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Original Research Article

Identification of Active Fatty Acid Mixture Extracted from Rice Bran Oil Using NMR Spectroscopy

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ABSTRACT

Rice bran oil (RBO) is oil obtained from rice bran. It contains fatty acids and some phytochemicals. RBO has been reported to have some biological activities. Previous publications showed the anticancer activity of rice bran oil fraction extracted from rice bran oil in LoVo, a colorectal cancer cell line through apoptosis via the MAPK pathway with the half-maximal effective concentration (EC_{50}) value of 155.50 ± 1.10 µg/ml. Thus, this study investigated the fatty acid composition of the active rice bran oil fraction purified by column chromatography and identified by ^{1}H and ^{13}C NMR spectroscopy. The result showed that the active fatty acid fraction consists of saturated fatty acid and unsaturated fatty acid in a ratio of 36:64. These findings may lead to the development of alternative cancer therapies in the future.

***Keywords*:** Rice bran oil; fatty acid; NMR spectroscopy

Introduction

Cancer is a growing threat to human health worldwide, in some cases, may involve mass morbidity and mortality.[1] Natural products such as rice bran oil offer biological benefits and have inspired the development of new medicines.[2]

Rice (*Oryza sativa L.)* is a vital staple worldwide.[3] Rice bran (RB) is a by-product of the rice grain milling process. It accounts for about 10% of the total weight of whole rice grain and is mainly comprised of aleurone, subaleurone layer, pericarp, and germ. Rice bran oil (RBO) extracts from RB's lipid part, and the specific content varies between 10-23%. RBO generally comprises unsaturated fatty acids (55-87%) such as oleic acid and linoleic acid, and saturated fatty acids (19-35%) such as palmitic acids.[4] RBO also contains functional components (2-3%) such as tocopherol (vitamin E), tocotrienol, γ-oryzanol, and phytosterol. RBO has been demonstrated to have cholesterol-lowing, antihypertension, hyperglycemia-lowering,[2] antidiabetic, antioxidant, and anticancer effects.[5]

In addition to rice bran oil, several studies showed the anticancer activity of natural compounds or oils that contain fatty acids. For example, Apple seed oil contained oleic acid (47%), linoleic acid (44%), palmitic (7%), stearic (2%), and arachidic acids (1%) showed good pharmacological potential against A549, a human lung carcinoma and SiHa, a human cervical cancer cell line.[6] Almond oil from Northern Cyprus and Turkey contained oleic acid (78%; 75%), linoleic acid (14%; 16%), and palmitic acid (7%; 6%), both exhibited significant anti-proliferative and anticancer activity in Colo-320 and Colo-741, human colon adenocarcinoma cell lines.[7]

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The essential oil extracted from *Pinus roxburghii* showed anticancer activity in many human cell lines, especially HCT-116, a human colorectal carcinoma, and KBM-5, a human myelogenous leukemia cell lines.[8] Moreover, Essential Oil extracted from *Arnica montana* L. showed anticancer activity by induced apoptosis in MOGGCCM, a human anaplastic astrocytoma, and T98G, a human glioblastoma multiforme cell lines .[9] Different types of coconut oil showed different anticancer efficiency in cell lines due to their fatty acid composition.[10] Oil extracted from *Zanthoxylum bungeanum* seed which has unsaturated fatty acid as a main component, showed anticancer activity by induction of apoptosis via MAPK pathway on A375, a human malignant melanoma cell line.[11] Besides direct anticancer activity, fatty acids are also used to produce fatty acid-conjugated therapeutic molecules.[12, 13] For example, the mixture of linoleic acid (CLA) reduced key enzymes in glycogenesis expression.[14] Other research found CLA have immunomodulatory effects on some functions of bovine monocytes.[15] Docetaxel, breast cancer therapeutic drug conjugated linoleic acid (DTX-LA) increased drug delivery efficiency and significant antitumor activity.[16] Fatty acids also show other biological activities, for example, Chloroform extraction of *Chrysophyllum albidum* leaf and stem-bark have antioxidant property.[17] Similar to essential oils extracted from *Haplophyllum tuberculatum*[18] and *Dennettia tripetala*.[19] Some oils, such as coconut and moringa, showed protection against cadmium-induced toxicity in rats.[19]

Nuclear magnetic resonance (NMR) is a tool used to characterize organic compounds' structures. It is a very reliable technique and does not require chemical standards.[20] NMR is also beneficial in providing structural information and quantifying chemical composition in mixture samples. Quantification can be determined by peak area per nuclei, which is proportional to the number of corresponding nuclei. Several precedents report quantifying fatty acid composition in vegetable oils by proton (^{1}H) NMR.[21-24] Integration values of relevant proton signals were used to determine the amounts of fatty acids in vegetable oils.

Previous study showed anticancer activity of fatty acid fraction extracted from rice bran oil.[25] The fraction induced apoptosis in LoVo, colorectal cancer cell line through MAPK pathway. Thus, in this study, we aim to determine the ratio of saturated and unsaturated fatty acids of the active RBO fraction from integration values of characteristic proton signals of each fatty acid.

Materials and Methods

Materials

Organic rice bran oil extract (RBOE) Lot. 27062231 (June 22, 2017) was obtained from the Bakrua farmer group in Yasothon province, Thailand. All the solvents were analytical reagent grade or distilled. Column chromatography was performed under gravity on Merck Kieselgel 60 silica gel (230-400 mesh). All fractions were monitored by thin-layer chromatography (Merck Silica gel 60 F_{254} with a thickness of 0.2 mm on an aluminum sheet). The separated bands were detected by UV lamp at 254 nm and 366 nm and visualization with iodine vapours. NMR spectra were recorded on a Bruker AVANCE III 400 NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C in deuterated chloroform using TMS as an internal standard.

Isolation and Characterization

The crude RBOE (62.63 g) was added with hexane (600 ml) and partitioned with 90% aqueous methanol (600 ml) three times. The combined aqueous methanol layers were concentrated in a vacuum at 40°C to give 6.85 g. The extract was subjected to silica column chromatography with chloroform-methanol with gradually increasing methanol concentrations to give 11 fractions (FAs). All fractions were monitored by thin-layer chromatography (TLC) and screening for cancer cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The FA3 (0.32 g) was eluted with 2-3% methanol in chloroform. It showed two spots on TLC, which was further purified by a silica column chromatography with 2% methanol in chloroform to afford an off-white soft wax in oil (0.04 g) for anticancer activity testing and chemical composition profile by NMR spectroscopic technique.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.32-5.35 (m, 6H, -CH=CH-), 2.75 (t, *J* = 4.0 Hz, 2H, =CHCH$_2$CH=), 2.33 (t, *J* = 8.0 Hz, 20H, CH_2COOH), 1.99-2.03 (m, 26H, CH_2CH=CH-), 1.61 (t, *J* = 8.0 Hz, 29H, CH_2CH_2COOH), 1.20-1.50 (m, 225H, -$(CH_2)_n$), 0.86 (t, *J* = 4.0 Hz, 38H, -CH_3).

^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.6, 22.7, 24.7 (2C), 25.7, 27.2 (3C), 29.0 (2C), 29.2, 29.3(2C), 29.4, 29.5, 29.6, 29.7, 29.8, 31.6, 31.9 (2C), 33.9, 127.9, 128.1, 129.8, 130.0, 130.2, 179.2.

Results and Discussion

MTT results from previous research showed that rice bran oil extract fraction (FA3) has the best effect on LoVo cells viability with the half-maximal effective concentration (EC_{50}) value of 155.50 ± 1.10 µg/ml. In contrast, it showed no inhibition against the control cells at the same concentration, Vero, with an EC_{50} of 258.60 ± 1.07 µg/ml. Then this fraction was used to determine the molecular mechanism against LoVo cells, and found that it able to induced apoptosis in LoVo cells through the MAPK pathway.[25]

Isolation of the FA3 fraction was obtained from silica column chromatography as an off-white oil wax showing only one spot on TLC with 1% methanol in dichloromethane. The nuclear magnetic resonance (NMR) spectroscopic data of the FA3 indicated that it contained a mixture of saturated and unsaturated fatty acids. Typically, fatty acids obtained from rice bran oil extract contained fatty acids (Figure 1), including palmitic acid as a major saturated fatty acid, oleic acid as a major monounsaturated fatty acid, and linoleic acid as a major polyunsaturated fatty acid.[26, 27]

The FA3 NMR data were identified by comparing the obtained ^{1}H and ^{13}C NMR spectra with those of previously reported spectra. The ^{1}H-NMR spectrum (Figure 2) had no signals for methylene and methine protons in *α*-position of glycerol backbone in the range of 4.0-4.5 ppm, which indicated that triglyceride was not present in this fraction. The spectrum showed signals assigned to olefinic protons of unsaturated fatty acids at 5.32-5.35 ppm. The signal at 2.75 ppm was assigned to the *bis*-allylic proton of polyunsaturated fatty acid, and the signal at 1.99-2.03 ppm was assigned to allylic protons of unsaturated fatty acid. The signals at 2.33 and 1.61 ppm were ascribed to *α*- and *β*-methylene protons adjacent to the carbonyl group, respectively. The signals assigned to methylene protons on saturated carbon atoms and terminal methyl protons were observed at 1.20-1.50 and 0.86 ppm, respectively.

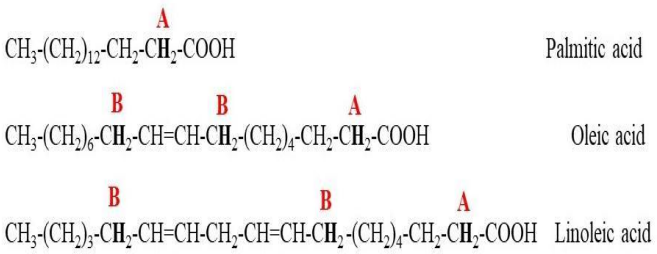

Figure 1: Major fatty acids composition of rice bran oil. [18]

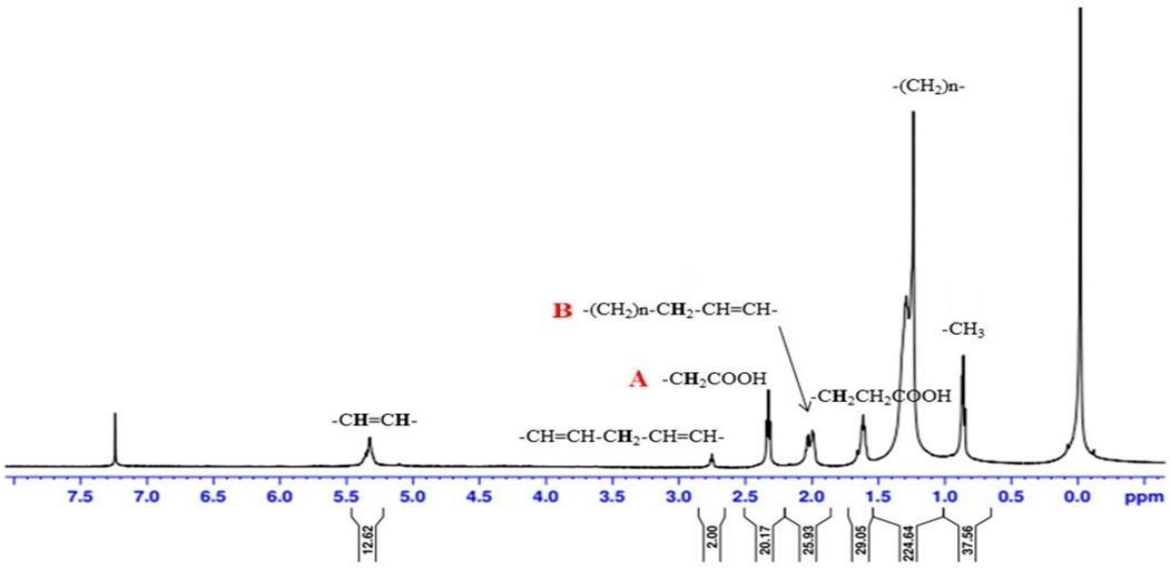

Figure 2: ^{1}H NMR spectrum of the FA3 with integration values.

The ^{13}C NMR spectrum showed a downfield signal attributed to the carbonyl carbon of a carboxylic acid at 179.2 ppm. The characteristic signals were ascribed to olefinic carbon of unsaturated fatty acid in the 127-131 ppm region, as shown in Figure 3.[28]

Each peak area in the ^{1}H-NMR spectrum is proportional to the number of protons of each signal. Therefore, the content of saturated and unsaturated fatty acids can be obtained by measuring the peak area of each fatty acid characteristic signal. *α*-methylene proton adjacent to the carbonyl group (signal A) and allylic proton (signal B) in Figure 2 is helpful for this purpose.[29] Therefore, the total amounts of saturated and unsaturated fatty acids can be determined by using signal A at 2.33 ppm, and that of unsaturated fatty acids can be determined by signal B at 1.99-2.03 ppm. Subsequently, the content of saturated fatty acids can also be determined by assuming that the total content (C) of fatty acids equals 100% and then subtracting the content of unsaturated fatty acids ($\%C_{sat} = 100 - C_{unsat}$). The integration value ratio of signal A of total fatty acids to signal B of unsaturated fatty acid is two *α*-methylene protons (integration value/2: 20.17/ 2 = 10.09) to two four allylic protons (integration value/4: 25.93/4 = 6.48). Then converting this integration value ratio into percentages and found a ratio of 64.2% unsaturated fatty acid to 35.8% saturated fatty acid is obtained.

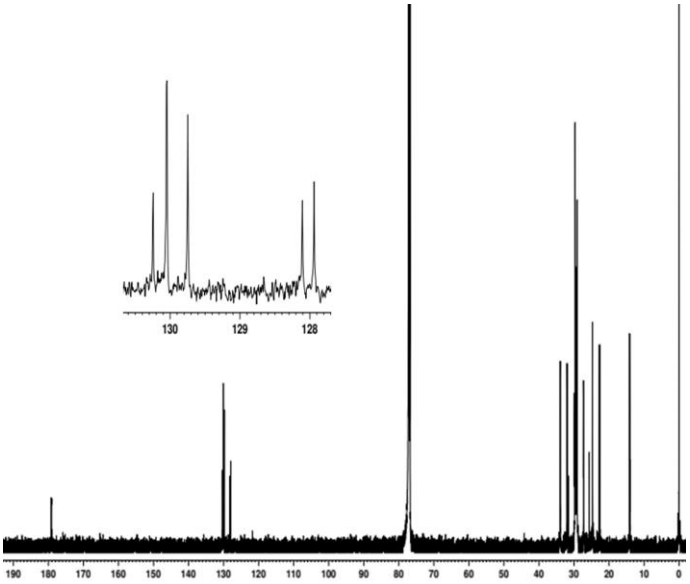

Figure 3: ^{13}C NMR spectrum of the FA3.

Conclusion

In the present study, NMR analysis of FA3 demonstrated that it contained amounts of saturated fatty acid: unsaturated fatty acid in the ratio of 36:64. This oil mixture exhibited anti-cancer activity as in the previous report.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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