



In-vitro evaluation of antimicrobial and insect repellent potential of supercritical-carbon dioxide (SCF-CO₂) extracts of selected botanicals against stored product pests and foodborne pathogens

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Revised: 19 September 2019 / Accepted: 24 October 2019 / Published online: 12 November 2019
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Abstract In the present study, the antimicrobial and the insect repellent activity of 16 botanical extracts obtained by supercritical CO₂ (SCF) extraction were evaluated. The present investigation was conducted as there is a necessity for exploration of natural botanical extracts that target both stored product insects and microbes. The antimicrobial activity was studied by disc diffusion and broth microdilution methods against ten microbial species, including Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogenes*), Gram-negative bacteria (*Escherichia coli* and *Salmonella enterica*), and fungi (*Aspergillus flavus*, *Aspergillus paraciticus*, *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium verrucosum*). Repellency assay was carried out by area preference method against three coleopteran insects (*Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus oryzae*). Among all the extracts, thyme and ajwain were effective against all the tested bacteria with a minimum inhibition concentration (MIC) of 256–1024 µg/mL. Hop extract resulted in better antibacterial activity against

all the tested Gram-positive bacteria with a MIC of 32–64 µg/mL. Oregano, thyme and ajwain extracts showed broad-spectrum antifungal activity against all the tested fungi with MIC of 128–1024 µg/mL. Most of the extracts exhibited class V (80.1–100%) repellency against *T. castaneum*. Extracts of hop, ajwain and thyme were found to have strong repellency against *T. castaneum* and *R. dominica*. Therefore, SCF extracts of ajwain and thyme can be explored further for the application of bio-extracts as a growth limiting factors in a microcosm where such consortia thrive.

Keywords Foodborne pathogens · Coleopteran insects · Super critical fluid extraction · Insect repellency · Microbial inhibition

Introduction

The food spoilage is a complex process, resultant of several physical, biological, chemical and environmental factors which cumulatively make the commodity unacceptable for human consumption. Spoilage of stored food grains is a major concern with almost 10 to 30% damage by stored product insects (Ferry et al. 2004; Bhavya et al. 2018). Adverse effects associated with stored-product insects can be classified broadly into two classes as either a direct or indirect effect on human food (Hubert et al. 2018). Direct effects by pests are through physical contamination of food by arthropod body parts/fragments and chemical contamination by carcinogenic or allergenic products related to their physiology. Complex indirect effects of stored product insects on food are through altering the humidity and temperature, in turn, creating favourable environmental conditions for the pathogenic bacteria and fungi. They also

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13197-019-04141-6>) contains supplementary material, which is available to authorized users.

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host/transmit microorganisms like *Escherichia*, *Serratia*, *Streptococcus*, *Bacillus*, and *Klebsiella* (Harein and De Las Casas 1968). Pathogenic fungi in stored product interact with the stored product pests resulting in mycotoxin contamination (Sinha and Sinha 1990; Hell et al. 2000). Toxic strains of *Aspergillus flavus* were isolated from insects like *Sitophilus oryzae* and *Tribolium castaneum* (Hell et al. 2000). *Aspergillus* species produce mycotoxins (secondary metabolites) like aflatoxin and ochratoxin, which are also associated with postharvest storage (Nidhina et al. 2017). Stored-product pests interact with symbiotic and parasitic bacteria by the transmission of bacteria (Channaiah et al. 2010) and hosting genes of antibiotic-resistance. Stored product insects serve as vectors for the human pathogens and antibiotic resistant strains (Larson et al. 2008). In the 21st century, foodborne diseases resulting from the ingestion of contaminated foods is a huge concern to public health.

Over the last few decades pest control measures involved the usage of toxic chemicals as insecticides and fumigants (Bomzan et al. 2018). Furthermore, public concern about food contamination with bactericidal and fungicidal residues has significantly increased, and the emerging industrialization of food manufacture catalyses the presence and spread of new or antibiotic-resistant pathogens (Tauxe et al. 2010). Considering all these factors, the development of new safe and biodegradable alternative strategy to combat the pest and microbial control is required. In this purview, the use of phytochemicals and plant extracts as insecticides, insect repellents, antiseptics and antimicrobial agents that are effective and economically feasible are of new interest to researchers from various fields. Apart from being used as pest control agents, these botanical extracts also have a wide range of application in culinary, human and veterinary medicine, cosmetic industry. Plants have an ability to synthesize a large pool of secondary metabolites capable of defending themselves against microbes, pests and herbivores. It is well established that plant extracts, and essential oils have a broad spectrum of antimicrobial activity against foodborne pathogens and spoilage microorganisms (Gyawali and Ibrahim 2014). Plants extracts/essential oils like basil, fennel, thyme, clove, cinnamon, oregano, mint, ajwain, and garlic have shown to have antimicrobial and insecticidal potential, which have advantages and disadvantages in maintaining human health (Pandey and Kumar 2013; Pandey et al. 2017; Messaoudi and Begaa 2018; Begaa and Messaoudi 2019). The plant extracts exhibiting antimicrobial property are mainly known by their bioactive molecules, such as phenolics, polyphenols, quinones, flavones, flavonoids, flavones, tannins, terpenoids, coumarins, alkaloids, lectins, polypeptides and other compounds (Cowan 1999). These bioactive compounds can be

extracted by several extraction procedures. However, the toxicity of the solvents used in extraction techniques, the degradation of the compounds, and the selectivity of the process are key points that are to be considered during phytochemical extraction. In this context, supercritical fluid extraction (SCF) procedure indeed is a novel competitive alternative for the extraction of valuable bioactive compounds by which the final product obtained are without toxic residues, with no degradation of active components, and high purity (da Silva et al. 2016). There is no comprehensive study determining the efficacy of SCF extracts of spices and herbs on both microbial and stored product insects that are serious issues in safety and food spoilage. Hence, the objective of the present study was to determine the biological activity of SCF extracts like fennel, basil, lemon myrtle, oregano, sage, thyme, cinnamon, curcuma xanthorrhiza, licorice, garlic, ajwain, hop, clove, margosa, rosemary and schisandra against stored product pests and foodborne pathogens.

Materials and methods

Preparation and characterisation of supercritical extracts

All supercritical extracts used in this study were commercial products of Flavex Naturextrakte GmbH (Rehlingen, Germany) conforming to established product specifications.

Dry leaf materials (basil, lemon myrtle, oregano, sage and thyme) and hop cones were passed through a cutting mill and then pelletized. All other materials like fruits, buds, bark, root and seeds were first passed through a cutting mill (cinnamon, curcuma xanthorrhiza and licorice) or directly passed through a pin or turbo mill (ajwain, clove, margosa, rosemary and schisandra) to give a powder with particle size of 200–600 μm . Only garlic cloves were freshly mashed and after addition of 2% olive oil blended with kieselgur in a ratio of 2 + 3 to give a powder for extraction.

All powders were processed in a high-pressure extraction plant with 3×300 L extractor volume, typical extractor filling was 90 kg of powder and extraction time was 1.5 h on the average with CO_2 mass flow of 600 kg/h according to a solvent ratio of 10 kg CO_2/kg input material. The solvent amount was adjusted to obtain an extraction degree of NLT 90% of marker constituents based on the mass balance of feedstock, extract and residue. Typical extraction conditions for selective CO_2 extracts containing mainly steam volatile mono- and sesquiterpenes (ajwain, basil, clove, fennel, lemon myrtle, oregano, thyme) were 90–120 bar and 40–60 $^\circ\text{C}$. Total CO_2 extracts which

contain in addition non-volatile lipophilic constituents and oils (cinnamon, curcuma xanthorrhiza, garlic, hop, marga and schisandra) were extracted at 250–350 bar and 50–60 °C. The antioxidative diterpene phenols of rosemary and sage as well as licorice phenols were extracted at 400–500 bar and 60 °C with a few percent of food grade ethanol as co-solvent.

All extracts apart from garlic, hop, licorice, rosemary and sage were composed by 100% of feedstock constituents; the others were standardised by addition of some vegetable oil—garlic with olive oil, licorice with MCT oil and hop, rosemary and sage with sunflower oil.

The composition of all supercritical CO₂ extracts was analysed by HPLC–DAD and GC–MS. Authentic reference substances were used for identification and quantification as far as they were available. Supplementary Table 1 gives the extract composition of all product batches used for efficacy testing. Data were taken from the batch related certificates of analysis provided by the manufacturer.

Bacterial, fungal strains and culture conditions

Bacillus subtilis (ATCC 6633), *Escherichia coli* (ATCC 11775), *Listeria monocytogenes* (ATCC 13932), *Staphylococcus aureus* (ATCC 12600) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 25241) were obtained from Food Safety and Analytical Quality Control Laboratory at CSIR-CFTRI, Mysuru, India and stored in 20% glycerol at – 80 °C. Vials containing frozen stock cultures were thawed at room temperature and were reactivated before use in 10 ml of sterile brain heart infusion (BHI) broth at 37 ± 2 °C for 24 h under static conditions. The bacterial strains were grown and maintained in BHI agar slants at 37 ± 2 °C and 4 °C, respectively. The bacterial suspension in the BHI broth at an initial

concentration of approximately 10⁹ colony-forming units (CFU)/mL was used for further experiments.

Aspergillus ochraceus (MTCC 1810), *Aspergillus flavus* (MTCC 277), *Aspergillus niger* (MTCC 281), *Penicillium verrucosum* (MTCC 1758), and *Aspergillus parasiticus* (MTCC 2796) were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. Fungi used in the experiment were cultured on potato dextrose agar (PDA) slants and incubated at 25 ± 2 °C and were maintained at 4 °C until use. The fungal spore suspension prepared in 0.9% saline solution containing approx. 10⁶ CFU/mL was used in the studies.

Rearing of stored product insects

Rhyzopertha dominica (lesser grain borer), *Tribolium castaneum* (red flour beetle) and *Sitophilus oryzae* (rice weevil) being maintained at Department of Food Protection and Infestation Control, CSIR-CFTRI, Mysuru, India were cultured on conditioned whole wheat (moisture content of 11.5–12.5%), and maintained at 28 ± 2 °C and 70 ± 5% RH and a 16:8 light: dark photoperiod. Adult insects of 1–2 weeks age were used in all the experiments. Cultures were maintained in the laboratory without exposure to any insecticide. All experiments were carried out under the same environmental conditions.

In vitro antibacterial activity by disc diffusion method

Antibacterial activity of sixteen supercritical extracts against five pathogenic bacteria (three Gram-positive and two Gram-negative) was investigated by the agar disk diffusion method as described by Rohinishree and Negi (2016). Briefly, the BHI agar medium was seeded with

Table 1 Minimum inhibitory concentration of supercritical critical fluid extracts of botanicals against selected bacteria cultures

Extract	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. enterica</i>
Minimum inhibitory concentration (µg/mL)					
Ajwain	1024	512	256	512	512
Oregano	256	128	256	ND	256
Lemon myrtle	> 2048	1024	512	ND	ND
Cinnamon	512	512	512	ND	ND
Thyme	512	256	256	256	512
Licorice	512	ND	ND	ND	ND
Rosemary	512	256	ND	ND	ND
Sage	512	512	ND	ND	ND
Hop	64	32	32	ND	ND
Basil	ND	> 2048	ND	ND	ND
Clove	ND	1024	ND	ND	ND

ND Not determined

0.1% of inoculum (10^7 log CFU/mL) and poured onto the plates. After solidification, the sterile filter discs (6 mm diameter, Hi-Media, Mumbai) were placed, and 10 μ L of the extract was added on the disc and incubated at 37 ± 2 °C for 24 h. The plates containing sterile discs without the addition of the extracts served as negative control. The sensitivity of the bacteria to the supercritical extracts was determined by measuring the size of the inhibitory zones (including the diameter of the disk) around the discs on the agar surface, and values < 10 mm were considered as not active (i.e., negative) against tested bacteria. Extracts with the inhibition zone of > 15 mm were tested for their minimum inhibition concentration (MIC) against the selected bacteria.

In vitro antifungal activity by disc diffusion method

Antifungal activity of ten supercritical extracts against five fungi namely *A. ochraceous*, *A. flavus*, *A. niger*, *P. verrucosum* and *A. parasiticus*, were investigated by the agar disk diffusion method as mentioned by Chandrasekaran and Venkatesalu (2004). The PDA agar medium was seeded with a spore suspension of approx. 10^6 CFU/mL by spread plate method. The sterile filter discs (6 mm diameter, Hi-Media, Mumbai) were placed, and 10 μ L of the extract was added on the disc and incubated at 25 ± 2 °C for 3–5 days. The in vitro antifungal activity was determined by measuring the zones of growth inhibition including the diameter of the discs. The inhibition zones with a diameter of < 10 mm are considered as not active (i.e., negative) and extracts with inhibition zones of > 20 mm were tested for their minimum inhibition concentration (MIC) against the selected fungi.

Determination of minimum inhibition concentration (MIC) against selected foodborne pathogens

Supercritical extracts exhibiting an inhibition zone ≥ 15 mm in diameter was screened to determine the MIC by standard two-fold microbroth dilution methodology given by Goyal and Kaushik (2011) with slight modifications. Bacterial suspensions were prepared as mentioned earlier and diluted with BHI broth to obtain a final inoculum of 10^7 CFU/mL in the assay for all the bacteria (based on optical density values at 630 nm previously calibrated against viable counts). A stock solution of each SCF extract was serially diluted in 96 well microtiter plate with BHI broth to obtain a concentration ranging from 4 to 2048 μ g/mL of dimethyl sulfoxide (DMSO). Wells containing BHI broth, inoculated broth and DMSO treated broth served as media, negative and diluent controls. A standardized inoculum of each bacterial strain was added (5 μ L) to each well and microtiter plates were then incubated at 37 ± 2 °C for 24 h.

Following incubation, MIC was calculated as the lowest concentration of the extract inhibiting the growth of bacterial strain by measuring optical density at 630 nm using Varioskan flash reader (Thermo scientific, Bengaluru, India). The MIC of the samples was determined from the absorbance by comparing to the controls. The MIC results are means of four replicates from two separate experiments.

Determination of minimum inhibition concentration against the test fungi

The MIC for all the selected SCF extracts was performed by the broth microdilution method (Gomez-Lopez et al. 2005). The fungal spore suspension was prepared into sterile 0.9% saline solution as stated earlier. The suspension was adjusted to yield final inoculum of 10^5 CFU/mL by diluting into RPMI-1640 media (with L-glutamine, without NaHCO_3 and supplemented with 2% glucose and 0.165 M MOPS (3-(N-morpholino) propanesulfonic acid), pH 7). Fungal spore suspensions were prepared as mentioned earlier and diluted with RPMI media to obtain a final inoculum of 10^5 CFU/mL in the assay for all the fungi (based on haemocytometer counts). A stock solution of each SCF extract was serially diluted in 96 well microtiter plate with RPMI media to obtain a concentration ranging from 4 to 2048 μ g/mL of DMSO. Wells containing RPMI media, inoculated broth and DMSO treated broth served as media, negative and diluent controls. The plates were incubated at 25 ± 2 °C for 48 to 72 h. Absorbance was measured at 492 nm using Varioskan flash reader (Thermo Scientific) at 48 and 72 h. Further, visual observation of hyphal growth of the fungi was also examined. The MIC of the samples was determined from the absorbance by comparing to the controls. The MIC results are means of four replicates from two separate experiments.

Determination of insect repellency of SCF-CO₂ extracts by contact assay with area preference method

Half filter paper discs (Whatman No. 1, diameter 9 cm) were prepared, and extracts were added to each half-disc, which were air-dried for 10 min. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a Petri dish (diameter 9 cm); the inner surface of the petri dish was smeared with liquid Teflon (insect anti-slip) to prevent insects escaping. The orientation of the seam was changed in the replicates to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Twenty adult insects were released in the middle of each filter-paper circle. Each concentration was tested 4 times, and insects that settled on each half of the filter paper disc were

counted at hourly intervals for 5 h. The equation of percentage repellency (PR) for a given treatment time is given as follows (1):

$$PR = [(Nc - Nt)/(Nc + Nt)] \times 100 \tag{1}$$

where Nc, the number of insects in the untreated (control); and Nt, treated areas, respectively. The averages were then assessed to the different class using the scale described by Jilani and Su (1983). PR > 0.01 to < 0.1 = class 0; 0.1 to 20 = class I; 20.1 to 40 = class II; 40.1 to 60 = class III; 60.1 to 80 = class IV; 80.1 to 100 = class V.

Statistical analysis

Values are expressed as mean ± standard error mean of three independent experiments done in triplicates. Statistical analysis was performed using SPSS 20 version (IBM SPSS Statistics 20), and significance between the groups was determined by one-way analysis of variance (ANOVA) with Tukey’s post *t* test. Cluster analysis was performed to group cases according to their similarity of the response. Based on the repellent activity, the hierarchical cluster analysis was performed with 48 data points corresponding to average PR values of the extracts. The Cluster Analysis was performed using SPSS 20 version with Hierarchical Cluster Between-groups linkage method and Squared Euclidean distance as a measurement. Generalized linear models (GLM) was fitted to assess the effects of extracts and concentrations on the repellency activity against insects. Value of *p* < 0.05 was considered as the significant difference between the groups.

Results and discussions

In vitro antimicrobial activity by disc diffusion method

Disc diffusion assay was performed as an initial screening process for selecting the extracts to determine the MIC

values. The diameter of inhibition zones (mm) of 16 extracts against five different bacteria was presented in supplementary Table 2. Most of the extracts exhibited an adequate inhibitory effect on *L. monocytogenes*, where ajwain and oregano showed 63 mm inhibition. Cinnamon, rosemary and Hop showed a positive impact on Gram-positive bacteria tested (*S. aureus*, *B. subtilis* and *L. monocytogenes*). In the present study, SCF extract of rosemary inhibited *S. aureus* with an inhibition zone of 19 mm but did not inhibit *E. coli*. In contrary, essential oil of rosemary was found to inhibit both *E. coli* and *S. aureus* with an inhibition zone of 19 mm at 1:4 dilution (Hosni et al. 2013). Santoyo et al. (2005) reported an inhibition zone of 18, 17 and 29 for *E. coli*, *B. subtilis* and *S. aureus*, respectively, when treated with 200 µL of SCF rosemary extract. In the present investigation, ajwain, thyme and oregano SCF extracts showed antibacterial effect on all the tested bacteria. The essential oil of thyme was found to inhibit both *E. coli* and *S. aureus* with an inhibition zone of 15 and 13 mm at 1:32 dilution, respectively (Hosni et al. 2013). While, Karakaya et al. (2011) reported that SCF extract of oregano didn’t show any inhibition zone against *E. coli*, *S. aureus* and *L. monocytogenes*. The difference in the results may be due to the variation in the bacterial strains used, method of extraction, concentration and difference in composition of the extract. However, our observations are in tune with Santoyo et al. (2006), who have also recorded an inhibition zone of 19, 20, 21 and 18 mm against *E. coli*, *B. subtilis*, *S. aureus* and *A. niger*, respectively, with SCF oregano extract. This inhibitory effect may be attributed to the major compound present in the oregano extract, carvacrol at 40% (Santoyo et al. 2006), while it is 50% in SCF extract used in the present study.

In the present study, inhibition zone was also determined against *A. ochraceous*, *A. flavus*, *A. niger*, *P. verrucosum*, and *A. parasiticus* using 10 supercritical extracts (Supplementary Table 3). Oregano, ajwain and thyme extracts were effective against all tested fungi. Curcuma extract had no inhibitory effect on any of the tested fungi. Basil and lemon myrtle extracts showed inhibition zone

Table 2 Minimum inhibitory concentration of supercritical critical fluid extracts of botanicals against selected fungal cultures

Extract	<i>A. niger</i>	<i>A. ochraceous</i>	<i>P. verrucosum</i>	<i>A. flavus</i>	<i>A. parasiticus</i>
Minimum inhibitory concentration (µg/mL)					
Ajwain	512	512	256	1024	1024
Oregano	512	512	128	512	256
Lemon myrtle	> 2048	2048	2048	ND	ND
Cinnamon	128	64	64	128	ND
Thyme	512	256	256	512	256
Clove	1024	1024	512	2048	512
Garlic	> 2048	ND	> 2048	ND	> 2048

ND Not determined

(> 12 mm) against all the tested fungi except for *A. parasiticus*. The essential oil (10 µL) of ajwain seeds showed 9 mm of inhibition zone against *A. niger* (Shokri et al. 2016), which was contrary to our results (> 80 mm inhibition) with SCF extract, which may be due to different extraction procedure. In hydrodistillation, essential oil may suffer chemical alteration if the compounds are sensitive (Sovilj et al. 2011). Moreover, recovery of essential oil is better in SCF compared to hydrodistillation (Khajeh et al. 2004).

Determination of minimum inhibition concentration against the test bacteria and fungi

Those extracts with an inhibition zone of above 15 mm were selected for determining the MIC against the food-borne pathogenic bacteria (Table 1). Hop extract was found to have least MIC of 32 µg/mL against *L. monocytogenes* and *B. subtilis*; 64 µg/mL against *S. aureus*. Even, Rój et al. (2015) reported a lower MIC value (20 µg/mL) for SCF extract of hop against *L. monocytogenes* and *S. aureus* and a higher MIC value of > 2560 µg/mL against *E. coli*. In the present study, sage, rosemary, licorice, thyme, and cinnamon showed 512 µg/mL of MIC against *S. aureus*. In contrast, with rosemary leaf SCF extract, Genena et al. (2008) have shown a MIC (µg/mL) of 1000 and a low MIC of 250 against *E. coli* and *S. aureus*, respectively. SCF extracts of oregano and thyme showed MIC of 200 µg/mL against *L. monocytogenes* (Gutierrez et al. 2009). Ivanovic et al. (2013) reported that a MIC (µg/mL) of 640 and 20 of clove bud and oregano SCF extract is required to inhibit the growth of *S. aureus*. While, SCF extract of Greek oregano was found to have MIC of 320 and 640 µg/mL against *S. aureus* and *E. coli*, respectively (Stamenic et al. 2014). Contrary to these reports, in our experiments, we found that a MIC of 256 µg/ml of oregano SCF extract is required to inhibit the growth of *S. aureus*. Lemon myrtle and cinnamon showed 512 µg/mL MIC of against *B. subtilis*. Ajwain and thyme showed 512 µg/ml of MIC, whereas, oregano showed 256 µg/mL of MIC against *S. enterica*. Ajwain showed MIC of 256 µg/mL against *E. coli*. SCF extracts of thyme and rosemary exhibited MIC of ≤ 40 and 640 µg/mL against *B. subtilis*, 640 and 2560 µg/mL against *E. coli*, respectively (Ivanovic et al. 2012). We observed inhibition with 256 µg/mL concentration against both *B. subtilis* and *E. coli* with SCF extract of thyme.

Cinnamon was found to be effective against all the tested fungi by MIC determination (Table 2). The MIC of 64 µg/mL for cinnamon was obtained against *P. verrucosum* and *A. ochraceous*, whereas, it was 128 µg/mL for *A. flavus* and *A. niger*. Cinnamon showed 128 µg/mL of MIC, whereas, oregano, ajwain and clove showed 512 µg/mL of

MIC against *A. niger*. The essential oil of *Trachyspermum ammi* showed MIC of 1500 µg/mL against *A. niger* (Shokri et al. 2016). In our study, ajwain and thyme showed 256 µg/mL of MIC, whereas, clove showed 512 µg/mL of MIC against *P. verrucosum*. Thyme and oregano showed 256 µg/mL of MIC, whereas, clove showed 512 µg/mL of MIC against *A. parasiticus*.

Repellent activity of supercritical extracts against three stored products insects

Supercritical fluid extracts were found to have a good insect repellent activity against all the three test insects used in the experiment, i.e., *R. dominica*, *T. castaneum*, *S. oryzae* (Figs. 1, 2, 3). Repellent effect of extracts significantly differed among insects (GLM, $p < 0.05$) and were positively associated with high concentration of extracts. In the present study, extracts exhibiting PR values > 40% were considered to be strong repellents. Hierarchical cluster analysis yielded different responses classes based on the similarity of behavioral response of the insect towards the extracts (Figs. 1, 2, 3). The PR values for almost all the tested extracts were higher than 80% at the test concentration of 0.06 µL/cm². At least 6 extracts did not exhibit a significant repellency even at the maximum test concentration against *R. dominica* and *S. oryzae*. Licorice, rosemary and garlic extracts did not cause any repellency against both *R. dominica* and *S. oryzae*. Extracts of margosa, basil, hop, schisandra, curcuma and clove bud, oregano, sage, hop were found to be repellent at least at one of the test concentration against *S. oryzae* and *R. dominica*, respectively. Fennel, ajwain, curcuma, thyme, lemon myrtle extracts had significant repellence at all the test concentration against *R. dominica* (Fig. 2). Whereas, *S. oryzae* was repelled by oregano, clove, cinnamon and lemon myrtle extracts (Fig. 3).

While testing the repellency activity, 11 out of 16 extracts showed Class-V repellency against *T. castaneum*. Hop, curcuma, thyme, ajwain and clove, cinnamon, lemon myrtle exhibited class IV repellency against *R. dominica* and *S. oryzae*, respectively (Fig. 4). Lemon myrtle and oregano showed good repellency against all the three tested coleopteran insects.

Conclusion

To summarize, hop extract resulted in better antibacterial activity against all the tested Gram-positive bacteria with a MIC of 32–64 µg/mL and oregano, thyme and ajwain extracts indicated broad-spectrum antifungal activity against all the tested fungi with MIC in the range of 128–1024 µg/mL. Though most of the extracts exhibited

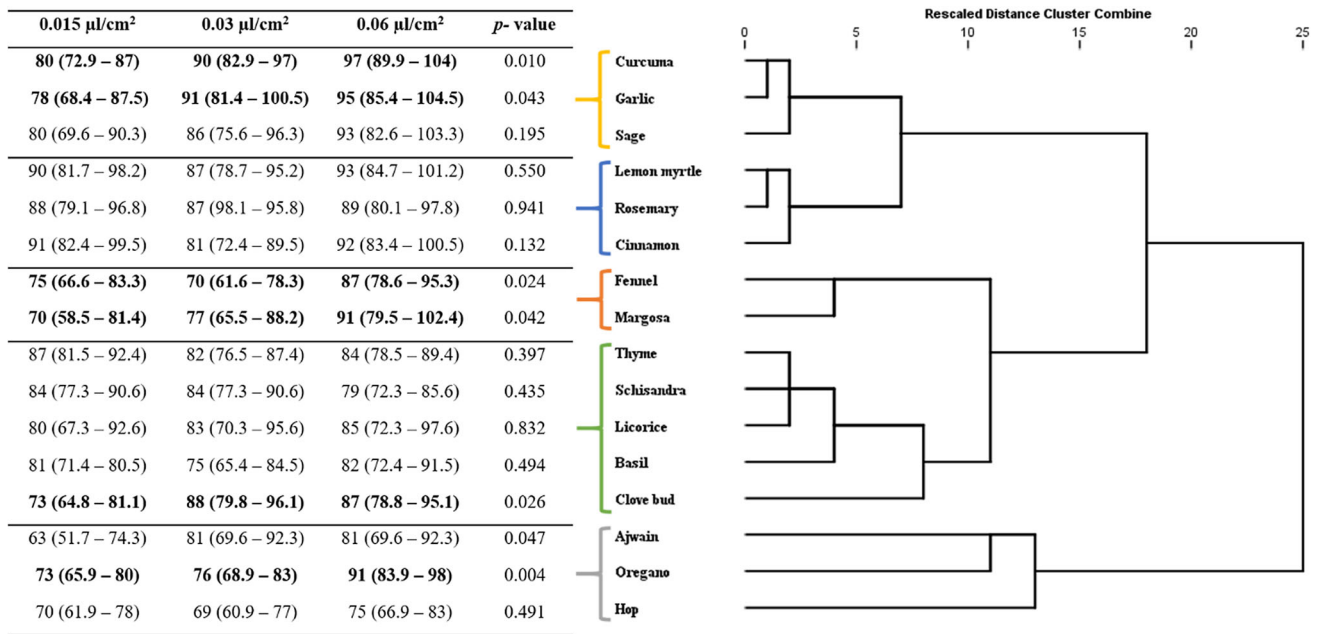


Fig. 1 Response of *T. castaneum* adults to the supercritical critical fluid extracts at 3 concentrations (0.015, 0.03, 0.06 $\mu\text{L}/\text{cm}^2$): dendrogram determined by hierarchical cluster analysis. Values in

bold lettering were significantly different with the Post Hoc Bonferroni correction method. *P* value of the GLM of the interaction concentration-product (dose-dependency) on the repellency

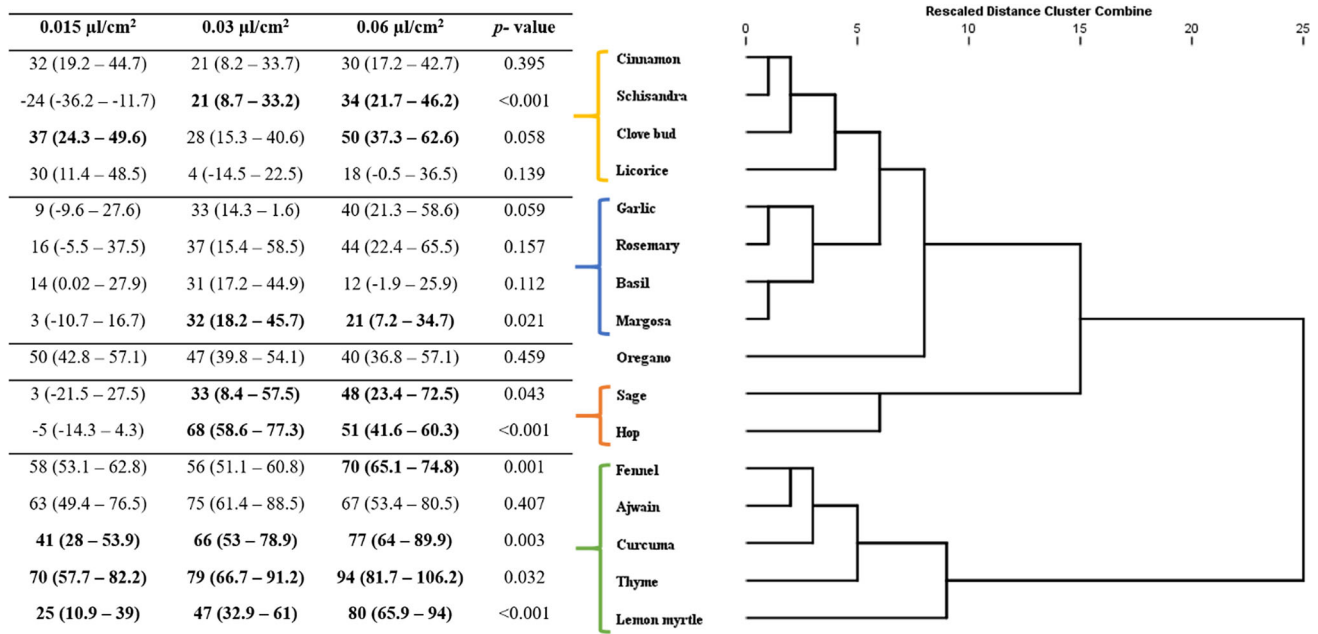


Fig. 2 Response of *R. dominica* adults to the supercritical critical fluid extracts of plant botanicals at 3 concentrations (0.015, 0.03, 0.06 $\mu\text{L}/\text{cm}^2$): dendrogram determined by hierarchical cluster analysis.

Values in bold lettering were significantly different with the Post Hoc Bonferroni correction method. *P*-value of the GLM of the interaction concentration-product (dose-dependency) on the repellency

class V repellency against *T. castaneum*, maximum repellency observed against *S. oryzae*, and *R. dominica* was limited to class IV. Extracts of hop, ajwain and thyme were found to have strong repellency against *T. castaneum* and *R. dominica*. Ajwain and thyme SCF extracts showed both insect repellent and antimicrobial activity, which can be

attributed to its high thymol content (> 50%). Since, natural compounds are always considered as safer food preservatives over synthetic chemicals, these SCF extracts have a potential scope in food grain storage application. Efficacy of these SCF extracts as fumigants and antimicrobials in food system can be explored in future studies.

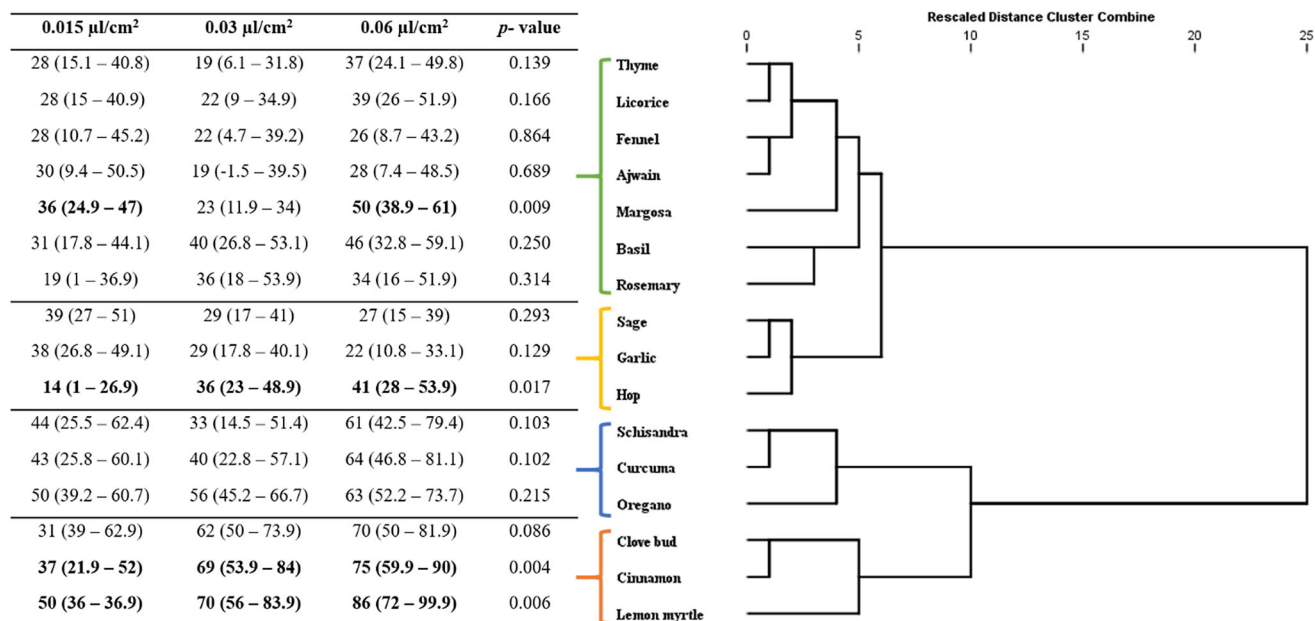


Fig. 3 Response of *S. oryzae* adults to the supercritical critical fluid extracts of plant botanicals at 3 concentrations (0.015, 0.03, 0.06 $\mu\text{L}/\text{cm}^2$); dendrogram determined by hierarchical cluster analysis. Values

in bold lettering were significantly different with the Post Hoc Bonferroni correction method. *P* value of the GLM of the interaction concentration-product (dose-dependency) on the repellency

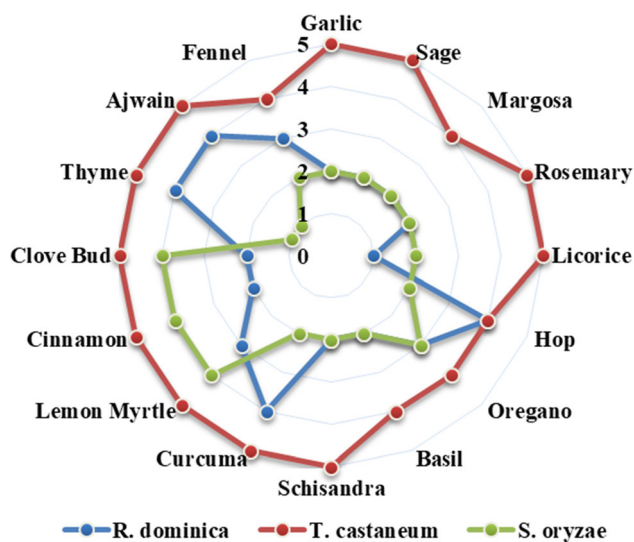


Fig. 4 Repellency response of *T. castaneum*, *R. dominica* and *S. oryzae* adults to the supercritical critical fluid extracts of plant botanicals. (PR values > 0.01 to < 0.1 = class 0; 0.1 to 20 = class 1; 20.1 to 40 = class 2; 40.1 to 60 = class 3; 60.1 to 80 = class 4; 80.1 to 100 = class 5)

Acknowledgements The authors thank Director, CSIR-Central Food Technological Research Institute, Mysuru for the support and facilities provided for this study. This research was financially supported by Department of Biotechnology, Government of India (BT/IN/Fin-nish/06/AP/2013).

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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