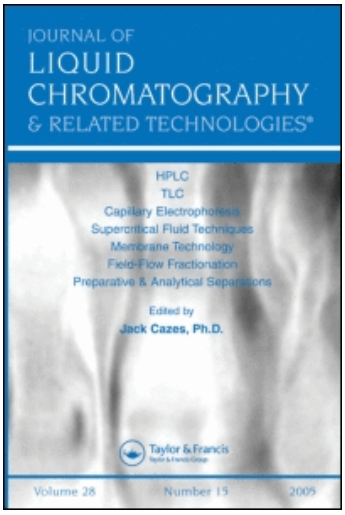

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Supercritical Fluid Extraction of Drug-Like Materials from Selected Myanmar Natural Plants and their Antimicrobial Activity

Dongjin Pyo[a]; Hlaing Hlaing Oo[b]
[a] Department of Chemistry, Kangwon National University, Chuncheon, Korea [b] Universities' Research Centre, Yangon University, Yangon, Myanmar

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Supercritical Fluid Extraction of Drug-Like Materials from Selected Myanmar Natural Plants and their Antimicrobial Activity

Dongjin Pyo
Department of Chemistry, Kangwon National University, Chuncheon, Korea

Hlaing Hlaing Oo
Universities' Research Centre, Yangon University, Yangon, Myanmar

Abstract: Supercritical fluid extracts of three Myanmar natural plants, namely, *Curcuma aeruginosa*, *Citrus hystrix*, and *Azadirachta indica* A. Juss were screened for antimicrobial activity against different strains of bacteria and yeast, which are known to cause various types of skin infections. The antimicrobial activity was tested on *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Propionibacterum acnes*, and *Malassezia furfur*. None of the plants tested produced consistent inhibition of the Gram-negative bacteria *E. coli* and yeast *M. furfur*. Three plants consistently inhibited bacterial growth of *Bacillus subtilis*. The antimicrobial activity profile showed that *Bacillus subtilis* was the most susceptible bacterial strain. According to the antimicrobial profile, SFE extracts of *C. aeruginosa* presented no significant difference in inhibitory activity on all Gram-positive bacterial strains. *A. indica* leaf extracts showed the highest antibacterial activity on *P. acness* and *S. aureus*, a moderate activity on *B. cereus*, *B. subtilis*, and *S. epidermidis*. The SFE extracts of *C. hystrix* (stem and bark) presented the highest antibacterial activity on *B. subtilis*, moderate activity on *B. cereus* and *S. epidermidis*, and weak activity against *S. aureus* and *P. acness.* Therefore, the SFE extracts of *C. aeruginosa*, *C. hystrix*, and *A. indica* have the possibility to be applied as a constituent of cosmetic products and medicines, because they exhibit antimicrobial activities. The most active extracts can be subjected to isolation

Address correspondence to Prof. Dongjin Pyo, Department of Chemistry, Kangwon National University, Chuncheon 200-701, Korea. E-mail: pyod@kangwon.ac.kr

of the therapeutic antimicrobials using SFE techniques and undergo further pharmacological evaluation.

Keywords: Supercritical fluid extraction, Myanmar natural plants, Antimicrobial activity, Skin infection

INTRODUCTION

Plants are not only important to the millions of people to whom traditional medicine serves as the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals. Several methods can be used for preparing extracts from plant materials including organic solvent extraction, steam distillation, and supercritical gas extraction. Thus, compelling regulations on the usage of hazardous, carcinogenic, or toxic solvents, as well as high energy costs for solvent recovery has resulted in a need for an alternative to the classical extraction methods.[1] This alternative emerged in the form of supercritical fluid extraction (SFE).

The SFE technique was found to be selective in the separation of desired compounds without leaving toxic residues in extracts and without risk of thermal degradation of processed products.[2] It also offers four major advantages over classical solvent extraction methods: easily adjustable selectivity (pressure, temperature), high mass transfer rate, online fractionation, and spontaneous elimination of the solvent. During its relatively short history, supercritical fluid chromatography (SFC) has become an attractive alternative to gas chromatography (GC) and liquid chromatography (LC) in certain industrially important applications.[3] Environmentally friendly supercritical fluid CO_2 and its associated technologies are being applied in many applications to replace hazardous solvents, lower costs, and improve efficiencies. Moreover, CO_2 (critical point T_c 31.1°C; P_c 72.0 bar; d_c 0.47 g cm^{-3}) is safe (generally recognized as safe, GRAS from the US Food and Drug Administration, FDA), residue free, non flammable, and inexpensive. In addition, supercritical CO_2, with it ability to mimic solvents and penetrate matrices easily, can replace Soxhlet extractions while reducing a chemist's exposure to hazardous chemicals, improving the environment, and reducing costs. Supercritical fluid extract is a nontoxic state-of-the-art technology originally commercialized for the food, fragrance, nutra-, and phyto- pharmaceuticals, and environmental industries. SFE products are superior in quality; higher yields, extracts with delicacy and freshness close to natural, no residual solvents, high potency of active components, free of biological contaminants, longer shelf life, ability to fractionate extract in single step, flexibility to extract multiple products by changing operating conditions; and also, carbon dioxide is safe as solvent for natural products.

Supercritical fluid extraction of herbal and natural products has also been identified as well.[4] Some commonly used herbs and spices such as garlic, black cumin, cloves, cinnamon, turmeric, thyme, bay leaves, mustard, and rosemary possesses antimicrobial properties and can be used as therapeutics.[5] The rediscovery of natural products like Neem (*Azadirachta indica*), that have long traditions of safety and efficacy offer the opportunity to get back to the basics of healthy and beautiful skin. Skin is our largest organ, which is constantly replacing old and damaged cells, so it needs a lot of care from the inside and the outside if it is to remain healthy. This amazing organ that protects us is also a visible display of how healthy we are and of how long and how wisely we have lived.

Turmeric is a well known indigenous herbal medicine having many biological activities. It is a well known spice, which is used as a dye, medicine, and flavoring agent, and exhibits a wide range of biological activities. *Curcuma aeruginosa*, which belongs to the family Zingiberaceae, native of Myanmar[6] and the warm, humid environments of South and South-East Asia is commonly known as turmeric. It is the rhizome or underground stem of a ginger like plant. A traditional Ayurvedic medicine, it is prescribed for treatment of many medicinal problems ranging from constipation to skin disease. It was used as a digestive aid and treatment for fever, wounds, infections, dysentery, arthritis, jaundice, and other liver problems.[7] Curcumas also exhibits anti-tumor activities and prevents cancer. It inhibits the topoisomerase enzyme, which is required for the replication of cancer. It has been used for thousands of years as an anti-inflammatory agent in the treatment of sprains, cramps, bruises, and muscle pain. Its anti-inflammatory properties may reduce inflammation and its possible anti-infective properties may help to prevent infection of skin injuries. Topical applications have been used to promote the healing of wounds and skin conditions.

Citrus hystrix DC., a member of the citrus family Rutaceae (Kaffir lime), is a very thorny tree that produces unique edible leaves. The genus *citrus* is native to the tropical and subtropical regions of South-East Asia, India, and Southern China, to northern Australia and New Caledonia. Recent findings indicate that *Citrus hystrix* leaves have the following properties: natural cleanser, deodorizer, stimulant, appetizer, astringent, antiseptic, antibacterial, antiamebic, antihistamine, antispasmodic, disinfectant, anti-tumor, and hypotensive activity.[8] Powdered kaffir limes leaves can be found in today's specialty markets. If available, the fruit, especially the skin, may also be used as a unique flavoring.

Scientists have seen what neem can do for skin conditions and have verified the marvelous healing properties of neem. It is in the family Meliaceae, native to India and Burma. Its origins lie in Asia, probably in the Indian subcontinent, but it is found in dry forest areas throughout South and South-East Asia.[9] It kills the bacteria that cause acnes and reduces the

inflammation that makes it so noticeable. Neem also inhibits allergic reactions when applied externally or consumed internally.[10] All parts of the neem tree (leaves, flowers, seeds, fruits, roots, and bark) are recognized to possess a wide range of pharmacological effects. Neem based creams have even improved the appearance of scarred and reddened skin from acnes, which actually occurred years ago.

Several experimental studies have demonstrated that extraction of drug materials from natural plants used classical solvent extraction techniques. However, to date, no study has been made of the supercritical fluid extraction of drug like material from Myanmar natural plants (MNPs), and antimicrobial activity of the supercritical fluid extracts from rhizomes of *Curcuma aeruginosa* and leaf and bark of *Azadrichta indica* A. Juss.

EXPERIMENTAL

Sample Collection and Preparation

The parts used of three different selected Myanmar natural plants, such as *Curcuma aeruginosa*, *Citrus hystrix*, and *Azadirachta indica* A. Juss are presented in Table 1. The specimens were identified at Universities' Research Centre, Yangon University, Yangon, Myanmar. The plant parts selected were dried in shade and protected from direct exposure to sunlight, pulverized in mixer-grinder, and powdered in motor and pestle before supercritical fluid extraction. The selected Myanmar natural plant samples were presented in Figures 1 to 3.

Reagents and Chemicals

Supercritical fluid extractions were preformed with carbon dioxide, SFC grade (Scott Specialty Gases, Plumsteadville, PA). All solvents (ethanol, methanol, 2-propanol, water) were HPLC grade from Aldrich (Milwaukee, WI).

Table 1. The parts used of the selected myanmar natural plants for the determination of antimicrobial activity

Selected myanmar natural plant		
Scientific name	Myanmar name	Parts used
Curcuma aeruginosa	Sa-nwin Tain Pyar, Gamone Tain Pyar	Rhizome
Citrus hystrix	Shauk Nu, Shauk Waing	Stem and bark
Azadirachta indica A. Juss	Tamar, Tamarkha, Tamarbin	Leaf, Bark

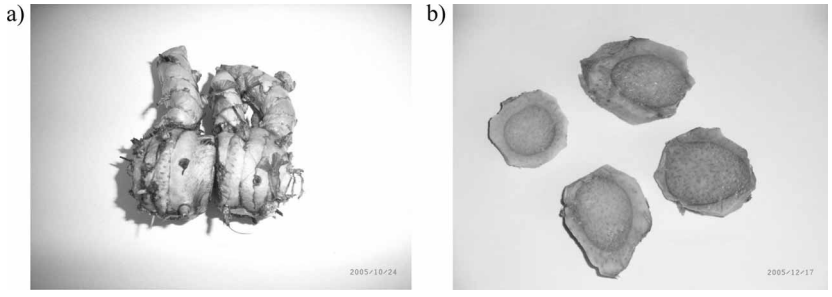

Figure 1. (a) *C. aeruginosa* rhizome, (b) horizontal peels of *C. aeruginosa*.

Extraction of Drug-Like Materials by SFE Technique

Supercritical fluid extraction of drug like material from selected Myanmar natural plants were performed using the JASCO (Tokyo, Japan) LC-900 SFE system. The schematic diagram of the system is shown in Figure 4. In a typical experimental run, about 10 g of each sample was placed into the extraction column. Light phase fluid (carbon dioxide) was supplied from the CO_2 cylinder by the Intelligent HPLC pump (Jasco, PU-980). Carbon dioxide was then fed into the system until the desired pressure was achieved and the desired system temperature was set in the gas chromatograph (Varian 3400). The system was then left to equilibrate for about 15 minutes. The modifier solvent was supplied to the system by means of a pump controller (ISCO Series D). The liquid CO_2 and modifier solvent were continuously delivered at their flow rate, and the extract was collected in a cold trap located at the back pressure regulator (JASCO 880–81). With the continuous flow of supercritical carbon dioxide to the system, active ingredients present in the raw material get selectively absorbed by the supercritical CO_2 and then get separated in the separation vessels. Each fraction took about 60 minutes. During extraction, 6 *C. aeruginosa* fractions, 4 *C. hystrix* fractions, 2 *A.*

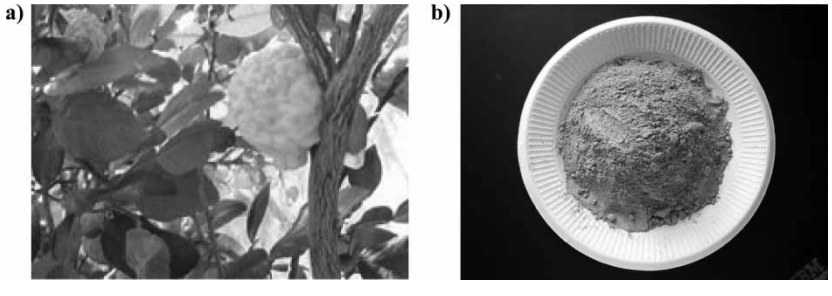

Figure 2. (a) *C. hystrix* plant, (b) powdered *C. hystrix*.

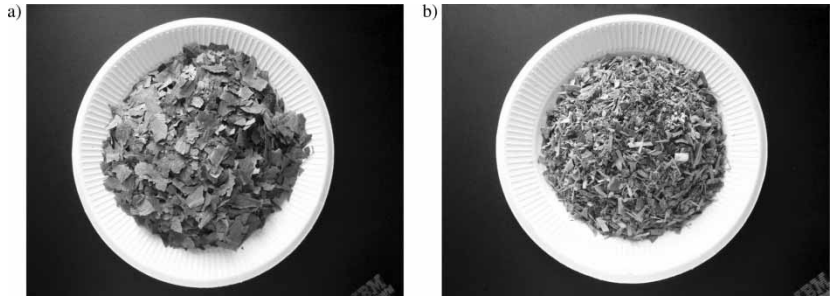

Figure 3. (a) *A. indica* leaves, (b) *A. indica* bark.

indica (bark) fractions, and 3 A. *indica* (Leaf) fractions were collected in the vials. Most of fractions collected were mainly liquid like nature, except one fraction of *A. indica* leaf used without a modifier solvent gave solid fraction. All extractions were performed in triplicate. The variation of extraction conditions were as follows: CO_2 pressure (100 to 250 bar), extraction temperature (40 to 60°C), CO_2 flow rate (3 mL min^{-1}), and different modifier solvents (ethanol and 1:1 ratio of ethanol: 2-propanol) were used.

SFE experiments were made three times for each fraction and the extract of all portions were combined in one vessel, and the organic solvent removed by means of rotary evaporator (Heidolph) under reduced pressure associated with liquid N_2 flow and water circulation pump at not more than 40°C. Then, the obtained residues were put into the micro tubes. These micro tubes, including SFE extract residues, were then placed on a hot plate at

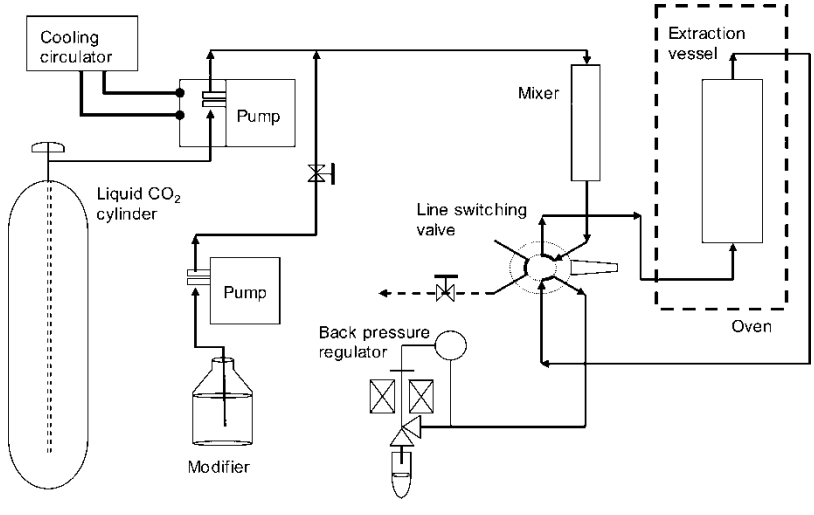

Figure 4. Schematic flow diagram of SFE modular system.

30°C, and a stream of nitrogen was passed over them. The resulting dried mass in the micro tubes was stored in a refrigerator until being tested for antimicrobial activity. Finally, the dried extracts were sent to Radiant Corporation to test their antimicrobial activity.

Antimicrobial Assay

The agar disk diffusion method was employed for the determination of antimicrobial activities following the above procedure. The supercritical fluid extracts were individually tested against seven strains of bacteria: *B. cereus*, *B. subtilis*, *E. coli*, *S. aureus*, *S. epidermidis*, *P. acnes*, and *M. furfur*. The characteristic of bacterial species are presented in Table 2. Each sample (500 μg) was dissolved with 100% dimethylsulfoxide (DMSO) and added to the discs. The discs were allowed to dry in a biological safety cabinet and then incubated. *B. cereus* and *B. subtilis* were cultured in Heuller-Hinton media at 30°C for 24 h. *E. coli*, *S. aureus*, and *S. epidermidis* were

Table 2. Characteristics of used bacteria for antimicrobial activity

Bacterial species	Gram reaction	Disease	Target organ	Source of infection
Staphylococcus epidermidis	+	Opportunistic infections	Skin, open wounds, urinary tract	Normal flora on skin, environment
Staphylococcus aureus	+	Skin and other infections	Skin, open wounds	Nasal/oral con tact, close human-to-human contact
Bacillus cereus	+	Food poisoning	Small intestine	Unrefrigerated foods
Bacillus subtilis	+	Food poisoning	Small intestine	Unrefrigerated foods
Malasseiza furfur	+	Skin infection	Dandruff, tinea versicolor	Sebaceous glands
Propionibacterium Acnes	+	Skin disorder	Crypts of the skin, contribute to acnes	Sebaceous follicles
Escherichia coli	−	Opportunistic infections	Skin, open wounds	Poor hygiene (hand washing)

also cultured in Heuller-Hinton media at 37°C for 24 h. *P. acnes* was cultured in reinforced Clostridial media at 37°C for 72 h. *M. furfur* was cultured in Pityrosporum media at 37°C for 48 h. Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented.

RESULTS AND DISCUSSION

The use of SFE to obtain bioactive substances from medicinal plants has been proven to be a viable alternative for the extraction of natural compounds. Herbs and spices have been used for generations by humans as food and to treat infection, because they have medicinal properties that prevent disease. Given the demand of natural products that are inherently safe and environmentally compatible, the advancement in supercritical fluid extraction has provided a better alternative for extracting products. This paper describes an investigation conducted to evaluate the applicability and efficiency of SFE methods for the extraction of drug like materials from selected Myanmar natural plants.

Supercritical Fluid Extraction of Selected Myanmar Natural Plants

The supercritical process is extraordinarily complex and high tech and easy to understand. This process uses compressed carbon dioxide that is simply one of the constituents of normal air, and plants absorb it every moment. The compressed gas has the density of a liquid, but is able to penetrate deeply into the plant and dissolve the lipophilic constituents. Then, the pressure is carefully released, the gas just harmlessly dissipates into the atmosphere, and all that is left behind is pure. No pollution, no heat stress or damage, and no solvent residue. The products gained by supercritical fluid extraction are different from the traditional ones, both in their appearance and composition. The supercritical fluid extraction parameter of selected Myanmar natural plants was presented in Table 3.

SFE parameters such as CO_2 pressure, extraction temperature, and modifier solvents were varied in order to predict the best extraction conditions. When neat CO_2 was used as the extraction fluid at desired temperature and pressure, *C. hystrix* (stem and bark) gave orange liquid extracts and *A. indica* leaf formed yellow precipitates, while no extracts could be obtained from *C. aeruginosa* rhizome. It can be explained that the constituent in *C. aeruginosa* rhizome is sparsely soluble in neat CO_2. In addition, the poor extraction result of this sample with neat CO_2 is probably caused by the fact that it consists of fairly polar functional groups. Consequently, the extraction of drug like materials depends on their distribution between the supercritical fluid and the sorptive sites in the sample matrix.

Table 3. Supercritical fluid extraction parameter of selected myanmar natural plants at various conditions

Sr. no	Lot number	Temperature (°C)	Pressure (atm)	Modifier ratio (%)	Color of extract
1	Cur 001	40	100	100% Ethanol	Light yellow liquid
2	Cur 002	40	200	100% Ethanol	Orange liquid
3	Cur 003	40	150	100% Ethanol	Orange liquid
4	Cur 004	50	200	100% Ethanol	Yellow liquid
5	Cur 005	60	200	100% Ethanol	Yellow liquid
6	Cur 009	50	250	50% Ethanol: 50% 2-propanol	Deep orange liquid
7	Citrus 001	40	200	100% Ethanol	Yellow liquid
8	Citrus 002	40	200	50% Ethanol: 50% 2-propanol	Yellow liquid
9	Citrus 003	40	250	50% Ethanol: 50% 2-propanol	Yellow liquid
10	Citrus 004	40	250	No modifier	Orange liquid
11	Neem 001 (leaf)	40	200	50% Ethanol: 50% 2-propanol	Greenish liquid
12	Neem 002 (leaf)	40	200	100% Ethanol	Greenish liquid
13	Neem 003 (bark)	40	200	100% Ethanol	Yellow liquid
14	Neem 004 (bark)	40	250	50% Ethanol: 50% 2-propanol	Yellow liquid
15	Neem 012 (leaf)	40	250	No modifier	Yellow precipitate

Supercritical fluid CO_2 flow rate: 3 ml min^{-1}, Modifier flow rate: 0.2 ml min^{-1}, Extraction time: 60 minute.

The supercritical fluid extraction of selected MNPs was successful when 100% ethanol and 1:1 ratio of ethanol and 2-propanol were used as modifier solvents at different temperatures and pressure. Therefore, the use of cosolvents can have a profound effect on increasing solubility levels of polar solutes in supercritical fluids. The kind of materials in natural plants can affect the solubility of drug like materials in modified supercritical fluid CO_2. Therefore, the use of cosolvents can have a profound effect on increasing solubility levels of polar solutes in supercritical fluids. Moreover, SFE techniques have a unique advantage over the classical solvent extraction techniques in terms of analysis time. Each fraction in an SFE technique takes place in 90 min, which consists of 60 min extraction time and 30 min for modifier evaporating time. In addition, sample handling steps are minimized, thus reducing possible loss of analytes, no organic solvent extractions are involved, and cleanup steps are not employed in this method. Thus,

the SFE technique could be a potential tool in an extraction of drug like materials from natural plants.

Antimicrobial Assay of SFE Extracts from Myanmar Natural Plants

Herbal medicine is used to treat various infectious diseases in most of the world's cultures. The antimicrobial screening tests of SFE extracts of three selected Myanmar natural plants against gram-positive bacteria and yeast are presented in Table 4. The six SFE extracts of *C. aeruginosa* rhizome and four SFE extracts of *C. hystrix* (stem and bark), and five SFE extracts of *A. indica* (leaf and bark) were tested using the Agar disk diffusion method. This method was used due to its simplicity, economy, reproducibility, and speed of performance. The extent of anti-bacterial activity was measured by the visual judging of the zone diameter of inhibition.

Curcuma aeruginosa

Turmeric (Curcuma) is used for the treatment of cut, scrapes, and skin conditions, such as acnes, dermatitis, diaper rash, and psoriasis. It is also known to have anti-inflammatory effects, which includes pain, redness, and swelling in the area of the damage, and it can occur within body tissues, as well as on the surface of the skin.

The antimicrobial activity of *C. aeruginosa* was illustrated in Figure 5. It clearly showed that SFE extracts of Cur 001 to 006 gave weak inhibitory activities against *B. cereus* and moderately active on *B. subtilis*, *S. epidermidis*, *S. aureus*, and *P. acnes*, and inhibition zone diameters had no significant differences. However, the treatment of *E. coli* and *M. furfur* showed no growth inhibition zones in these extracts. Therefore, variation of temperature and pressure were not affected on the inhibitory activity for SFE extracts of *C. aeruginosa* rhizome. An interesting finding in this experiment was the effect of a cosolvent (modifier solvent) on the extraction process. The SFE extract Cur 009 gave a weak inhibitory activity in *B. cereus* and *P. acnes*, and moderate inhibitory activity in *B. subtilis* and *S. epidermidis* when modifier solvent 1:1 ratio of ethanol and 2-propanol was used. Cur 009 showed quite a decreased inhibition zone diameter compared with Cur 001 to 006, and weak inhibition activity on *P. acnes*. Therefore, SFE extracts of *C. aeruginosa* using 100% ethanol modifier solvent is a more convenient extract for the application of related cosmetic products and medicines. Moreover, the variation of inhibition zone diameter with different modifier solvent indicates that modifier solvent plays an important role in the supercritical fluid extraction of drug like materials from natural plants.

Table 4. Antimicrobial activity of SFE extracts of selected myanmar natural plants using agar disk diffusion method

	Inhibition zone diameter (mm)						
Sample	*B. cereus*	*B. subtilis*	*E. coli*	*S. epidermidis*	*S. aureus*	*P. acnes*	*M. furfur*
Cur 001	14[W]	13	—	14	13	12	—
Cur 002	12[W]	13	—	14	12	12	—
Cur 003	12[W]	12	—	13	13	12	—
Cur 004	13[W]	12	—	14	12	12	—
Cur 005	13[W]	14	—	14	13	13	—
Cur 009	11[W]	12	—	9.5	—	11[W]	—
Citrus 001[a]	—	11	—	13	—	13	—
Citrus 002[b]	11	17	—	13	10[W]	10[W]	—
Citrus 003[b]	11	18	—	12	—	—	—
Citrus 004[c]	9.5	14	—	13	—	—	—
Neem 001[b] (Leaf)	13[W]	11	—	—	10 [W]	11	—
Neem 002[a] (Leaf)	11	14	—	10[W]	11	14	—
Neem 003[a] (Bark)	10 [W]	10	—	—	—	12	—
Neem 004[b] (Bark)	10[W]	12	—	—	—	12	—
Neem 012[c] (Leaf)	13	14	—	12	15	17	—

[a]100% Ethanol as modifier solvent.
[b]1:1 ratio of ethanol and 2-propanol as modifier solvent.
[c]Without modifier solvent.

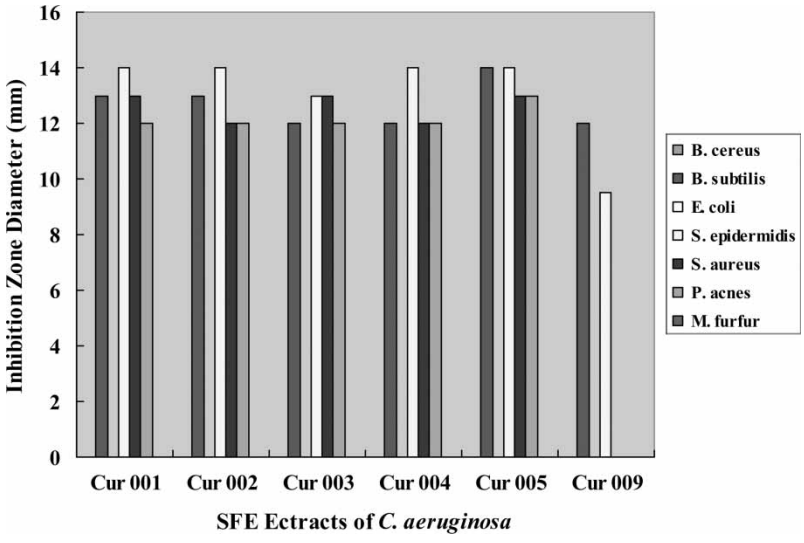

Figure 5. Antimicrobial activity of SFE extracts of *C. aeruginosa* rhizome.

Citrus hystrix

The methanolic extracts from the stem, sac, seed, peel, leaves, and callus of *C. hystrix* have antibacterial, antiamebic, anti-tumor, hypotensive activity, *etc*. The volatile oil from the callus of *C. hystrix* inhibited only *S. aureus*.[11] Most of the studies reported the leaf and fruits of *C. hystrix* and for its stem and bark in literature.

The antimicrobial activity profile of *C. hystrix* was described in Figure 6. From the antimicrobial profile, all four SFE extracts of *C. hystrix* was inactive against *E. coli* and *M. furfur*. The SFE extract of *C. hystrix* (Citrus-001) presented moderate activity against the gram positive bacteria *B. subtilis*, *S. epidermidis*, and *P. acnes*, while inactive against *B. cereus*, and *S. aureus*. The anti-microbial activity of *C. hystrix* was significantly changed when 1:1 ratio of ethanol and 2-propanol was used as the modifier solvent (Citrus-002) and other conditions, such as Citrus 001. Citrus-002 showed good activity on *B. subtilis*, a moderated activity on *B. cereus* and *S. epidermidis*, and weak activity against *S. aureus. C. hystrix* afforded the SFE extract Citrus-003 good activity against gram positive bacteria *B. subtilis*, moderated activity on *B. cereus* and *S. epidermidis*, and was inactive on *S. aureus* and *P. acnes*. The SFE extract of Citrus-004 (without modifier solvent) presented moderate activity on *B. cereus*, *B. subtilis*, and *S. epidermidis*, and was inactive against *S. aureus* and *P. acnes*. The different antimicrobial activity results were obtained in the four SFE extracts of *C. hystrix*. The SFE extracts of Citrus 002 and 003, using 1:1 ratio of ethanol and 2-propanol as modifier solvent, showed the highest

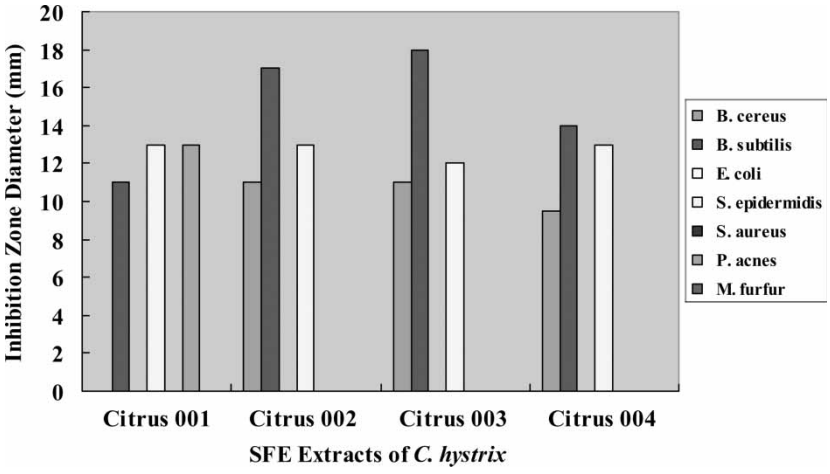

Figure 6. Antimicrobial activity of SFE extracts of *C. hystrix* stem and bark.

inhibition activity on *B. subtilis*. It is the first finding of antimicrobial activity of stem and bark of *C. hystrix* from the native Myanmar.

Azadirachta indica

Neem is a herb of choice for skin diseases because of its bitter principles and has proven to be highly effective in treating chronic disorders like psoriasis, acnes, eczema, itching, dandruff, and warts. The seed, bark, and leaf contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer, and antifungal uses.[12] The neem leaf extract is said to be good for the skin and has been used as a moisturizer in several lotions, creams, and gels. It has been used successfully in face products to control and eliminate the outbreak of severe acnes.

The SFE extracts of *A. indica* leaf (Neem 001 to 004, and 012) were totally inactive against *E. coli* and *M. furfur*. The effect of a modifier solvent was interesting in the SFE application of Neem. Figure 7 clearly showed that the SFE extract of Neem leaf using 1:1 ratio of ethanol and 2-propanol as modifier solvent (Neem 001) resulted in mild activity on *B. subtilis* and *P. acnes*, weak activity on *B. cereus* and *S. aureus*, and also was inactive on *S. epidiermidis*. The SFE extract of Neem leaf (Neem 002) using 100% ethanol as modifier solvent showed mild inhibitory activity on *B. cereus*, *B. subtilis*, *S. aureus*, *P. acnes*, and weak activity on *S. epidermidis*. The SFE extract of Neem leaf NL 012 (without modifier solvent) presented the highest inhibition activity on *P. acnes* and *S. aureus*, and then moderate inhibition activity on *B. cereus*, *B. subtilis*, *S. epidermidis*. Figure 7 clearly showed that the SFE extract of the *A. indica* leaf without a modifier solvent gave more pronounced inhibition activity than others, especially *P. acnes* and *S. aureus*. The SFE extracts of

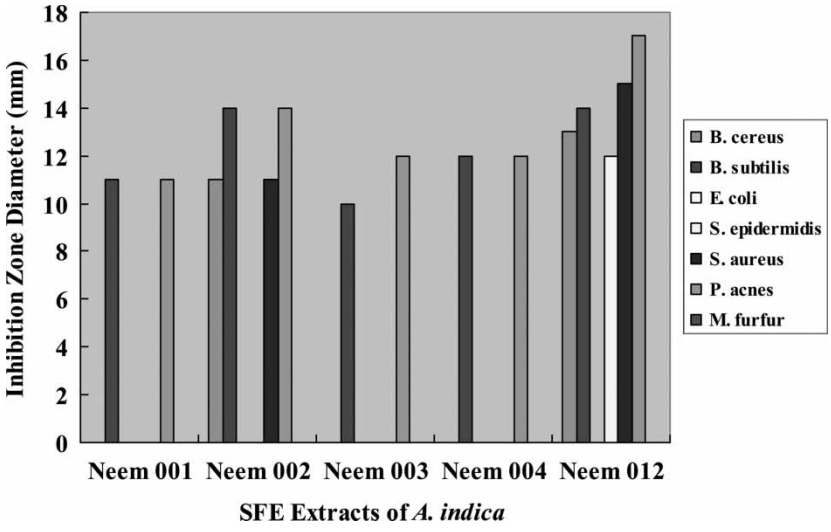

Figure 7. Antimicrobial activity of SFE extracts of *A. indica* leaf and bark.

Neem bark (Neem 003 and 004) were moderately active on *B. subtilis* and *P. acnes*, had weak activity on *B. cereus*, and was inactive against *S. epidermidis* and *S. aureus*. The results clearly showed that SFE extracts of the neem leaf using 100% ethanol as modifier solvent (Neem 001–002) and without modifier solvent (Neem 012), possess antimicrobial activity with notable effects on *B. subtilis*, *S. aureus*, and, especially, the highest inhibition activity on *P. acnes*. Thus, the SFE extracts of *A. indica* without modifier solvent and using 100% ethanol as modifier solvent, were more convenient for the applications.

According to the antimicrobial activity profiles, the SFE extracts of the selected three Myanmar natural plants were totally inactive against Gram negative bacteria *E. coli*. The reason for the difference in sensitivity between Gram(+) and Gram(−) bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, Gram(−) bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram(+) bacteria are more susceptible, having only an outer peptidoglycan layer, which is not an effective permeability barrier. Therefore, the cell walls of Gram(−) organisms, which are more complex than the Gram(+) ones, act as a diffusional barrier making them less susceptible to the antimicrobial agents than the Gram(+) bacteria.[13]

In Myanmar, *C. aeruginosa*, *C. hystrix*, and *A. indica* are used to treat skin effective bacteria. From the antimicrobial screening experiment, SFE extracts of *C. aeruginosa* presented no significant differences in inhibitory activity on all Gram positive bacterial strains. The SFE extracts of *C. aeruginosa* gave slightly increased inhibition activity on *S. epidermidis*, while *A. indica* leaf extracts showed the highest antibacterial activity on *P. acnes* and *S. aureus*,

and a moderate activity on *B. cereus*, *B. subtilis*, and *S. epidermidis*. The SFE extracts of *C. hystrix* (stem and bark) presented the highest antibacterial activity on *B. subtilis*, moderate activity on *B. cereus* and *S. epidermidis*, and weak activity against *S. aureus* and *P. acnes.* The results of this study indicated that the selected Myanmar medicinal plants commonly used by traditional medical practitioners are able to cure skin infections such as *P. acnes*.

The antimicrobial activity results were shown to be mostly dependent on the SFE operation conditions. The SFE process utilizes carbon dioxide at critical temperatures and pressures to extract the active ingredient of the natural plants, without the usual high temperatures or harsh chemicals, thus, yielding a more concentrated extract which resembles the herb more closely. The supercritical fluid extraction technique has several advantages other than extraction techniques, such as minimizing sample handling steps, thus reducing the possible losses of analytes and saving analysis time. No cleanup steps are employed, and no organic solvent extractions are involved in this method. All of these advantages combine to make the supercritical fluid extraction method a potential tool for the extraction of drug materials from natural plants.

As a result of this study, the SFE extracts of the selected Myanmar natural plants suggested that some of the SFE extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

CONCLUSION

According to the investigation of SFE extracts on selected Myanmar natural plants, the results confirmed the therapeutic potency of these plants (*C. aeruginosa*, *C. hystrix*, and *A. indica*) used in traditional medicine. In addition, these results form a good basis for selection of candidate plant species for further phytochemical and pharmacological investigations. The antimicrobial activity could be enhanced if the SFE conditions are varied appropriately, temperature, pressure, and modifier solvent. The most active extracts can be subjected to isolation of the therapeutic antimicrobials, using SFE techniques and undergoing further pharmacological evaluation. Additionally, the SFE extracts of *C. aeruginosa* rhizome, *C. hystrix* stem and bark, and *A. indica* leaf have the possibility to be applied as a constituent of cosmetic products and medicines, because they exhibit antimicrobial activities.

This is the first report on the inhibitory effect of supercritical fluid extracts from selected Myanmar medicinal plants on the growth of bacteria. Although, quantization and characterization of individual SFE extracts was not carried out, the results of the present study substantiate the potential of Myanmar medicinal plants in treating microbial infections of the skin, the majority of which are of infectious origins. However, further study has to be conducted to obtain more convenient drug like materials from these plants using SFE techniques.

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REFERENCES

1. Kaplan, M.; Simmonds, M.R.; Davidson, G. Comparison of supercritical fluid and solvent extraction of feverfew (*Tanacetum parthenium*). Turk. J. Chem. **2002**, *26*, 473–480.
2. Mohamed, R.S.; Mansoori, G.A. *The Use of Supercritical Fluid Extraction Technology in Food Processing, Featured Article-Food Technology Magazine*; The World Markets Research Centre: London, UK, June 2002.
3. Pyo, D.; Lim, C. Supercritical fluid chromatographic separation of dimethylpolysiloxane. Polymer. Bull. Korean Chem. Soc. **2005**, *26* (2), 312–314.
4. Singh, H.; Hasan, M.; Kang, L.J. Supercritical carbon dioxide extraction of sarawakbalck pepper oil. National Symposium of Science and Technology **2003**.
5. Lai, P.K.; Roy, J. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem. **2004**, *11*, 1451–1460.
6. Kress, W.J.O.; Prince, L.M.; Willams, K.J. The phylogeny and a new classification of the gingers (Zingiberaceae). Amer. J. Botany **2002**, *89* (10), 1682–1696.
7. Gul, N.; Mujahid, T.Y.; Samia Ahmad, N.J. Studies on the antibacterial effect of different fractions of *Curcuma longa* against urinary tract infection isolates. Pakistan. J. Biol. Sci. **2004**, *7* (12), 2055–2060.
8. Kandawaswami, C.; Perkins, E.; Soloniuk, D.S.; Middleton, E., Jr. Antiproliferative effects of citrus flavonoids on a human squamous cell carcinoma in vitro. Cancer Lett. **1991**, *41*, 714–719.
9. Schmutterer, H. Ed. *The Neem Tree; Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes*; VCH: Weinheim, Germany, 1995.
10. (a) Vohora, S.B. What is purification of blood? *XXVIII* (1), 72–84; (b) Wagh, S.Y. Clinical studies in viral hepatitis. Deerghayu Intl. **1986**, *4* (4), 17–19.
11. Suri, R.; Radzali, M.; Mohd, A.; Marziah, S.; Arif, Z.J.; Samsumaharto, R.A. Antibacterial assay of *citrus hystrix* extracts. J. Trop. Med. Plants **2002**, *3* (1), 35–41.
12. Prasad, H.C.; Mazumder, R.; Chakcaborty, R. Research on two medicinal plants from 'Ayurvedic' system of medicine *Azadirachta indica* A juss and Melia Azadirach Linn. Their past, present and future. Deerghayu Intl. **1993**.
13. Tadeg, H.; Mohammed, E.; Asres, K.; Gebre-Mariam, T. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J. Ethnopharmacol. **2005**, *100* (1–2), 168–175.

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