinicus EO showed quite remark-able antibacterial potency against almost all the bacterial strains tested (MIC 0.5 2 mg/mL and MBC 0.5 4 mg/mL), with the excep-tion of E. faecalis and P. aeruginosa. Moreover, P. amboinicus EO was seen to exert bactericidal effect at its MIC values against E. coli, K.

pneumoniae, B. spizizenii, and MRSA (MIC=MBC). As reported, MIC equivalent to MBC is indicative of the bactericidal potential, along with the wide spectrum and great therapeutic potential of the plant ([Olajuyigbe and Afolayan, 2012](#page1)). Interestingly, GC MS analysis revealed P. amboinicus EO to contain carvacrol (17.9%) as the major component ([Table 1](#page1)), which has been widely documented to possess remarkable antimicrobial action ([Magi et al., 2015](#page1); [Memar et al.,](#page1) [2017](#page1); [Marinelli et al., 2018](#page1)). Other studies have also highlighted the antibacterial potential of P. amboinicus leaf EO containing carvacrol as the principal compound ([Hassani et al., 2012](#page1)). In fact, in the case of Gram-negative bacteria, carvacrol has been reported to cause the dis-integration of the polysaccharidic capsule, thus resulting into an increase in the fluidity and permeability of the cytoplasmic mem-brane ([Helander et al., 1998](#page1)). In addition, it has been found to act as a proton exchanger, affecting the cytoplasmic pH and proton motive force, followed by a decrease in ATP and DNA synthesis and inhibition of cytoplasmic enzymes causing bacterial death ([Ultee et al., 2002](#page1); [Fadli et al., 2012](#page1)).

Moreover, while [Hassani et al. (2012)](#page1) showed that P. amboinicus EO was more effective against S. aureus than E. coli (0.1 and 0.2% respectively), the opposite was observed in the present study. For instance, not only the same MIC values were obtained for S. aureus and E. coli (2 mg/mL), but P. amboinicus EO was found to have bacteri-cidal effect on E. coli at its MIC value, while for S. aureus, it showed the same at MICx2. [Erny et al. (2014)](#page1) also reported the EO of P. amboini-cus to have antibacterial effect against microbial strains such as E.coli, S. aureus, MRSA, and S. epidermidis, amongst others (MIC: 780, 12,500, 6250, and 25,000 mg/mL respectively), but was inactive against P. aeruginosa. However, in the present study, the antibacterial effect of P. amboinicus EO was more prominent against the

|  |  |
| --- | --- |
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Table 3

Antibiotic potentiating activity of Plectranthus amboinicus (PA) EO.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| EO/Antibiotic | | E. coli | |  | K. pneumoniae | |  | S. aureus | | | MRSA | |  | S. epidermidis | |  | B. spizizenii | | |  |
| (1:1) | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| FIC | FICI |  | FIC | FICI |  | FIC | FICI | | FIC | FICI | FIC | | FICI |  | FIC | FICI | |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | PA | 0.02 | 0.52[b](#page1) | 0.06 | | 0.56[b](#page1) | 0.03 | | 0.09[a](#page1) | | 0.25 | 0.50[a](#page1) | 0.25 | | 0.75[b](#page1) | 0.06 | | 0.31[a](#page1) | |  |
|  | CIP | 0.50 |  | 0.50 | |  | 0.06 | |  |  | 0.25 |  | 0.50 | |  | 0.25 | |  |  |  |
|  | PA | 0.50 | 1.50[d](#page1) | 0.50 | | 0.75[b](#page1) | 0.13 | | 0.25[a](#page1) | | 0.50 | 1.00[c](#page1) | - | | - | 0.25 | | 0.50[a](#page1) | |  |
|  | STR | 1.00 |  | 0.25 | |  | 0.13 | |  |  | 0.50 |  |  |  |  | 0.25 | |  |  |  |
|  | PA | 0.25 | 0.75[b](#page1) | 0.50 | | 0.75[b](#page1) | 0.25 | | 0.50[a](#page1) | | 0.50 | 1.00[c](#page1) | 0.25 | | 0.50[a](#page1) | 0.50 | | 1.00[c](#page1) | |  |
|  | CHL | 0.50 |  | 0.25 | |  | 0.25 | |  |  | 0.50 |  | 0.25 | |  | 0.50 | |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

FIC: fractional inhibitory concentration; FICI: fractional inhibitory concentration index; CHL: chloramphenicol; CIP: ciprofloxa-

cin; STR: streptomycin; -: not determined.

1. : synergy
2. : partial synergy
3. : additive
4. : indifferent

Staphylococcus strains. Additionally, antibacterial effect was noted against P. aeruginosa herein, although it was weak (MIC= 32 mg/mL) ([Table 2](#page1)).

Morinda citrifolia EO showed comparable antibacterial potency against all bacteria tested with MIC 4 or 8 mg/mL. Unlike the other EOs, M. citrifolia did not display MIC greater than 8 mg/mL on any of the tested bacteria. Interestingly, the antimicrobial activity of M. citri-folia EO in the present study can be attributed to its main components given that it was largely dominated by the presence of alkanoic acids, especially octanoic acids (78.9%) ([Table 1](#page1)), which is in agreement with previous studies ([Brophy et al., 2008](#page1); [Yang et al., 2016](#page1)).

In the present study, MIC values 8 32 mg/mL were obtained for C. aurantium leaf EO against the tested bacteria except E.coli which was resistant at even the highest concentration of the EO used. Besides, C. aurantium leaf EO was found to have only bacteriostatic effect at MIC for all tested bacteria. However, the bactericidal action of C. auran-tium leaf EO was determined only on P. aeruginosa and B. spizizenii at MIC£2 (MBC 16 and 32 mg/mL respectively). [Ellouze et al. (2012)](#page1) also reported the antibacterial activity of C. aurantium leaf EOs whereby the EOs prepared from the leaves of C. aurantium obtained from different seasons containing linalool (43.2 65.97%), linalyl ace-tate (0.77 24.77%), and a-terpineol (9.29 12.12%) in the highest concentrations, were found to be active against some Gram-positive bacteria such as B. subtilis (MIC= 2.7 mg/mL) and S. aureus (MIC= 4.8 mg/mL). These findings differed from those obtained in the pres-ent study whereby higher MIC values were noted. This can be due to the variation in the chemical composition of the EO, which rather contained sabinene (38.1%), citronellal (13.7%), and (E)-b-ocimene (11.6%) as major components ([Table 1](#page1)).

Moreover, unlike in the present study, [Teneva et al. (2019)](#page1) reported C. aurantium zest to possess antibacterial property against both Gram-positive bacteria (MIC==60 ppm) and Gram-negative bacteria (MIC> 600 ppm). Remarkably, the EO was also found to contain limonene (85.22%) as major component similar to the one studied here, which contained 84.3% limonene. This variation in antibacterial activity could possibly be due to the antagonistic effects of minor components pres-ent in the EO of the present study, as they have been reported to have a crucial role along with major components ([Bassole and Juliani,](#page1) [2012](#page1)). However, [Ouedrhiri et al. (2015)](#page1) reported bacterial strains such as S. aureus, E. coli and P. aeruginosa to be resistant to C. aurantium zest EO, which was also the case in the present study and was rather active against B. subtilis (MIC and MBC= 2% v/v). In fact, the authors attributed the moderate activity of C. aurantium zest EO to its main compound limonene, which has previously been found to have weak antibacterial effect ([Sonboli et al., 2005](#page1)). Besides, in the same study of [Ouedrhiri](#page1) [et al. (2015)](#page1), the antibacterial effect of EO from C. aurantium leaf was reported to be more effective than its zest. For instance, the leaf EO was observed to have MIC and MBC of 1 to >2% v/v. Besides, while C.

aurantium leaf EO was ineffective against P. aeruginosa, antibacterial potential was noted against P. aeruginosa in the present study, although it was relatively weak (MIC= 8 mg/mL and MBC= 16 mg/mL).

On the other hand, C. camphora EO was found to exert weak anti-bacterial effect on the tested microorganisms (MIC= 32 mg/mL), while it was inactive against P. aeruginosa and E. faecalis. Conversely, C. cam-phora leaf EO containing camphor as major component, was seen to exhibit greater antibacterial capacity in the study of [Satyal et al. (2013)](#page1), which differed from the C. camphora EO in the present study which was composed of 1,8-cineole predominantly (54.0%) ([Table 1](#page1)).

Additionally, C. longa EO was found to exhibit weaker antibacte-rial effect against Gram-negative bacteria (MIC 16 mg/mL) com-pared with Gram-positive bacteria (MIC: 4 16 mg/mL). This was consistent with the results obtained by [Cuc et al. (2010)](#page1) which also showed Gram-positive bacteria to be more sensitive to C. longa EO than Gram-negative bacteria.

While no antibacterial activity was observed against P. aeruginosa by P. crispum (parsley) EO, MIC of 4 32 mg/mL against the other bacte-ria was noted. Moreover, P. crispum EO was seen to have bactericidal effect on E. coli and B. spizizenii, although relatively high values were obtained (MBC: 32 and 16 mg/mL respectively). Other studies have also shown parsley EO to be ineffective against some of the bacteria investigated herein ([Gutierrez et al., 2008](#page1); [Teixeira et al., 2013](#page1)). Fur-thermore, it has been reported that the high antibacterial action of phenolic components may be described in terms of the alkyl substitu-tion into the phenol nucleus ([Dorman and Deans, 2000](#page1)). However, the formation of phenoxyl radicals, which interact with alkyl substituents, does not take place with more stable compounds such as anethole and myristicin, which could possibly explain the relative lack of activity of fennel, nutmeg, or parsley EOs, as reported by [Gutierrez et al. (2008)](#page1). In fact, myristicin (40.3%) was also revealed to be the major compo-nent of P. crispum EO studied herein ([Table 1](#page1)) and hence could explain its relatively moderate antibacterial action. On the contrary, in the study of [Linde et al. (2016)](#page1), parsley EO was found to inhibit bacterial growth to a much greater potency and accordingly, MIC ranging from 0.04 to 1.00 mg/mL and MBC ranging from 0.15 to 10.00 mg/mL were obtained. Furthermore, P. aeruginosa, S. enterica, and S. aureus were reported to be the most susceptible bacteria.

Nonetheless, S. samarangense EO showed varied antibacterial activity. For instance, poor activity was noted against Gram-negative bacteria, E. faecalis, S. aureus, and S. epidermidis. Conversely, relatively high (MIC= 0.25 mg/mL) and moderate activity (MIC= 4 mg/mL) were reported against B. spizizenii and MRSA, respectively. Remarkably, S. samarangense EO was also seen to be bactericidal at MIC (MIC=MBC= 0.25 mg/mL) against B. spizizenii. Thus, B. spizizenii was observed to be particularly sensitive to S. samarangense EO in the present study. The antimicrobial potential of S. samarangense EO has also been highlighted in other studies ([Reddy et al., 2015](#page1)).

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Several studies have demonstrated the inactivity or weak antimi-crobial activity of terpenes such as p-cymene, b-myrcene, b-caryo-phyllene, a-terpinene, g-terpinene, d 3-carene, b-pinene, a-pinene, sabinene, and limonene against a wide range of pathogens ([Dorman](#page1) [and Deans, 2000](#page1); [Koutsoudaki et al., 2005](#page1)). This was in agreement with the present study which also reported the inactivity of C. auran-tium fruit peel and P. senacia EOs which were found to contain as major components limonene (84.3%) and myrcene (62.2%) respec-tively ([Table 1](#page1)). Similarly, the presence of sabinene (38.1%) in C. aur-antium leaf could have contributed to its low antimicrobial activity

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(MIC: 8 32 mg/mL) ([Table 3](#page1)). Furthermore, in the study of [Iscan](#page1) [(2017)](#page1), monoterpene hydrocarbons were found to possess weak anti-bacterial effects with MIC values of 4.0 to >16.0 mg/mL, while previ-ous reports have established that monoterpene hydrocarbons were generally inactive compounds due to their limited water solubility ([Griffin et al., 1999](#page1)).

Several mechanisms of actions of EOs against bacteria have been revealed. For instance, lysis of cell wall, cell membrane leakage, increased permeability of the cell membrane together with its direct association with ions loss and reduced membrane potential, collaps-ing of the proton pump, as well as diminution of the ATP pool, and cell death are among the various EOs’ mechanisms of actions that have been acknowledged ([Lambert et al., 2001](#page1); [Oussalah et al., 2006](#page1); [Di Pasqua et al., 2006](#page1); [Turina et al., 2006](#page1); [Li et al., 2019](#page1)).

Most studies conducted regarding the antimicrobial properties of EOs have revealed Gram-positive bacteria to be usually more suscep-tible than Gram-negative bacteria. This is due to the presence of an outer membrane which contains hydrophilic lipopolysaccharides encompassing the bacterial peptidoglycan layer in gram-negative bacteria which creates a barrier towards macromolecules as well as hydrophobic compounds and restricts the diffusion of hydrophobic compounds into the cytoplasm ([Nikaido, 2003](#page1); [Yap et al., 2014](#page1)a, [2014b](#page1)). In fact, the outer membrane barrier is thought to be an important mechanism in multi-drug resistant Gram-negative bacte-ria ([Yap et al., 2014](#page1)a, [2014b](#page1)). Interestingly, in the present work also, the EOs showed greater antibacterial effect on Gram-positive com-pared with Gram-negative bacteria ([Table 2](#page1)).

Although the MIC values of EOs reported in the present study were higher than those obtained for the antibiotics, these results can still be deemed to be of interest considering that EOs are multi-com-ponent in nature with a mixture of bioactive compounds unlike anti-biotics ([Dorman and Deans, 2000](#page1)). Besides, the varying degrees of sensitivity of the bacteria may be due to both the inherent tolerance of the microorganisms as well as the nature and combinations of phy-tocompounds found in the EOs ([Parveen et al., 2013](#page1)). In fact, several studies have shown whole EOs to usually have greater antibacterial potential than the mixtures of their major constituents, suggesting that the minor constituents are critical to the synergistic activity of EOs’ constituents, even though additive and antagonistic effects have also been pointed out ([Bassole and Juliani, 2012](#page1)).

3.2. Antibiotic potentiating activity of EOs

Undoubtedly, numerous studies have been conducted in order to discover new antimicrobial agents that can be useful in combination therapies to address the phenomenon of antibiotic resistance. Addi-tionally, with increasing health-consciousness of the general public, the mining of novel antimicrobials have shifted its direction from synthetic chemicals to greener plant-based compounds such as EOs, taking into consideration the relatively lower side effects and cost effectiveness of natural products, when developed at a commercial scale ([Yap et al., 2014](#page1)a, [2014b](#page1); [Yang et al., 2017](#page1)).

Indeed, combination therapy, combining conventional antibiotics and natural products, represents a promising strategy to surmount antibiotic resistance ([Wolska et al., 2012](#page1); [Silva et al., 2019](#page1)). Such combinations of antimicrobials may display synergistic, additive,

indifferent or antagonistic effects. In particular, the synergistic com-binations demonstrate higher efficacy and reduced toxicity due to their multi-target activity which may prevent the emergence of anti-biotic resistance and hence can be effective against multidrug-resis-tant microbial strains ([Van Vuuren and Viljoen, 2011](#page1); [Bassole and](#page1) [Juliani, 2012](#page1)).

The results of the combined effects of the antibiotics and EOs in the present study were expressed in terms of fractional inhibitory concentration index (FICI) which is equivalent to the sum of the FICs of the antibiotic and EO. Accordingly, the results were considered synergistic if the FICI of the combination is 0.5, partial synergy if it was 0.5 0.75, additive for FICI= 0.76 1.0, indifferent (non-interac-tive) for FICI>1.0 4.0, or antagonistic for FICI> 4.0 ([Rakholiya et al.,](#page1) [2013](#page1); [Ouedrhiri et al., 2015](#page1)).

As seen in [Table 3](#page1), synergistic interactions were produced for the combination of P. amboinicus EO with each of the three antibiotics; ciprofloxacin, streptomycin and chloramphenicol against S. aureus (FICI= 0.09, 0.25, and 0.50 respectively). On the other hand, only cip-rofloxacin in combination with P. amboinicus EO was seen to exert a synergistic activity against MRSA, while an additive effect was noted when P. amboinicus EO was combined with both streptomycin and chloramphenicol against MRSA (FICI=1). Synergy was also obtained for the combination of P. amboinicus EO with ciprofloxacin and strep-tomycin against B. spizizenii (FICI= 0.31 and 0.5 respectively), while chloramphenicol and P. amboinicus EO together showed an additive interaction (FICI= 1). Nevertheless, for K. pneumoniae, only partial synergy was observed for P. amboinicus EO and the antibiotics combi-nations. For E. coli, while a partial synergistic activity was obtained by P. amboinicus EO and the two antibiotics; ciprofloxacin and chloram-phenicol (FICI= 0.52 and 0.75 respectively), an indifferent behavior was noted for the combination of P. amboinicus EO and streptomycin (FICI= 1.5).

Furthermore, a synergistic behavior was obtained by S. coriaceum EO combined with ciprofloxacin against P. aeruginosa and B. spizizenii (FICI: 0.19 and 0.38 respectively), as well as with chloramphenicol against S. aureus (FICI= 0.25). On the other hand, partial synergy was achieved by S. coriaceum EO with ciprofloxacin against MRSA and S. aureus (FICI= 0.75), whereas with streptomycin against P. aeruginosa and B. spizizenii (FICI= 0.75). In contrast, the combination of S. coria-ceum EO with streptomycin against MRSA, while the combination of S. coriaceum EO with chloramphenicol against MRSA and B. spizizenii indicated an indifferent behavior (FICI= 1.5). Nonetheless, an additive interaction was noted for the combination of S. coriaceum EO with streptomycin against S. aureus (FICI= 1) ([Table 4](#page1)). Additionally, syner-gistic interactions were exhibited by S. samarangense EO with each of the three antibiotics against B. spizizenii ([Table 5](#page1)). Interestingly, none of the combinations of EOs and antibiotics indicated antagonism.

Table 4

Antibiotic potentiating activity of Syzygium coriaceum (SC) EO.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | EO/Antibiotic | MRSA | |  | S. aureus | | |  | P. aeruginosa | | |  | B. spizizenii | | |  |
| (1:1) | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| FIC | FICI |  | FIC | FICI | | FIC | | FICI | | FIC | | FICI | |  |
|  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | SC | 0.50 | 0.75[b](#page1) | 0.25 | | 0.75[b](#page1) | | 0.06 | | 0.19[a](#page1) | | 0.13 | | 0.38[a](#page1) | |  |
|  | CIP | 0.25 |  | 0.50 | |  |  | 0.13 | |  |  | 0.25 | |  |  |  |
|  | SC | 1.00 | 1.50[d](#page1) | 0.50 | | 1.00[c](#page1) | | 0.50 | | 0.75[b](#page1) | | 0.50 | | 0.75[b](#page1) | |  |
|  | STR | 0.50 |  | 0.50 | |  |  | 0.25 | |  |  | 0.25 | |  |  |  |
|  | SC | 1.00 | 1.50[d](#page1) | 0.13 | | 0.25[a](#page1) | | - | | - |  | 1.00 | | 1.50[d](#page1) | |  |
|  | CHL | 0.50 |  | 0.13 | |  |  |  |  |  |  | 0.50 | |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

FIC: fractional inhibitory concentration; FICI: fractional inhibitory concentration index; CHL: chloramphenicol; CIP: ciprofloxacin; STR: streptomycin, -: not determined.

1. : synergy
2. : partial synergy
3. : additive
4. : indifferent

|  |  |
| --- | --- |
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Table 5

Antibiotic potentiating activity of Syzy-

gium samarangense (SS) EO.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | EO/Antibiotic (1:1) | B. spizizenii | | |
|  |  |  |  |  |
|  |  | FIC | FICI | |
|  |  |  |  |  |
|  | SS | 0.06 | 0.31[a](#page1) | |
|  | CIP | 0.25 |  |  |
|  | SS | 0.25 | 0.38[a](#page1) | |
|  | STR | 0.13 |  |  |
|  | SS | 0.13 | 0.38[a](#page1) | |
|  | CHL | 0.25 |  |  |
|  |  |  |  |  |

FIC: fractional inhibitory concentration;

FICI: fractional inhibitory concentration

index; CHL: chloramphenicol; CIP: cip-

rofloxacin; STR: streptomycin.

1. : synergy

Thus, based on these findings, the combinations of antibiotics with the EOs in the ratio of 1:1 were found to result mostly into syn-ergistic and partial synergistic effects. Synergistic combinations are usually the main focus for downstream investigation as they are con-sidered to be the most effective and tend to reduce the effective dos-age of the antimicrobial agents required for treating an infection. However, comparable effects of additivity and synergistic interac-tions between EO and antibiotics have been reported in the study of [Yang et al. (2017)](#page1), where in both cases they showed similar severity in terms of the disruption of the bacterial membrane.

The screening of medicinal plants with antimicrobial activity with the aim to discover synergistic interactions with antimicrobial drugs could provide a significant source of bioactive compounds that could be used in combination therapies. Such active compounds or frac-tions may not essentially possess strong antibacterial properties themselves, but may synergize with conventional antibiotics, as seen in the present study, via known or novel modes of action ([Rakholiya](#page1) [et al., 2013](#page1)).

4. Conclusion

In the present investigation, varied antibacterial potency of the EOs was demonstrated. Among them, P. amboinicus, M. citrifolia, S. samarangense, S. coriaceum, and C. longa EOs were identified as most active against the tested bacteria. Moreover, the EOs were found to be more effective against Gram-positive bacteria compared with the Gram-negative bacteria. In particular, B. spizizenii was found to be the most sensitive to the active EOs. In contrast, C. aurantium fruit peel and P. senacia EOs were inactive against all the tested bacteria. Given that this study is the first attempt to evaluate the antibacterial prop-erties of the EOs from the endemic plants, S. coriaceum and P. senacia, these findings obtained in the present study can be used as prelimi-nary information for establishing future studies. Additionally, this study highlighted the antibiotic potentiating effects of the two Syzy-gium EOs, including P. amboinicus EO which in combination with the antibiotics, showed improved antibacterial efficacy at reduced con-centrations. Interestingly, mostly synergistic and partially synergistic interactions were obtained for the different EO/antibiotic (1:1) com-binations. Thus, with increasing antibiotic resistance among patho-genic microorganisms and the renewed interest in plant-derived extracts in search for prospective candidates against them, the inves-tigated EOs can be regarded as valuable natural antibacterial agents for their potential application in the pharmaceutical as well as cos-metic industries. Nonetheless, further assessments are required to establish the safety of the EOs.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.sajb.2020.05.001](https://doi.org/10.1016/j.sajb.2020.05.001).

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