Research Article

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Synthesis of multi-template molecularly imprinted polymers (MT-MIPs) for isolating ethyl para-methoxycinnamate and ethyl cinnamate from *Kaempferia galanga* L., extract with methacrylic acid as functional monomer

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Abstract: *Kaempferia galanga* L. extract contains ethyl p-methoxycinnamate (EPMC) and ethyl cinnamate (EC), which have several pharmacological activities. EPMC and EC have been successfully isolated, but the %yield was low. Therefore, developing an isolation method to increase the %yield result of EPMC and EC is essential. The molecularly imprinted polymers have been applied to separate lot of active compounds from natural products with excellent results. MIP synthesis is usually performed using a single template with high selectivity for the target analyte but only detect single chemical compounds. Hence, this study synthesized multi-template molecularly imprinted polymers (MT-MIPs) for isolating EPMC and EC simultaneously using methacrylic acid as a functional monomer and ethylene glycol dimethacrylate or trimethyl propane trimethacrylate (TRIM) as a crosslinker. The study results indicate that MT-MIP produced with TRIM is more effective in separating EPMC and EC simultaneously in *K. galanga* L. extracts. However, the yields of EPMC and EC were still low. The yields of EPMC and EC in *n*-hexane extracts were 1.557 and 1.929%, with purity of 66.330 and 61.510%, respectively. Further research is necessary to determine the ideal functional monomer and its ratio to template molecules to obtain the excellent selectivity of the MT-MIPs used for simultaneously isolating EPMC and EC.

Keywords: multi-template molecularly imprinted polymers, *Kaempferia galanga* L., ethyl p-methoxycinnamate, ethyl cinnamate

Abbreviations

AIBN	azobisisobutyronitrile
CD	cinnamaldehyde
EC	ethyl cinnamate
EGDMA	ethylene glycol dimethacrylate
EPMC	ethyl p-methoxycinnamate
IF	imprinting factor
K_d	distribution coefficient
MAA	methacrylic acid
MC	methyl cinnamate
MIPs	molecularly imprinted polymers
MT-MIPs	multi-template molecularly imprinted polymers
MT-NIPs	multi-templates non-imprinted polymers
MT-MIP-1	multi-template molecularly imprinted polymer with EGDMA
MT-MIP-2	multi-template molecularly imprinted polymer with TRIM
MT-NIP-1	multi-template non-imprinted polymer with EGDMA
MT-NIP-2	multi-template non-imprinted polymer with TRIM
TRIM	trimethyl propane trimethacrylate

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1 Introduction

Kaempferia galanga L., known as kencur in Indonesia, is a tropical plant often used as a traditional medicinal herb [1,2]. *K. galanga* L. contains 2–4% essential oil [3], with the highest content being ethyl cinnamate (EC) (29.56%) and ethyl p-methoxycinnamate (EPMC) (43.35%) [4,5]. EC is a compound that has sedative, vasorelaxation, and anti-carcinogenic activities. EPMC is a compound that has antimicrobial, anti-inflammatory, anti-tuberculosis, and hypopigmentation activities [6,7].

EPMC and EC have excellent potential in the pharmacological field. However, isolating these two compounds is difficult because of the many other matrices in the *K. galanga* L., extract [8]. The methods commonly used for EPMC and EC isolation from *K. galanga* L., are liquid–liquid and liquid–solid extraction [9,10]. Both methods are less effective because they produce low percent yield values, less than 2% [11]. Therefore, a more effective technique with a high percent yield is needed. Molecular imprinted polymers (MIPs) are a promising method that uses a sorbent. MIPs are created through a molecular imprinting process, which involves forming polymers using template molecules and functional monomers. These polymers are then cross-linked (three-dimensional) by adding a crosslinker [12]. The template produces a cavity that acts as a binding site for molecules and can effectively recognize them [13]. MIPs have several advantages, including being lightweight, having good physical and chemical stability, being easy to prepare, and being cost-effective [14]. Extraction using MIPs also produces a high yield percentage (>80%) [15,16].

MIPs synthesis are usually performed using a single template. Single-template MIPs have high selectivity for the target analyte. However, its use has various limitations, including only being able to detect single chemical compounds, having low reusability and reproducibility, and poor affinity [17]. Therefore, MIPs synthesis using multi-template MIP (MT-MIPs) have been developed [18]. MT-MIPs have several advantages, including the simultaneous extraction of several chemical compounds, lower costs, and shorter time requirements [19].

Until now, MT-MIPs have never been made to extract EC and EPMC compounds simultaneously from *K. galanga* L. This MT-MIPs techniques is a novel and promising method in increasing EPMC and EC yield percentage on *K. galanga* L., reducing time, and simplifying isolation procedures. Therefore, in this research, MT-MIP with two kind of crosslinkers were synthesized using methacrylic acid (MAA) monomer, ethylene glycol dimethacrylate (EGDMA) or trimethyl propane trimethacrylate (TRIM) as a crosslinker, and azobisisobutyronitrile (AIBN) as an initiator in *n*-hexane as a porogen solvent via bulk polymerization. The characterization of the MT-MIPs including its analytical performances was performed in order to have alternative method for simultaneously isolating EC and EPMC compounds from *K. galanga* L. extract.

2 Materials and methods

2.1 Materials

The materials required include EPMC and cinnamaldehyde (CD) obtained from Markherbs. EC, MAA, EGDMA, TRIM, AIBN, and methyl cinnamate (MC) were obtained from Sigma Aldrich. *n*-Hexane methanol, acetone, acetic acid, ethanol, isopropanol, acetonitrile, and ethyl acetate were obtained from Merck. The distilled water was obtained from PT. IPHA Laboratories and *K. galanga* L. extract were obtained from Herbal Study Center, Universitas Padjadjaran. Unless otherwise stated, all materials used in the study were pro-analytical grade.

2.2 Synthesis of MT-MIPs and MT-non-imprinted polymers (NIPs)

MT-MIPs were synthesized using the bulk method with a molar composition ratio of template molecule: functional monomer: crosslinker of 1:7:20 (Table 1).

EC and EPMC were dissolved in 4 mL *n*-hexane. Then, MAA was added to the vial and sonicated for 10 min. After sonication, crosslinker (EGDMA or TRIM) was added, and the mixture was sonicated again for 10 min. AIBN was then added to the mixture, and it was sonicated for another 5 min. Polymerization was carried out in an oven at 70°C for 18 h. The resultant polymer was crushed and then

Table 1: Composition of material used to synthesize MT-MIPs and MT-NIPs

Polymers	Template molecules (mmol)		MAA (mmol)	EGDMA (mmol)	TRIM (mmol)
	EPMC	EC			
MT-MIP-1	0.67	0.33	7	20	—
MT-NIP-1	—	—	7	20	—
MT-MIP-2	0.67	0.33	7	—	20
MT-NIP-2	—	—	7	—	20

sieved using mesh number 80. The polymer was then washed with methanol and distilled water. In order to make the size uniform, 20 mL of acetone was added to the polymer and shaken. Any small particles found on the surface of the acetone solution were removed. Finally, the polymer was dried again using an oven at 50°C for 18 h. The same procedure was followed to synthesize MT-NIPs sorbents without adding a template [20].

2.3 Template extraction from MT-MIPs

Two different methods were used for template extraction on MT-MIP-1 and MT-MIP-2. For MIP1, the Soxhlet extraction method was employed, which involved using 250 mL of acetic acid: methanol (2:8, v/v) solvent to remove the molecule templates over 24 h. On the other hand, for MT-MIP-2, ultrasonic extraction was used to remove the molecule templates for 3 h, using the same solvent and filtered using filter paper. After extraction, the MIP was washed with methanol and water and dried in an oven for 18 h at 50°C. To ensure complete extraction of EPMC and EC templates, 20 mg of MT-MIPs were added to 5 mL of methanol, and aliquots were analyzed using HPLC.

2.4 Physical characterization of sorbents with Fourier transform infrared (FTIR), scanning electron microscope (SEM), and Brunauer–Emmett–Teller (BET)

FTIR analysis was used to observe its infrared spectrum. The process involved crushing 2 mg of MT-MIPs or MT-NIPs with 200 mg of KBr and forming pellets. The transmission was measured at various wavenumbers ranging from 4,000 to 400 cm^{-1}. The functional group of the MT-MIPs was identified before and after extraction, following the same method for the MT-NIPs. SEM was used to study the morphology of the MT-MIPs and MT-NIPs, while BET was employed to observe the surface area of the MT-MIPs and MT-NIPs.

2.5 Evaluation of MT-MIPs and MT-NIPs adsorption ability

To evaluate the adsorption ability of the synthesized sorbent on EC and EPMC, 5 mL of 6 µg/mL the mixture solution of EPMC and EC with concentration ratio EC:EPMC 2:3 was added to a vial containing 20 mg MT-MIPs sorbent. The mixture was then shaken for 20 min using a shaker and left at room temperature for 24 h to reach equilibrium. This process was carried out in various solvents, including ethanol, isopropanol, ethyl acetate, acetonitrile, and *n*-hexane. HPLC was used to measure the filtrate, and the difference between the initial and final concentrations of EC and EPMC in the filtrate was used to calculate the amount of EC and EPMC adsorbed (LOQ of EPMC: 0.12 µg/mL; LOQ of EC: 0.41 µg/mL). The exact process was repeated to evaluate the adsorption capacity of MT-NIP.

2.6 Evaluation of MT-MIP and MT-NIP adsorption capacity

The adsorption capacity was evaluated using variations of total EC and EPMC concentrations (9, 12, and 15 µg/mL) with concentration ratio EC:EPMC 2:3 to total. About 5 mL of EPMC and EC solution was added to the vial containing 20 mg of MT-MIPs or MT-NIPs. The mixture was then shaken using a shaker for 20 min and left at room temperature for 24 h to reach equilibrium. The filtrate was analyzed using HPLC. The data obtained were plotted into the Freundlich and Langmuir isotherm adsorption curves.

2.7 Evaluation of MT-MIPs and MT-NIPs adsorption selectivity

The adsorption selectivity was evaluated by analyzing a mixed solution of EPMC, EC, MC, and CD. About 5 mL of the solution was put into a vial containing 20 mg of MT-MIPs or MT-NIPs. The mixture was then shaken using a shaker for 20 min and left at room temperature for 24 h to reach equilibrium. The filtrate was analyzed using HPLC. The distribution coefficient (K_d) and imprinting factor (IF) were calculated.

2.8 Application of MT-MIPs and MT-NIPs for EPMC and EC extraction

A solution of *K. galanga* L. extract at 100 µg/mL concentration was prepared using *n*-hexane solvent. Next, 5 mL of that solution was added to a centrifuge tube containing either 20 mg of MT-MIPs or MT-NIPs. The mixture was shaken for 20 min and left for 24 h. The mixture was then

separated using centrifugation at 6,000 rpm for 15 min. The supernatant was separated, and the precipitated polymer was mixed with a solution of acetic acid in methanol (2:8 v/v) to extract the EPMC and EC adsorbed in the polymer. Then, the supernatant was separated using centrifugation and evaporated until dry. The resulting residue was resuspended using 5 mL of *n*-hexane, and the resuspension was analyzed using HPLC [21].

3 Results

3.1 Synthesis of MT-MIPs and MT-NIPs

Bulk polymerization was chosen for this study due to its simplicity and low solvent usage. EGDMA was used as a crosslinker for MT-MIP-1 and MT-NIP-1, while TRIM was used for MT-MIP-2 and MT-NIP-2. The formation scheme of MT-MIPs is shown in Figure 1.

3.2 Physical characterization of sorbents with FTIR, SEM, and BET

Characterization using FTIR was performed on MT-MIPs before extraction, MT-MIPs after extraction, and MT-NIPs (Figure 2). Based on Figure 2(a) and (b), it can be seen that the peak for the vinyl group (C═C stretching) at 1,658–1,648 cm^{-1} is absent, and there are no twin peaks due to the absorption of the C–H bending group at wave numbers 1,000–900 cm^{-1} [22]. The vinyl group is a functional group found in MAA monomers. The absence of vinyl groups in the MT-MIPs and MT-NIPs sorbents indicates that the polymerization process was successful because if the vinyl groups are present, it suggests that MAA monomers remain in the polymer, showing the polymerization reaction was incomplete [22].

EPMC and EC have aromatic groups in their structures [23]. In the IR spectrum, the aromatic group peak was shown at wave numbers 3,100–3,000 cm^{-1} (C–H sp^2 stretching aromatic). After extraction, the characterization results of the MT-MIP sorbent showed the absence of peaks at wave numbers 3,100–3,000 cm^{-1}, indicating that the EPMC and EC templates were extracted perfectly. However, the FTIR spectrum of MT-MIPs before extraction showed the same as the FTIR of MT-MIPs after extraction. Theoretically, the FTIR spectrum of MT-MIPs before extraction should present the aromatic group indicated by the EPMC and EC present. The absence of aromatic group peaks in the MT-MIPs sorbent before extraction can be due to the low concentration of EPMC and EC used for MT-MIPs synthesized and nonhomogeneous EPMC and EC in the MT-MIPs [24].

Polymer characterization using SEM aims to observe the morphology and identify the particle size of the polymer that has been synthesized [25]. The results of characterization using SEM at 5,000× magnification is shown in Figure 3.

Based on Figure 3, the particle size of MT-MIP-1 and MT-NIP-1 were smaller than MT-MIP-2 and MT-NIP-2. This could be because TRIM has three vinyl groups, while EGDMA is a diene molecule, so TRIM can participate more in the polymerization process than EGDMA [26].

Physical characterization with BET aims to determine a polymer's surface area, pore volume, and pore radius. BET characterization is based on the adsorption isotherm of non-reactive gas molecules, such as nitrogen [27]. The results of the characterization of surface area, pore volume, and pore radius is seen in Table 2.

Table 2 shows that the surface area and pore volume of MT-MIP-1 is smaller than MT-NIP-1, while the surface area and pore volume of MT-MIP-2 is larger than MT-NIP-2. In most cases, MIPs have higher surface area and pore volume than NIPs, which results in stronger adsorption ability. However, there are several cases that show otherwise, the surface area and pore volume of MIPs is lower than NIPs [28]. Therefore, it is necessary to carry out analytical performance measurements to prove it.

3.3 Evaluation of MT-MIPs and MT-NIPs adsorption ability

The adsorption abilities were evaluated to determine the optimal solvent required by MT-MIPs and MT-NIPs to provide optimal adsorption performance [29]. Polymers' adsorption abilities can differ because they can swell differently in different solvents [30]. The choice of solvent for this evaluation was based on its ability to dissolve EPMC and EC, which can dissolve in both polar and non-polar solvents. Polar protic solvents such as acetonitrile, ethanol, and isopropanol were used, along with polar aprotic solvent ethyl acetate and nonpolar solvent *n*-hexane [31]. The results of the adsorption ability is shown in Figure 4.

Based on Figure 4, in MT-MIP-1, the solvent suitable for adsorbing EPMC and EC simultaneously is *n*-hexane with EPMC and EC adsorption ability values of 9.00 ± 1.60% and 3.96 ± 1%, respectively. In MIT-MIP-2, the highest adsorption was when *n*-hexane solvent was used. The adsorption percentages of EPMC and EC were 22.92 ± 2.26 and 4.85 ± 2.12%, respectively. However, the adsorption capacity of MT-NIP-2 for EPMC and EC is greater than that of

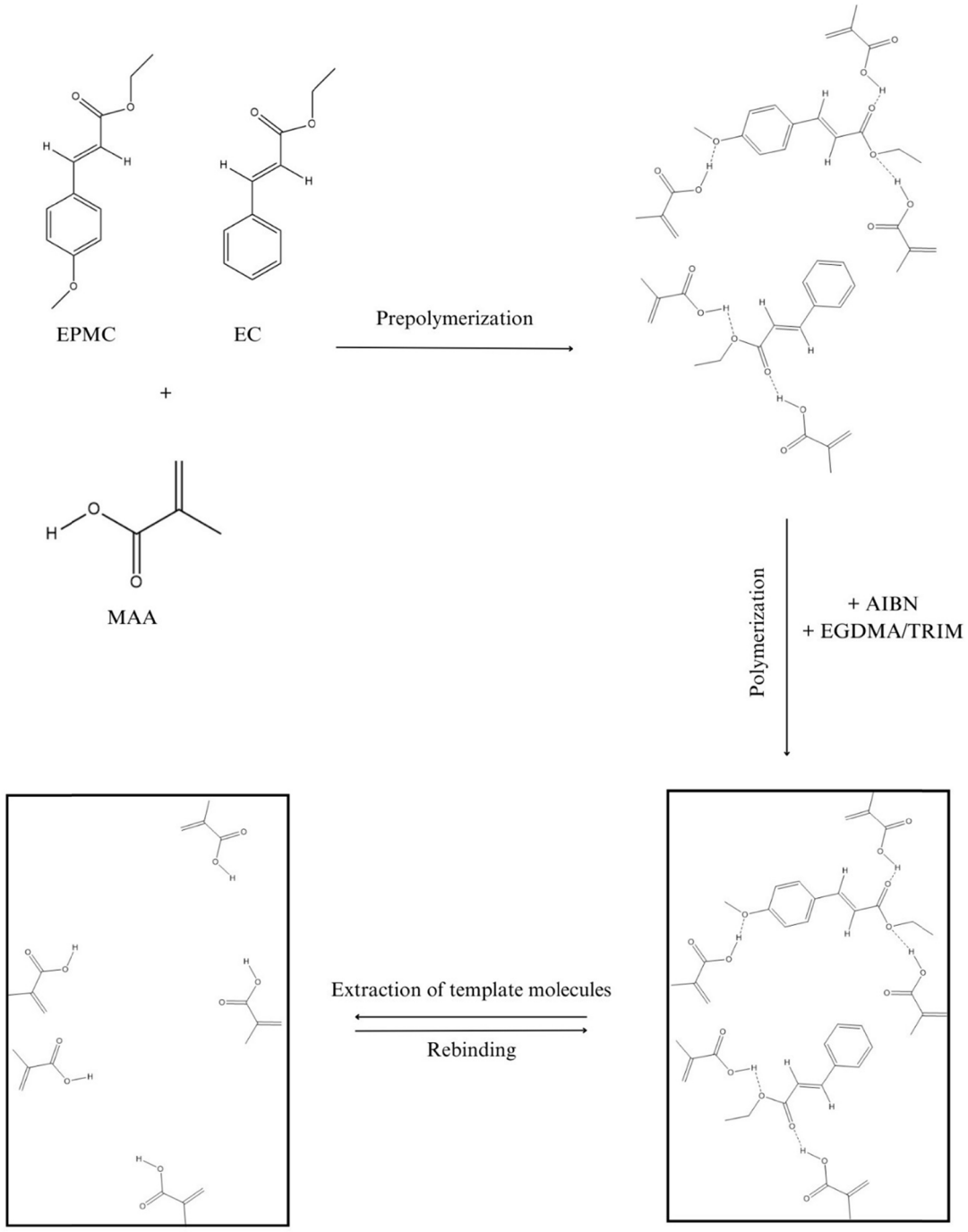
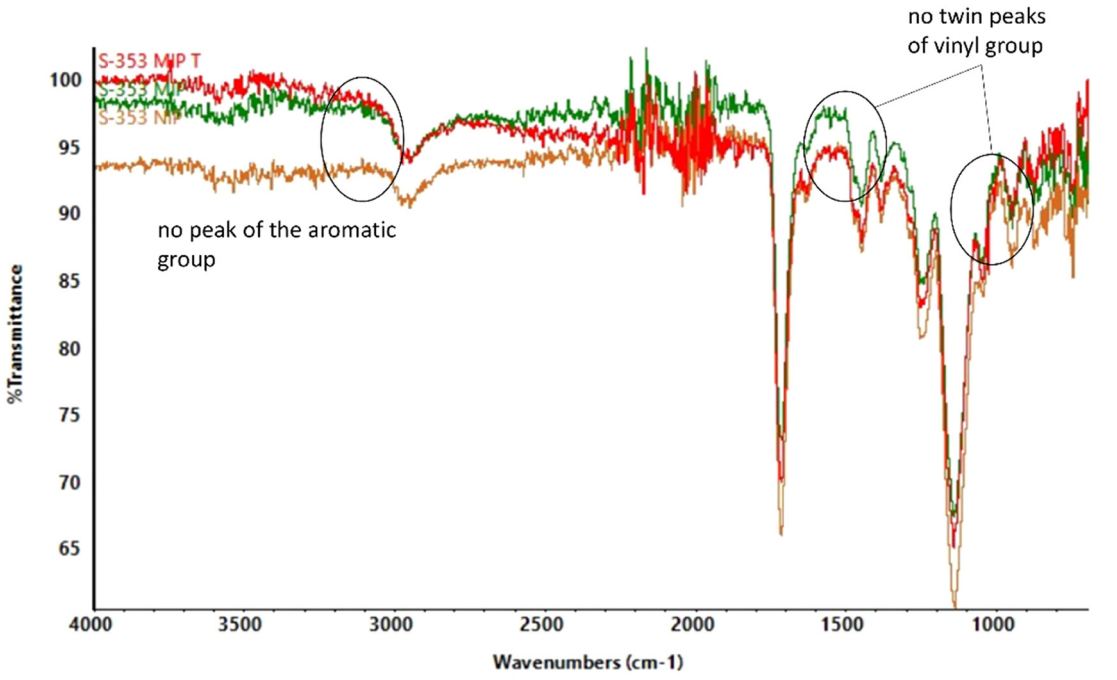


Figure 1: Synthesis scheme of MT-MIPs with MAA monomer.

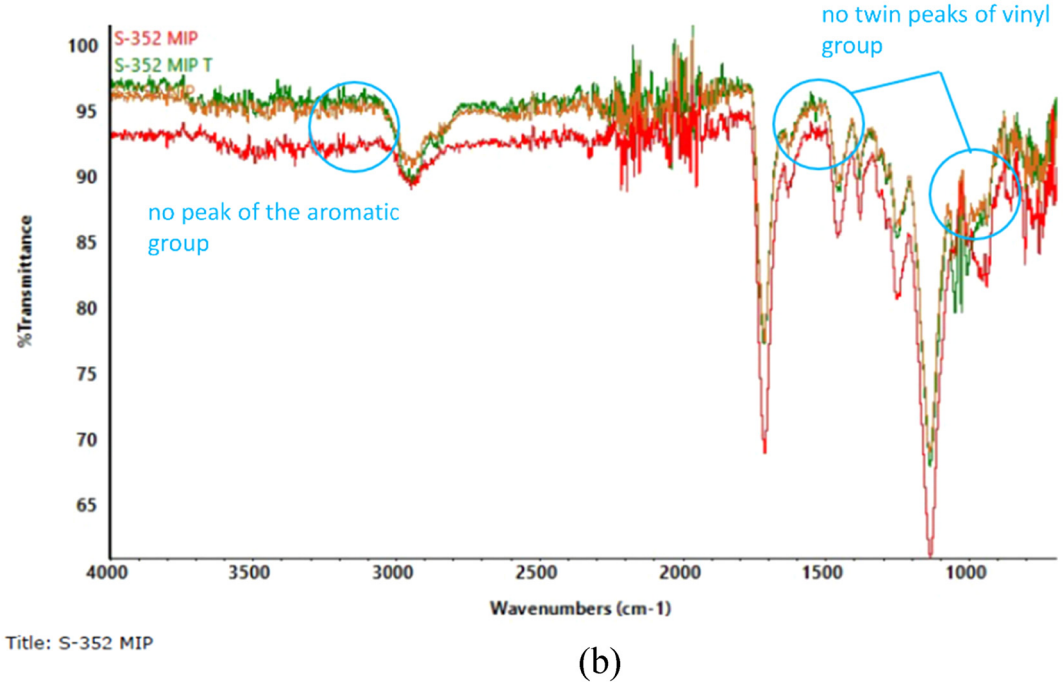
MT-MIP-2. Therefore, the *n*-hexane cannot be used because the interactions that may occur are non-specific [32]. Isopropanol was chosen as the optimum solvent compared to the others because the adsorption percent value of MT-MIP-2 was slightly greater than MT-NIP-2. The adsorption percentage of EPMC in isopropanol solvent was 6.69 ± 3.19 for MT-MIP-2 and 6.17 ± 2.87 for MT-NIP-2. Meanwhile, EC is 1.47 ± 2.07 for MT-MIP-2 and 1.47 ± 2.36 for MT-NIP-2.

3.4 Evaluation of MT-MIPs and MT-NIPs adsorption capacity

The adsorption capacity was evaluated to determine the polymer's binding sites and affinity for the EPMC and EC. The distribution of MT-MIPs binding sites can be determined by applying the Freundlich or Langmuir isotherm equations. These equations help determine polymer adsorption sites'



(a)



(b)

Figure 2: IR spectrum of (a) MT-MIP-1 before extraction (red), MT-MIP-1 after extraction (green), and MT-NIP-1 (brown). (b) MT-MIP-2 before extraction (green), MT-MIP-2 after extraction (red), and MT-NIP-2 (brown).

affinity, capacity measurement, and homogeneity index [33]. The result of this evaluation is shown in Table 3.

Based on the Table 3, the MT-MIP-1 and MT-NIP-1 have Freundlich isotherms for both EPMC and EC. The MT-MIP-2 and MT-NIP-2 sorbents for EPMC and EC also follow the Freundlich isotherm model. The adsorption capacity value indicates the affinity or capacity of the sorbent to bind the analyte, where the higher the value, the more the analyte

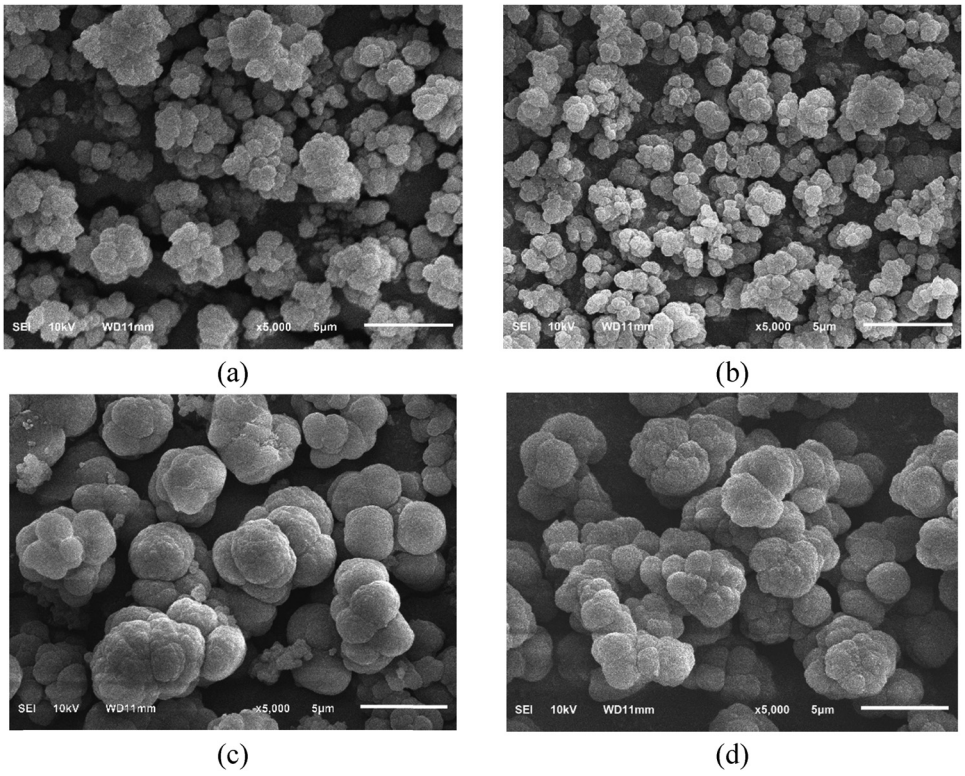


Figure 3: Characterization using SEM (a) MT-MIP-1, (b) MT-NIP-1, (c) MT-MIP-2, and (d) MT-NIP-2.

Table 2: Surface area, pore volume, and pore radius of MT-MIPs and MT-NIPs

Polymers	Surface area (m^2/g)	Pore volume (cc/g)	Pore radius (Å)
MT-MIP-1	187.204	0.493	20.074
MT-NIP-1	735.145	1.272	15.641
MT-MIP-2	191.852	0.586	20.046
MT-NIP-2	176.039	0.333	16.818

adsorbed to the sorbent [34,35]. The adsorption capacity for EPMC on MT-MIP-1 has a more excellent value than MT-MIP-2 (0.42521 mg g^{-1} for MT-MIP-1 and 0.0008 mg g^{-1} for MT-MIP-2). Meanwhile, the EC adsorption capacity of MT-MIP-1 has a lower value than MT-MIP-2 (0.4742 mg g^{-1} for MT-MIP-1 and 7.5875 mg g^{-1} for MT-MIP-2).

3.5 Evaluation of MT-MIPs and MT-NIPs adsorption selectivity

Selectivity determination was carried out to determine the ability of MT-MIPs to adsorb the EPMC and EC selectively when compared to their analogous compounds, namely CD and MC. The selection of CD and MC compounds was based on the structural similarity of the two compounds to EPMC and EC. Apart from that, CD and MC are also essential oil compounds contained in the *K. galanga* L. extract [36].

The distribution coefficient (K_d) is a parameter that shows the distribution of the analyte fraction adsorbed by the sorbent compared to the analyte fraction remaining in the solution [37]. The IF value is a value that shows the analytical performance of the sorbent in separation analysis. The IF value is obtained from ratio of the distribution coefficient between MT-MIP and MT-NIP, which shows the selectivity of MT-MIP toward the analyte when compared to MT-NIP [38]. Based on Table 4, MT-MIP-1 has the highest *K* and IF values for EPMC and EC compounds compared to CD and MC compounds.

3.6 Application of MT-MIPs and MT-NIPs for EPMC and EC extraction

Two extracts of *K. galanga* L. extracted using two different solvents were used in this study which are *n*-hexane and ethyl acetate. The percentage yield of EPMC and EC successfully extracted from *K. galanga* L. extract using MT-MIP and MT-

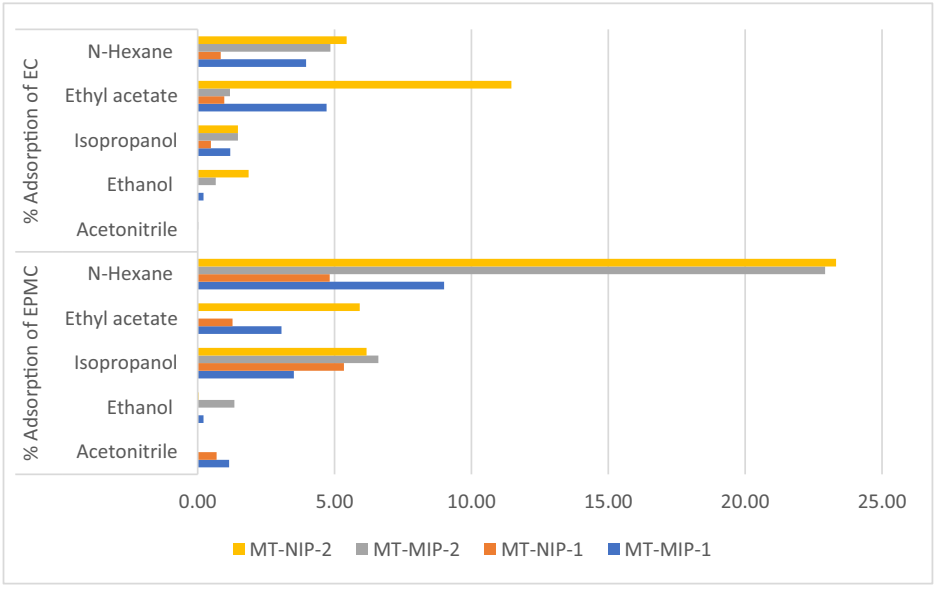


Figure 4: Evaluation results of MT-MIPs and MT-NIP adsorption abilities on EPMC and EC.

Table 3: Evaluation result of the adsorption capacity of MT-MIPs and MT-NIPs

Isotherm	Parameter	EPMC				EC			
		MT-MIP-1	MT-NIP-1	MT-MIP-2	MT-NIP-2	MT-MIP-1	MT-NIP-1	MT-MIP-2	MT-NIP- 2
Langmuir	Q_m	0.1146	0.1108	−0.0268	−0.0292	0.0558	0.1120	0.0835	−0.0038
	K_L	−0.2662	−0.2867	−0.1062	−0.0941	−0.1961	−0.2867	0.9339	−0.1059
	R^2	0.8781	0.9148	0.9286	0.9772	0.8609	0.9494	0.1159	0.8897
Freundlich	1/*n*	−0.3641	−0.3457	2.4590	2.1257	−0.6202	0.3388	−2.2965	3.5418
	n	−2.7465	−2.8927	0.4067	0.4704	−1.6124	2.9516	−0.4354	0.2823
	K_f	0.42521	0.37749	0.0008	0.0010	0.4742	0.03388	7.5875	0.0000
	R^2	0.3092	0.4109	0.9988	0.9925	0.4529	0.7889	0.4818	0.7852

Note: Q_m: adsorption capacity (mg/g), K_L: Langmuir constant (L/mg), R^2: linear equation regression, 1/*n*: homogeneity index, *n*: adsorption intensity, K_f: adsorption capacity (mg/g).

NIP in *n*-hexane extract and ethyl acetate extract is shown in Table 5, respectively.

Table 5 shows that EPMC extracted from *K. galanga* L. ethyl acetate extract using MT-MIP 1 had a greater yield percentage (9.75 ± 0.004%) than MT-NIP-1 (3.86 ± 0.41%). Meanwhile, the results for EC extraction from both extracts show the same value. For MT-MIP-2, the percent yield for both extracts was not too different.

Based on the adsorption capacity result, MT-MIP-1 has a better value in separating EPMC and EC simultaneously. In addition, based on the IF value, MT-MIP-1 is better than MT-MIP-2. However, as in Table 5, the comparison of

Table 4: Result of selectivity MT-MIPs dan MT-NIPs

Analyte	K_d (mL g^{-1})		IF	K_d (mL g^{-1})		IF
	MT-MIP-1	MT-MIP-1		MT-MIP-2	MT-NIP-2	
EPMC	147.5	143.2	1.03	129.5851	135.7835	0.9544
EC	522.57	498.38	1.05	522.2680	537.4456	0.9718
SD	35.67	42.10	0.85	7.3165	5.6864	1.2867
MS	14.06	16.39	0.86	4.6870	1.9364	2.4204

Table 5: Analysis of percent yield of EPMC and EC extracted from *K. galanga* L. extract using MT-MIPs and MT-NIPs

Extract	Compound	Polymer	Yield (%)	%Yield MIP/NIP	Purity (%)
n-Hexane	EPMC	MT-MIP-1	1.47 ± 0.20	0.860	95.24 ± 4.76
		MT-NIP-1	1.71 ± 0.49		98.93 ± 1.07
		MT-MIP-2	1.557 ± 0.10	1.009	66.330 ± 2.45
		MT-NIP-2	1.543 ± 0.09		69.674 ± 6.95
	EC	MT-MIP-1	0.64 ± 0.02	1.143	37.07 ± 5.44
		MT-NIP-1	0.56 ± 0.04		17.93 ± 0.00
		MT-MIP-2	1.929 ± 0.00	1.102	61.510 ± 0.00
		MT-NIP-2	1.751 ± 0.00		36.322 ± 0.00
Ethyl acetate	EPMC	MT-MIP-1	9.75 ± 0.004	2.526	98.88 ± 0.13
		MT-NIP-1	3.86 ± 0.41		96.36 ± 0.33
		MT-MIP-2	1.687 ± 0.14	1.205	79.692 ± 0.22
		MT-NIP-2	1.400 ± 0.05		59.381 ± 4.27
	EC	MT-MIP-1	0.54 ± 0.05	0.947	31.97 ± 0.00
		MT-NIP-1	0.57 ± 0.07		31.92 ± 0.00
		MT-MIP-2	2.099 ± 0.15	∞	72.027 ± 0.00
		MT-NIP-2	0.000 ± 0.00		0.000 ± 0.00

percent yield MT-MIPs results and percent yield MT-NIPs results, MT-MIP-2 has a greater value >1, indicating the printing process's success. It can be concluded that in extracting EPMC and EC in extract, MT-MIP-2 has a better ability to separate EPMC and EC than MT-MIP-1 in both kind of extracts.

4 Conclusions

Two kinds of EPMC and EC MT-MIPs have been synthesized by bulk polymerization for isolating EPMC and EC simultaneously. MT-MIP made using TRIM as crosslinker has better performances compared to EDGMA to isolate EPMC and EC simultaneously. The yields of EPMC and EC in *n*-hexane extracts were 1.557 and 1.929%, with purity of 66.330 and 61.510%, respectively. Meanwhile, the yields of EPMC and EC in ethyl acetate extract were 1.687 and 2.099%, with purity of 79.692 and 72.027%, respectively. The results show that the application of MT-MIPs for isolating EPMC and EC in *K. galanga* L. extracts still needs improvement for higher yields. Therefore, further study is required, such as selecting the functional monomer and determining the functional monomer's ratio to template molecules.

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Conflict of interest: Authors state no conflict of interest.

Ethical approval: The research related to animals' use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

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