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ORIGINAL ARTICLE

HPLC-DAD and GC-MS identification of pesticides compound from diethyl ether extract of mangosteen peel

Anwar Kasim* and Daimon Syukri
Agricultural Product Technology
Faculty of Agricultural Technology, University of Andalas 25163
Coresponding author:Email : Anwar_ks@yahoo.com

ABSTRACT
Previous studies have shown that diethyl ether extracts from samples of mangosteen peel have the effect of pesticides. This study was a preliminary study to identify the groups of compounds that exert these effects by high performance liquid chromatography with diode array detection (HPLC-DAD) and gas chromatography–mass spectrometry (GC-MS). The data obtained revealed that diethyl ether extracts of mangosteen contains compounds that have a chromophore group that can absorb UV light and compounds that are sensitive to heat.
Keywords: preliminary study, pestisides compound, mangosteen peel extract.

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INTRODUCTION

Currently, there is widespread development in the use of plants as traditional medicine. This has led to the belief that the use of medicinal plants does not cause adverse effects although this needs to be scientifically proven. One of the plants that has medicinal properties in the fruit peel is the mangosteen. Mangosteen peel contains some compounds that have been found to exhibit pharmacological activity such as anti-inflammatory, antihistamine, antibacterial, anti-heart disease, and antifungal properties and has been found to be useful in treating human immunodeficiency virus infection [1]. Today, there is a demand for mangosteen rind powder preparations in the form of capsules and juice of mangosteen peel due to its medicinal properties. According to studies by Poeloengan *et al* [2] and Heyne [3], mangosteen peel contains secondary metabolites, such as alkaloids, saponins, tannins, and flavonoids (xanthone, mangostin, and garsinon). Sundaram *et al* [4] have isolated mangostin from mangosteen, a xanthin, as well as 4 of its derivatives. In 1987, Mahabusarakam *et al* [5] isolated and identified 8 xanthones derived from the peel and four compounds of the mangosteen fruit.

To obtain active compound(s) from the mangosteen peel, an extraction process is necessary. Selection of a suitable solvent can increase the efficiency of the extraction. Factors that need to be considered in the selection of the solvent include selectivity, toxicity, polarity, easy evaporation, and price of the solvent. Based on the polarity, the choice of the solvent will affect the compound to be extracted since the nature of the solvent used will affect the polarity of the compounds extracted.

This study extends the investigations of Eunuchs [6], who showed that the diethyl ether extract of mangosteen peel has a strong pesticide-like effect. In this study, we further explored the content profile of the diethyl ether extract of mangosteen peel by using a chromatography method with high performance liquid chromatography with diode array detector (HPLC-DAD) and gas chromatography–mass spectrometry (GC-MS)

MATERIALS AND METHODS

Materials.

Mangosteen fruits as the main material used in this study were obtained from local market in the city of Padang, West Sumatra. Chemicals for extraction and analysis were obtain from different sources: petroleum benzene, diethyl ether (technical quality), demineralised water, methanol (quality HPLC (Merck-Germany), proanalisa Formic acid (Merck-Germany).

The research was conducted in the Laboratory of Central Instrumentation, Faculty of Agricultural Technology Universitas Andalas. The equipments were ultra sonic (elma), Rotary Evaporator (Buchi),

Rechirculating Chiller (Buchi), HPLC with a detector diode array SPD-M20A (Shimadzu), analytical balance (Kern), Hot Plate stirrer (Velp), ultra GC-MS QP2010 (Shimadzu), and other glassware.

Sample preparation.

Sample preparation for analysis was done by cutting the mangosteen peel into small pieces, dried, grinded and sieved to 20 mesh sieve. Extraction of the active substance was done by petroleum benzene with successive extraction method followed by solvent diethyl ether. All extracts from diethyl ether extracts obtained are collected, then, the solvent was evaporated by using a rotary evaporator at a temperature of 40˚C. Viscous extract obtained is weighed and stored at 4C for further analysis.

Analytical procedure

High performance liquid chromatography (HPLC-DAD).

HPLC measurement was performed on liquid chromatography Shimadzu (Kyoto, Japan) using a diode array detector (SPD-M20A, Shimadzu, Kyoto, Japan). Data is processed through a personal computer using the program Lab Solution (Shimadzu LC solution). Analytical column used was Shimadzu-ODS (250 x 4 mm internal diameter, 5 µm particle size; Shimadzu, Japan) with the column temperature was set at 30 °C.

HPLC is conditioned based upon a polarity gradient step method. The solvents were (A) 3 % formic acid, and (B) Methanol. The elusion system was from isocratic of B at 10% for 4 min, 10 to 40% B for 6 min, 40 to 70% of B for 5 min, isocratic 70% of B for 8 min, from 70 to 10% of B for 2 min and finally 10% of B for 5 min. Flow rate of solvent was set at 1.0 mL/min. Injection volumes were 100 µL, and the detection wavelength was set on the range 200-800 nm.

Gas Chromatograph-Mass Spectrometer (GC-MS).

Semi polar compound in diethyl ether extract of mangosteen peel was identified with gas chromatography analysis using GC-MS instrument (Shimadzu QP2010). Injection temperature was set at 150 °C. Helium as the carrier gas is set at a constant rate of 10 ml / min. For separation, RTX-5 column (Restek) was used. Separation condition was set from 50°C -250°C at a speed of temperature rise was 5°C / min. Obtained spectra were identified by comparing with the Wiley and NIST Library.

RESULTS AND DISCUSSION

The mangosteen peel samples were prepared by separating the peel of the mangosteen fruit, downsizing, and then sieving with a 20 mesh. Thereafter, the samples were dried at a temperature of 50°C with moisture content of up to ±8–10% water content. Downsizing aims to increase the contact between solids and solvent in the extraction process, while drying is aimed at ensuring durability of the raw materials and prevention of the mushrooming process and facilitating extraction by the solvent. In this study, non-polar solvent, namely petroleum benzene, and semi-polar solvent, namely diethyl ether; therefore, if the raw material still has a high moisture content, the interaction between the solvent and raw materials may not be appropriate since the polarity of water would be different from that of the solvents used. However, high drying temperatures should not be used since this may cause damage to flavonoids or alkaloids at temperatures of 60°C–70°C.

Selection of the extracting solvent is based on the targeted active substances to be retrieved or analysis. In this experiment, the extracting solvent was semi-polar, namely diethyl ether. Petroleum benzene was used in the early stages to remove sap contained in the sample. This is in accordance with previous reports by Kasim [6], which reported that the diethyl ether extracts of the mangosteen peel have a strong pesticide-like effect against insects,*Reticulitermes* spp and *Aspergillus* spp.

The identification of alkaloid profiles can be done by HPLC with Dode array Detector (DAD) applications. The use of the DAD is an advancement of the use of detectors based on the absorption of UV and visible light with scanning measurement at long ranges of wavelength (200–800 nm in a single analysis with a very short time. This method is very useful for qualitative analysis to identify active substances in natural products and resources.

UV-Vis spectrophotometry (Figure 1) showed that the active ingredient(s) in the diethyl ether extract has the ability to absorb light in the UV region, i.e. in a wavelength of 217, 243, 259, 314 and 349 nm. The ability of UV absorption indicates that the compounds present in the extract has a double bond conjugation or chromophore group.

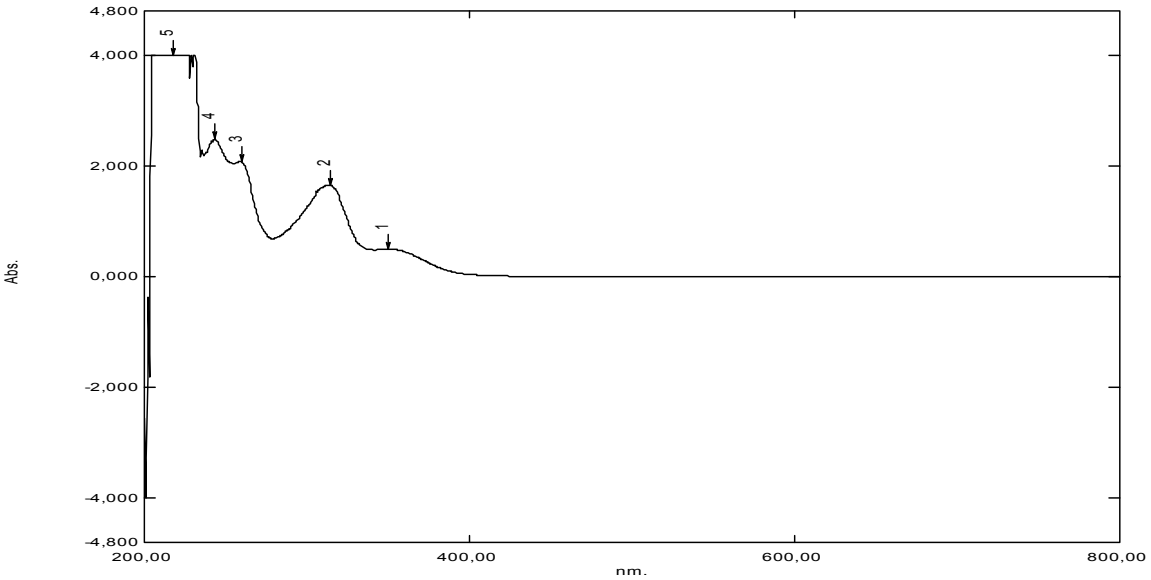
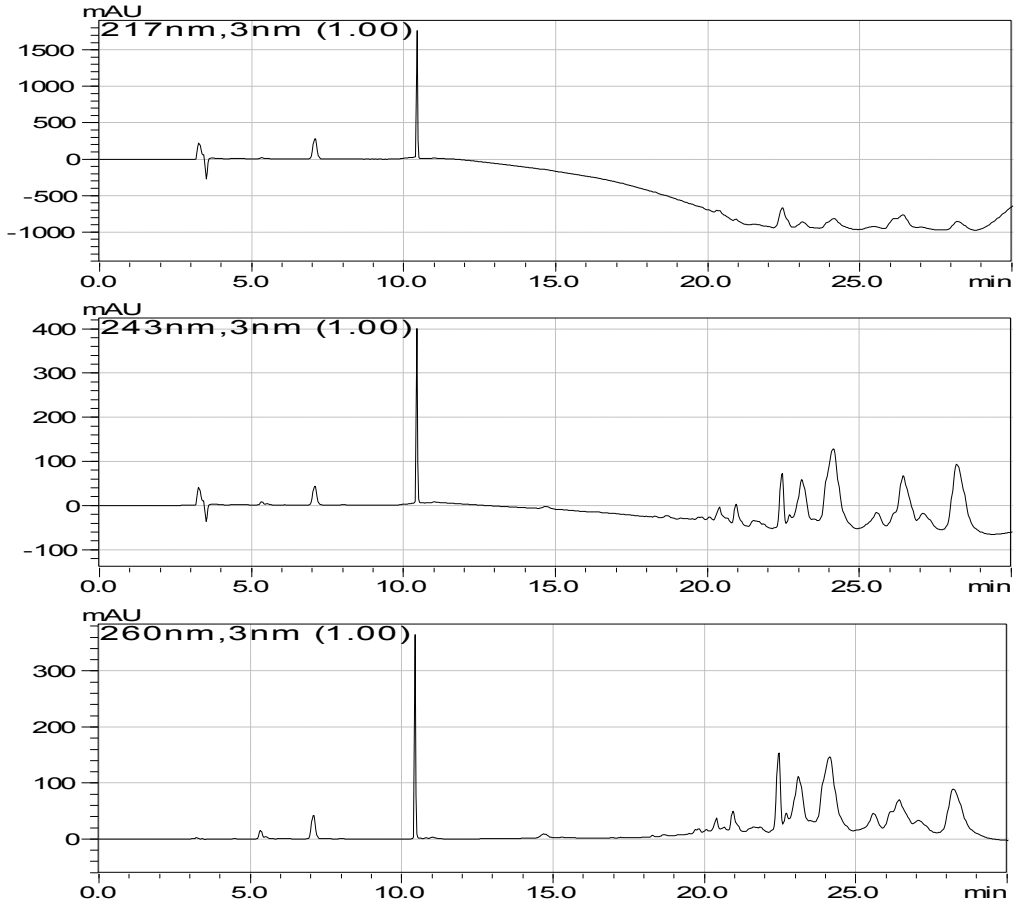


Fig. 1 UV-VIS spectrum of diethyl ether extract of mangosteen peel

HPLC analysis is performed with the principle of reverse phase, where the target test compounds are compounds that have semi-polar properties. Therefore, from the results of HPLC-DAD chromatograms conducted in multiple wavelengths, it appears that the diethyl ether extract of mangosteen peel has several compounds thought to belong to the class of alkaloids.

Analysis of the profile peaks of the chromatogram with the DAD can help determination of the uptake of each of these peaks in a single analysis, as shown in Figure 2. The data show two groups of peaks, namely the chromatogram peaks formed in the mobile phase composition b (methanol) during ascent and that in the mobile phase composition b during isocratic conditions. Surveillance data of the chromatogram peaks at wavelengths of 217 to 350 nm showed that the more visible wavelength peaks covered a large area. This implied that the components of the diethyl ether extract of mangosteen peel samples were mostly composed of compounds with maximum absorbance wavelength of 300 nm.



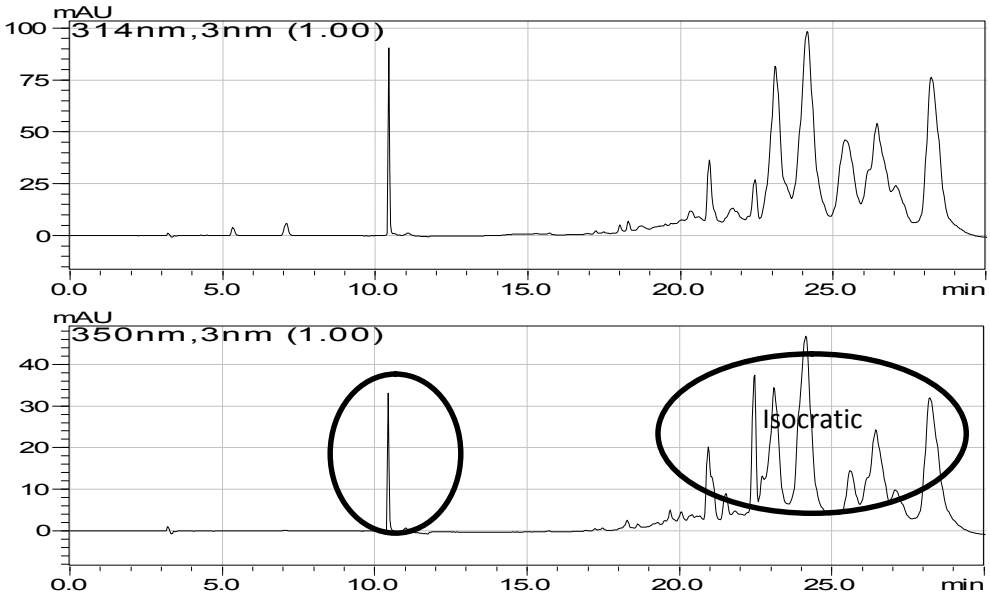
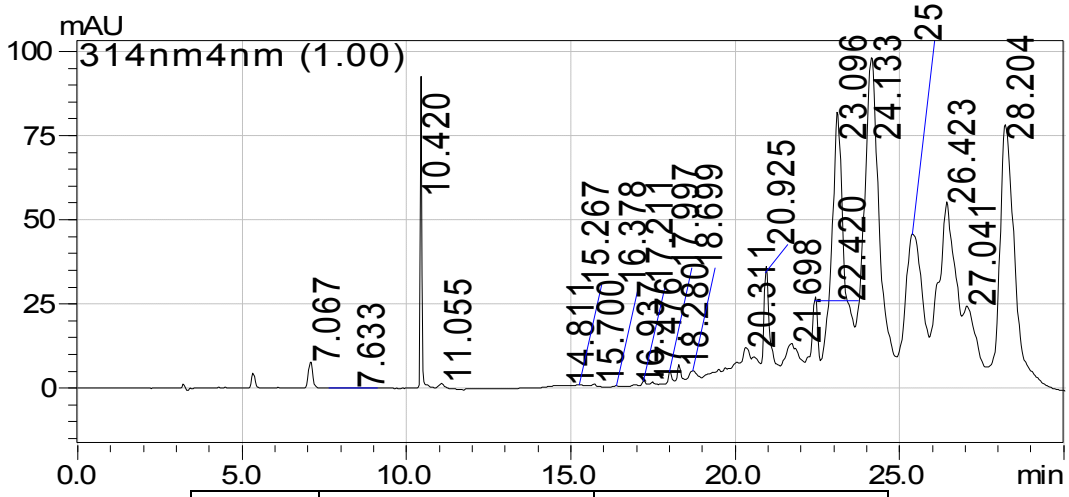


Fig. 2 DAD chromatogram of diethyl ether extract of mangosteen peel

The DAD can provide information about the maximum wavelength of each peak that exists in the data chromatogram. Figure 3 provides information on the maximum wavelength of six dominant peaks detected at various retention times: 10.39; 22.47; 23.08; 24.18, 26.47; 28.25 minutes, respectively.



RT (min)	lamdha max	lamdha min
10.39	210/656	409/583
22.47	266/388/295/194/656	283/333/648/604/769
23.09	196/254/312/357/485	281/342/474/605/658
24.18	197/251/314/389/438	283/369/431/605/668
26.47	250/196/304/527/628	274/487/605/658/769
28.25	196/250/306/485/568	275/460/605/658/769

Fig.3 Information about the maximum wavelength of the dominant peaks in the DAD chromatogram of diethyl ether extract of mangosteen peel.

Figure 4 displays three-dimensional (3D) graphics that provide additional data in the form of energy levels contained in each peak existing on the chromatogram, which are represented by different colors. The red color indicates high energy that possibly represents a double-bond conjugation aromatic ring.

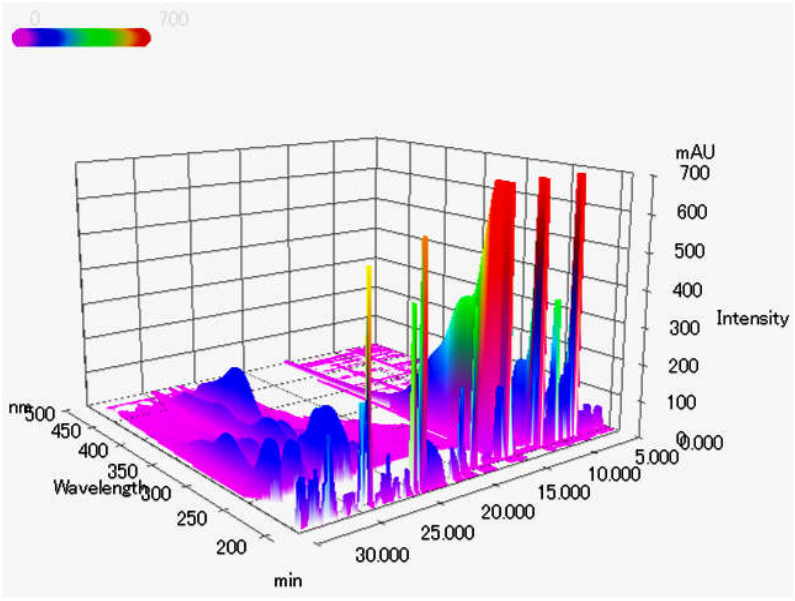


Fig. 4 3-D chart of the diethyl ether extract chromatogram of mangosteen peel

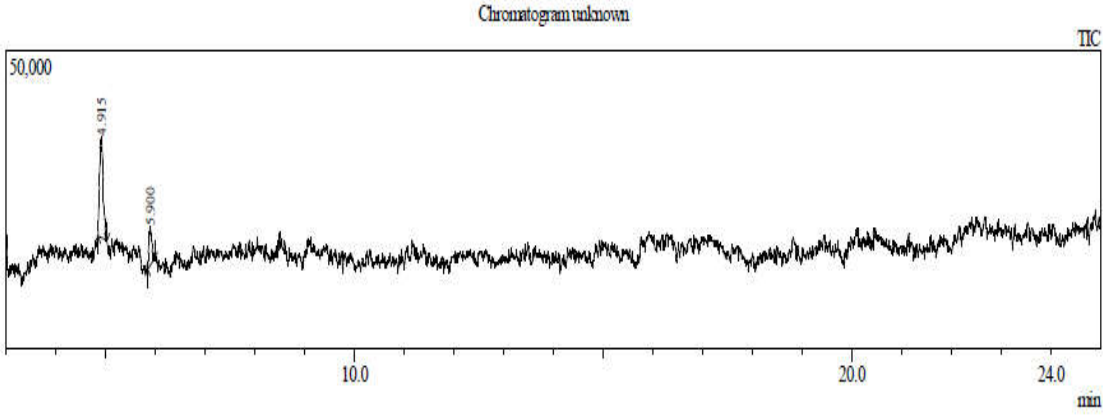
For further confirmation, comparative analysis is necessary with comparison with standard compounds, identification of molecular weight using mass spectroscopy, and finally the isolation of the pure compounds.

Compound contained in mangosteen peel extract when identified by the chromatogram was found to be semi-polar in nature, with the ability to absorb UV light wave or visible light since the extraction was carried out with a semi-polar solvent of diethyl ether with 2.8 index of polarity [7]. The use of a semi-polar solvent will extract semi-polar compounds. Based on the analysis of HPLC performed with inverted phase to the target separation of analytes that are polar still be the data obtained chromatogram, O shows still occurs the separation process within the column that is semi-polar to the compound which has a different polarity to the column. Compounds that can be separated by columns have polarity from that of the column and can be dissolved by the mobile phase; in other words, semi-polar compounds may still remain undetected in this process.

The existence of a group of alkaloids in the diethyl ether extract of mangosteen peel may explain the antimicrobial activity/pesticide-like effects of the extract. Alkaloids exert their antimicrobial effects by interfering with peptidoglycan in the cell constituent microbes, which interrupts the cell lining and causes cell death [8].

Unlike the case with HPLC analysis, which targets heat-sensitive analytes, GC-MS is used to target compounds that are resistant to heat treatment. GC-MS could add further information after analysis by HPLC-DAD by providing a chromatogram that also provides information on the molecular weights of the separated compounds, which would in turn help identify the compound based on a comparison of the fragmentation pattern of compounds, as detected by the Data Library contained in the GC-MS software tool itself.

The results of compound identification by GC-MS chromatography of diethyl ether extract of mangosteen peel are presented in Figure 5. As shown in the figure, no active substances were detected; this indicates that none of the compounds have heat stability. These findings suggest that the pesticide-like effects may be attributed to the presence of unidentified heat-sensitive substances that have a chromophore group. Further analysis using the gas chromatography system would not be possible since the system is suitable only for heat-resistant analytes, which do not seem to be present on the diethyl ether extracts of mangosteen peel.



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	4.915	83533	76.27	16867	72.49	4.95	MI	Ethyl ether
2	5.900	25993	23.73	6401	27.51	4.06	MI	Hexane
		109526	100.00	23268	100.00			

Fig. 5 GC-MS chromatogram of diethyl extract of mangosteen peel

CONCLUSION

The diethyl ether extract of mangosteen peel samples known to exert pesticide-like effects are likely to originate from compounds that have semi-polar properties. These compounds have a chromophore group that absorbs UV light at a wavelength of 200–350 nm, which makes these compounds susceptible to heat. These compounds are predicted to be of no less than six different types.

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