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DNA-binding Activity and Partial Characterization by Fourier Transform Infrared Spectroscopy (FTIR) of *Curcuma longa* L. SC-CO_2 Extracts

Magdalene Mae L. Del Socorro*, Franco G. Teves and Ma. Reina Suzette B. Madamba
Dept. of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, PHILIPPINES

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Abstract

Nowadays, an increasing interest in a number of plants for their medicinal properties had been shown by a large number of scientific studies conducted. Turmeric (Curcuma longa Linn.) is among those studied plants demonstrating potential medicinal properties. In this study, SC-CO_2 extracts of C. longa L. using a local variety were characterized and studied for their DNA-binding activity using the Biomolecular-chemical screening method. Results showed Rf-ratio values ranges from 0.50-0.83 which indicate the presence of compounds with strong to moderate affinity towards DNA. Fourier Transform Infrared Spectroscopy was employed for the detection and characterization of the compounds present in the extracts. FTIR results of C. longa L. extracts at 10MPa, 20MPa and 30MPa revealed almost the same high peak levels which indicate the presence of the same functional groups O-H stretching in phenols, -C-H stretching of alkanes and C=O stretch of carbonyl groups. Presence of these functional groups and DNA-binding affinity exhibited by C. longa L. towards DNA suggests that the plant has a potential pharmacological property.

Keywords: *Curcuma longa* L., Biomolecular-chemical screening, DNA-binding affinity, fourier transform infrared spectroscopy (FTIR), SC-CO_2 extraction.

Introduction

Numerous studies demonstrated the preventive and curative properties of several plants to certain diseases. Diseases that could either be genetically inherited or those that are commonly caused by unhealthy lifestyle. However, the potential use of plants as a source of new drugs is still poorly explored of the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and an even smaller percentage has been properly studied in terms of their pharmacological properties[1]. Turmeric (*Curcuma longa* Linn.) is among those significantly studied plants demonstrating potential medicinal properties. It is a low growing shrubby species in the family Zingiberaceae[2]. Turmeric is used as a food additive (spice), preservative and colouring agent in Asian countries, including China and South East Asia. It is also considered as auspicious and is a part of religious rituals. It has been used as an ethnomedicine from time immemorial in Ayurvedic system, in which practice of it for thousands of years has alleviated illnesses and attributed overall positive health[3]. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, cough, diabetic wounds and hepatic disorders[4].

Supercritical fluid extraction (SFE) process is a rapidly developing method used to produce bioactive compounds by pure technology, under mild conditions. Unlike other extraction methods, long extraction time, low yield, toxic solvent residue, labour-intensive operation and degradation of thermo-sensitive compounds are avoided in SFE. The unique characteristic of this system is usage of gases above their critical point to extract selective soluble components from a raw material. Carbon dioxide (CO_2) is the most widely used solvent in SFE, since it is physiologically harmless, environmentally safe, non-explosive, and readily available and it can be easily removed from products. In recent years, several researchers studied the extraction of natural compounds from plant matrix by using supercritical carbon dioxide (SC-CO_2)[5].

DNA-binding activity can be assessed using a screening strategy called the Biomolecular-chemical screening method. It is a novel approach which combines the chemical screening strategy with binding studies of biological relevance. It allows examining binding properties of low molecular weight metabolites to certain bio-macromolecules. Biomolecular-chemical screening method is useful to screen binding behavior towards DNA of both, pure metabolites by one-dimensional TLC, and crude extracts by two-dimensional TLC[6].

Fourier Transform Infrared Spectroscopy (FTIR) is use to detect and characterize compounds present in plant extracts. It is a technique that uses the approach of metabolic fingerprinting to understand the composition, chemical structure and discrimination of biomolecules in medicinal plants. The technique was being successfully explored for the study of biological materials and eventually became an accepted tool for the characterization of biomolecules[7].

Due to the broad usage, displayed potential medicinal properties and accepted use of *Curcuma longa* L. in traditional medicine,

this study aimed to study the DNA-binding properties of the biologically active compounds of the plant using Biomolecular-chemical screening method and to characterize its extracts using Fourier Transform Infrared Spectroscopy (FTIR) for the detection of the biomolecules present in the plant sample.

Even though several studies have already detected a range of bioactive compounds from this plant *Curcuma longa* Linn., further screening for biologically active compounds from this plant may aid in the thorough and substantial research studies on the look for new drugs. This may provide information for future studies aiming to search for drugs needed for the treatment of cancer. Furthermore, this will also provide a baseline data for advanced scientific research in addition to the preceding studies conducted.

Material and Methods

Preparation of Sample: Fresh native *Curcuma longa* L. rhizomes were obtained from Marawi, Lanao del Sur, cleaned, sliced thinly and subjected to modified cryogenic grinding method. Thinly sliced *C. longa* L. rhizomes were submerged to liquid nitrogen for approximately 5 minutes and were grinded fast using a mortar and pestle to achieve the desired powder form of the rhizomes. The liquid nitrogen used was provided by the Northern Mindanao Agricultural Research Center, Malaybalay Stockfarm, Dalwangan, Malaybalay City, Bukidnon, Philippines.

Extraction Procedure: *Curcuma longa* L. samples were subjected to super critical carbon dioxide extraction using a Supercritical Fluid Extractor (Akico) in the Hydraulics and Fluid Mechanics Laboratory at the College of Engineering, MSU-Iligan Institute of Technology, Iligan City.

Biomolecular-chemical Screening: Biomolecular-chemical screening was conducted according to the method described by Maier and colleagues with a chromatographic solvent system composed of toluene:ethyl acetate [9.3:0.7].

Fourier Transform Infrared Spectroscopy: Fourier Transform Infrared Spectroscopy was performed using Perkin-Elmer System (Perkin-Elmer Inc. USA) consisting of a spectrum 100 FTIR spectrometer monitored at 4000-550 cm^{-1}.

Results and Discussion

Extraction of *C. longa* L. was done using Super Critical Carbon dioxide Fluid Extraction. In determining the DNA-binding properties of *Curcuma longa*, a novel screening strategy called the Biomolecular-chemical Screening was used which is an approach advantageously used to detect the interaction of pure compounds with DNA. Biomolecular-chemical screening combines the analysis of the chromatographic and chemical behavior of secondary metabolites on TLC plates with the binding studies of these molecules with biomolecules like DNA. Biomolecular-screening and studies on DNA-binding properties of secondary metabolites from complex crude extracts make use of two-dimensional TLC analysis. Two TLC plates were prepared for each turmeric sample at SC-CO_2 10MPa, 20MPa and 30MPa, the measuring plate and the reference plate. Both chromatoplates were expected to produce the same chromatogram. However, this was not always the result since samples sometimes produce varying Rf values due to some external factors such as moisture and position of the plates inside the chamber.

As shown in table 1, *C. longa* has a DNA-binding property towards DNA based on obtained Rf-ratio values (Rf_2/Rf_1) less than 1. *C. longa* SC-CO_2 extract at 10MPa dissolved in acetone gave Rf value range at 0.50-0.83 which indicates strong-moderate affinity of the sample towards DNA. *C. longa* SC-CO_2 extract at 20MPa and 30MPa dissolved in acetone showed Rf values ranging from 0.68-0.80 and 0.66-0.82 respectively suggests moderate-strong affinity of sample towards DNA. *C. longa* SC-CO_2 extract at 20MPa and 30MPa dissolved in chloroform revealed Rf values 0.69-0.84 and 0.50-0.79, respectively. SC-CO_2 extract at 10MPa showed Rf values at 0.70-0.82. From the results obtained we can state that *C. longa* SC-CO_2 extracts contain compounds with DNA-binding properties (R^/Rf^O^) which clearly support the idea that Curcumin is often cited as pleiotropic, meaning it has the ability to interact with many cell targets and has also been known to exert anti-inflammatory and growth-inhibition by inhibiting expression of certain growth factors in cancer cells[9]. It has been shown to inhibit the activity of lipoxygenase[10] and inhibit the activation of various transcription factors that play a key role in inflammation, cell survival and proliferation, and angiogenesis[11].

Fourier Transform Infrared Spectroscopy: Results of functional group analysis using FTIR revealed the existence of various characteristic functional groups in *C. longa* at SC-CO_2 pressures 10MPa, 20MPa and 30MPa (figure-1). *Curcuma longa* Linn. SC-CO_2 extracts showed a range of 16-20 absorption bands and three strong peaks in the FTIR spectra. A very strong absorption band was observed at a range 3445.2-3448.78 cm^{-1} may be due to the presence of bonded O-H stretching of phenols and the strong absorption band observed at 2923.95-2926.37 cm^{-1} may represent bonded –C-H stretching of alkanes. The peak at 1682.75-1683.45 cm^{-1} can be attributed as bonded C=O stretching of carbonyls. Absorption bands at 1617.68-1617.99 cm^{-1}, 1514.58-1514.96 cm^{-1}, 1377.25-1377.35 cm^{-1} and 1208.98-1216.35 cm^{-1} are asymmetrical stretching of Nitro Compounds. The band at 1446.18 cm^{-1} may indicate the presence of scissoring and bending –C-H- group of alkanes. Peaks at 1122.86-1123.32 cm^{-1} and 1034.01-1034.33 cm^{-1} can be due to the presence of stretching C-O alcohols, ethers, carboxylic acids and ester group of compounds. As shown in figure-1, *Curcuma longa* Linn. samples obtained at 10MPa, 20MPa and 30MPa SC-CO_2 extraction showed almost the same high peak levels and thus indicate the presence of the same functional groups O-H stretching in phenols, -C-H stretching of alkanes and C=O stretch of carbonyl groups, respectively.

The observed presence of bonded O-H stretching of phenols in the plant and DNA-binding affinity exhibited by *C. longa* L. towards DNA suggests that the plant has a displayed potential medicinal property. Some phenolic compounds are believed to be cancer chemopreventives[12], these are compounds that may decrease the risk of developing cancer[13], which is believed to become the main cause of death in worldwide and also play role in the prevention of cardiovascular diseases[14]. Phenolic compounds found in plants may also have antioxidant effects by reacting with and capturing dangerously reactive compounds before it can react with other biomolecules and cause serious damage. Moreover, it may help promote healthy aging by reducing DNA damage caused by these free radicals. In which overproduction of such free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases, including cancer[15]. On the other hand, several studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects[16].

Presumably, these observed groups O-H stretching in phenols, -C-H stretching of alkanes and C=O stretch of carbonyl groups comprise Curcumin the bioactive substance of *C. longa* which is mainly made up of –C-H stretching of alkanes and bonded O-H. Curcumin is a polyphenol compound and can exist in at least two tautomeric forms, keto and enol[17]. It is thought to be the most active pharmacological agent of turmeric. Curcumin (diferuloylmethane) is responsible for the yellow colour, and comprises curcumin I, curcumin II and curcumin III[18]. It has been shown that curcumin have a wide spectrum of biological actions such as anti-inflammatory, antioxidant, anticancer, antidiabetic, antiallergic, antiviral, antiprotozoal, and antifungal activities[19]. All these shown multitude beneficial medicinal effects of *C. longa* make it an excellent candidate for development of new drugs. One of the biochemical mechanisms attributed to the anticarcinogenic activity of curcumin is related to its carbonyl group[20].

Table-1
Corresponding Rf-ratio values of separated bands of *Curcuma longa* subjected to 2D-TLC

Samples			**2D-TLC**					**Rf Values**
	bands	b	a	Rf1	b	a	Rf2	Rf2/Rf1
A_1	1	0.6	5	0.12	-	5	-	-
	2	1.4	5	0.28	0.7	5	0.14	0.50
	3	2.0	5	0.40	1.1	5	0.22	0.55
	4	2.6	5	0.52	1.7	5	0.34	0.65
	5	2.9	5	0.58	2.1	5	0.42	0.72
	6	3.6	5	0.72	3.0	5	0.60	0.83
A_2	1	0.6	5	0.12	-	5	-	-
	2	1.3	5	0.26	-	5	-	-
	3	2.5	5	0.50	1.7	5	0.34	0.68
	4	2.8	5	0.56	2.1	5	0.42	0.75
	5	3.5	5	0.70	2.8	5	0.56	0.80
A_3	1	0.7	5	0.14	-	5	-	-
	2	1.2	5	0.24	-	5	-	-
	3	2.4	5	0.48	1.6	5	0.32	0.66
	4	2.7	5	0.54	1.9	5	0.38	0.75
	5	3.4	5	0.70	2.8	5	0.56	0.80
C_1	1	1.2	5	0.24	-	5	-	-
	2	2.4	5	0.48	1.7	5	0.34	0.70
	3	2.7	5	0.54	2	5	0.40	0.74
	4	3.4	5	0.68	2.8	5	0.56	0.82
C_2	1	1.1	5	0.24	-	5	-	-
	2	2.3	5	0.46	1.6	5	0.32	0.69
	3	2.6	5	0.52	2	5	0.40	0.76
	4	3.3	5	0.66	2.8	5	0.54	0.84
C_3	1	1.6	5	0.32	-	5	-	-
	2	1.2	5	0.24	0.6	5	0.12	0.50
	3	2.4	5	0.48	1.6	5	0.32	0.67
	4	2.8	5	0.56	2.0	5	0.40	0.71
	5	3.4	5	0.68	2.7	5	0.54	0.79

A_1 – *C. longa* SC-CO_2 at 10MPa in acetone 1mg/ml ; A_2 - *C. longa* SC-CO_2 at 20MPa in acetone 1mg/ml ; A_3 - *C. longa* SC-CO_2 at 30MPa in acetone 1mg/ml ; C_1 - *C. longa* SC-CO_2 at 20MPa in chloroform 1mg/ml ; C_2 - *C. longa* SC-CO_2 at 20MPa in chloroform 1mg/ml ; C_3. *C. longa* SC-CO_2 at30MPa in chloroform 1mg/ml ; (-) indicate that chromatograms appeared no separation during 2D-TLC analysis

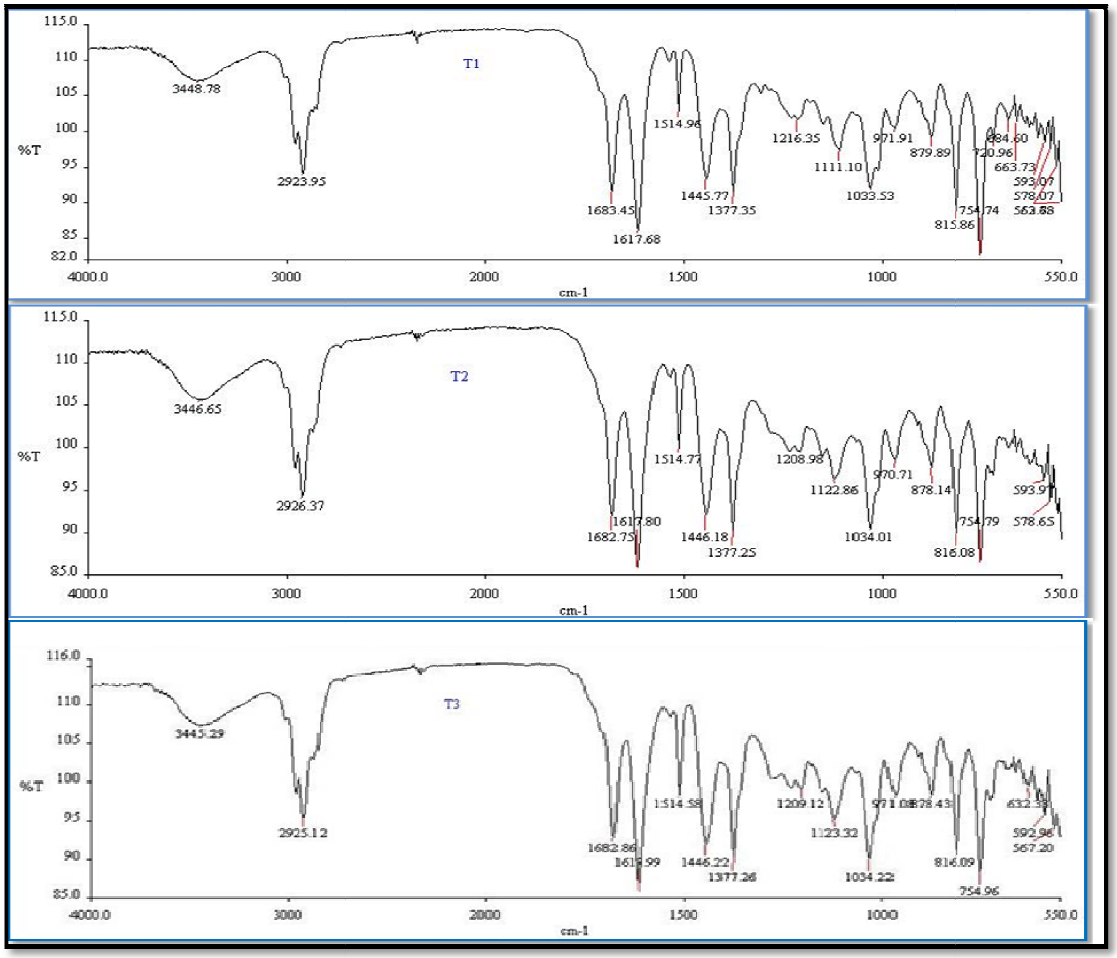

Figure-1
FTIR spectra of *Curcuma longa* L. SC-CO_2 extracts. T_1 - *C. longa* L. extract at SC-CO_2 10MPa; T_2 - *C. longa* L. extract at SC-CO_2 20MPa; and T_3 - *C. longa* L. extract at SC-CO_2 30MPa

Conclusion

Biomolecular-screening of *C. longa* SC-CO_2 extracts obtained Rf-ratio values (Rf_2/Rf_1) less than 1 which suggests moderate to strong affinity of sample towards DNA. FTIR spectra of *C. longa* L. SC-CO_2 10MPa, 20MPa and 30MPa extracts showed almost the same high peak levels and indicate the presence of the same functional groups O-H stretching in phenols, -C-H stretching of alkanes and C=O stretch of carbonyl groups, respectively, thus all three extracts contain the same compounds which may differ only in their content level. Observed presence of these functional groups and DNA-binding affinity exhibited by *C. longa* L. towards DNA suggests that the plant has a potential medicinal property. Further biochemical screening on this plant for the observed biological activities is recommended to validate and improve the observed bioactivities. Further elucidation and identification is recommended specifically on the separated bands exhibiting DNA affinity to further investigate the potential medicinal property of *Curcuma longa* L.

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