ORIGINAL ARTICLE

Essential oil components and biological activities of *Coleus parvifolius* leaves

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Abstract

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The essential oil distilled from the leaves of *Coleus parvifolius* Benth. (Labiatae) was studied by gas chromatography and mass spectrometry (GC-MS). The main components were found to be (*E*)-phytol (42.77%), followed by eicosatrienoate (16.39%), *n*-tetradecanoic acid (14.42%), octoil (6.54%), 2-methyl-7-octadecyne (5.97%), nonadecane (3.25%), germacrene-D (2.19%) and α-humulene (1.42%), respectively. Regarding biological activities, the ethanolic extract of *C. parvifolius* showed potent antimicrobial activity against gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis* and *B. cereus*) with inhibition zones of 7-11 mm at a concentration of 10 mg/disc and moderate activity against gram negative bacteria (Salmonella typhi, *S. enteritidis* and *Escherichia coli*) with inhibition zones of 9-11 mm at 100 mg/disc, whereas it was inactive against fungus, *Candida albicans* at a concentration of 100 mg/disc. The extract also exhibited strong antioxidant activity (ED_{50} = 5.87±0.03 μg/ml) three times higher than that of butylated hydroxytoluene (BHT, ED_{50} = 18.08±0.43 μg/ml). Moreover, it was non-toxic to brine shrimp with LC_{50} value > 1,000 μg/ml.

Key words : essential oil components, biological activities, *Coleus parvifolius* Benth.

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บทคัดย่อ

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องค์ประกอบเคมีในน้ำมันหอมระเหยและฤทธิ์ทางชีวภาพของใบมันขี้หนู (*Coleus parvifolius*)

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การศึกษาองค์ประกอบเคมีในน้ำมันหอมระเหยใบมันขี้หนู โดยวิธีแก๊สโครมาโตกราฟีพบสาร (*E*)-phytol 42.77%, eicosatrienoate 16.39%, n-tetradecanoic acid 14.42%, octoil 6.54%, 2-methyl-7-octadecyne 5.97%, nonadecane 3.25%, germacrene-D 2.19% และ α-humulene 1.42% ตามลำดับ สารสกัดด้วยเอทานอลจากใบมันขี้หนู มีฤทธิ์ต้านเชื้อแบคทีเรียแกรมบวกได้ดี โดยให้ค่า inhibition zone อยู่ในช่วง 7-11 มม. ที่ความเข้มข้น 10 mg/disc (*Staphylococus aureus, Bacillus subtilis* และ *B. cereus*) และมีฤทธิ์ต้านเชื้อแบคทีเรียแกรมลบได้ปานกลาง โดยให้ค่า inhibition zone 9-11 มม. ที่ความเข้มข้น 100 mg/disc (*Salmonella typhi, S. enteridis* และ *Escherichia coli*) ในขณะที่ไม่มีฤทธิ์ต้านเชื้อรา *Candida albicans* สารสกัดจากใบมันขี้หนูยังมีฤทธิ์ต้านการเกิดออกซิเดชันได้ดี (ED_{50} = 5.87±0.03 μg/ml) โดยมีฤทธิ์สูงกว่า BHT ถึง 3 เท่า (ED_{50} = 18.08±0.43 μg/ml) นอกจากนี้ยังไม่พบความเป็นพิษต่อไรน้ำเค็ม

ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Coleus parvifolius Benth. (Syn *C. tuberculosus* Benth., so called Mun-Khee-Nhoo in Thai) is one of the plants in Labiatae or Mint family; and is widely distributed in Asia and tropical areas, especially in the southern part of Thailand. In Thailand, tubers of this plant have been used in cooking as one of the ingredients in Thai curry. Preparations of *Coleus* spp. have long been used in Ayurvedic traditional medicine particularly in treatment of heart disease, abdominal pain and convulsions (Evans, 1989). Many *Coleus* spp. such as *C. aromaticus, C. barbatus, C. forskohlii, C. blumei* and *C. scutellaroides* were reported for their constituents and biological activities. However, *C. parvifolius* still lacks of scientific information on both chemical components and bioactivities. The present study, therefore, reports the essential oil components and biological activities (cytotoxicity, antimicrobial and antioxidant activities) of this plant.

Materials and Methods

Plant collection

The fresh leaves of *C. parvifolius* (Figure 1) 50 kg were collected from Amphur Rattaphoom,

Figure 1. Coleus parvifolius Benth.

Songkhla province, Thailand in 2002. The specimen (No. SKP. 0950316) was deposited in the herbarium of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Essential oil extraction and determination

Fresh leaves of *C. parvifolius* 5 kg were extracted by steam distillation for 3 hrs. After that, the obtained volatile oil was injected to GC-MS, using GC-MS HP-5890 series II plus gas chromatography and HP 5972 series mass selective detector from Hewlett-Packard.

The oil of *C. parvifolius* was analyzed by GC-MS in the following conditions: helium carrier gas flow rate 1 ml/min; injector temperature 250ºC; initial column temperature 80ºC, held at this temperature for 2 minutes; final temperature 280ºC, rate 15ºC/min; detector temperature 280ºC ; sample injection volume 1 µl. The system was connected with a 30 m x 0.25 mm. (I.D.) capillary column coated with HP-1. The spectra were recorded and compared with the library.

Ethanolic extraction of *C. parvifolius*

Fresh leaves 40 kg of *C. parvifolius* were extracted with ethanol (10 L, each) three times and obtained 408 g of ethanolic extract (% yield = 1.02 %w/w). The solvent was removed under reduce pressure to give the respective dry extract and was further determined for cytotoxicity (brine shrimp lethality assay), antioxidant and antimicrobial activities.

Biological assays

Brine shrimp lethality assay (Andre *et al*., 1995)

Brine shrimp lethality test was carried out following the method described by Andre *et al*., 1995. Briefly, eggs of brine shrimp (*Artimia salina*) were hatched in artificial sea water for 36-48 hrs. Crude extract of *C. parvifolius* was dissolved in artificial sea water by dilution to 1,000, 500, 100, 50 and 10 µg/ml, respectively. 10-15 Brine shrimps in 100 µl of sea water were transferred into a 96-well plate. Then, sample solutions in various concentrations of *C. parvifolius* were added into each well (n=3). After 24 hrs, the dead and living brine shrimps were counted. Lethality concentation at 50% inhibition (LC_{50}) was calculated using the Finney probit analysis program.

Antioxidant assay (Hatano *et al*., 1989)

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was carried out as described by Hatano *et al*., 1989. Briefly, a portion of sample solution was mixed with the same volume of 6×10^{-5} M DPPH in ethanol and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 520 nm. BHT, which is one of the well-known antioxidants, was used as positive control.

Antimicrobial assay (Griggs *et al*., 2001)

The antimicrobial assay was carried out as described by Griggs *et al*., 2001. Briefly, 1-2 colonies of each microbe [gram-positive *Bacillus subtilis, B. cereus, Staphylococcus aureus* (normal and resistant strain); gram-negative *Salmonella typhi, S. enteritidis* and *Escherichia coli*; and fungus *Candida albicans*] were suspended in saline solution and the turbidity adjusted to equal 0.5 McFarland standard. Prepared microbe culture was then swabbed on the plate of Tryptic Soy Agar (TSA) separately, whereas *C. albicans*, was swabbed onto the Sabouraud Dextrose Agar (SDA). The sterile disc (6 mm diameter disc) containing 10 µl of sample solution in water was placed on the prepared agar plate. Sterile distilled water without sample was used as the control. The plates were incubated for 24 hrs at 35ºC and then the inhibition zones (in mm) were determined for antimicrobial activity. (The microorganisms without strain codes were derived from Songklanakarin Hospital)

Results and Discussion

Essential oil components of *C. parvifolius*

The 0.015 % w/w of typical odor with pale yellow color of *C. parvifolius* oil was extracted. As shown in Table 1 and Figure 2, eight compo-

Table 1. Essential oil components of *C. parvifolius* leaves separated by GC-MS. Compound numbers and names of the oil constituents correspond to those shown in Figure 2.

Compound No.	Compound name	Retention time (min)	Peak area %
1.	α-Humulene	8.72	1.42
2.	Germacrene-D	8.97	2.19
3.	*n*-Tetradecanoic acid	12.43	14.42
4.	(*E*)-Phytol	13.38	42.77
5.	Eicosatrienoate	13.56	16.39
6.	2-Methyl-7-octadecyne	13.95	5.97
7.	Octoil	15.83	6.54
8.	Nonadecane	18.36	3.25
	Unidentified	-	7.05
	Total		**100.00**

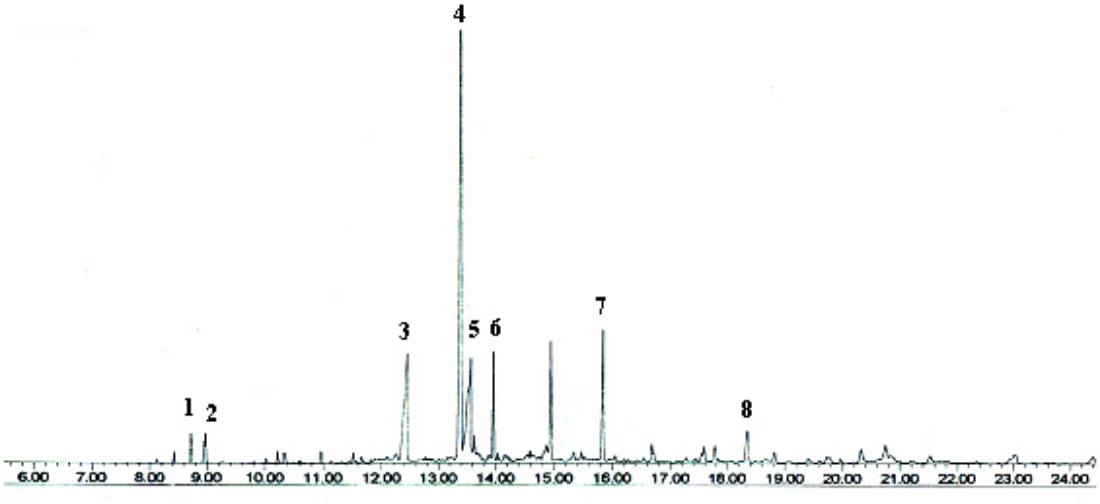

Figure 2. GC chromatogram of essential oil obtained from *C. parvifolius* leaves.

nents of *C. parvifolius* oil were well separated within 25 minutes. The main components were found to be (*E*)-phytol (42.77%), followed by eicosatrienoate (16.39%), *n*-tetradecanoic acid (14.42%), octoil (6.54%), 2-methyl-7-octadecyne (5.97%), nonadecane (3.25%), germacrene-D (2.19%) and α-humulene (1.42%), respectively. The oil of this plant contains some components that have been reported to exhibit various activities. (*E*)-phytol is one part of chlorophyll which is important for plant biosynthesis. (*E*)-Phytol and eicosatrienoic acid were also reported for their anticancer activity against HT-29 human colon cancer cells, MG-63 osteosarcoma cells and AZ-521 gastric cancer cells (Lee *et al*., 1999). (*E*)-Phytol and its derivatives, (*E*)-phytol acetate and (*E*)-phytol epoxide were found to show anti-mycobacterial activity against *Mycobacterium tuberculosis* (Rajab *et al*., 1998). α-Humulene, a kind of monoterpene, has been found in many kinds of plant species such as hops and clove. These two plants were reported to exhibit sedative effect (Evans, 1989). The oil components of *C. parvifolius* are somewhat different from that of *C. aromaticus*,

Table 2. DPPH radical scavenging activity of *Coleus parvifolius* extract

Sample	% Inhibition at various concentrations of the extract (µg/ml)				ED_{50} (µg/ml)
	25	2.5	6.25	2.5	
Ethanolic extract of *C. parvifolius*	86.32±0.64	82.88±1.34	52.94±1.34	28.81±1.74	5.87±0.03
BHT (positive control)	59.84±0.97	48.25±2.97	26.35±2.49	-	18.08±0.43

The results are mean±S.D (n=3)

Table 3. Antimicrobial activity of *C. parvifolius* extract by agar disc diffusion assay (+ : active, - : inactive).

Microorganisms	Activity/Inhibition zone (mm.)		
	100 mg/disc	10 mg/disc	1 mg/disc
A) *Gram-positive bacteria*			
1. *Staphylococcus aureus* ATCC 25923	+ /15	+ / 8	-
2. *S. aureus* SK 5 (MRSA)	+ /20	+ /11	-
3. *Bacillus subtilis* BGA	+ /14	+ /9	-
4. *B. cereus*	+ /13.5	+ /7	-
B) *Gram-negative bacteria*			
5. *Salmonella typhi*	+ /11	-	-
6. *S. enteritidis*	+ /9	-	-
7. *Escherichia coli* ATCC 25922	+ /10.5	-	-
C) *Fungus*			
8. *C. albicans*	-	-	-

which is the well-known one. The main components of *C. aromaticus* are thymol (79.6%), other monoterpenes (8.3%) and sesquiter-penes (4.5%) (Pak, 1988), whereas those of *C. parvifolius* are (*E*)-phytol (42.7%), followed by other aliphatic hydrocarbons (46.5%) and mono-terpenes (4.6%). This result indicated that *C. parvifolius* contains mainly aliphatic hydrocarbons, whereas those of *C. aromaticus* are monoterpenes.

Biological assays

Brine shrimp lethality assay

Brine shrimp lethality test is the method which was carried out for screening cytotoxicity of plant extracts or compounds. The result indicated that the ethanolic extract of *C. parvifolius* was non-toxic to brine shrimp with LC_{50} value > 1,000 µg/ml. This is indicated that the leaves of *C. parvifolius* might be safe to be used as foods.

Antioxidant assay

Antioxidant activity was carried out using DPPH radical scavenging assay. The result (Table 2) showed that the ethanolic extract of *C. parvifolius* exhibited potent DPPH scavenging activity with ED_{50} value of 5.87±0.03 µg/ml, whereas that of butylated hydroxytoluene (BHT) was 18.08±0.43 µg/ml. The extract of this plant possessed strong antioxidant activity about three times higher than that of BHT. This result is compatible with the use of *Coleus* spp. preparation in traditional medicine for treatment of heart diseases and inflammation disorders, since free radical or reactive oxygen radicals usually induce cellular damage and play a

crucial role in many diseases such as heart disease, cancer, rheumatoid arthritis, hepatic disorder and aging diseases (Aruoma, 1994). Therefore, the extract of *C. parvifolius* that exhibited potent activity against DPPH radical, may have some benefit in treatment of those diseases.

Antimicrobial assay

As shown in Table 3, the result indicated that ethanolic extract of *C. parvifolius* exhibited potent activity against gram-positive bacteria [*S. aureus* ATCC 25923, *S. aureus* SK 5 (MRSA), *B. subtilis* and *B. cereus*], especially to *S. aureus* SK 5, the methicillin resistant strain (+ ve, ϕ 20 mm at 100 mg/disc and + ve, ϕ 11 mm at 10 mg/disc of *C. parvifolius* extract), whereas it showed moderate activity against gram-negative bacteria causing diarrhea with inhibition zone of 9-11 mm at 100 mg/disc (*S. typhi, S. enteridis* and *E. coli* 25922). However, the extract of this plant could not inhibit the growth of *C. albicans* at concentration 100 mg/disc. This result indicated that ethanolic extract of *C. parvifolius* can be used for treatment the infectious diseases caused by gram-positive bacteria such as *S. aureus* and its resistant strain (MRSA) including *B. subtilis* and *B. cereus*, and can be used for treatment of infectious diseases caused by *S. typhi, S. enteridis* and *E. coli*, which are gram-negative bacteria. However, it may not be used for treatment the diseases caused by a certain fungi, *C. albicans*.

Regarding the biological activities of *C. parvifolius*, we previously reported its anti-HIV1-integrase activity and active principles were found to be rosmarinic acid and methyl rosmarinate. Other compounds found in this plant were luteolin, luteolin glucuronide, luteolin glucoside, luteolin 7-methyl ether, α- and β-amyrin and daucosterol (Tewtrakul *et al*., 2003). The isolation of active principles for antimicrobial and antioxidant activities of this plant are now in progress.

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