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Original Article

Chemical constituents of the essential oil, antioxidant and antibacterial activities from *Elettariopsis curtisii* Baker.

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Abstract

Elettariopsis curtisii Baker, the culinary and medicinal herb, was investigated to elucidate its chemical constituents and determine antioxidant and antibacterial activities. The essential oil of *E. curtisii* was obtained by steam distillation of fresh rhizomes in a maximum yield of 0.63%. GC-MS data indicated the presence of six compounds, of which *trans*-2-decenal (78.03%) was the principal constituent. The essential oils and also the hexane, dichloromethane and methanol extracts from the rhizomes and leaves were assessed for antioxidant and antibacterial activities. In an evaluation of antioxidant activity, the crude dichloromethane extract of the leaves exhibited the highest scavenging effect on the DPPH radical with an EC_{50} of 0.28±0.01 mg/mL. The leaf dichloromethane extract also had the highest total phenol concentration, (73.4± 2.80 mg GA/g of extract) whereas the crude methanol extract from the rhizomes had the highest reducing power with an EC_{50} of 2.07±0.06 mg/mL. In terms of antibacterial activity, the essential oil (distilled from either the leaves or the rhizomes) displayed the highest inhibitory activity, with the same MID value of 1 mg/disc against 5 strains of bacteria, *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Sarcina* sp. and *Pseudomonas aeruginosa.*

Keywords: *Elettariopsis curtisii*, essential oil, antioxidant, antibacterial activity

1. Introduction

Elettariopsis curtisii Baker belongs to the family Zingiberaceae. The genus of Elettariopsis includes 30 species, of which 3 are found in Thailand, namely *E. curtisii, E. smithiae and E. tribola* (Sirirugsa, 1989; Kharukanunt and Promchum, 2001). *E. curtisii* is a perennial rhizomatous herb that is widely distributed in the south of Thailand, to a lesser extent in the north of the country, (Bumrungthai *et al.*, 2004) and in Malaya and Borneo (Skinner, 1999; Cowley, 2006). *E. curtisii* has rhizomes that are white, creepy and slender, with pseudo stems at intervals of about 90 cm. In Thailand, *E. curtisii* is commonly known as "Putsing" and is highly prized in folk medicine for its carminative properties. The herb Putsing has a strong odor and the whole plant is used medicinally, either in the form of a decoction or a bath (ARCBC, 2004). In rural areas of Thailand the fresh leaves are also eaten as salad vegetables, and are added to chilli paste to enhance its flavor and stimulate gastric secretion.

In recent years there has been an upsurge of interest in herbs, spices and vegetables that contain natural substances with health-promoting or pharmaceutical properties. Many vegetables are sources of dietary antioxidants (Suhaj, 2006; Miean and Mohamed, 2001; Velioglu *et al*., 1998) that may help to mitigate a range of chronic health problems by reacting with free radicals. Free radicals, especially reactive oxygen and nitrogen species have been implicated as mediators of chronic deteriorative inflammatory and autoimmune diseases, including rheumatoid arthritis, cancer, diabetes and cardiovascular disease etc (Tsao and Deng, 2004). Furthermore, microorganisms are causative agents in both food poisoning and food deterioration, and so antimicrobial agents from plants are of interest as natural preservatives. Various plants in the family Zingiberaceae, including *Zingiber*

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officinale, *Z. zerumbet*, *Z. cassumunar*, *Curcuma xanthorrhica* (Habsah *et al*. 2000; Chanwithesuk *et al*., 2005; Hinneburg *et al*., 2005), *C. longa* (Selvam *et al*., 1995; Monkolsilp *et al*., 2004) and *C. zedoria* (Mau *et al*., 2003) show antioxidant and antimicrobial activity.

However, apart from an isolated study into the insecticidal properties of *E. curtisii*, (Naovanit, 1998) to our knowledge the phytochemistry and antimicrobial properties of this plant remain unreported. Hence, this is the first publication to describe the composition of the volatile oil from *E. curtisii*, and disclose its *in vitro* antioxidant and antibacterial activities.

2. Materials and Methods

2.1 Plant materials

Plant materials were collected from Pattani province and the specimen was identified by Mr Bamroong Kharukanunt and deposited at Chulabhorn Development Project, Number 7, Yala. The plants were separated into rhizomes, roots, leaves and pseudo stems. All parts of the plant were cleaned and washed with water. Some fresh material was used immediately to extract the essential oils. The remainder of the plant was chopped into small pieces and dried in a hot air fan-oven before solvent extraction.

2.2 Isolation of essential oils

The fresh plant material, which consisted of rhizomes, (382 g) leaves, (207 g) roots (202 g) and pseudo stems (388 g) was chopped into small pieces and ground in a blender. The ground materials were suspended in water and hydrodistilled using a Dean-Stark apparatus to obtain volatile oils in 2.4, 0.24, 0.48 and 0.78 g yield, respectively. These oils were dried over anhydrous sodium sulfate and stored under nitrogen in a freezer at -20°C prior to analysis. Constituents of the rhizome essential oil were analyzed by GC-MS.

2.3 Solvent extraction

The dried rhizomes (464 g) and leaves (640 g) were ground to fine powders separately, by milling. The resulting materials were extracted successively with hexane, dichloromethane and methanol for 48 h, extracting three times with each solvent. Each combined extract was evaporated under reduced pressure to yield crude hexane, (32.1 g) dichloromethane, (10.0 g) and methanol extracts (19.4 g) of the leaves and the crude hexane, (55.0 g) dichloromethane (34.0 g) and methanol extracts (29.8 g) of the rhizomes, which were used for determination of the total phenol concentration, as well as antioxidant and antibacterial activities.

2.4 GC-MS analysis

GC-MS analysis was carried out on a HP 5890 Gas Chromatograph-HP 6972 Mass Selective Detector. The GC was equipped with a HP-5 column (10 m × 0.17 mm × 0.1 mm i.d.). The injector temperature was 200°C and the oven temperature was programmed at a rate of 10°C/minute from 60°C (1 minute) to 200°C (5 minutes). The spectrometer was run in electron impact mode, scanning at 33-550 amu with a solvent delay time of 3.0 minutes and a transfer line temperature of 200°C. The relative proportion of each individual component of the oil was expressed as a percentage of the total peak area.

2.5 Bacterial cultures

Five strains of bacteria, *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Sarcina* sp. and *Pseudomonas aeruginosa* were obtained from the Biology Laboratory of the Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus. Each microorganism was cultured in nutrient agar (NA) at 37°C for 24 h. The cultures were adjusted to a concentration of 10^6-10^7 cells/mL in 0.1% peptone by counting with a haemacytometer. The cultures of bacteria were maintained on nutrient agar at 4°C throughout the study and used as stock cultures.

2.6 Antibacterial assays

The antibacterial activity of the essential oils and crude extracts, including the hexane, dichloromethane and methanol extracts of the leaves and rhizomes was evaluated by the standard disc diffusion method (Habsah *et al*., 2000). Each sample was adjusted to a concentration of 0.1-1.5 g/mL using dichloromethane as solvent. A 10 mL aliquot of each sample was injected into sterilized discs of 6 mm diameter. Each disc contained a different extract at a concentration of 1-15 mg/disc. The discs were allowed to dry in a biological safety cabinet and then transferred to a solid agar medium and incubated at 37°C for 24 h. The minimal inhibition dose (MID) for each extract was recorded in mg/disc. Next, three discs were impregnated with one of each of the extraction solvents, (hexane, dichloromethane, and methanol) allowed to dry, and then used as negative controls. A further three discs were treated with the reference antibiotics from Oxoid, ciprofloxacin (5 µg), tetracycline (30 µg) and penicillin G (10 units), which were used as positive controls. All experiments were performed in triplicate.

2.7 Total phenol concentrations

Total phenol concentrations were determined using a Folin-Ciocalteu assay, as described by Amin (2006). A 0.1 mL aliquot of sample (5 mg/mL in methanol) was added to 0.75 mL of Folin-Ciocalteu reagent, which was previously diluted 10-fold with distilled water. The mixture was allowed to stand at room temperature for 5 min, and then 0.75 mL of 10 % sodium bicarbonate was added, followed by 10 mL of

distilled water. After standing for 90 min at room temperature, absorbance was then measured at 725 nm. Gallic acid was used as the standard phenol and the total phenol concentration was expressed as gallic acid equivalents/g of extract.

2.8 Antioxidant assays

The crude extracts and essential oils from the leaves and rhizomes were analyzed the total phenol concentration and evaluated for antioxidant activity by 2 methods, as follows.

Scavenging of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radicals

The antioxidant activity of each extract was evaluated on the basis of its activity in scavenging the stable DPPH radical, using a slight modification of the method described by Shimada (1992). Each extract was diluted in methanol to give at least 5 different concentrations. An aliquot (2 mL) of the extract at each concentration was mixed with 2 mL of 0.2 mM DPPH. The mixture was then homogenized and left to stand for 30 min in the dark. The absorbance was then measured at 517 nm against a blank using a spectrophotometer. The scavenging effect was calculated as follows: scavenging effect (%) = [(ΔA_{517} of control - ΔA_{517} of extract) / ΔA_{517} of control]. The EC_{50} value is the concentration of extract at which DPPH radicals were scavenged by 50%. Results were expressed as a mean standard deviation from three replicate measurements; butylated hydroxyl anisole (BHA) was used as a reference synthetic antioxidant.

Reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). The crude extract (1-20 mg/mL) or essential oil (1-50 mg/mL) in methanol (1.0 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer, (pH 6.6) and 2.5 mL of 1 % aqueous potassium ferricyanide solution. After incubation at 50°C for 30 min, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride, and then the absorbance at 700 nm was measured against a blank. The EC_{50} value represents the concentration of extract at which the absorbance was 0.5. Results were expressed as a mean standard deviation of three replicate measurements, with ascorbic acid as a reference reducing agent.

3. Results and Discussion

3.1 Constituents of the essential oil

Extracts of all parts of *E. curtisii* afforded colorless essential oils with a strong odor. However, the best source of oil was the rhizome, which gave a yield of 0.63%. The leaves, roots and pseudo-stems, afforded essential oil in lower yields of 0.12, 0.20 and 0.24%, respectively. According to GC-MS analysis under the conditions described above, 6 compounds, including various of aldehydes, alcohols, esters and alkanes were identified from the essential oil of the rhizomes. *Trans*-2-decenal was the major component of the oil, being present at a level of 78%. The remaining constituents were present at much lower concentrations, as shown in Table 1. *Trans*-2-decenal is found in a number of other herbs, including *Coriandrum sativum* (20%), (Delaquis *et al*., 2002; Potter and Fagerson, 1990) olive oil and the avocado mesocarp (6.3%) (Sinyinda and Gramshaw, 1998), watermelon (Kemp, 1975) and orange essential oil (Högnadóttir and Rouseff, 2003) and has well-documented antimicrobial activity.

3.2 Antioxidant activity

Total phenol concentrations of extracts from *E. curtisii* were estimated as gallic acid (GA) equivalents. The concentration of total phenols varied from 5.4 to 73.4 mg GA/g of extract as shown in Table 2. The crude extracts of the leaves had a slightly higher total phenol concentration than those from the rhizomes. The highest concentration of phenols was found in the dichloromethane extract of both the leaves and rhizomes, while the lowest concentration was recorded in the essential oils.

All extracts showed some radical-scavenging effect when tested with DPPH. Amongst them, the dichloromethane and methanol extracts of the rhizomes had the highest activity, 93.47 and 92.56% respectively, at a concentration of 1 mg/mL. Slightly lower activity (73.23 and 85.55%) was shown by the corresponding leaf extracts at the same concentration. In contrast, the hexane extracts and essential oils from the rhizomes and leaves had much lower antioxidant activity. EC_{50} values for the various extracts are recorded in Table 2, with lower EC_{50} values corresponding to higher antioxidant activity. All extracts from the leaves exhibited higher activity than those from the rhizomes, and the dichloromethane extracts from both leaves and rhizomes proved most effective with EC_{50} values of 0.28 and 0.34 mg/mL. However, they were still less active than BHA, the reference synthetic antioxidant, which has an EC_{50} value of 0.01 ± 0.001 mg/mL In general, the DPPH scavenging activity of the extracts showed a correlation with total phenol concentration. Extracts with

Table 1. Constituents of the essential oil from rhizomes of *E. curtisii*

Peak	Compound	% of Total
1	Octenal	6.45
2	1-Methyl-2-cyclopenten-1-ol	1.96
3	*Trans*-2-decenal	78.03
4	2-Decen-1-ol	4.32
5	2-Dodecenyl acetate	7.00
6	n-Heptadecane	2.24

Table 2. Total phenols, EC_{50} values of DPPH and reducing power activity of extracts from *E. curtisii*

Crude Extracts	Total phenols (mg GA/g of extract)	EC_{50} (mg/mL)	
		DPPH	Reducing Power
Leaves			
Essential oil	15.5±1.63	2.30±0.10	-
Hexane	41.9±5.19	0.99±0.12	42.67±1.53
Dichloromethane	73.4±2.80	0.28±0.01	19.00±1.00
Methanol	21.2±0.20	0.33±0.01	2.07±0.06
Rhizomes			
Essential oil	5.4±0.8	34.0±0.10	-
Hexane	15.8±1.21	12.13±0.90	-
Dichloromethane	53.9±0.8	0.34±0.03	2.27±0.15
Methanol	19.9±0.31	0.68±0.07	4.20±0.20
Reference antioxidants			
BHA	NT	0.01±0.00	NT
Ascorbic acid	NT	NT	0.28±0.01

(-) = no significant reduction detected
NT = not tested

higher total phenol concentration exhibited greater activities, except for the hexane crude extract from the leaves. This result is possibly due to the different phenols of the extracts. It has been reported that the radical scavenging efficiency depended on the structural conformation and the number of available hydroxyl groups of phenols (Brand-Williams *et al.*, 1995)

The reducing power of all extracts increased with concentration. For the leaves of *E. curtisii*, the reducing activity of the methanol extract (EC_{50} 2.07 mg/mL) was the highest, but was still lower than that of the reference reducing agent, ascorbic acid (EC_{50} 0.28 mg/mL). The dichloromethane and hexane extracts showed lower reducing activities, and the crude essential oil was least effective, having a reducing power of 0.17 at a concentration of 10 mg/mL and only 0.35 at 50 mg/mL. With the rhizomes, the dichloromethane extract had the highest reducing activity, but was slightly less active than the methanol extract from leaves. Again, EC_{50} values were not recorded for the hexane extract and the essential oil from the rhizomes, because their reducing activities were significantly lower, at just 0.12 and 0.15 respectively at 40 mg/mL. A correlation between the radical scavenging activity and the reducing power was found in extracts of rhizomes. However, in leaves, the methanol extracts showed the highest reducing power but did not showed the highest scavenging property. Apart from phenolic compounds, the methanol extract might contain other phytochemicals contributing to reducing activity.

3.3 Antibacterial activity

The antibacterial activity of eight different extracts from *E. curtisii* has been evaluated *in vitro* at the concentration of 1-15 mg/disc against 5 strains of bacteria. Each of the extracts showed a different antibacterial activity. The extracts inhibited the growth of 3 to 5 bacterial species tested. The minimum dose per disc to inhibit the growth of the test microorganism is recorded in Table 3. None of the solvent controls showed any inhibitory effect on any of the test bacteria. On the other hand, the antibiotic drugs,

Table 3. Minimum inhibitory dose (mg/disc) for antibacterial activity of *E. curtisii*

Bacteria	MID of leaf extracts				MID of rhizome extracts			
	Essential oil	Hexane	Dichloromethane	Methanol	Essential oil	Hexane	Dichloromethane	Methanol
B. subtilis	≤1	>15	15	>15	≤1	5	>15	15
S. aureus	≤1	15	15	15	≤1	≤1	5	15
Sarcina sp.	≤1	15	≤1	5	≤1	≤1	≤1	2
P. aeruginosa	≤1	15	>15	≤1	≤1	2	4	15
E. coli	≤1	15	15	>15	≤1	2	5	>15

ciprofloxacin and tetracycline were active against all bacteria. In addition, Penicillin G was active against 3 strains of bacteria including *B. subtilis, S. aureus,* and *Sarcina* sp. but showed no effect on *P. aeruginosa* and *E. coli*.

Of the eight extractions, both essential oils from the rhizomes and leaves were found to be most effective. They inhibited the growth of all bacteria at the concentrations tested with MID value of 1 mg/disc. The crude hexane extract from the rhizome was also active against all bacteria but was slightly less effective against *E. coli, P. aeruginosa* and *B. subtilis* (MID 2-5 mg/disc) than the essential oils. Additionally, the crude dichloromethane extract from the rhizomes exhibited moderate antibacterial activity against 4 of the bacterial strains, namely, *E. coli, P. aeruginosa, S. aureus* and *Sarcina* sp., but not against *B. subtilis*. Its most potent inhibitory activity was recorded for *Sarcina* sp., with a MID of 1 mg/disc. The methanol extract of the rhizomes displayed much lower activity than other extracts. It was inhibitory to *P. aeruginosa, S. aureus* and *B. subtilis* with a highest MID of 15 mg/disc and was active against *Sarcina* sp. (MID 2 mg/disc) but inactive against *B. subtilis*.

The antibacterial properties of the crude extracts from leaves of *E. curtisii* were less pronounced than those of the crude extracts from the rhizomes. The crude hexane extract was the least active against *E. coli, P. aeruginosa, S. aureus* and *Sarcina* sp (MID 15 mg/disc) and was inactive against *B. subtilis*. The crude dichloromethane extract also showed poor activity against bacteria, except for *Sarcina* sp. (MID 1 mg/disc) and failed to inhibit *P. aeruginosa*, while the crude methanol extract inhibited the other 3 strains of bacteria, but not *E. coli* or *B. subtilis*.

The antibacterial activity of the essential oils of *E. curtisii* may be attributable to the presence of aldehyde constituents. There has been some evidence that α, β-unsaturated aliphatic aldehydes possess growth inhibitory and microbicidal activity. This property depends on the length of the aliphatic carbon chain and the group of microorganism (Pauli, 2001). For example, the olive aldehydes, (*E*)-2-heptenal, (*E*)-2-nonenal, (*E*)-2-decenal and (*E, E*)-2, 4-decadienal exhibit a broad spectrum of antibacterial activity against Gram-positive and Gram-negative microorganisms (Bisignano *et al*., 2001). These aldehydes have also been shown to exhibit significant antifungal activity against *Trichophyton mentagrophytes* and *Microsporum canes* strains (Battinelli *et al*., 2005). Furthermore, the volatile oils from both coriander leaves and the seeds of *Coriandrum Sativ*um, which contain (*E*)-2-decenal and linalool as major components, exhibit antibacterial activities (Delaquis *et al*., 2002). Not only aldehydes but also aliphatic alcohols inhibit microbial growth, although the alcohols are less effective. Furthermore, it has been reported that minor components of volatile oils may produce a synergistic antibacterial effect when combined with other active components. Thus, it is possible that minor components of the essential oil of *E. curtisii,* such as 2-decan-1-ol may enhance its antibacterial activity.

Interestingly, the essential oils showed high antibacterial activities but were ineffective as antioxidants. This suggests that aldehydes, the major constituents of the essential oils, were inactive to DPPH radical. In contrast, the dichloromethane and methanol extracts exhibited low antibacterial activities but high antioxidant activities.

4. Conclusion

In conclusion, this study provides a preliminary analysis of the essential oil from the rhizomes and leaves of *E. curtisii*, together with a brief investigation of their antioxidant and antibacterial activities. Also, it has been shown that the antioxidant / antibacterial properties of the herbal extract can be adjusted by varying the method of extraction. This analysis of interest to both consumers and food scientists, as *E. curtisii* may provide health benefits to the public and also act as either a preservative or antioxidant. Further isolation and identification of biological active compounds is in progress to clarify the mechanism of antioxidant / antibacterial activities.

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