RJC

RASĀYAN *J. Chem.*
Vol. 10 | No. 3 |707 - 715 | July - September | 2017
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

CHARACTERIZATION, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF MANGOSTEEN (*Garcinia mangostana* L.) PERICARP NANOSIZED EXTRACT

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ABSTRACT

The difference of crude size was reported to give impact over magosteen pericarp nanosized extract (ME) antibacterial and antioxidant activity. Nano-sized ME (213,6 nm) showed the best antibacterial activity in inhibiting the growth of bacteria compared to extract from crude with a particle size of 20 mesh and 40 mesh. In the other hand, its antioxidant activity was the lowest. Meanwhile, the chemical compound contained in the ME supposed to support both activities. This research was conducted to characterize the fractions from nano-sized ME based on its antibacterial as well as antioxidant activity. The fractionation of extract provided 6 fractions. Fraction 5 and 6 had the highest antibacterial activity over *B. cereus*, *S. aureus*, and *S. flexneri* and also for antioxidant activity. The result of GC-MS for fraction 5 showed that the fraction contained a high abundance of fatty acid, but fraction 3 was identified to contain 1,3,7-trihydroxy-2,8-diisoprenylxanthone and (R)-(-)-mellein.

Keywords: antibacterial, antioxidant, mangosteen pericarp nanosized extract, xanthone

INTRODUCTION

Mangosteen pericarp has been used as traditional medicine to heal several diseases such as flu, cystitis, diarrhea, dysentery, eczema, fever, pruritus, gut and skin disease[1]. Extract and the isolated compound from mangosteen pericarp was reported to have varried phamarcologic activities such as antibacterial and antioxidant[2]. The antibacterial agent is a chemical compound that is able to inhibit the growth of bacteria by interfering its metabolism[3]. In the pharmaceutical field, the antibacterial agent is required to prevent or heal infectious disease that might be caused by a pathogen and resistant bacterial[4]. Meanwhile, the antioxidant agent can postpone or inhibit substrate oxidation[5]. Oxidation reaction possibly produces a free radical compound that is able to initiate any other oxidation reaction. It can cause cell damage or worse, death. Therefore, the antioxidant agent is also necessary to stop the intermediate free radical compound to inhibit the other oxidation reaction and prevent the damage[6].

Ibrahim *et al*.[7] reported that ME has activity variation such as antioxidant, anticancer, anti-inflammation, anti-allergic, antibacterial, antifungal, antiparasite, anti-obesity, and has potency as Alzheimer drug. Parhusip *et al*.[8] and Putra[9] reported that mangosteen pericarp ethanol extract can inhibit *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum* growth zone. Mangosteen pericarp ethanol extract also had effective concentration value in inhibiting 50% (IC_{50}) 1,1-diphenyl-2-picrylhydrazyl (DPPH) of 7.48 µg/mL[10]. Siampa[11] researched the effect of mangosteen pericarp extract sample size to antibacterial and antioxidant activity. The mangosteen pericarp extract was prepared from a sample having a size of 213.6 nm, 40 mesh, and 20 mesh. The mangosteen pericarp (ME) nanosized extract had a bit higher antibacterial activity average in inhibiting *S. aureus*, *B. Cereus*, and *Shigella flexineri* growth than 40 and 20 mesh extract, but its antioxidant activity in DPPH inhibition was the opposite and the difference was significant enough. The ME IC_{50} value was higher (99.4 ppm) than 40 (60.3 ppm) and 20 mesh (49.4 ppm) extract. The phytochemical analysis result from those three extracts did not show any differences. Those three extracts in ethanol extract showed the positive result to flavonoid, tannin and polyphenol, triterpenoid, alkaloid, and saponin compound[11]. The aim of this research was to

http://dx.doi.org/10.7324/RJC.2017.1031766

identify a compound having a role as antibacterial and also antioxidant from mangosteen pericarp ethanol extract. The chosen extract used in this research was mangosteen pericarp (ME) nanosized extract.
Putra[9] identified that compound having a dominant role in antibacterial and antioxidant activity in mangosteen pericarp was secondary metabolites from xanthone group. Govindachari *et al*.[12] succeed to isolate xanthone compound from mangosteen pericarp, that is α-mangostin, gartanin, and 8-desoxygartanin. Obolskiy *et al*.[2] summarized some other xanthone compounds that were isolated from mangosteen pericarp that was β- and γ-mangostin, 1- and 3-isomangostin, mangostinon, mangostanol, mangostanin, garsinon B, garsinon D, garsinon E, 9-hidroxycalabaxanthone, and demethylcalabaxanthone.

EXPERIMENTAL

Apparatus was used in this research were analytical balance, autoclave, oven blower, distillation apparatus, rotary evaporator, laminar, 90 well microplate, ultraviolet (UV) lamp 254 and 366 nm, vacuum liquid chromatography (VLC), flash column chromatography (FCC), UV-visible (UV-Vis) spectrophotometer UVmini-1240 Shimadzu, GC-MS Waters, and common glassware in laboratory. The materials used were mangosteen pericarp (ME) nanosized extract from previous research by Siampa[9], dimethyl sulfoxide (DMSO), chloroform p.a., ethanol, methanol, acetone, dichloromethane (DCM), *n*-hexane, ethyl acetate, thin layer chromatography (TLC), silica gel 60 GF_{254} for p-TLC, silica gel 60 H for VLC, silica gel 60 (250 - 400 mesh) for FCC and pre-adsorption, disc paper, ampicillin, nutrient agar (NA), nutrient broth (NB), some bacterial: *S. aureus*, *B. cereus*, *E. coli*, and *S. flexneri* obtained from IPBCC (Institut Pertanian Bogor Culture Collection), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid p.a.

Fractionation using VLC[13] and Identification

The eluent was chosen from the combination of *n*-hexane:ethyl acetate 9:1 to 1:9. Combination giving the best separation profile would be chosen to be used in fractionation using VLC. As much as 25 g ME was dissolved in acetone with the addition of 50 g silica gel 60 for pre-adsorption. The mixture was concentrated using rotary evaporator. The result was placed on 250 g of silica gel 60 H for VLC that had been packed in VLC column. Then, the extract was eluted using 250 mL of each chosen eluent. The eluates separation profile was identified using TLC with *n*-hexane:ethyl acetate 8:2 eluent, eluates having similar separation profile were combined into a new fraction. All fractions were tested for antibacterial and antioxidant activity. The ME fractions with highest antibacterial and antioxidant activity were characterized by GC-MS. The GC condition: front inlet using split mode, with initial temperature 270 °C, pressure 16.38 psi, and using helium (He) eluent. Oven condition that was used is initial temperature 60 °C and final temperature 50°C. Flow time 39 minutes. The size of the column used was 60 m × 0.25 mm × 0.25 µm with static flow rate, 0.9 mL/minutes. Ionization at mass spectrometer was done using electron ionization (EI). The mass spectrum that was obtained, would be matched with Wiley 9th library.

Antibacterial assay[8]

The antibacterial assay used disc diffusion method. Each bacteria (*S. aureus*, *B. cereus*, *E. coli*, and *S. flexneri*) were inoculated into NB media and incubated until its optical density reaches 0.6–0.8. As much as 100 µL inoculated media was added into 20 mL NA media in petry dish. The paper disc was placed on the mixture and inoculated with 10 µL fraction with concentration variation of 3.12, 6.25, 12.5, 25, and 50% (b/v). The dishes were covered and incubated at room temperature for 24 hours. After that, the colorless zone around the disc was measured and inhibition index was calculated using the following equation:

$$\text{Inhibition Index} = \frac{\text{total inhibition zone - disc diameter}}{\text{disc diameter}} \quad (1)$$

Antioxidant assay[14,15]

The antioxidant assay used DPPH scavenging method. Each fraction was dissolved into ethanol with a concentration of 50 ppm stock solution and was then diluted into 25, 12.5, 6.25, and 3.12 ppm. As much as

500 µL fraction solution from each concentration was added to 500 µL DPPH (125 µM in ethanol). The mixture was shaken and incubated in dark room at room temperature for 30 minutes. The absorbance was measured at wavelength 517 nm using UV-Vis spectrophotometer. Ascorbic acid was used as positive control. Antioxidant activity in scavenging DPPH was calculated using the following equation 2. The relation between concentration and activity concentration was determined, and IC_{50} was measured using interpolation. Antioxidant activity appears in the value of IC_{50}.

$$\text{Inhibition (\%)} = \frac{(A-B)}{A} \times 100 \qquad (2)$$

A: Blank absorbance (ethanol and DPPH)
B: Sample absorbance (fraction and DPPH)

RESULTS AND DISCUSSION

ME fractionation

VLC fractionation was aimed to classify compounds based on polarity degree while decreasing compounds amount. This way was the first stage to purify a crude extract in advance. The ME fractionation was started by determining eluent from *n*-hexane: ethyl acetate solvent combination with the ratio of 9:1 to 1:9 through TLC. The solvent combination chosen as eluent was *n*-hexane:ethyl acetate having a ratio of 9:1, 8:2, 7:3, 6:4, and 0:10. The chosen eluent was eluent having the ability to separate a lot of spots with the best separation. The ME fractionation used eluent gradation of *n*-hexane: ethyl acetate by eluting with 250 mL of following eluents: 9:1 (2 times), 8:2 (4 times), 7:3 (2 times), 6:4 (2 times), and 0:10 (2 times) ratio, respectively. The ME fractionation gave 12 eluates. Every eluates separation pattern was identified by TLC and eluates having similar pattern were collected as one fraction. Its collection gave 6 fractions. Weight and chromatogram of 1 - 6 fraction data were shown in Table-1 and Figure-1 respectively.

Table-1: Fraction weight and yield percentage of ME fractionation result using VLC

Fraction	Weight (g)	Yield (%)
1	0.2690	1,08
2	4.0837	16,33
3	5.3195	21,28
4	5.2582	21,03
5	3.6015	14,41
6	5.7700	23,08

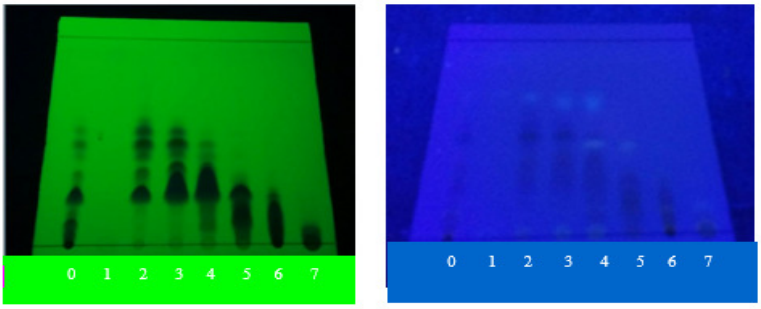


Fig.-1: TLC chromatogram of ME fraction with eluent of *n*-hexane: ethyl acetate (7:3) from (0) ethanol extract, (1–6) fraction 1 - 6, (7) methanol fraction under (a) UV (λ_{254nm}) light and (b) under UV (λ_{366nm}) light

Antibacterial activity of ME fractions

Every fraction gave different IHD based on colorless zone diameter around the disc. Yet, concentration variation did not influence fraction IHD value. Fraction 1 inhibited *E. coli* growth, but did not inhibit *B. cereus*, *S. aureus*, and *S. flexneri* growth. While, fraction 2 until 6 did not inhibit *E. coli* growth, but inhibit

B. cereus, *S. aureus*, and *S. flexneri* growth (Fig.-2). From the antibacterial activity assay, it showed that fraction 6 gave the stronger inhibition than the other 5 fractions. Fraction 6 hampered the bacterial growth with diameters for *B. cereus* (15 mm with IHD 0.50), *S. aureus* (13 mm with IHD 0.18), and *S. flexneri* (15 mm with IHD 0.50). The IHD value of those six fractions was still lower than ME IHD to *B. cereus* (1.58), *S. aureus* (1.72), and *E. coli* (1.35) at concentration of 50% (b/v)[8], and also to *S. flexneri* (0.08) at 0.12% (b/v)[11]. The high antibacterial activity of the extract can be caused by antagonist effect; effects produced by several fractions were lower than extract before fractionated[16]. The antibacterial activity of this extract also lower than the other plant such as 70% ethanol extracts from *H. Suaveoelens* inhibiting the bacterial growth with diameters varying from 16 ± 0 to 24 ± 0 mm and from 15 ± 0 to 24 ±0 mm for *L. Multiflora*. While, the essential oils hampered the bacterial growth with diameters varying from 0 ± 0 to 16 ± 0 mm for *H. Suaveoelens* and from 20 ± 0 to 28 ± 0 mm for *L. Multiflora*[17].

One thing affecting sample ability in inhibiting bacteria growth was the compound content in that sample and its ability to interact with bacteria membrane. Besides, bacteria type also affected compounds ability to inhibit that bacteria growth. Bacteria can be distinguished into gram positive and gram negative bacteria based on cell membrane structure[18]. The Gram-positive bacterial cell membrane is thicker (30–80 nm) than gram negative, but it is dominated by peptidoglycan. While gram-negative bacteria has thinner cell membrane layer with more layers amount. It causes gram-negative bacteria to be more resistant to antibacterial and osmotic pressure than gram-positive bacteria. *B. cereus* and *S. aureus* are belonged to gram-positive bacteria ,while *S. flexneri* and *E. coli* are gram negative bacteria. Even though *S. flexneri* and *E. coli* are gram negative bacteria, inhibition of both bacteria is different, showed by IHD value difference of every fraction. Siampa[11] stated that the tougher *E. coli* cell wall structure than other gram-negative bacteria so that the growth inhibition by antibacterial become more difficult.

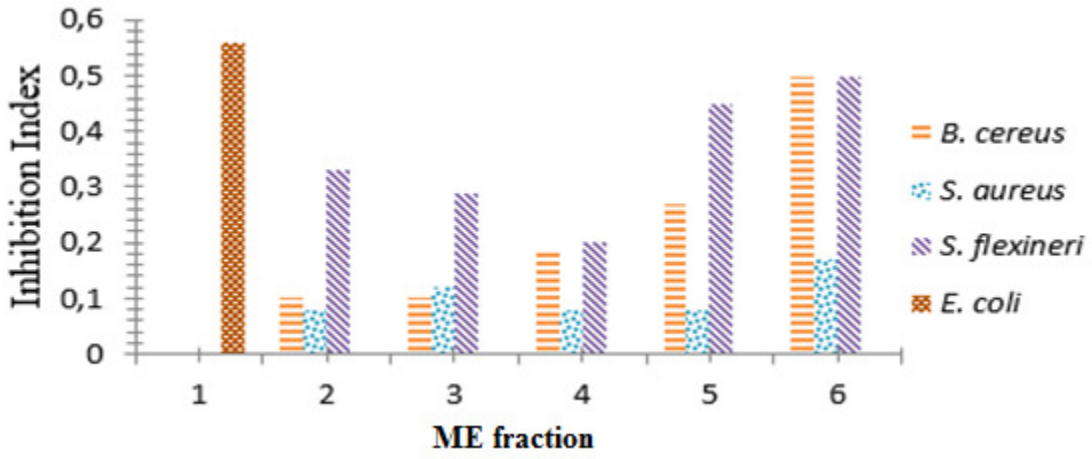


Fig.-2: The ME fraction IHD to several tested bacteria

Antioxidant Activity of ME Fraction

Fraction 1 until 4 had IC_{50} value more than 50 ppm, while fraction 5 and 6 has IC_{50} of 28.19 ppm and 26.86 ppm respectively (Table-2). The IC_{50} of fraction 5 and 6 were lower than mangosteen pericarp nano extract IC_{50} that was 99.4 ppm[11]. It showed that fraction antioxidant activity was better than nano ME. Yet, this value was still lower than Tjahjani *et al.*[10] Research reported that mangosteen pericarp ethanol extract had effective concentration value in inhibiting 50% (IC_{50}) to DPPH compound of 7.48 µg/mL. The differences of these researches were from sample size and the used solvents. This research used ethanol solvent with 213.6 nm sample size, while Tjahjani *et al.*[10] used methanol solvent with sample size around 40 mesh. The phytochemical analysis result from those extracts did not show any differences. Those extracts showed the positive result to flavonoid, tannin and polyphenol, triterpenoid, alkaloid, and saponin compounds. The IC_{50} is a sample effective concentration in inhibiting 50% of DPPH[19] radical. IC_{50} value is determined from

linear regression curve of % DPPH inhibition with fraction concentration. DPPH is a stable radical compound having purple color if dissolved in ethanol. Compound addition having ability to donate a proton to DPPH is able to change DPPH into non-radical compound, 1,1-diphenyl-2-picrylhydrazyl which is yellow[20]. Purple color intensity decrement indicated the reduced DPPH amount which is able to be determined by UV-Vis spectrophotometer at 517 nm[14]. Antioxidant activity of fraction 5 and 6 are better than fraction 1 to 4. Yet, if it is compared to positive control (ascorbic acid), antioxidant activities of both fractions are still low.

Table-2: The IC_{50} value of ME fraction in inhibiting DPPH radical

Sample	IC_{50} (ppm)
Fraction 1	>50
Fraction 2	>50
Fraction 3	>50
Fraction 4	>50
Fraction 5	28.19
Fraction 6	26.86
Ascorbic acid	4.45

Many types of research regarding antioxidant were reported from the plants such as *Lagenaria siceraria, Bacopa monniera, Jasminum auriculatum, Kalanchoe pinnata and Oxalis corniculata*. Durgawale *et al.*[21] reported that *O. corniculata* methanol extract contained vitamin E and squalene. These compounds were expected to have an important role in its activity as an antioxidant. Those compounds were also identified through GC-MS in fraction 6 of ME.

Fraction 5 and 3 Identification

The chosen ME fractions characterized were fraction 5 and 3. Fraction 5 was identified for it had the second highest antibacterial and antioxidant activity after fraction 6 and showed similarity TLC pattern. While fraction 3 was chosen as it has the highest yield among the other 4 fractions and qualitatively showed higher phenolic compound content than the others. The characterization of these fractions used GC-MS.

Fraction 5 Identification

Fraction 5 chromatogram showed a lot of peaks from retention time of 0 until 20 minutes (Figure 3). At a retention time of 0.00 - 5.59 minutes, there were several peaks of short chain aliphatic hydrocarbon groups such as pentanal (R_t 4.85 minute), hepta-1,3-diene (R_t 5.07 minute), propane (R_t 5.29 minute), and 2-pentanone (R_t 5.59 minute). While at a retention time of 6.00 - 13.00 minutes, lactone group peak that is 2-furanone (R_t 7.39 minute) was identified, the rest peaks belonged to fatty acid groups, that were hexadecanoic acid or palmitic acid (R_t 14.69 minute) and octadecanoic acid or stearic acid (R_t 16.147 minute). Compounds supporting antioxidant activity from fraction 5 did not appear. Perhaps, it was caused by fraction 5 impurities dominated by short chain aliphatic hydrocarbon and several fatty acid compounds. The fatty acid compound was reported to be one of the antibacterial agents that can effectively inhibit the growth of bacterial because its nonpolar side which can interact with cell membrane that contains phospholipid layer[22]. Interactions of fatty acid with the cell membrane of bacteria can cause the formation of pores on the bacterial cell membrane. This is one of antibacterial agent mechanism in damaging bacterial's cell[23].

Fraction 3

Fraction 3 had the fourth highest antibacterial and antioxidant activity after fraction 6 and 5. Fraction 3 was chosen to be characterized for its weight was higher than fraction 2 having the 3rd highest antibacterial and antioxidant activity. A lot of peaks were identified in fraction 3 chromatogram from retention time of 0 to 55 minute (Fig.-4). Based on retention time, compounds in fraction 3 were classified into short chain aliphatic hydrocarbon group at a retention time of 0.00 - 6.13 minute such as cyclopentanol (R_t 5.18 minute),

2-pentanone (R_t 5.53 minute), and ethanol (R_t 5.97 minute). At *Rt* 15.37 minute, Isopropyl palmitate, a fatty acid compound was also identified, while at a retention time of 12.00 to 47.13 minute, phenolic group compounds, that are (R)-(-)-Melein and 1,3,7-Trihydroxy-2,4-diisoprenylxanthone (Figure 5) were identified.

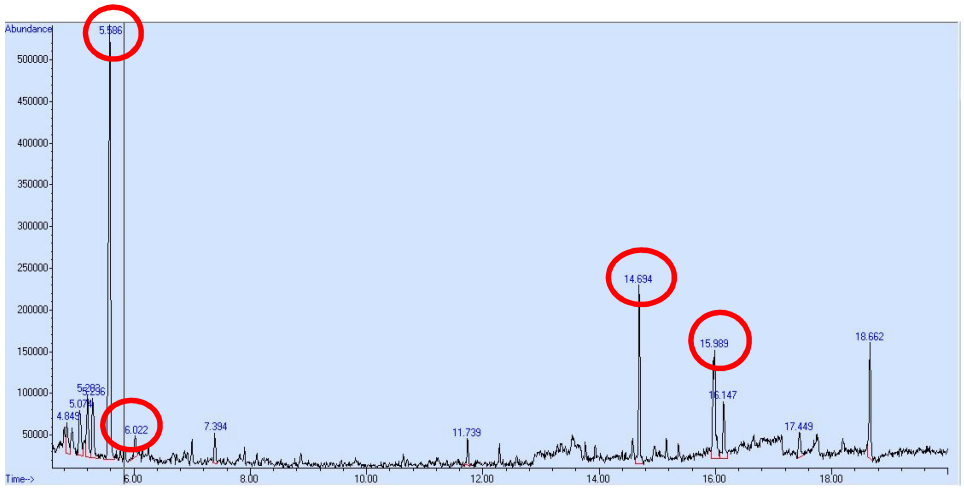


Fig.-3: The fraction 5 of ME chromatogram

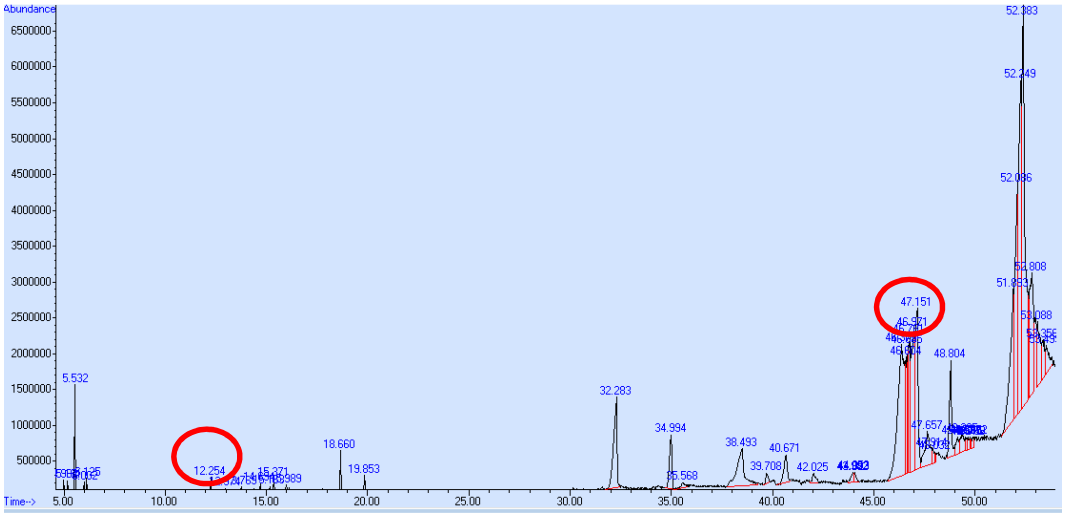
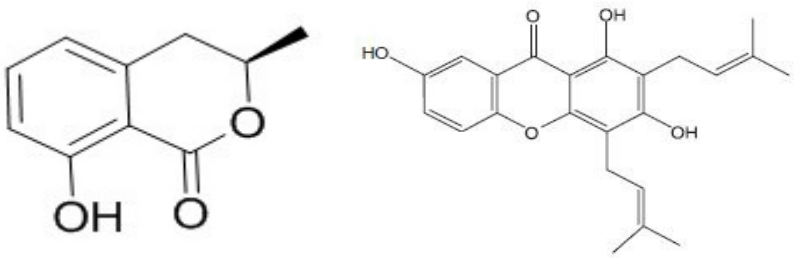


Fig.-4: Fraction 3 of ME chromatogram

(R)-(-)-Melein (Fig.-5a) was identified at R_t 12.25 minute. This compound belongs to phenolic compound, dihydroisocoumarin derivative[24]. While 1,3,7-trihydroxy-2,4-diisoprenylxanthone (Fig.-5b) was identified at 46.43 minutes. It is phenolic compound from xanthone group which is typical in mangosteen pericarp[2]. The advanced study of a peak having R_t 46.43 minute by using Wiley 9th database showed that 1,3,7-trihydroxy-2,4-diisoprenylxanthone was a compound with a molecular weight of 380 and had $C_{23}H_{24}O_5$ molecular formula. Mass spectrum of that compound showed similarity to a database that was characterizing fragment appearance at *m/z* 325 and 269 whether in fraction 3 spectrum or that standard compound spectrum (Fig.-6). The ion at *m/z* 325 was reduced as $[C_{19}H_{17}O_5]^{+\cdot}$ releasing $[C_4H_7]^{+\cdot}$. While ion at *m/z* 269 was reduced as $[C_{17}H_{18}O_3]^{+\cdot}$ releasing $[C_6H_6O_2]^{+\cdot}$ (Fig.-7).

Characterization result was conducted by compound abundance approach which can be detected by GC-MS based on Wiley 9th database. The advance purification is required to prove the actual structure in order

to determine the actual compound having antioxidant and antibacterial activity. The advance purification such chromatotron is required so the fraction in a small amount can be separated well. Results from this research support empirical information stating that mangosteen pericarp extract has potential as antibacterial and antioxidant.



(a) (b)

Fig.-5: Structure of (a) (R)-(-) Melein and (b) 1,3,7-trihydroxy-2,4-diisoprenylxanthone

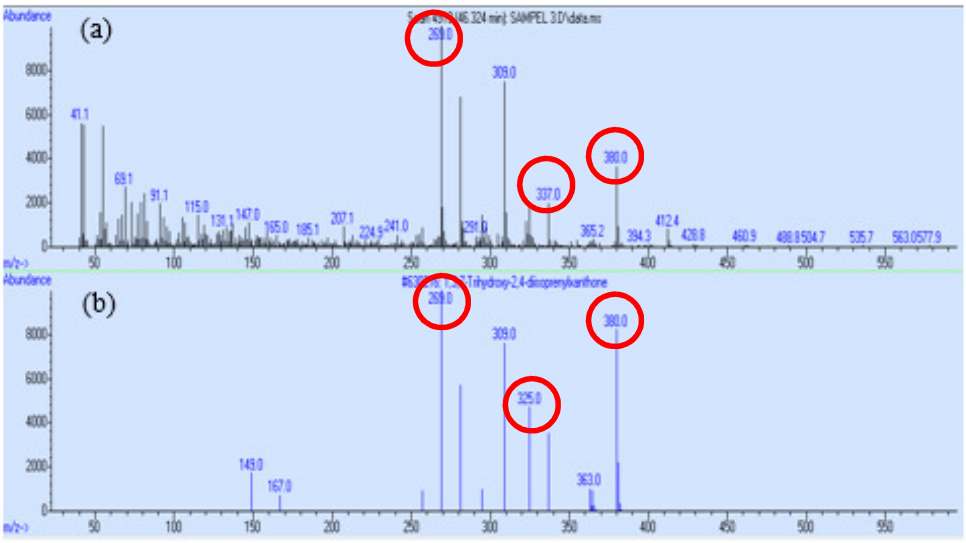


Fig.-6: (a) Mass spectrum of compound having R_t 46.43 minute and (b) reference mass spectrum of 1,3,7-trihydroxy-2.4-diisoprenylxanthone

CONCLUSION

The fraction 5 of ME has antibacterial and antioxidant, even though from GC-MS characterization result showed chromatogram with peaks dominated by fatty acid group compounds, while the phenolic derivative compound was identified in fraction 3. Phenolic compounds identified are dihydroxyisocoumarin group, that is (R)-(-)-mellein and xanthone, that is 1,3,7-trihydroxy-2,8-diisoprenylxanthone.

ACKNOWLEDGEMENT

The author expresses deepest appreciation to Direktorat Jenderal Pendidikan Tinggi Kementerian Pendidikan dan Kebudayaan No. 083/SP2H/PL/Dit.Litabmas/II/2015, tanggal 5 Februari 2015 who funded this research through BPPTN Bogor Agriculture University research program 2015 on behalf of Prof. Dr. Purwantiningsih Sugita, MS.

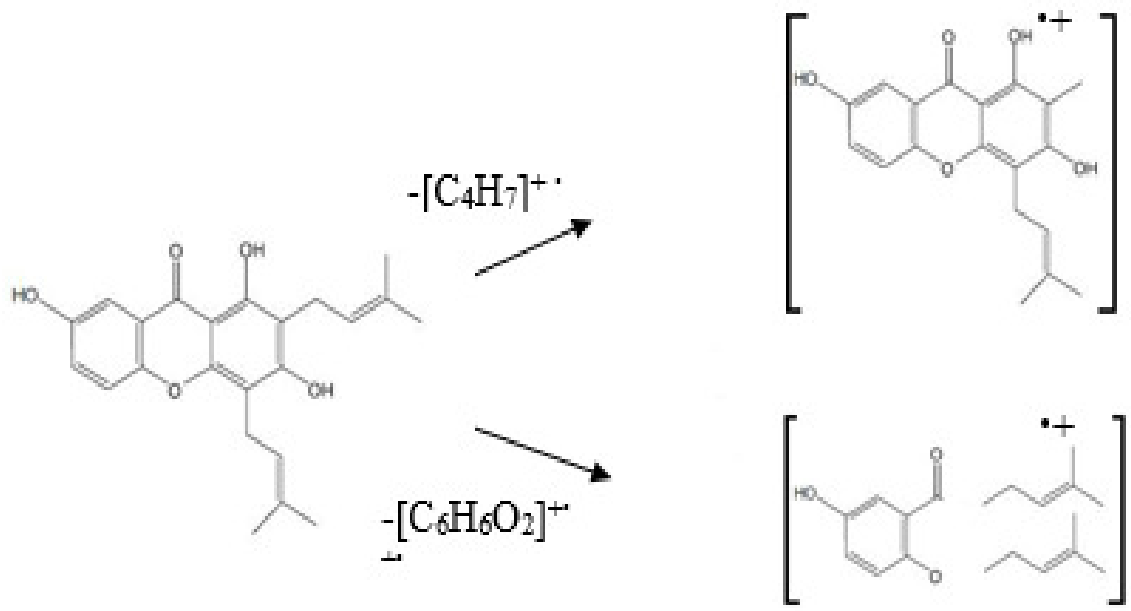


Fig.-7: Fragmentation of 1,3,7-trihydroxy-2,4-diisoprenylxanthone

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[RJC-1766/2017]