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Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains

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T. MANGENA AND N.Y.O. MUYIMA. 1999. Essential oils are frequently used for flavour and fragrance in the perfume, pharmaceutical, cosmetic and food industries. The antimicrobial activities of the essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* were tested against 41 microbial strains. The test organisms were selected on the basis of their significance as food spoilage and/or poisoning, common human and plant pathogens. The agar diffusion assay was performed using nutrient agar and antibiotic medium. All the oils tested displayed some antimicrobial activities. However, the efficiency differed and depended both on the type and concentration of the oil, as well as the test microbial strain. *Artemisia afra and R. officinalis* showed similar and higher antimicrobial activity than *P. incana*. Due to their broad antimicrobial activities, the essential oils of the above plants growing in Eastern Cape may have preservative potential for the food and cosmetic industries.

INTRODUCTION

The Eastern Cape region of South Africa has veld types with arguably the richest composition of indigenous aromatic plant species in the whole of Southern Africa (Graven *et al*. 1987). Prominent among these aromatic plants are indigenous species such as *Artemisia afra* and *Pteronia incana*, as well as foreign species such as *Rosmarinus officinalis*.

Artemisia afra is one of the oldest known medicinal plants in Southern Africa. It is used to cure diseases such as the common cold, diabetes mellitus, bronchial complaints and stomach disorders (Graven *et al*. 1990). The volatile oil of *A. afra* has been reported to have several biological activities, notably antibacterial, antifungal and antioxidative properties. According to Graven and collaborators, the oil has value as a biological agent with greater antimycotic than antibacterial activity (Graven *et al*. 1992).

Pteronia incana produces a yellowish oil with a strong fragrance. Interest in this oil has been primarily due to its unique odour properties which make it suitable for use as a fragrance (Bruns and Meiertoberens 1987). *Pteronia incana*, compared with *A. afra* and *R. officinalis*, is not well documented. Apart from the chemical composition, there is no further information, for example on antimicrobial activity, available about this oil.

Rosmarinus officinalis produces a colourless or pale yellow oil with the characteristic of rosemary and a warm camphoraceous taste. It is used in the perfume industry and as a flavour agent. Several compounds found in this oil have been reported to be inhibitory to micro-organisms (Guenther 1974; Tyler *et al*. 1976; Deans and Svoboda 1993). It is, however, known that variants of the same species can differ in their constituent essential oils (Janssen *et al*. 1987). Although the essential oil of *R. officinalis* from the Mediterranean region appears to be well studied, its composition, as well as its antimicrobial properties, could differ from those of the variants growing in South Africa because of the differences in the two environments.

Several components of essential oils have been reported to possess biological activities. Camphene, for instance, was found to be effective against *Staphylococcus aureus* and *Escherichia coli*. α-Pinene significantly reduced growth of *Erwinia amylovora* while β-pinene was inhibitory to bacteria, especially at higher bacterial populations (Scortichini and Rossi 1991). Large amounts of monoterpene hydrocarbons and/or sesquiterpenes were, however, found to lower the antimicrobial activity of essential oils (Chalchat *et al*. 1997).

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In general, Gram-negative bacteria have been found to be more resistant to essential oils than Gram-positive bacteria, possibly because of their cell wall lipopolysaccharide (Farbood *et al.* 1976; Outtara *et al.* 1997). A number of factors hamper the evaluation of the antimicrobial activity of essential oils, namely, their volatility at room temperature, their water insolubility, and their complexity (Janssen *et al.* 1987).

Essential oils have many uses in day-to-day life as well as in industry. They are widely used as flavouring for foods and confections, and as spices. They are also used in the perfume and cosmetic industry for fragrance. They are incorporated in the production of modern skin products because of the complexity of their active compounds, strong fragrant properties and better marketing value. They have been proposed as natural conservation agents for cosmetic preparations because of their antimicrobial activities (Manou *et al.* 1998).

In view of the increasing use of essential oils in the food, cosmetic and pharmaceutical industries, it is important to examine the oils from indigenous plants for antimicrobial activities. A comparison of their activities with that of a well known oil such as rosemary from the same environment should give a better indication as to whether essential oils of aromatic plants native to the Eastern Cape have any value as candidate biological control agents in the above industries. The aim of this study was to carry out a comparative analysis of the antimicrobial activities of essential oils of two aromatic plants indigenous to the Eastern Cape, namely *A. afra* and *P. incana*, and *R. officinalis*, an aromatic plant from the Mediterranean region grown in the Eastern Cape.

MATERIALS AND METHODS

Preparation and chemical analysis of essential oils

Artemisia afra and *R. officinalis* were collected from the University of Fort Hare Research farm in Alice (26°50′E, 332°47′S) while *P. incana* was collected near Doubledrift game reserve, a distance of about 90 km from Alice, where it grows in the wild. The oils were extracted from the fresh leaves of the plants by water distillation. They were then kept in bottles covered in aluminium foil at 4 °C to prevent the negative effect of light, especially direct sunlight. GC-MS analysis (using the 5890 A Gas Chromatograph and 5970 series Mass Selective Detector from Hewlett Packard, Pennsylvania, USA) was carried out to determine the chemical composition of the oils.

Cultures of bacteria and yeasts

Bacteria and yeast strains were selected on the basis of their significance in food spoilage and/or poisoning, and as common human and plant pathogens. *Acinetobacter* cultures were obtained from the Environmental Biotechnology Laboratory, University of Pretoria. The other bacterial strains were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, unless indicated otherwise. The bacterial stock cultures were maintained on nutrient agar slants. A loopful of bacterial cells from the agar slant was inoculated in 100 ml nutrient broth or antibiotic medium 2 (Difco) contained in a 250 ml side-arm Erlenmeyer flask. Incubation was done at 25 °C for 16–20 h. After incubation, the cultures were diluted to give an $O.D._{600}$ of 0·2 using 0·1 mol l^{-1} phosphate buffer for agar seeding.

The yeast stock cultures were obtained from the University of Orange Free State Yeast Collection (UOFS Y), Bloemfontein. These cultures were maintained on yeast-malt-peptone-glucose (YMPG) agar slants (Conner and Beuchat 1984). A loopful of yeast cells from an agar slant was inoculated in 100 ml YMPG broth contained in a 250 ml side-arm Erlenmeyer flask and incubated at 30 °C, 150 rev min^{-1} for 44–48 h. Sterile vitamin solution (thiamine hydrochloride, riboflavin, inositol and calcium pantothenate at final concentrations of 0·5 mg l^{-1} each, and para-aminobenzoic acid at 0·25 mg l^{-1}) was added to a *Hyphopichia burtonii* culture. Yeast cultures were diluted to give an $O.D._{600}$ of 0·3 using 0·1 mol l^{-1} phosphate buffer for agar seeding.

Testing of essential oils for antibacterial activities

The agar diffusion assay was used for the determination of antimicrobial activities of the oils. Nutrient and antibiotic medium agars were used according to the growth requirements of the test organism previously determined. A base layer of 5–6 ml was prepared (same media as above) and 4 ml of the appropriate molten agar were inoculated with microbial suspensions of known density for the seed layer. Discs 6 mm in diameter were soaked with 15 μl of the test oil and placed on the set agar. Bacterial plates were incubated at 25 °C for 48 h and yeasts were incubated at 30 °C for 96 h, after which zones of inhibition were measured. All the plates were incubated in an upright position to prevent the disc from falling as well as to allow the oil to sink into the agar. Ethanol (95%) was used as a negative control in all the plates while novobiocin (0·5 μg ml^{-1}) and cycloheximide (0·2 μg ml^{-1}) were used as positive controls for bacteria and yeasts, respectively. All the tests were replicated four times.

RESULTS

The main components of the essential oils identified by the GC-MS analysis are given in Table 1 while the results for the antimicrobial activity tests are given in Tables 2 and 3 for bacteria and yeasts, respectively.

Table 1 Composition of the essential oils from *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* as identified by GC-MS analysis

Compound	Concentration (% peak area)		
	A. afra	*P. incana*	*R. officinalis*
Bornylacetate	—	—	3·17
Camphene	—	—	6·08
Camphor	—	—	30·12
1,8-Cineole	8·19	—	31·12
o-Cymene	—	4·47	—
p-Cymene	—	18·90	—
Limonene + 1,8 cineole	—	20·82	—
Mycerene	—	0·49	—
α-Pinene	—	19·08	18·18
β-Pinene	—	32·20	2·58
α-Thujone	78·68	—	—
β-Thujone	13·13	—	—
Verbenone	—	—	4·12
Unidentified	—	4·04	3·88

—, Compound not identified.

DISCUSSION

Chemical analysis revealed that all the oils tested contained 1,8-cineole, with *R. officinalis* containing the highest percentage. The major constituents of *A. afra*, α-and β-thujone, were not found in any of the other oils. *Pteronia incana* was the only oil having o- and p-cymene amongst its constituents.

Artemisia afra oil had a higher α-thujone and a lower 1,8-cineole content than that reported by Graven *et al.* (1992) and the oil did not contain any camphor. The differences can probably be attributed to the different genotypes of the plant material used (Graven *et al.* 1990).

Pteronia incana oil had a higher content of *p*-cymene as well as limonene + 1,8 cineole, and lower content of mycerene than that reported by Bruns and Meiertoberens (1987). Moreover, the oil contained *o*-cymene, which had not been reported. The α- and β-pinene components were present in percentages similar to those reported by several investigators (Piprek *et al.* 1982). Overall, chemical composition indicates that *P. incana* and *R. officinalis* oils have a higher diversity of chemical compounds than *A. afra* oil.

The results of the antibacterial tests indicated that *A. afra* has a broad spectrum of inhibitory activity. *Streptococcus pyogenes*, *Listeria monocytogenes* and *Acinetobacter johnsonii* showed the highest sensitivity to *A. afra* oil. *Acinetobacter calcoaceticus* and *Pseudomonas aeruginosa* had lower zones of inhibition than those reported by Graven and collaborators for the same organisms. Similar zones of inhibition were obtained for *Staph. aureus* and *Bacillus subtilis*, while *Erwinia carotovora* and *Micrococcus luteus* had higher zones of inhibition (Graven *et al.* 1992). The differences can possibly be explained by the different methods used to assess the antimicrobial activity.

Pteronia incana oil also displayed a fairly broad spectrum of antibacterial activity, particularly at higher concentrations. However, it was less efficient than *A. afra* and *R. officinalis* oils. *Enterobacter cloacae* and *Staph. aureus* showed the highest sensitivity to *P. incana* oil.

Rosmarinus officinalis oil displayed similar antibacterial activity to *A. afra* oil. *Acinetobacter lwoffi, Shigella flexneri* and *Strep. pyogenes* showed the highest sensitivity to *R. officinalis* oil. *Enterobacter aerogenes*, *Ac. calcoaceticus*, *B. subtilis*, *Erw. carotovora, Staph. aureus* and *Yersinia enterocolitica* showed a lower sensitivity to this oil compared with the findings of Deans and Ritchie (1987). *Salmonella enteritidis* and *Salm. typhi* displayed the lowest sensitivity, while *Ps. aeruginosa* and *Ps. fluorescens* PSE showed no sensitivity to any of the oils tested. *Streptococcus pyogenes* was the most affected by the essential oils. In general, Gram-positive bacteria seemed to be more sensitive to the oils than Gram-negative bacteria as the former displayed 100% sensitivity to all the oils tested undiluted. This is in agreement with observations by other authors that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria (Farbood *et al.* 1976; Outtara *et al.* 1997). Although all the oils tested displayed some antibacterial activities, the efficiency differed and depended both on the type and concentration of the oil as well as on the bacterial strain used.

For the yeasts, *A. afra* oil displayed notable inhibitory activity against all the strains tested. *Hanseniaspora vinae* UOFS YO151, *Saccharomyces cerevisiae* UOFS Y150 and UOFS Y0154 were most affected by the oil while *Zygosaccharomyces bailii* UOFS YC757 and *Dekkera bruxellensis* UOFS YC506 were least affected. All the yeasts tested showed sensitivity to *R. officinalis* oil. *Dekkera bruxellensis* UOFS YC506 and *H. vinae* UOFS Y151 were most affected by *R. officinalis* oil while *H. burtonii* UOFS YC608, *S. cerevisiae* UOFS Y0149 and *T. delbruckii* UOFS Y0159 were most affected by *P. incana* oil. However, only half the yeasts tested were sensitive to the latter oil in an undiluted form. *Artemisia afra* oil seemed to produce zones of inhibition with larger diameters than those of *P. incana* and *R. officinalis* oils. With respect to their inhibitory properties towards yeasts at the lower dilution, *A. afra* oil showed a much broader activity than either *R. officinalis* or *P. incana*, suggesting that this essential oil may have a lower minimum inhibitory concentration value. The inhibitory activity depended on both the type and concentration of the oil, as well as the yeast strain.

Although *A. afra* oil may be more inhibitory at lower concentration towards yeasts, its overall antimicrobial activity was found to be similar to that of *R. officinalis* oil; *P. incana*

Table 2 Antimicrobial activity of undiluted, 1 : 1 and 1 : 2 dilutions of the essential oil of *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* expressed as diameter of zone of inhibition in millimetres (including disc diameter of 6 mm) against selected bacterial strains

	Diameter of zone of inhibition (mm)								
	Artemisia afra			*Pteronia incana*			*Rosmarinus officinalis*		
Test Organism	Neat	1 : 1	1 : 2	Neat	1 : 1	1 : 2	Neat	1 : 1	1 : 2
Acinetobacter calcoaceticus ATCC23055	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	12·0 ± 2·8	8·0 ± 0·0	8·0 ± 0·0
Ac. lwoffii ATCC21130	11·0 ± 1·4	9·0 ± 1·4	6·0 ± 0·0	13·0 ± 1·4	10·0 ± 0·0	6·0 ± 0·0	20·0 ± 1·4	14·0 ± 1·4	ND
Ac. johnsonii ATCC17906	23·0 ± 1·4	17·0 ± 1·4	6·0 ± 0·0	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	16·0 ± 0·0	12·5 ± 1·7	9·5 % 1·9
Bacillus cereus ATCC10876	9·5 ± 1·0	8·0 ± 0·0	6·0 ± 0·0	13·0 ± 1·4	10·0 ± 2·3	6·0 ± 0·0	11·5 ± 2·1	10·0 ± 0·0	8·0 ± 0·0
B. subtilis	14·5 ± 2·4	10·0 ± 1·5	9·0 ± 1·2	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	14·0 ± 0·0	10·0 ± 1·9	9·0 ± 1·4
Enterobacter aerogenes	10·5 ± 1·0	9·0 ± 1·2	8·5 ± 1·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	11·0 ± 1·2	9·0 ± 1·2	8·0 ± 1·2
Ent. cloacae	10·5 ± 1·9	10·0 ± 0·0	8·5 ± 1·2	20·0 ± 0·0	10·0 ± 0·0	10·0 ± 0·0	13·6 ± 1·4	9·5 ± 1·2	8·0 ± 0·0
Erwinia chrysanthemi	11·0 ± 1·4	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0
Erw. carotovora	18·5 ± 1·2	14·0 ± 0·0	10·0 ± 0·0	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	14·0 ± 1·6	11·5 ± 1·4	9·0 ± 2·0
Escherishia coli K12	17·5 ± 2·0	16·5 ± 2·0	10·5 ± 1·2	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	17·5 ± 2·3	11·5 ± 1·2	8·0 ± 0·0
E. coli (SR)*	9·5 ± 1·2	10·0 ± 1·2	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	12·0 ± 0·0	8·0 ± 1·9	9·0 ± 1·4
Listeria monocytogenes ATCC7644	25·0 ± 1·4	18·0 ± 2·8	ND	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	17·0 ± 1·4	14·0 ± 0·0	9·0 ± 1·4
Micrococcus luteus	12·0 ± 0·0	12·0 ± 0·0	10·0 ± 0·0	10·0 ± 0·0	10·0 ± 0·0	6·0 ± 0·0	16·0 ± 0·0	16·0 ± 0·0	10·0 ± 0·0
Proteus mirabilis ATCC43071	12·0 ± 2·0	10·0 ± 0·0	8·0 ± 0·0	10·0 ± 2·8	10·0 ± 0·0	6·0 ± 0·0	12·5 ± 1·0	9·0 ± 0·0	9·0 ± 1·2
Pseudomonas aeruginosa	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0
Ps. fluorescens PSE	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0
Salmonella enteritidus	14·0 ± 2·8	12·0 ± 0·0	6·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	10·0 ± 0·0	9·0 ± 1·4	6·0 ± 0·0
Salm. newport	14·5 ± 1·2	13·5 ± 2·6	10·5 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	16·0 ± 2·5	10·0 ± 2·0	8·0 ± 1·2
Salm. poona NCTC4840	12·0 ± 2·3	10·0 ± 1·2	6·0 ± 0·0	11·0 ± 1·2	9·0 ± 1·2	6·0 ± 0·0	12·0 ± 1·6	11·5 ± 1·0	8·0 ± 0·0
Salm. thompson	13·5 ± 1·4	9·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	16·0 ± 0·0	12·0 ± 0·0	10·0 ± 0·0
Salm. typhi	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	9·0 ± 1·4	8·0 ± 0·0	6·0 ± 0·0
Salm. typhimurium (SR)*	11·0 ± 1·8	10·5 ± 0·8	8·5 ± 1·6	13·0 ± 1·4	6·0 ± 0·0	6·0 ± 0·0	12·0 ± 2·5	10·5 ± 2·3	8·0 ± 1·0
Salm. typhimurium (WT)†	11·6 ± 4·9	9·5 ± 0·6	8·0 ± 0·0	12·0 ± 0·0	10·0 ± 0·0	6·0 ± 0·0	11·7 ± 1·5	8·7 ± 1·2	8·0 ± 0·0
Shigella flexneri ATCC12022	13·0 ± 3·4	9·0 ± 1·2	6·0 ± 0·0	13·0 ± 1·4	10·0 ± 0·0	6·0 ± 0·0	15·5 ± 2·3	10·5 ± 1·0	8·0 ± 0·0
Sh. sonnei ATCC9290	9·5 ± 1·0	9·0 ± 1·2	6·0 ± 0·0	10·5 ± 1·0	9·5 ± 1·0	8·0 ± 0·0	12·5 ± 1·0	10·0 ± 0·0	8·5 ± 1·2
Staphylococcus aureus	14·0 ± 0·0	10·0 ± 0·0	6·0 ± 0·0	24·0 ± 0·0	15·5 ± 1·4	6·0 ± 0·0	12·0 ± 2·3	9·0 ± 1·2	8·0 ± 0·0
Staph. epidermidus	10·0 ± 0·0	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	10·0 ± 0·0	10·0 ± 0·0	6·0 ± 0·0
Streptococcus pyogenes ATCC19615	28·0 ± 0·0	24·0 ± 0·0	12·0 ± 0·0	14·0 ± 0·0	12·0 ± 0·0	6·0 ± 0·0	12·0 ± 0·0	12·0 ± 0·0	10·0 ± 0·0
Yersinia enterocolitica ATCC9610	15·5 ± 2·5	10·5 ± 1·2	6·0 ± 0·0	11·0 ± 1·4	9·0 ± 1·4	8·0 ± 0·0	16·0 ± 0·0	13·0 ± 1·4	10·0 ± 0·0
Total sensitive organisms	27	27	13	19	15	3	27	27	22
% sensitive organisms	93	93	45	66	52	10	93	93	76

* Streptomycin resistant strain.

† Wild type.

ND, not determined; Neat – undiluted oil; 1 : 1 and 1 : 2 – oil dilutions (v/v) with 95% ethanol. Results are the mean diameter of zone of inhibition followed by the standard deviation.

Table 3 Antimicrobial activity of undiluted, 1 : 1 and 1 : 2 dilutions of the essential oil of *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* expressed as diameter of zone of inhibition in millimetres (including disc diameter of 6 mm) against selected yeast strains

	Diameter of zones of inhibition (mm)								
	Artemisia afra			*Pteronia incana*			*Rosmarinus officinalis*		
Test organism	Neat	1 : 1	1 : 2	Neat	1 : 1	1 : 2	Neat	1 : 1	1 : 2
Dekkera bruxellensis UOFS YC506	12·0 ± 0·7	9·0 ± 1·4	8·0 ± 0·0	9·5 ± 1·9	8·0 ± 1·2	6·0 ± 0·0	14·0 ± 2·3	10·0 ± 2·3	8·0 ± 1·2
Hanseniaspora vinae UOFS Y0151	21·0 ± 1·4	16·5 ± 0·7	13·0 ± 1·4	9·0 ± 1·2	8·0 ± 0·0	8·0 ± 1·2	18·0 ± 2·3	12·5 ± 2·0	9·0 ± 1·2
Hyphopichia burtonii UOFS YC608	19·5 ± 1·9	15·8 ± 0·5	12·3 ± 0·5	10·8 ± 3·2	8·0 ± 0·0	8·0 ± 1·2	12·0 ± 1·2	9·5 ± 1·2	6·0 ± 0·0
Pichia anomala UOFS Y0157	14·5 ± 0·7	12·0 ± 0·0	11·5 ± 0·7	8·5 ± 1·2	6·0 ± 0·0	6·0 ± 0·0	11·5 ± 3·1	10·5 ± 0·6	9·5 ± 1·2
P. fabianii UOFS Y0152	12·3 ± 3·2	9·5 ± 1·9	8·3 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	10·5 ± 1·0	8·0 ± 1·4	8·0 ± 1·9
P. jadinii UOFS Y0156	13·8 ± 2·7	12·0 ± 2·3	10·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	11·5 ± 2·2	10·0 ± 2·8	9·0 ± 0·7
Saccharomyces cerevisiae UOFS Y0149	18·5 ± 2·5	15·3 ± 0·9	10·5 ± 2·5	11·5 ± 4·3	8·5 ± 1·6	8·0 ± 1·2	11·5 ± 2·5	10·0 ± 2·3	6·0 ± 0·0
S. cerevisiae UOFS Y150	20·0 ± 1·5	15·5 ± 0·6	13·5 ± 0·6	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	12·5 ± 1·2	10·5 ± 0·6	10·0 ± 0·0
S. cerevisiae UOFS Y0154	22·3 ± 2·8	15·5 ± 2·8	13·0 ± 2·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	12·8 ± 1·5	9·8 ± 0·6	8·0 ± 1·4
Torulaspora delbruckii UOFS Y0159	12·0 ± 0·0	10·0 ± 2·3	8·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	13·5 ± 1·5	10·5 ± 1·2	10·0 ± 0·0
T. delbruckii UOFS Y160	10·0 ± 0·0	10·0 ± 0·0	8·0 ± 0·0	12·0 ± 0·0	10·0 ± 0·0	8·0 ± 2·8	10·0 ± 0·0	8·0 ± 0·0	8·0 ± 0·0
Zygosaccharomyces bailii UOFS YC757	11·5 ± 0·6	9·0 ± 1·2	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	6·0 ± 0·0	11·2 ± 1·0	9·3 ± 1·4	9·0 ± 1·9
Total sensitive organisms	12	12	11	6	5	4	12	12	10
% sensitive organisms	100	100	92	50	42	33	100	100	83

Neat = undiluted oil; 1 : 1 and 1 : 2 oil dilutions with 95% ethanol (v/v).
Results are the mean diameter of zone of inhibition followed by the standard deviation.

oil had a greater antibacterial than antiyeast activity but its overall antimicrobial activity was lower compared with *A. afra* and *R. officinalis* oils. Nevertheless, *Ent. cloacae*, *Erw. chrysanthemi* and *Staph. aureus* appeared to be more sensitive to *P. incana* oil than to *A. afra* and *R. officinalis* oils. *Bacillus cereus*, *Erw. chrysanthemi*, *Salm. typhimurium* (wild type) and *Staph. aureus* seemed to be more affected by oils from the two indigenous plants than by the Mediterranean species. Although *A. afra* compared favourably with *R. officinalis*, its high composition of α- and β-thujone might hamper its use as a food preservative. However, the fact that *A. afra* oil was active at lower concentrations could possibly alleviate the limitations posed by thujone toxicity. All three oils had some antimicrobial activity although the extent differed. The higher the concentration of the oil, the more antimicrobial activity it showed. It is interesting to note that the oils showed antimicrobial activity towards organisms of importance to food spoilage and/or poisoning, as well as to those of interest to the medical field, such as the salmonellas, shigellas and staphylococci. In view of their broad antimicrobial activity, the essential oils of the above plants may have interesting preservative potential for industrial applications. The oils can be suggested as candidate natural conservation agents in the cosmetic and/or food industries, and as active ingredients in medical preparations.

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