Molecular Dynamics Simulation of *Mahkota Dewa (Phaleria Macrocarpa)* Extract in Subcritical Water Extraction Process

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Abstract. *Mahkota Dewa (Phaleria Macrocarpa)*, a good source of saponin, flavanoid, polyphenol, alkaloid, and mangiferin has an extensive range of medicinal effects. The intermolecular interactions between solute and solvents such as hydrogen bonding considered as an important factor that affect the extraction of bioactive compounds. In this work, molecular dynamics simulation was performed to elucidate the hydrogen bonding exists between *Mahkota Dewa* extracts and water during subcritical extraction process. A bioactive compound in the *Mahkota Dewa* extract, namely mangiferin was selected as a model compound. The simulation was performed at 373 K and 4.0 MPa using COMPASS force field and Ewald summation method available in Material Studio 7.0 simulation package. The radial distribution functions (RDF) between mangiferin and water signify the presence of hydrogen bonding in the extraction process. The simulation of the binary mixture of mangiferin:water shows that strong hydrogen bonding was formed. It is suggested that, the intermolecular interaction between $O_{H2O} \cdot \cdot H_{MR4(OH1)}$ has been identified to be responsible for the mangiferin extraction process.

1. Introduction

Plants with therapeutic performances or known as herbs have been used as an alternative health care since ancient time. This practice has been recognized by the World Health Organization (WHO) [1,2]. *Mahkota Dewa* or scientifically known as *Phaleria macrocarpa* is one of the popular herbal plants in Malaysia due its medicinal properties [3]. *Mahkota Dewa* originated from *Thymelaceae* family and growth in the topical areas of Papua Island, Indonesia [3,4]. The bioactive ingredients in this plant contains antihistamine, antioxidant, antidiabetic and anticancer effect [5]. The example of bioactive ingredients in *Mahkota Dewa* fruits are alkaloid, saponin, flavonoid, polyphenol and mangiferin [4]. Among these compounds, mangiferin has a broad range of pharmacological effects including antidiabetic, anti-HIV, anticancer, immunomodulatory and antioxidant [4,6]. Solvent extraction, conventional methods which often used to extract the bioactive compounds from various plants. The organic solvents employed during the process are as hexane, methanol or ethanol [7]. However, this process reported as impractical due the need of further purification step as well as low extraction yield [7]. Subcritical water extraction (SWE), involve the use of water as solvent under a pressurized condition reported as a non-toxic and environmental friendly technique. As water heated under pressure to subcritical temperatures, there are significant changes to its polarity, often expressed simply as the dielectric constant. When temperature increased to sufficiently high temperatures, the dielectric constant of water decreases and observed to mimic a range of organic and hydro-organic solvents [8]. The application of SWE can be found for extraction of various herbal products such ginger, algae, olive leaves and also oregano [9-11]. However, to the best of our knowledge, the

mechanism of mangiferin extraction using water is yet to be reported. This mechanism is quite impossible to be seen using experimental approach, unless model using a suitable software.

Molecular dynamics simulation is a powerful tool use to model the molecules and generate information at the microscopic level [12]. Recently, molecular dynamics simulation has been employed in various studies such as extraction, crystallization, and membrane development [2,13-15]. This approach is ideally suitable to examine the intra and intermolecular interaction with different materials based on radial distribution function (RDF) analysis [12,16-18]. Adam and co-workers has applied molecular dynamic simulation to simulate the patchouli oil extraction behaviour in different solvents namely acetone, ethanol and hexane. The study suggested that different solubility behaviour was due to the different of hydrogen bonding arrangement between it solute and solvent molecules [2]. This paper is aimed to model the intermolecular interaction, mainly hydrogen bonding, during the extraction of bioactive compound namely mangiferin from *Mahkota Dewa* fruits in water using molecular dynamic simulation. The simulation was performed at 373 K and 4.0 MPa [19] using COMPASS force field and Ewald summation method available in Material Studio 7.0 simulation package.

2. Methods

2.1. Simulation details
The MD simulation of mangiferin extraction were performed using Accelrys Material Studio® 7.0 software (Accelrys, Inc., San Diego, USA) which installed in HP Z420 Workstation with Windows 7 Professional operating system. The three-dimensional (3D) molecules structure of mangiferin and water downloaded from Chemspider databases and imported into the software. The partial labeling of molecules shown in Figure 1 was used in this simulation work for the purpose of molecular recognition.
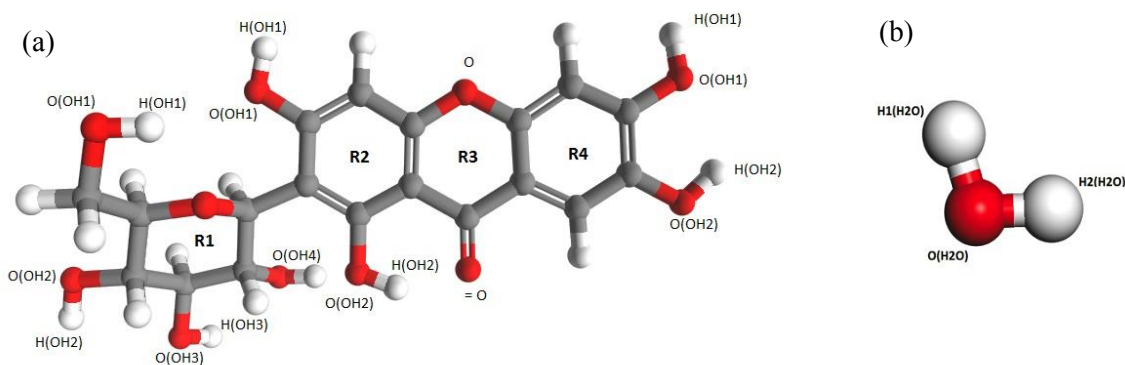
Figure 1. Chemical structure of (a) Mangiferin and (b) Water. Color representation: White - hydrogen, Red - oxygen and Grey - carbon [23].

Each molecule was optimized using geometry optimization to produce a stable molecular geometry for subsequent simulation steps. A smart algorithm was used to calculate the energy and atomic force with a fine convergence quality. The cubic periodic boundary simulation box for pure water and mixture of water and mangiferin were constructed and minimize at 373 K and 4.0 Mpa by using amorphous cell calculation module. The minimization step is crucial in minimizing the energy produce and to ensure simulation box ready for the dynamic stage. The number of molecules, densities of pure components and binary system as well as size of the simulation boxes is tabulated in Table 1. The density of binary mixture was calculated using the following equation:

$$Density, \rho = A \, B^{-\left(1-\frac{T}{C}\right)^{n}} \tag{1}$$

where A, B, C and n is the regression coefficient of chemical compound and T represent the temperature in Kelvin (K). The simulation was initiated by equilibrating the system under constant

number of molecules, volume and temperature (NVT) ensemble for 1000 ps where molecules are allowed to evolve from random starting to stable configuration with energy conservation. The simulation was continued by dynamic mode with constant number of molecules, pressure and temperature (NPT) ensemble using Nose thermostat [2] and Berendsen barostat [2] for 1000 ps. The time step was set at 1.0 fs. The cut-off distance for Lennard-Jones potential was set to 12 Å which was close to half of the cell length. A condensed-phase optimized molecular potentials for atomistic simulation studies (COMPASS) force field and Ewald summation techniques with an accuracy of 0.0001 kcal/mol were used to model the intermolecular interaction in the system [14-15,18].

Table 1. Simulation data.

System	Number of Molecule	Density [g/cm^3]	Equilibrated box size, A × B × C
Pure system:			
Water	1000	0.9529 [18]	32.56 × 32.56 × 32.56
Mangiferin	50	1.8430 [18]	31.54 × 31.54 × 31.54
Binary system:			
Mangiferin: Water	50:1000	0.9953	40.26 × 40.26 × 40.26

2.2. Radial distribution analysis

Atoms from the trajectory files of the simulation labeled and then each pair of labeled atoms was analyzed for its radial distribution functions (RDF). The labeled atom was based on the atom that has the ability to initiate the hydrogen bond. RDF is essential to describe the micro-structure of the material and describes the variation of specific atomic density as a function of a distance from one reference atom [13,20]. In other words, RDF counts the number of two-atom species with specific distances and can be defines as:

$$g_{xy}(r) = \frac{[N_y(r, r + dr)]}{\rho_y 4\pi r^2 dr} \tag{2}$$

where r is spherical radius distance from the reference atom [13], ρ_y is a density of a y atom, $[N_y(r, r + dr)]$ is number of y atoms in a shell of width r at distance r and x is reference atom [18].

3. Results and discussion

3.1. Validation of MD simulation method

The Simulation validation method is essential to determine whether the applied simulation method and COMPASS force able to produce reliable results. Figure 2 compares the RDF of pure water obtained in this work with those reported in literature [16]. The possible bonding formation between O_{H2O}••$H1_{H2O}$, O_{H2O}••O_{H2O} and $H1_{H2O}$••$H2_{H2O}$ obtained in this work shows first peaks at 1.75 Å, 2.75 Å, and 2.25 Å, respectively. It can be seen that, both simulated and literature RDF pattern show quite a similar trend. Based on these values, it can be affirmed that O_{H2O}••$H1_{H2O}$, which shows the nearest neighbor interaction, is stronger and more intense compared to O_{H2O}••O_{H2O} and $H1_{H2O}$••$H2_{H2O}$, thus suggested to represent the strength of hydrogen bonding in pure water. A slight difference in RDF intensity, g(r) for O_{H2O}••O_{H2O} was probably due to differences in number of water molecules used during simulation as Abdul Mudalip et al. [16] simulate 250 water molecules meanwhile, this study used 1000 water molecules.

Table 2 shows the comparison between simulated data and the setting parameters of water and mangiferin:water. The percentage of deviation for average density is low, which is less than 6%. Sun [21] who had performed a MD for 150 organic structures using COMPASS force field reported a maximum absolute error of 6% for the simulated density. Since the deviation obtained in this work is quite small, it can be suggested that the use of the COMPASS force field and the Ewald summation method can generate reliable simulation results.
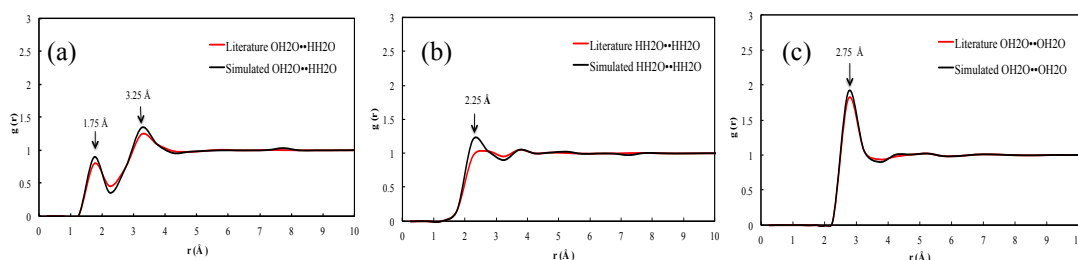
Figure 2. Comparison of RDF for (a) $O_{H2O}\cdot\cdot H1_{H2O}$ (b) $H1_{H2O}\cdot\cdot H2_{H2O}$ (c) $O_{H2O}\cdot\cdot O_{H2O}$ from this study and Mudalip et al. [16] .

**Table 2.** Simulation result of *Mahkota Dewa* extraction.

System	Water	Mangiferin/water
Average Density [g/cm^3]		
Simulated Value	0.8970	0.9960
Setting Value	0.9529	0.9953
Deviation [%]*	5.87	0.07

*Deviation = [(Simulated value - Calculated value)/ Calculated value] x 100

### 3.2. RDF analysis in binary mixture

The strength of the intermolecular interactions between solutes and solvents determines the solubility and amount of a solute extracted in a particular solvent. In this study hydrogen bond is one of the intermolecular interactions that present since it is the primary intermolecular forces present in water. Hydrogen bond is an attractive force exists in between hydrogen in a molecule with an electronegative atom of different molecule. The electronegative atom includes oxygen, nitrogen, and fluorine, which have a partial negative charge. Analysis of RDF can provide atomistic representation of intermolecular interaction that exists in binary mixture [14]. In binary mixture, the intermolecular interactions exist between molecules of solvent-solvent, solute-solvent, and solute-solute. During solvation, the solute must be surrounded, or solvated, by the solvent. Solutes are successfully dissolved into solvents when solute-solvent interactions are stronger than the solute-solute or solvent-solvent interactions [2].

Figure 3 (a) shows the RDF of solvent-solvent, which is $O_{H2O}\cdot\cdot H1_{H2O}$ and $O_{H2O}\cdot\cdot O_{H2O}$ in binary mixture of mangiferin:water during subcritical extraction process. The RDF of $O_{H2O}\cdot\cdot H1_{H2O}$ obtained shows strong hydrogen bonding interaction at 1.75 Å and 3.25 Å, with intensities of 2.33 and 2.02 respectively. Meanwhile, for $O_{H2O}\cdot\cdot O_{H2O}$ RDF observed at radial distance of 2.75 Å with an intensity of 3.91. Both $O_{H2O}\cdot\cdot H1_{H2O}$ and $O_{H2O}\cdot\cdot O_{H2O}$ RDF pattern was in agreement with the RDF pattern for the simulated pure water with slight different of intensity, g(r). The same RDF pattern indicates that the presence of a mangiferin as solute in the binary system does not modify the long-range pure solvent structure and higher intensities indicates that there was strong hydrogen bonding interaction between water molecules in the binary system. The solute-solute interaction in between $O_M\cdot\cdot H_M$ and $O_M\cdot\cdot O_M$ shows in Figure 3 (b).
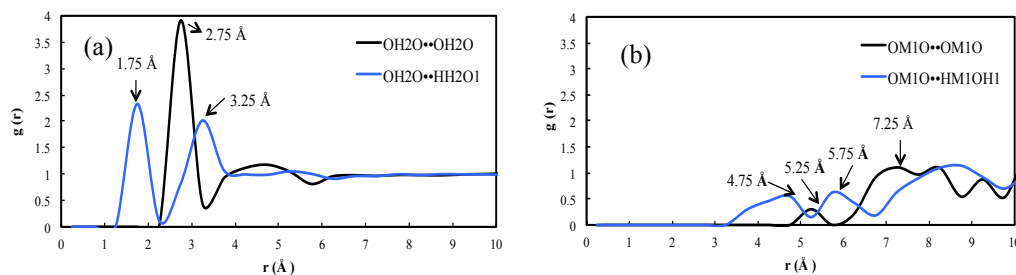
Figure 3. (a) Solvent-solvent, $O_{H2O}\cdot\cdot H1_{H2O}$ and $O_{H2O}\cdot\cdot O_{H2O}$ interactions in binary system (b) Solute-solute interaction in binary system with $O_M\cdot\cdot H_M$ and $O_M\cdot\cdot O_M$.

The first peaks observed at 4.75 Å and 5.75 Å for O_M••H_M and 5.25Å and 7. 25 Å for O_M••O_M. The radial distribution function of both interaction are less structured with no sharp peak and thus this interaction is not significant in this simulation and experimental extraction process. The RDF for the interaction of O_{H2O}••H_M and H_{H2O}••O_M illustrated in Figure 4 (a) and (b). The interactions between water atoms which is O_{H2O} with the hydrogen atom from mangiferin $H_{MR2(OH1)}$, $H_{MR2(OH2)}$, $H_{MR4(OH1)}$ and $H_{MR4(OH2)}$ recorded RDF of 1.75 Å and 3.25 Å. The RDF of O_{H2O}••$H_{MR4(OH1)}$, recorded the sharpest peak with the intensity of 2.76 at radial distance of 1.75 Å and RDF of O_{H2O}••$H_{MR2(OH1)}$ shows intensity of 2.70, which is 0.06 less from O_{H2O}••$H_{MR4(OH1)}$. The second peak of 3.25 Å recorded the intensity of 1.2. As the radial distance became longer, the intensity reach 1.0 that indicates there is no long-range orders interaction [22]. Since the O_{H2O}••$H_{MR4(OH1)}$ shows the sharpest peak, it can be suggested that it can represent the strength of solute-solvent interaction in the mangiferin extraction. Meanwhile in the Figure 4(b) interaction between $H1_{H2O}$ with $O_{MR1(OH1)}$, O_{MR1O}, $O_{MR2(OH2)}$, O_{MR3O}, and $O_{MR3=O}$ shows lower intensity, g(r) with RDF of 1.75 Å and 3.25 Å. The highest intensity shows by interaction of $H1_{H2O}$••$O_{MR1(OH1)}$, with intensity of 1.42 at 1.75 Å radial distance and 1.43 intensity at 3.25 Å. Overall, the interaction between of $H1_{H2O}$•• O_M shows that the intensity of the interaction is low compared to interaction between O_{H2O}••H_M. Thus, this study was focusing more on interaction of O_{H2O}••H_M.
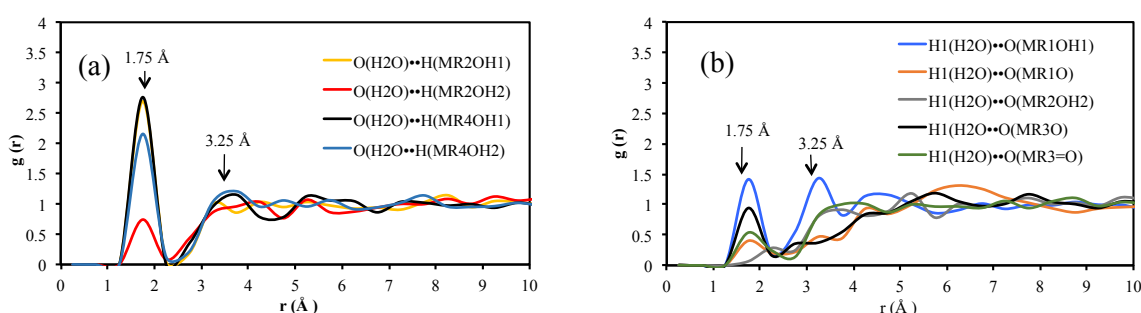
Figure 4. (a) and (b) Solvent-solvent interactions and radial distribution function (RDF) between O_{H2O} with H_M in the binary system at 373 K

4. Conclusion
The molecular point of view of mangiferin in subcritical water extraction process has been successfully revealed using molecular dynamics simulation technique. The simulated RDF pattern for pure water using the COMPASS force field and Ewald summation method show an agreement with literature with slight differences in RDF intensity. The simulation of the binary mixture of mangiferin:water shows that strong hydrogen bonding formed and the intermolecular interaction between O_{H2O}••$H_{MR4(OH1)}$ has been identified to represent the strength of solute-solvent interactions in mangiferin extraction from *Mahkota Dewa* fruits.

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