International Journal of Phytomedicine 3 (2011) 198-203
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Constituents of the rhizome of *Curcuma aeruginosa* and its DNA fingerprint

Chantana Aromdee[1]*, Senee Polrat[2], Preeya Wangsomnuek[3]

***Corresponding author:**

Chantana Aromdee
Associate Professor,
Faculty of Pharmaceutical Sciences, Khon Kaen. University, 40002, Thailand.
chaaro@kku.ac.th
Phone: 6643-362095
Fax: 4443202379
2Graduate School, Khon Kaen University, 40002, Thailand.
3Department of Biology, Faculty of Sciences, Khon Kaen University, 40002, Thailand.

Abstract

Identity of the rhizhome of *Curcuma aeruginosa* Roxb. was established by three techniques: (1) the DNA fingerprint, (2) the chemical constituents of its volatile oils by using gas chromatograph-mass spectrometer, and (3) thin-layer chromatography (TLC) of the methanol extract. These three techniques were used to differentiate *C. aeruginosa* from its similar species. Result from the polymerase chain reaction (PCR) amplification, different polymorphic bands between the two specimens were found. The relative amounts of camphor, curzerenone and epicurzerenone in the *C. aeruginosa* rhizome were 16.85, 16.81 and 3.5% of total peak areas, whereas 6.04, 0 and 62.84% of total peak areas were found in the Curcuma sp. The thin-layer chromatogram revealed that Curcuma sp. contained curcumine, whereas only traces were detected in C. aeruginosa.

Key words: Curcuma aeruginosa, Zingiberaceae, TLC, GC-MS and DNA fingerprints.

Introduction

C. aeruginosa, Zingiberaceae, is used for carminatives, analgesia and uterus antiinflammation [1]. In Thailand *C. aeruginosa* is called Wahn Mahamek, the colour of its rhizhome is purple. There is another C. sp of which its rhizhome is greenish purple which was incorrectly handled as Wahn Mahamek, either intentionally or unintentionally. Fresh rhizomes of the two species can be distinguished easily. However, when they are dried and powdered for medicinal purpose, they are more difficult to differentiate. This work is part of the establishment of a standard for *C. aeruginosa* rhizome (Wahn Mahamek). The two species were grown at the Ubonrachathani Regional Medical Science Center, Thailand. They were investigated comparatively by DNA fingerprint and chemical constituents, the latter by gas chromatography and thin-layer chromatography.

Material and Methods

Chemicals

Camphor, borneol, curcumene and alkanes (decane to docosane) were purchased from Sigma Aldrich, WI, USA. Solvents were purchased from BDH, England. TLC precoated silica gel plate GF 254 was purchased from Merck Darmstadt, Germany.

Plant Material

Voucher specimens of the two plants (SN001 and SN002), collected by Senee Polrat, were deposited at the Biology Department, Faculty of Science, Khon Kaen University. They were identified by Mrs. Jaree Bansiddhi of the

This work is licensed under a Creative Commons Attribution 3.0 License.

Institute of Medicinal Plant Research, Department of Medical Sciences, the Ministry of Public Health, Bangkok, Thailand.

DNA extraction and amplification

DNA from fresh Curcuma rhizomes was isolated by a modified method of Wangsomnuk et al. [2]. DNA quantity and quality were detected by using ethidium bromide stained agarose gel electrophoresis and observed under UV light. Approximately 40 ng of genomic DNA was used as a template for PCR with trnL-trnF specific primers (PZ2 and PZ3) and six random primers OPA02, OPA03, OPA13, OPA20, OPE4 and OPE7. The reaction was carried out using a thermal cycle programmer (Corbett Research, Mortlake, NSW, Australia) with 3 min at 95 °C for initial DNA denaturation, followed by 40 cycles of denaturation at 93 °C for 30s; annealing temperature at 50 °C for 40s; extension at 72 °C for 30s. The final cycle was followed by 5 min at 72 °C. The PCR amplification products were resolved in 1.2% agarose gels in 0.5x TBE buffer at 50 V for 90 min and stained with ethidium bromide. The 100 bp DNA Ladder Plus (Gene Ruler TM, Fermentas, MD, USA) was used as a molecular size marker. The PCR profiles were visualized under UV light, photographed and stored for further analysis. The PCR products amplified from trnL-trnF chloroplast genes were isolated and purified from agarose gel, and cloned into pGEM-T vector (Promega, CA, USA). Their sequences were analysed and then compared to available chloroplast genes in the NCBI database [3].

Distillation of volatile oil

Specimens were sliced and cut into small pieces. Samples (100 g) were boiled with water (300 mL). The volatile oil collected in a volatile oil apparatus complied with the British Pharmacopoeia Standard [4].

GC-MS analysis

Identification of the volatile oils from the rhizomes of *C. aeruginosa* and estimation of the relative amounts of the constituents was determined by a Gas-chromatograph-Mass Spectroscopy (GC-MS). A solution containing a series of aliphatic alkanes (decane to docosane) was used to determine the linear retention indices. The GC-MS was acquired at Fortune Scientific Ltd for the use of a Trace Ultra GC (Italy) equipped with a DSQ MS (USA). The GC was fitted with a 30m x 0.25mm column coated with a 25 μm film of 5% phenyl-95% dimethylsiloxane film thick, (TR-5, Thermo Fischer Scientific, UK) and the oven temperature programmed to increase from 60 – 240°C (4 C.min^{-1}) and 240 – 270°C (10°C.min^{-1}) then held for 2 min. The injection port was maintained at 270°C and 1 μL of sample was injected in split mode (1:100). The MS was operated in positive ion mode and scanned from 35–650 m/z with an interface temperature of 275°C and the ion source at 220°C. Helium was used as a carrier gas at a flow rate of 1 mL.min^{-1}. The constituents were identified by comparing their mass spectra with the NIST Mass Spectral library and by comparing their linear retention indices [5]. Two authentic standards, camphor and borneol were injected to confirm their identities.

Thin-layer chromatography

A thin-layer silica gel plate GF 254 was used as a stationary phase, the mobile phase composed of benzene:chloroform:methanol (49:49:2 v/v). Samples were prepared by sonicating 0.5 g of dried powdered plant with 5 mL methanol for 30 minutes. A 0.1% w/v curcumine solution in methanol was used as a reference. A portion of 40 μL of each sample solution and 5 μL of the reference solution were applied to the plate and developed. The separated components were detected visually and under a UV light at wavelengths of 254 and 366 nm.

Results and Discussion

C. aeruginosa and Curcuma sp. were characterised macroscopically. The shape of the rhizomes of the two plants are similar. However, the color of the rhizome of *C. aeruginosa* was bluish purple whereas the other was blue at the edge and yellowish green in the middle as can be seen in Figure1. It is not difficult to identify these two types of the fresh rhizomes. However, once it was dried and ground into powder, they are difficult to distinguish.

The DNA fingerprints of the two Curcumas are documented (Figure2, Table 1). The DNA isolation method employed was simple and rapid. Good yields of good quality of DNA were obtained from mature rhizomes of both samples. Random amplified polymorphic DNA (RAPD) and related techniques require less DNA, but purity is necessary to ensure repeatability and confidence [6], [7]. The isolated DNA was amenable to DNA fingerprinting using RAPD analysis and trnL-trnF gene-specific cloning in which good amplifications were obtained. Five of the six random-decamer primers selected (OPA02, OPA03, OPA13, OPA20, OPE4 and OPE7), proved to be useful for characterization of the DNA from the curcuma rhizomes due to production of clear, reproducible fragments and amplification of several polymorphisms between two accessions (Figure 2), whereas one (OPA02) was excluded due to amplification of the same fragments in both samples. The useful primers gave a total of twelve polymorphic fragments, ranging from approximately 578 to 1,620 bp (Table 1). Genetic structuring was evident due to the detection of specific bands in each sample examined. These should be useful as SCAR markers to distinguish between the two specimens and for identification as can be seen in Figure 2 and Table 1.

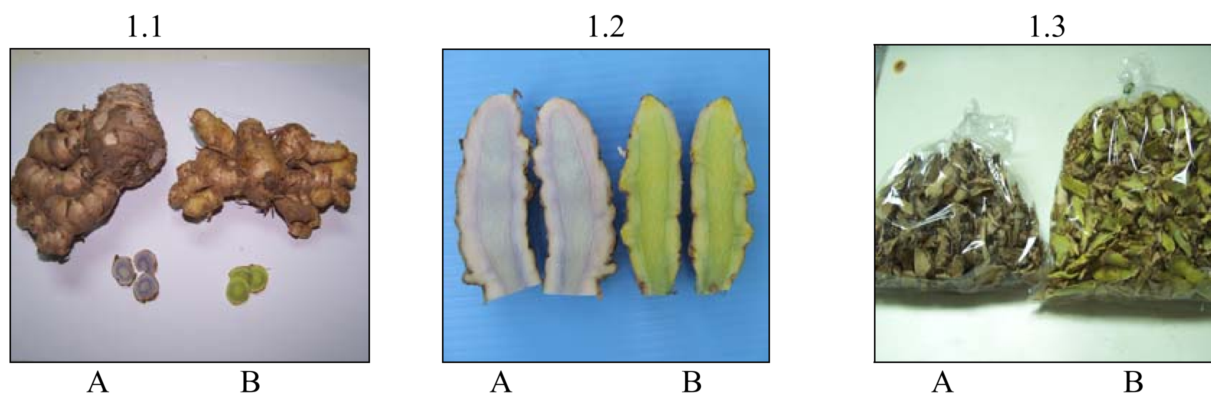

Figure 1 Rhizomes of *C. aeruginosa* A) and Curcuma spp B): 1.1) intact and cross section, 1.2) transverse sections and 1.3) dried sliced samples.

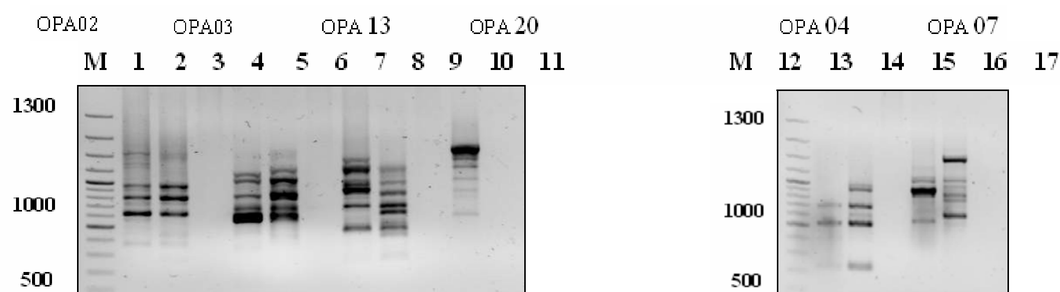

Figure 2 PCR amplification electrophoretic patterns obtained with random amplified polymorphic DNA (RAPD). 1, 4, 7, 10, 12 and 15 are PCR products from rhizomes of C. aeruginosa; 2, 5, 7, 8, 11, 13 and 16 are PCR products from rhizomes of Curcuma sp. and 3, 6, 9, 14 and 17 are negative controls and M is a 100 bp DNA ladder plus (Fermentas).

From the experiment, the contents of volatile oil obtained by steam distillation of were 0.44% for *C. aeruginosa* and 0.31% from Curcuma sp.. The GC-MS chromatogram of the *C. aeruginosa* extract is shown in Figure 3. The GC-FID chromatograms of the two samples are shown in Figures 4 and 5. The constituents of the two volatile oils, their retention times, and linear retention indices are listed in Table 2. The relative amounts of camphor, curzerenone and epicurzerenone in volatile oil of *C. aeruginosa* were 16.85, 16.81 and 3.5%, and 6.04, 0 and 62.84% of total peak areas of the Curcuma sp.. The difference is useful for identification of the two curcumas. The TLC chromatograms of the methanol extracts from the curcumas are shown in Figure 6. A significant amount of curcumine was found in Curcuma sp. but hardly any was detected in C. aeruginosa.

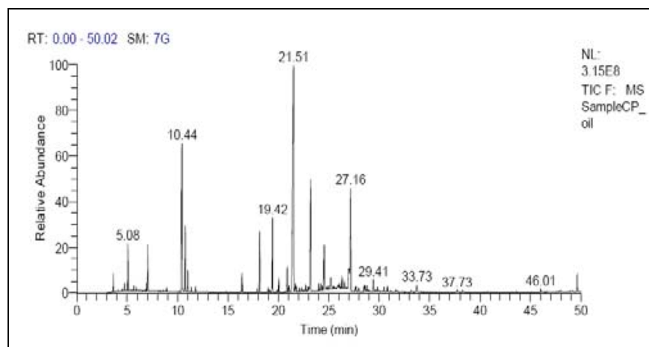

Figure 3. Gas Chromatogram of volatile oil from rhizomes of C. aeruginosa, 1 μL of volatile oil was injected (split mode 1:100) at 270 °C onto a TR-5 column using helium as carrier gas at a flow rate of 1 mL.min-1. The oven temperature was programmed for 60 – 240 °C (4 °C.min-1) and 240 – 270 ° C (10 °C.min-1) then held for 2 min. The detector and interface were maintained at 275 °C and the ion source at 220oC and the MS scanned in positive ion mode over 35 – 650 m/z.

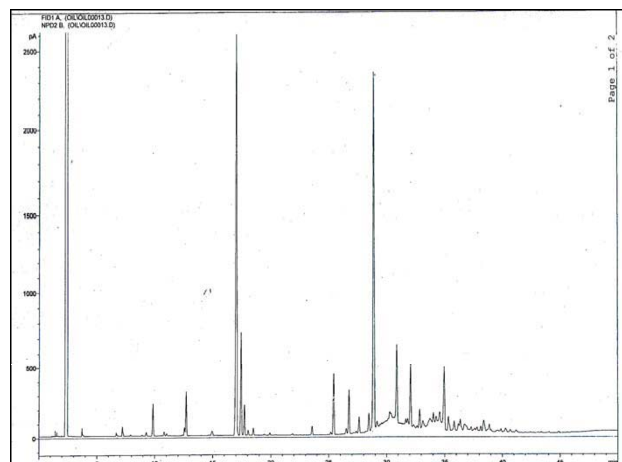

Figure 4 Gas Chromatogram of volatile oil from rhizomes of C. aeruginosa, 1 μL of volatile oil was injected (split mode 1:50) at 270 °C onto a HP-5 column using nitrogen as carrier gas at a flow rate of 2 mL.min-1. The oven temperature was programmed for 60 – 240° C (4 °C.min-1) and 240 – 270 °C (10 °C.min-1) then held for 2 min. The FID detector was maintained at 275 °C.

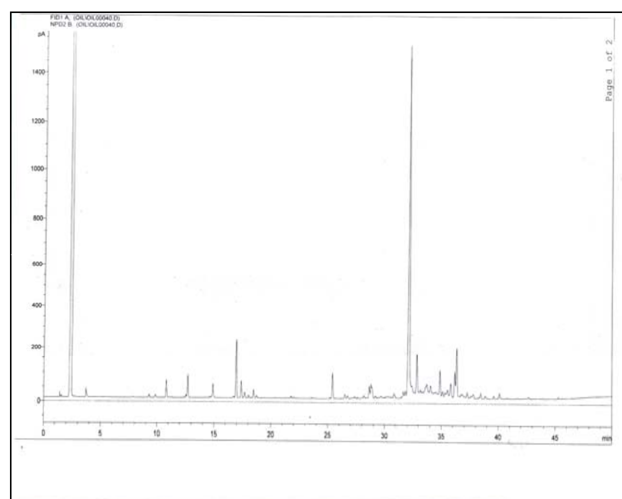

Figure 5 Gas Chromatogram of volatile oil from rhizomes of Curcuma sp. The conditions were as described in Figure 4.

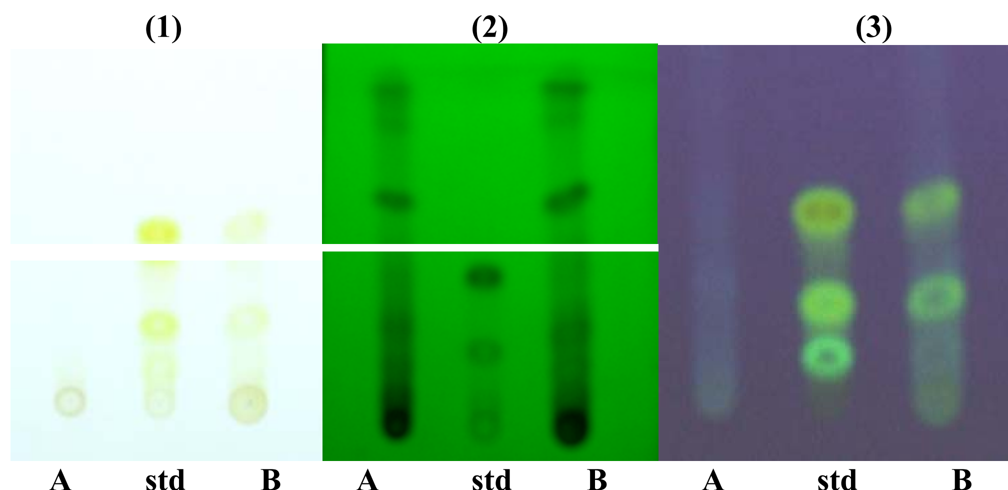

Figure 6 Thin-layer chromatograms of Curcumine (std) and methanol extracts from the rhizomes of *C. aeruginosa* (A) and Curcuma sp. (B). Stationary phase: a precoated silica gel plate GF 254; Mobile phase: benzene: chloroform: methanol (49:49:2); Detection: in daylight (1), and under the UV light at the wavelengths of 254 nm (2) and 366 nm (3).

Conclusions

These 3 tecniques can be carried out in most laboratories. They are now widely used and easy to perform. Five random-decamer primers can be used as DNA fingerprints as they are useful to distinguish the two species. From the GC-MS method curzerenone was not found the Curcuma sp.. Whereas traces of curcumine was detected in *C. aeruginosa* by TLC. These three techniques are applicable to distinguish other similar curcuma probably with slight modifications.

Authors' contribution

Mr. Polrat was a post-graduate student at the Faculty of Pharmaceutical Sciences. Aromdee is the main advisor responsible for the chemical identities. Wangsomnuek is the coadvisor responsible for the DNA fingerprints.

Acknowledgements:

We thank Mr. Kittiwongsunthorn, the director of Ubonratchathanee Medical Science Center and Ms. Dechachartiwongse, a herbal medicine specialist, at the Ministry of Public Health, for let us know the problem from public hearing about differentiating these two curcumas. We also thanks Professor R. ledger my exsupervisor for kindly editing this manuscript.

References

1. Jarikasem S, Thubthimthed S, Chawanannoraseeth K, Suntrontarasat T: Essential Oil from Three Curcuma species Collected in Thailand. In Proceedings of WOCMAP III: 3-7 February 2003; Chiangmai. Edited by E. Brovelli et al. International Society for Horticultural Science (ISHS); 2005;37-41.
2. Wangsomnuk PP, Wangsomnuk P, Maza B: Biodiversity and molecular aspects of Curcuma species from North-Eastern Thailand. In Proceedings of the 3rd Symposium on the Family Zingiberaceae: 7-12 July 2002; Khon Kaen. Edited by Chantaranothai P, Larsen K, Sirirugsa P, Simpson D: Applied Taxonomic Research Center; 2002;109-19.
3. The National Center for Biotechnology Information. Available at http://www.ncbi.nlm.nih.gov/. Accessed April 20, 2011.

4. British Phamacopoeia 2010: Essential oils in herbal drugs. London, England: The Stationary Office at the University Press. 2009;vol.4:p A289.
5. Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phase. J. of Chromatography. 1990:503(1):1-24.
6. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucl Acids Res. 1990;18:7213-8.
7. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res.1990;18:6531-5.