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Antimicrobial activity of chemically characterized plant essential oils against plant pathogenic *Xanthomonas* pathovars, and some foodborne human pathogenic bacteria and yeast

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Abstract
The continuous emergence of multidrug resistance (MDR) microbes and the negative effect of synthetic pesticides and food preservatives have directed us to search for essential oils (EOs) as alternate food preservatives. The present study evaluated the antimicrobial activity of EOs of *Artabotrys odoratissimus* (AOEO), *Boswellia serrata* (BSEO), *Cymbopogon citratus* (CCEO), and *Schefflera actinophylla* (SAEO) against selected pathogenic bacteria and yeast. The gas chromatography-mass spectrometry (GC-MS) analysis confirms the presence of 14, 29, 14, and 15 different phytochemicals in the AOEO, BSEO, CCEO and SAEO, respectively. The combinations of AOEO+BSEO, AOEO+CCEO, AOEO+SAEO, BSEO+CCEO and CCEO+SAEO (1:1 v/v) showed dose-dependent antimicrobial activity with ZOIs and MICs ranged between 07.51-22.76 mm and 0.125-4.0 µl/ml, respectively. Hence, these EOs combinations could be utilized as antimicrobial agents for the management of plant and human pathogenic microbes.

Keywords: plant essential oils, antimicrobial activity, GC-MS, phytochemicals, *Xanthomonas*, foodborne pathogens

Introduction
The *Xanthomonas* pathovars are common plant pathogenic bacteria belong to the phylum proteobacteria affecting a wide range of crop species and are responsible for significant yield and quality losses of agricultural foodstuffs (Doddaraju *et al*., 2019) [7]. Many synthetic antibiotics/pesticides are used in the form of spray, slurry, dusting to control disease caused by *Xanthomonas* spp., but many agrochemical pesticides are toxic to non-target organisms, including humans, and also associated with environmental hazards due to their xenobiotic properties (Mansfield *et al*., 2012) [9]. On the other side, the foodborne diseases caused by many multidrug-resistant (MDR) human pathogenic bacteria and yeast are the most critical threats to public health around the world (Xu *et al*., 2014; van Duin and Paterson 2016) [24, 21]. Some of the MDR microbes commonly reported worldwide are *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, and *Candida albicans* (van Duin and Paterson, 2016) [21]. As per the Centers for Disease Control and Prevention (CDC) estimate, around two million people are suffering from a foodborne infection in the USA alone, and nearly 23,000 patients die per annum due to the continuous emergence of MDR microbes (Prestinaci *et al*., 2015) [14]. Even though many agricultural and pharmacological industries have produced many new pesticides/antibiotics in the last two decades, the incessant increase of MDR microbes is a significant task for treating infectious diseases (Zheng *et al*., 2018) [25].
Many literatures report the potentiality of plant EOs as a source of antimicrobial agents, and the bioactivity depends on their functional group, nature, composition, and orientation (Mishra and Dubey 1994; Calo *et al*., 2015; Chouhan *et al*., 2017; Kumar *et al*., 2018) [11, 5, 6, 8]. The EOs are widely used in the preparation of medicine, perfumes, cosmetics, and bath products (Pandey *et al*., 2017) [12]. But, only a smaller percentage of identified EOs was exploited in agricultural and pharmacological usage. Hence, the present investigation was undertaken to assess the antimicrobial activity of EOs of *A. odoratissimus* (Annonaceae), *B. serrata* (Burseraceae), *C. citratus* (Poaceae), and *S. actinophylla* (Araliaceae) against different *Xanthomonas* pathovars and some foodborne bacteria and yeast.

Materials and Methods
EOs extraction and phytochemical analysis
Fresh leaves of *A. odoratissimus*, *B. serrata*, *C. citratus* and *S. actinophylla* were collected in the region of Bangaluru, Karnataka (India) during 2017-20 and subjected for essential oil extraction using hydrodistillation using Clevenger apparatus (Srivastava *et al*., 2009) [18]. The extracted AOEO, BSEO, CCEO, and SAEO were subjected to chemical profile analysis using gas chromatography coupled to mass spectrometry (GC-MS) (DSQ II model, Thermo Scientific, CA, USA). The method of plant identification, oil extraction, and conditions used for GC-MS analysis were published in our previous paper (Venkatesh *et al*., 2017) [22]. The individual chemical components were identified by comparing the spectral peaks and retention times with the spectral libraries of the National Institute of Standards and Technology (NIST), U.S Department of Commerce, Gaithersburg (Adams, 2007) [1].

Antimicrobial activity evaluation
The cultures of *Xanthomonas* pathovars *viz*., *Xanthomonas axonopodis* PV. *malvacearum* (*X. a. pv. m*), *Xanthomonas axonopodis* PV. *Phaseoli* (*X. a. pv. p*) and *Xanthomonas campestris* PV. *Vasicatoria* (*X. c. pv. v*) were collected from the Department of Microbiology, University of Mysore, Mysore (India). The foodborne human pathogenic microbes *viz*., *Escherichia coli* (NCIM 2065), *Pseudomonas aeruginosa* (NCIM 5031), *Salmonella typhi* (NCIM 2051), *Staphylococcus aureus* (NCIM 2079), and *Candida albicans*

(NCIM 3471) were procured from the National Collection of Industrial Microorganisms (NCIM), Pune (India). These tested microbes were maintained on Mueller-Hinton agar (MHA) for bacteria, and malt extract-glucose-yeast extract-peptone-agar (MGYPA) for yeast.

Different combinations of EOs *viz*., AOEO+BSEO, AOEO+CCEO, AOEO+SAEO and BSEO+CCEO (1:1, v/v) were prepared and subjected for antimicrobial activity evaluation using the disc diffusion method. The zone of inhibitions (ZOIs) around the disc was measured in millimeters (mm). Bacterimycin (20µg/disc), erythromycin (15mcg/disc), and fluconazole (25mcg/disc) were used as positive controls for *Xanthomonas* pathovars, foodborne bacteria, and yeast, respectively. The two-fold dilution technique was employed to determine the minimum inhibitory concentrations (MICs) using a 96-well microtiter plate. In MICs estimation, 200 µl of two-fold successively diluted EOs (AOEO+BSEO, AOEO+CCEO, AOEO+SAEO, BSEO+CCEO and CCEO+SAEO (1:1 v/v)) in MHB or MGYPB was taken and separately inoculated with 15 µl of a microbial cell suspension (10^8 CFU/ml of bacteria or 10^6 CFU/ml of yeast), and incubated at 37°C for bacteria (24 h) and 30°C for yeast (48 h). Then 50 µl of iodo-nitro-tetrazolium chloride (INT, 2 mg/ml) was mixed in each well and incubated additional 30 min. The change of pale yellow-colored INT into pink colour indicates cells viability, and the yellow colour unchanged designates as inhibition of microbial growth. The lowermost concentration at which the yellow colour continued was considered as MIC. The complete procedure of disc diffusion method and MICs determination was presented in our previous paper (Thippeswamy *et al*., 2015) [20]

Results and Discussion

In the present investigation, the chemical composition of AOEO, BSEO, CCEO, and SAEO were analyzed using GC-MS, and individual chemical components present in these EOs were identified (Table 1). The GC-MS data confirms the presence of 14, 29, 14, and 15 different chemical compounds in the AOEO, BSEO, CCEO, and SAEO, respectively. The major compounds identified were 3-carene (44.91%) and β-caryophyllene (19.17%) from AOEO, 3-carene (34.74%) and β-ocimene (13.78%) from BSEO, 2,6-octadienal, 3,7-dimethyl-, (E) (47.21%) and 2,6-octadienal, 3,7-dimethyl-, (Z) (32.60%) from CCEO, and caryophylene (49.23%) and humulene (26.17%) from SAEO. The obtained results confirm that the AOEO, BSEO, CCEO, and SAEO having a complex mixture of chemical components, but only a few, as mentioned above, were recorded as major chemical components (>10%), whereas most of the components are present at a trace quantity. Some of the chemical compounds of AOEO, BSEO, CCEO, and SAEO recorded by the earlier researchers were similar, but some of the major differences were observed in their percent abundances (Wang *et al*., 2012; Ajayi *et al*., 2016) [23, 3]. As per Pandey *et al*., (2017) [12] statement, antimicrobial activities directly depends on differences in chemical constituents of EOs. Our phytochemical profile analysis showed that the major chemicals mentioned above play an important role in the antimicrobial activity of AOEO, BSEO, CCEO, and SAEO.

The antimicrobial activity of EOs combinations were evaluated by measuring ZOI and MICs, and the obtained data are presented in Tables 2 & 3. The results revealed that the combinations of EOs showed organism's dependent low, moderate, and high antimicrobial activity against bacteria and yeast tested with ZOIs and MICs ranged between 07.51-22.76 mm and 0.125-4.0 µl/ml, respectively. In statistical analysis, *S. aureus* was observed as a more susceptible organism, followed by *X. axonopodis* pv. *malvacearum* with the least MICs, whereas *E. coli* was more resistant with the highest MICs. The order of antimicrobial activity was BSEO+CCEO > AOEO+BSEO > AOEO+CCEO > AOEO+SAEO.

The review of the literature revealed that the extracts of *A. odoratissimus, B. serrate, C. citratus* and *S. actinophylla* showed one or more bioactivities such as anticancer, antimicrobial, antioxidant, antimalarial, anthelmintic, antifertility, anti-inflammatory, anti-arthritic renal and cardioprotective properties (Srivastava *et al*., 2009; Pereira *et al*., 2009; Sharma *et al*., 2010; Aman *et al*., 2010; Tamgue *et al*., 2010; Raja *et al*., 2011; Ranitha *et al*., 2014; Banerjee *et al*., 2014; Ajayi *et al*., 2016) [18, 13, 17, 2, 19, 15, 16, 4, 3]. The solvent extract of *A. odoratissimus*, *B. serrata*, *C. citratus*, and *S. actinophylla* showed antimicrobial activity (Mishra and Dubey 1994; Sharma *et al*., 2010; Raja *et al*., 2011; Venkatesh *et al*., 2017) [11, 17, 15, 22]. Although there are some reports are available for the antimicrobial activity of AOEO, BSEO, CCEO, and SAEO, but only a few reports are known for their inhibitory activities EOs against human pathogenic microbes, and none of the reports are available for their inhibitory activities against phytopathogenic *Xanthomonas* pathovars. So, we are the first time to report the antimicrobial activities different EOs against plant pathogenic *Xanthomonas* pathovars. The promising antimicrobial activity of AOEO, BSEO, CCEO, and SAEO might be prospective sources as alternative antimicrobial agents for the control of diseases caused by phytopathogenic *Xanthomonas* species and foodborne bacteria and yeast.

Table 1: The major chemical compounds of AOEO, BSEO, CCEO, and SAEO.

AOEO		BSEO		CCEO		SAEO	
Compounds	**%RA**	**Compounds**	**%RA**	**Compounds**	**%RA**	**Compounds**	**%RA**
3-Carene	44.91	3-Carene	34.74	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl	0.04	Pinene	2.76
β-Thujene	1.72	β-Pinene	2.95	Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl	0.17	Limonene	3.12
D-Limonene	1.59	α-Phenylcyclo pentane acetic acid	1.24	Camphene	0.56	Cyclodecadiene	0.36
α- Copaene	6.59	D-Limonene	8.25	5-Hepten-2-one, 6-methyl	0.65	Benzenepropanal	1.19
β-Elemene	0.74	β-Ocimene	13.78	D-Limonene	0.14	Caryophylene	49.23
β- Caryophyllene	19.17	Terpinolene	5.39	β-Ocimene	0.08	Aristolene	0.92
α-Humulene	8.78	Pinocarveol	1.36	1-Cyclohexene-1-acetaldehyde, α, 2-dimethyl	0.57	Humulene	26.17

γ-Muurolene	2.71	α-Terpineol	2.25	Carveol	4.06	Selinene	1.24
α-Muurolene	1.29	γ-Elemene	1.77	cis-Verbenol	6.12	Naphthalene	6.44
Naphthalene	4.37	β-Caryophyllene	6.65	2,6-Octadienal, 3,7-dimethyl-, (Z)-	32.60	Cadina-1(10),6,8,triene	0.98
Caryophyllene oxide	5.55	α-Bergamotene	1.64	β-Myrcene	3.55	Caryophyllene oxide	2.54
Cubenol	0.74	Elixene	2.05	2,6-Octadienal, 3,7-dimethyl-, (E)-	47.21	Humulene epoxide 2	0.96
δ-Cedrol	1.35	Spathulenol	2.67	Caryophyllene	3.46	Selina-6-en-4-ol	2.57
γ-Gurjunene	0.52	Globulol	3.58	Caryophyllene oxide	0.79	Naphthalenol	0.86
		Viridiflorol	2.39			Tau. Cadinol	0.65

AOEO: *A. odoratissimus* essential oil; BSEO: *B. serrata* essential oil; CCEO: *C. citratus* essential oil; SAEO: *S. actinophylla* essential oil. %RA- Percentage of relative abundance. All 29 phytoconstituents of BSEO have been reported in our previous paper (Venkatesh *et al*., 2017) [22]

Table 2: Zone of inhibition (ZOIs) of EOs against *Xanthomonas* pathovars and some foodborne bacteria and yeast at 15 µl/disc.

Test organisms	AOEO+BSEO	AOEO+CCEO	AOEO+SAEO	BSEO+CCEO	CCEO+SAEO	Positive controls
E. coli	10.63±0.7	11.32±0.4	7.51±0.4	12.93±0.6	10.78±0.3	11.36±0.5
P. aeruginosa	9.24±0.6	10.53±0.6	8.58±0.4	11.52±0.3	10.65±0.5	11.32±0.3
S. typhi	14.52±0.8	16.41±0.4	12.62±0.7	17.34±0.6	14.29±0.7	20.18±0.7
S. aureus	17.23±0.6	21.89±0.8	15.81±0.5	22.76±1.2	16.34±0.5	18.62±0.6
X. a. pv. *m.*	16.23±0.9	18.36±0.9	13.48±0.5	20.21±1.5	14.452±0.7	14.43±0.8
X. a. pv. *p.*	12.74±0.5	14.45±1.0	12.16±0.5	16.48±0.4	13.83±0.4	11.58±0.4
X. c. pv. *v.*	14.38±1.9	18.16±1.2	13.49±0.4	20.58±1.4	13.46±0.8	12.25±0.8
C. albicans	11.81±0.6	13.36±0.5	9.64±0.3	15.41±0.7	12.53±0.4	15.45±0.6

The presented results are the mean of triplicates ± standard error, $p \leq 0.05$. AOEO: *A. odoratissimus* essential oil; BSEO: *B. serrata* essential oil; CCEO: *C. citratus* essential oil; SAEO: *S. actinophylla* essential oil. Erythromycin (15mcg/disc) (for human pathogenic bacteria), Bacterimycin (20 µg/disc) (for phytopathogenic bacteria), and fluconazole (25mcg/disc) (for yeast) served as a positive control.

Table 3: MICs of EOs against *Xanthomonas* pathovars and some foodborne human pathogenic bacteria and yeast.

Test microbes	AOEO+BSEO	AOEO+CCEO	AOEO+SAEO	BSEO+CCEO	CCEO+SAEO
E. coli	4.0	2.0	4.0	1.0	2.0
P. aeruginosa	4.0	2.0	4.0	1.0	2.0
S. typhi	2.0	1.0	1.0	0.25	0.5
S. aureus	0.5	0.5	0.5	0.125	0.25
X. a. pv. *m.*	0. 5	0. 5	0. 5	0.125	0.25
X. a. pv. *p.*	1.0	0.5	1.0	0.25	1.0
X. c. pv. *v.*	0.5	0.5	2.0	0.5	1.0
C. albicans	2.0	1.0	4.0	1.0	2.0

The values are the mean of triplicates ± standard error, $p \leq 0.0$. The presented MICs values are represented in µl/ml.

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