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DNA-binding property and FT-IR spectroscopic characterization of SC-CO_2 extracts from leaves, seeds, and roots of *Moringa oleifera* Lam.

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Abstract. A total of nine SC-CO_2 extracts were obtained from leaves, seeds, and roots of *Moringa oleifera* Lam. at 10 MPa, 20 MPa, and 30 MPa atmospheric pressures. Extracts were screened for its binding behavior towards DNA by two-dimensional TLC analysis in solvent system of toluene and ethyl acetate (9.3:0.7 mL v/v). All extracts showed DNA binding properties except the leaf extracts at 30 MPa where the computed Rf ratio-value was 1.023 only those with Rf ratio <1.0 has DNA binding affinity based on published reports. The extracts from seeds and roots at 10 MPa and seeds at 20 MPa showed moderate to strong DNA affinity with Rf ratio-values of 0.647, 0.789, and 0.818 respectively. The FT-IR characterization of all the extracts showed almost the same spectra with identical absorption bands that presumably belonging to fatty acids such as oleic, linoleic, erucic which dominate significantly. The results of the study serve as a basis for further exploration on the pharmaceutical potential of *M. oleifera* particularly that of its seeds and roots.

Key Words: SC-CO_2 (Supercritical Carbon Dioxide) extracts, two-dimension TLC (Thin Layer Chromatography) analysis, FT-IR (Fourier Transform Infrared Spectroscopy).

Introduction. There are many researches today that look into possible potentials of plants for pharmacological and even agricultural advancements. Heinrich & Gibbons (2001) attested that natural products derived from medicinal plants might play an interesting role for the identification of novel drugs for treating several diseases. The broad spectra of structurally diverse metabolites of biological activities in plants can be best screened through biomolecular-chemical screening as formulated by Maier et al (1999); which is a novel screening strategy that combines the analysis of the chromatographic and chemical behavior of metabolites on thin layer chromatography (TLC) plates in different solvent systems with binding studies of low molecular weight metabolites towards DNA. This method makes use of two-dimensional TLC analysis. In the first dimension (1-D TLC), extracts are separated; and in the second dimension (2-D TLC), binding towards DNA are being analyzed.

The Philippine government through the Department of Health has recently intensified the campaign on the use of medicinal plants to address the high cost of pharmaceutical products. One of the medicinal plants that was highlighted – due to its claim for its multiple benefits is *Moringa oleifera* Lam. ("malunggay", in the local vernacular).

M. oleifera is the most widely cultivated species of *Moringaceae* (order Brassicales), and one of the world's multi-functional tree. The plant was reported to contain various amino acids, fatty acids, vitamins, and nutrients (Nesamani 1999). The seed oil is also high in tocopherols (Tsaknis et al 1999). The leaf extracts were found to regulate thyroid status and cholesterol levels (Tahiliani & Kar 2000; Ghasi et al 2000). The roots contain active antibiotic principle, pterygo-spermin which inhibits the growth of many gram positive and gram negative bacteria (Paul & Didia 2012).

This study employed Supercritical Carbon Dioxide (SC-CO_2) extraction because it uses carbon dioxide which are harmless physiologically, safe environmentally, non-

explosive and can be easily removed from the natural products (Simandi et al 2002; Cavero et al 2006). This extraction method is a rapidly developing method to produce bioactive compounds as compared with traditional extraction methods such as steam distillation and organic solvent extraction using percolation, maceration, and Soxhlet techniques, in which they have long extraction time, low yield, toxic solvent residue, laborious operation, and degradation of thermo-sensitive compounds (Bimakr et al 2009; Lang & Wai 2001).

In addition, the FT-IR spectroscopy was also performed in order to identify the chemical functional groups present in the SC-CO_2 extracts from *M. oleifera* leaves, seeds, and roots; wherein infrared spectroscopy can result in a positive identification (qualitative analysis) in every fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atom making up the samples (Kumari et al 2006; Alves et al 2010).

This study generally aims to screen for the binding behavior towards DNA of the SC-CO_2 extracts from of leaves, seeds, and roots of *M. oleifera* and further identify its extracts through FT-IR spectroscopy. Moreover, the study aimed to contribute new data to the existing knowledge and may provide insight to the suggested high-value nutritious and healing properties of *M. oleifera* by highlighting the importance of DNA-binding property and possible chemical functional groups present in the SC-CO_2 extracts from leaves, seeds, and roots of *M. oleifera*.

Material and Method

Preparation of Samples. Once *M. oleifera* trees were established for sampling, seeds, leaves, and roots were harvested. Leaves were stripped off from the stems as well as the seeds from the pods. Any damaged samples (e.g. discolored leaves) were set aside and not included. The samples were rinsed in sterile water and also in a very weak bleach solution (1:100) to remove dirt and germs. In this sample preparation, the process made used of cryogenic grinding which is a very effective technique for taking hard substances like plants and turning them into powder without changing its color and other properties as well such as the flavor and nutrition (Shimo et al 1991). It uses liquid nitrogen (-196°C) as cryogen and so the samples were first frozen with liquid nitrogen and were pulverized using a mortar and pestle. The liquid nitrogen was provided by the Stockfarm Mini-plant of Region X - Department of Agriculture, Malaybalay, Bukidnon. The samples were then dried up to approximately 15% - 20% moisture content.

Extraction of M. oleifera seeds, leaves, and roots. The extraction of *M. oleifera* seeds, leaves, and roots were conducted using Supercritical Carbon Dioxide (SC – CO_2) Fluid Extractor (Akico brand) donated by the Research Center of Supercritical Fluid Technology, Tohuko University, Japan. It is located at the Hydraulics and Fluid Mechanics laboratory of the College of Engineering, MSU-IIT.

Preparation of the salmon sperm DNA. The salmon sperm DNA was purchased from Sigma Laboratories, U.S.A. through Chemline Scientific Enterprise, Philippines. A 2 mg of DNA sample was diluted with 1 mL of Tris- EDTA (TE) buffer to obtain a concentration of 2 mg/mL solution. This stock solution was used for the binding affinity procedures of *M. oleifera* seeds, leaves, and roots extracts; and stored in the refrigerator to guarantee stability of the DNA until used.

Two-dimensional TLC analysis. The *M. oleifera* seeds, leaves, and roots extracts that dissolved in chloroform which were used in 1-D TLC were applied in 2-D TLC. Toluene and ethyl acetate (9.3:0.7 mL v/v) was found to be a solvent system which produced a better development of the extracts.

The measuring plate was spotted with DNA while the reference plate was spotted without DNA. The plates were run in two developments, wherein the first development was for the purpose of separating molecules constituting the compound produced. The separations were viewed under UV light at 254 nm. As for the second development,

homogenized salmon sperm DNA was spotted above to the chromatogram using the capillary tube and viewed under UV light at 254 nm also.

Evaluation of thin layer chromatography measured in Rf (Retention factor) value expressed as:

$$\text{Rf value} = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent front}}$$

Moreover, the Rf ratio-value of each metabolite represented in the reference and measuring plates were measured. The reference plate was the source of Rf_1 value and the measuring plate was the source of Rf_2 value. The ratios of the Rf_2 (with DNA) with Rf_1 (without DNA) values were computed to determine the affinity of each metabolite to DNA molecule.

Fourier Transform-Infrared (FT-IR) spectroscopy. A FT-IR spectrometer was used to identify the chemical functional groups of SC-CO_2 extracts from leaves, seeds, and roots of *M. oleifera* using the PerkinElmer - FT-IR system (PerkinElmer, Inc., USA) consisting of a spectrum 100 FT-IR Spectrometer. Each spectrum was registered from 4000 to 550 cm^{-1}.

Results and Discussion. The findings revealed that the chromatograms of all SC-CO_2 extract from leaves, seeds, and roots of *M. oleifera* at different atmospheric pressures produced by two-dimensional TLC analysis had binding affinity towards DNA except the leaf extracts at 30 MPa with Rf ratio-value of 1.023 as measured and recorded in Table 1.

Table 1

Two-dimensional TLC analysis of SC-CO_2 extracts from leaves, seeds, and roots of *Moringa oleifera* with the corresponding Rf ratio-values of the chromatograms produced

M. oleifera extracts	*1-D TLC*	*2-D TLC*		*Rf_2/Rf_1 ratio*
		without DNA	*with DNA*	
Seeds at 10 MPa	0.156	*	*	-
	0.467	0.362	0.234	0.647**
Leaves at 10 MPa	0.170	*	*	-
	0.447	0.174	0.152	0.875**
	0.553	0.304	0.304	1.000
	0.957	0.957	0.913	0.995**
Roots at 10 MPa	0.391	0.422	0.333	0.789**
	0.978	0.889	0.911	1.025
Seeds at 20 MPa	0.500	0.489	0.400	0.818**
	0.938	*	*	-
Leaves at 20 MPa	0.958	1.000	0.909	0.909**
Roots at 20 MPa	0.313	*	*	-
	0.500	0.489	0.447	0.913**
	0.958	0.957	0.936	0.978**
Seeds at 30 MPa	0.489	0.512	0.488	0.955**
	0.979	0.977	0.977	1.000
Leaves at 30 MPa	0.500	*	*	-
	0.783	*	*	-
	0.978	0.978	1.000	1.023
Roots at 30 MPa	0.391	0.435	0.457	1.050
	0.696	*	*	-
	0.913	0.957	0.913	0.977**

***** Chromatograms appeared no separation during 2-D TLC analysis, ** Chromatograms revealed binding affinity towards DNA.

DNA binding affinity was known when the values of the fractions were significantly below one. As discussed by Maier et al (1999), all the bands produced by the extracts in the second dimension were considered potential molecules as antibiotics with affinity to DNA binding. Metabolites with moderate to strong DNA binding property were with Rf_2/Rf_1 ratios less than 0.85, while as for the weak affinity to DNA binding was observed at Rf ratios-value greater than 0.85. The extracts from seeds and roots at 10 MPa showed moderate to strong affinity to DNA binding with Rf ratio-values of 0.647 and 0.789 respectively; as well as with the seeds extracts at 20 MPa with Rf ratio-value of 0.818.

The interpretation of functional group analysis by FT-IR involves the correlation of the absorption bands in the spectrum (cm^{-1}) of an unknown compound with the known absorption frequencies of the types of bonds. In this work, the results of the FT-IR spectra (Figure 1) of *M. oleifera* leaves showed absorption bands attributed to C-H, N-O, C-N stretching which possibly comprise to unsaturated fatty acids that are aliphatic carboxylic acid with varying hydrocarbon lengths at one end of the chain joined to terminal carboxyl (-COOH) group at the other end, which presumably belonging to oleic, erucic acids. As for example, oleic acids are monounsaturated (cis-9-octadecanoic acid) omega-9 fatty acid (Barkley & Wang 2005), which are known to reduce blood cholesterol levels in non-hypertriglyceridemic individuals (Mattson & Grundy 1985; Mensink & Katan 1990).

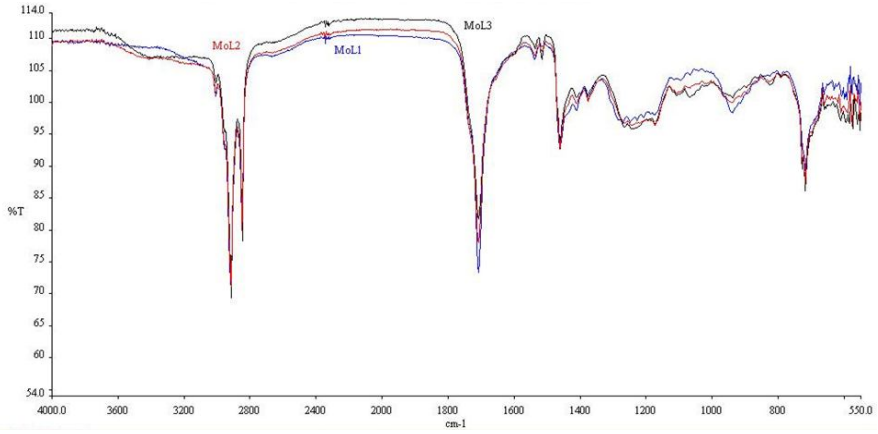

Figure 1. FT-IR spectra profile of SC-CO_2 extracts (4000 to 550 cm^{-1} absorbance) of leaves of *Moringa oleifera* at 10 MPa, 20 MPa, 30 MPa atmospheric pressures.

In the wave numbers of 1742.63 cm^{-1} and 1709.67 cm^{-1}, this set can be attributed to the C=O connection stretching which can be linked to esters, saturated aliphatic groups and α,β-unsaturated aldehydes, ketones. Due to the high intensity of the bands revealed by the FT-IR of the seeds (Figure 2), it is possible to assign them to the predominantly lipid component of the seed that it is present in high proportion similar to the proportion of protein (Brito et al 2006). A strong absorption such as of 1709.97 cm^{-1} can be attributed to the C=O stretching which may be also linked as part of the fatty acid portion of lipid and protein portion of the amides.

Moreover, in the SC-CO_2 roots extracts of *M. oleifera*, which is among the plant parts are not that well-studied. The FT-IR spectra results of these extracts generally linked identically to benzylamine and or as fatty acid alkyl esters. This fatty acid alkyl esters defined as biodiesel which are biodegradable, renewable, non-toxic, possesses inherent lubricity, and reduces most regulated exhaust emissions in comparison to petrodiesel (Rashid et al 2008).

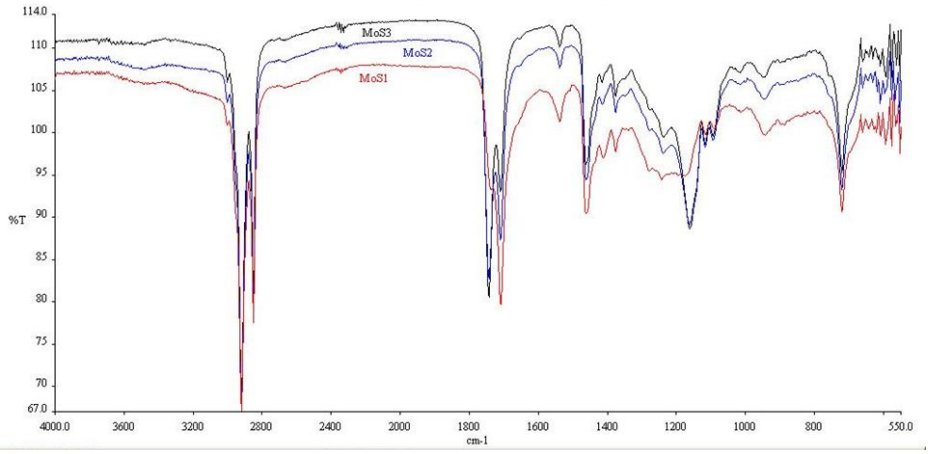

Figure 2. FT-IR spectra profile of SC-CO_2 extracts (4000 to 550 cm^{-1} absorbance) of seeds of *Moringa oleifera* at 10 MPa, 20 MPa, 30 MPa atmospheric pressures.

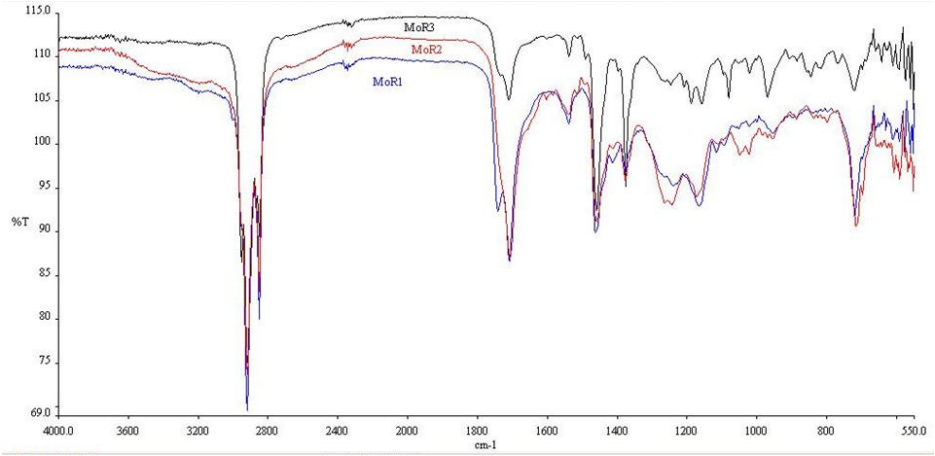

Figure 3. FT-IR spectra profile of SC-CO_2 extracts (4000 to 550 cm^{-1} absorbance) of roots of *Moringa oleifera* at 10 MPa, 20 MPa, 30 MPa atmospheric pressures.

Conclusions. The SC-CO_2 extracts obtained at 10 MPa from both seeds and roots and at 20 MPa from seeds of *M. oleifera* exhibit moderate to strong DNA-binding affinity. The leaf extracts at 30 MPa expressed no binding behavior towards DNA since the computed Rf-value (1.023) is greater than one.

The FT-IR spectroscopy was used for elucidating possible functional groups of the SC-CO_2 extracts from leaves, seeds, and roots of *M. oleifera*, in which the results of the absorption bands of the spectra of all extracts presumably connected to high contents of fatty acids such oleic, linoleic, erucic which must be consumed for good health because the body requires them for immeasurable biological processes.

This study contributes new data to the existing knowledge of *M. oleifera* leaves, seeds, and roots and may serve as a basis for further pharmaceutical investigation. The findings also lend support for the use of this plant as traditional and conventional medicine.

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