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Solvent Role in Molecular Recognition of Patchouli Extraction Process

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Patchouli or *Pogostemon cablin Benth* is an aromatic plant of importance to the fragrant and cosmetic industries. Its secondary metabolites present interesting pharmacological benefits such as antioxidant and antimutagenic properties. This work is an extended study of the published work in ethanol and water solvents using Ewald summation method and mass spectra characterization of patchouli essential oil extracted with three different polar and non-polar solvents. Ewald summation method has reproduced a better radical distribution function (rdf) intensity in the polar ethanol and water solvents using COMPASS force-field. This work concludes that the complex molecular interaction particularly hydrogen bonding play a significant role to affect the solubility of patchoulol solute either in polar or non-polar solvents during the extraction process.

Keywords: *Pogostemon cablin Benth*, Ewald, Hydrogen bonding.

INTRODUCTION

In medical applications, patchouli essential oil has been reported [1] to inhibit neurogenic pain and also prevents nausea and vomiting [2]. This study aims to recognize the role of the different solvent types in the extraction process of patchouli leaves through molecular dynamics simulation and further correlate infrared and mass spectral data from laboratory experiments.

Ethanol solvent demonstrated a higher content of patchulol marker compound due to the establishment of hydrogen bonding which increased the patchulol solute solubility in the solvent through the OH group [3]. Infrared spectroscopy is able to measure the "free" and "associated" OH-stretch bands and through changes in the relative intensities of the absorption bands, changes in the equilibrium between the free and associated species due to temperature and concentration are attributed [4]. In this work, the hydrogen bonding region in their spectra is of interest as it reflects the solubility of the marker and main solute extracted from the patchouli. Meanwhile, gas chromatography mass spectroscopy (GCMS) is able to identify and provide quantitative and qualitative information on the composition and chemical structures of each compound [5] in the patchouli oil. It combines the power of high resolution separation of components with selective and sensitive mass detection [6].

EXPERIMENTAL

The solvent extraction method is used to extract the patchouli essential oil from samples supplied by Syarikat Nilam Suling Sdn Bhd, Miri, Sarawak, Malaysia. Acetone, ethanol and *n*-hexane were used as the solvents were supplied by Merck (99.7 % purity). Patchoulol leaves (10 g) were macerated in solvent (100 mL) and left overnight. Separation was affected with Buchi Evaporator (R100) and the experiment was replicated three times for each solvent.

The Agilent 7890A Network Sytem gas chromatography equipped with an Agilent 5975C mass spectrometer was used to characterize the extracted oil [3]. Patchoulol (0.02 g) was taken up in 10 mL of each solvent. Then the sample was analyzed using a Perkin Elmer ATR-FTIR spectrometer (Frontier) with spectra range of 4000-500 cm^{-1}. A total 16-32 scans were acquired at 0.15 s/scan and spectral resolution of 2-4 cm^{-1}. The time step of spectral measurement was 2.4-19.2 s/spectrum. The spectra was then analyzed using OMNIC software.

Molecular dynamics simulation: The simulations were carried out in Acceryls Materials Studio (MS), version 5.5, using a HP Z400 workstation. Each solute and solvent molecules geometry was optimized prior to the creation of the simulation boxes. In this study, the condensed-phase optimized molecular potentials for atomistic simulation studies [COMPASS] force field [7] was used to model the system and Ewald summation

method was chosen for electrostatic and van der Waals interactions. The Ewald summation, similar to lattice method is a technique for efficiently summing the interaction between a molecule and all its periodic images. This method over emphasizes the continuum nature of a polar fluid and require a priori estimate of the relative permittivity [8,9]. The simulation begins with equilibration of the system under constant number of moles, volume and energy (NVE) ensemble for 100 ps. After equilibration, the systems were run in the NPT ensemble which is constant number of moles, pressure and temperature for a total simulation time of 5 ns with 1 fs stepsize [3]. The molecular labelling of patchoulol, ethanol and water is illustrated in Fig. 1 for further molecular recognition purpose. Table-1 summarises the number of molecules and density of simulation boxes generated for pure and binary systems at 298 K.

TABLE-1
LIST OF SIMULATED SYSTEMS APPLYING EWALD SUMMATION METHOD [Ref. 9]

Systems	Number of molecules	Density (g/cm^3)
Pure system at 298 K		
Ethanol	1000	0.780
Water	1000	1.000
Patchoulol	50	1.001
Binary system at 298 K		
Ethanol:Patchoulol	1000:20	0.795
Water:Patchoulol	1000:20	1.000

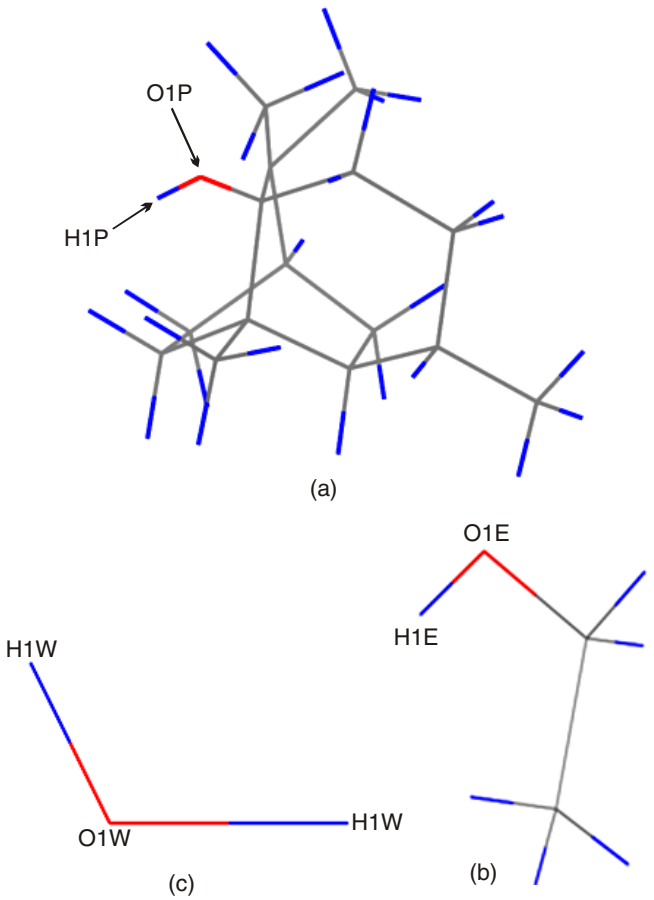

Fig. 1. Schematic labelling of patchoulol (a), ethanol (b) and (c) water molecular structures

RESULTS AND DISCUSSION

Hydrogen bonding intermolecular interaction: This work applied the Ewald summation method for the long-range correction summation method particularly in the polar ethanol compound simulation to reproduce more accurate radical distribution function (rdf) property compared to the published work [3]. Ewald summation method is efficient to describe the electrostatic interaction in a polar molecule such as acetone and ethanol for $N \leq 1000$ [10]. The radical distribution function result for pure ethanol solvent O1E---O1E (Fig. 2) and the hydrogen bonding radical distribution function structure of O1E---H1E (Fig. 3) show an improvement on the g(r) intensity value compared to a previous report [11]. The location of the first neighbour atom at 1.75 Å is still in agreement to the report [11] and the report by Adam *et al*. [3]. Similarly the radical distribution function of hydrogen bonding O1W---H1W in pure water is in agreement with published work [12] using TIP3P.

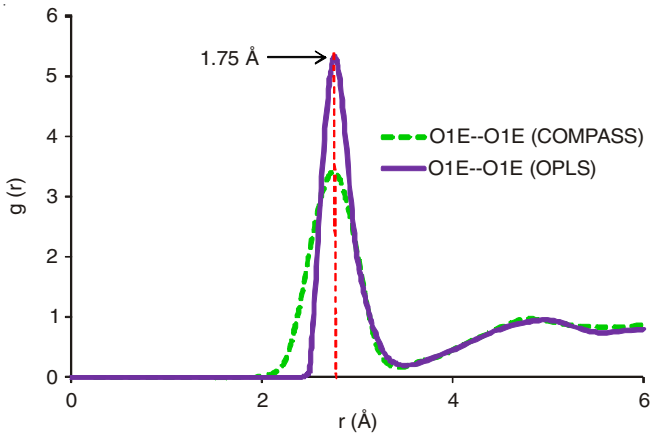

Fig. 2. Comparison of O1E---O1E in pure ethanol liquid system using COMPASS and OPLS force fields [Ref. 11]

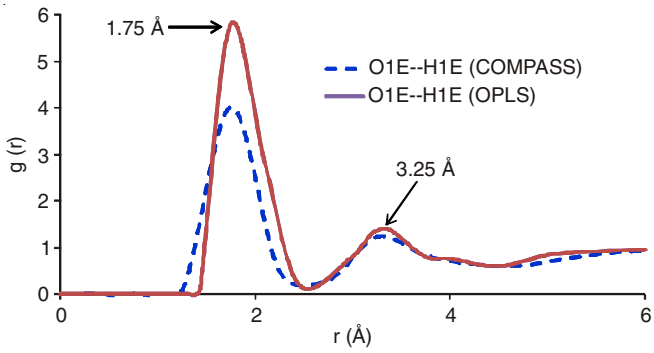

Fig. 3. Comparison of O1E---H1E in pure ethanol liquid system using COMPASS and OPLS force fields [Ref. 11]

The first and second neighbour atoms which represent the hydrogen bonding O1W---H1W in pure water system is at 1.75 and 3.25 Å respectively is in agreement to the literature value, but the g(r) intensities deviate slightly (Fig. 4). This concludes that the radical distribution function structure is suitable to be reproduced using COMPASS force field with Ewald summation in polar solvent molecule system to measure the intermolecular interaction strength. Meanwhile in binary ethanol system, a strong pattern of hydrogen bonding in Fig. 5 is recognized from O1P---H1E, O1E---H1P and O1P---O1E

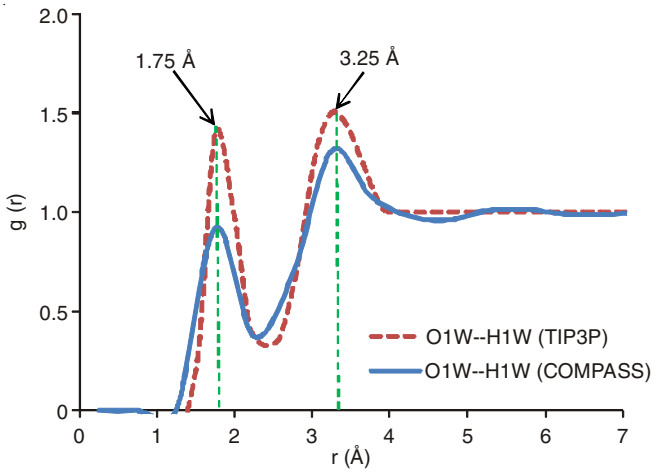

Fig. 4. Comparison of O1W---H1W in pure water system using COMPASS and TIP3P force fields [Ref. 12]

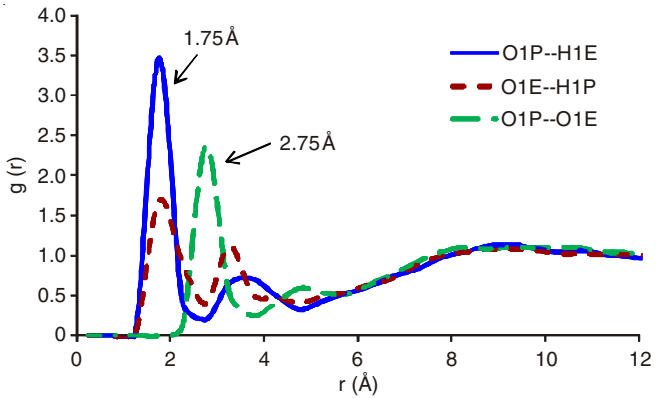

Fig. 5. Solute-solvent interaction in solvent extraction (binary ethanol-patchoulol system) with oxygen and hydrogen atoms as reference atoms

which has increased the extraction yield of patchulol in the ethanol solvent. The first neighbour atoms were found at 1.75 and 2.75 Å, which reflects the strong interaction between solute and solvent to increase the mass transfer process during the extraction process.

In the binary water-patchoulol system (Fig. 6) at similar temperature (298 K) to ethanol solution, only first neighbour atom of the hydrogen bonding synthon was established at 1.75 and 2.25 Å for O1P—H1W and O1W---H1P with lower probability compared to the binary ethanol-patchoulol system for the first neighbour atom interaction strength in Fig. 5. This may further reflect the lower patchouli oil yield when extracted using the water as solvent such as during hydrodistillation [13]. Different hydrogen bonding propensities in the solvents have contributed to different solubility values of the solute [14]. As an example, the solubility of an organic compound in subcritical water is influenced by the properties of water, the structure of the solute and the complex interactions between the water and the solute [15]. The weak repulsion of O1P---O1E and O1P---O1W were found both at 2.75 Å in ethanol-patchoulol and water-patchoulol systems (Figs. 5 and 6) suggesting during hydrodistillation, the rate of extraction is determined by the partition of the compound between the plant material and water, rather than the rate of diffusion of the compound out of the plant matrix [16]. Carr *et al.* [15] extensively reviewed

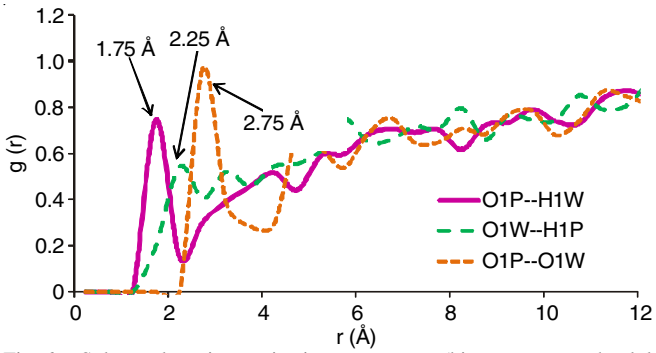

Fig. 6. Solute-solvent interaction in water process (binary water-patchoulol system) with O and H atoms as reference atoms

and emphasized that between 100 and 300 °C, the subcritical water is an effective solvent for both polar and non-polar compounds because of the tunable polarity of water. Water polarity is inversely dependent upon temperature increment. Above 100 °C, the dielectric constant of water becomes similar to that of organic solvents, like DMSO at ambient conditions.

Hydrogen bonding recognition: Reilly *et al.* [4] used Fourier transformed infrared (FT-IR) spectroscopy in their work to characterize mixtures of associating components qualitatively, when hydrogen bonding occurs in a mixture of alcohol at very low to low superficial concentrations in *n*-alkanes. It is important to recognize that the measurements reflect the association bonds of the system and not the associated species present in the system. Workers previously reported OH stretching vibration in amorphous cellulose [17] and lignin [18] to be around 3750-3000 cm^{-1}. A non-bonded or 'free' OH groups absorbed infrared in 3650-3584 cm^{-1} region and concluded that a shoulder around 3580 cm^{-1} which may due to 'free OH' group [17].

This work identified a sharp peak with stretching frequency 3500 cm^{-1} in pure amorphous patchoulol solid and attributed it to the OH stretching vibration of O1P----H1P (Fig. 7). In pure acetone and hexane solvents, there is no capability of forming hydrogen bonds, therefore peaks is in the region 3750-3000 cm^{-1} were not found. In pure ethanol solvent, a broad peak in the region 3600-3100 cm^{-1} suggests hydrogen bonding formation amongst ethanol solvent molecules O1E---H1E or the presence of free 'OH' groups in the liquid state. When comparing patchoulol solute in dilute concentration of acetone, hexane and ethanol solvents, only ethanol solution demonstrates the presence of hydrogen bonding. There is a slight change on peak broadness in the hydrogen bonding frequency region of pure ethanol and binary patchoulol-ethanol systems. This change indicate to the possibility of several hydrogen bonding arrangement in the binary system such as O1P---H1P, O1E---H1E, O1P---H1E and O1E---H1P vibrations relating to changes in charge distribution. This is in agreement with Carr *et al.* [15], suggesting that when a solute is added to a water system, more complex molecular interactions can take place. The complex molecular interactions can cause different solubility trends over a temperature range. It is also noteworthy that hydrogen bonding was not detected in the acetone solvent (Fig. 7).

Chemical constituent characterization: On the other hand, Fan *et al.* [19] extracted the patchouli in ethanol solvent,

TABLE-2
CHEMICAL CONSTITUENTS OF PATCHOULI ESSENTIAL OIL EXTRACTED USING ACETONE, ETHANOL AND HEXANE SOLVENTS

No.	Chemical compound	Area (%)			CAS number	Hydrogen bond donor/acceptor
		Acetone	Ethanol	Hexane		
1	β-Patchoulene	4.93	5.69	0.67	331-09-0	None
2	α-Patchoulene	2.80	3.21	3.71	560-32-7	None
3	Caryophyllene oxide	1.51	1.53	1.75	1139-30-6	Acceptor
4	Longifolenaldehyde	–	1.48	1.45	19890-847	Acceptor
5	Ledol	–	1.87	1.76	577-27-5	Acceptor/Donor
6	Patchouli alcohol	58.04	66.17	63.71	5986-55-0	Acceptor/Donor
7	Aristolone	1.64	1.68	1.74	6831-17-0	Acceptor
8	*n*-Hexadecanoic acid	1.76	2.09	1.17	57-10-3	Acceptor/Donor

Constituents are listed in the order of their relative content 1.0 %; MS by comparison of the MS with those of the NIST98 library (80 % matching from the library).

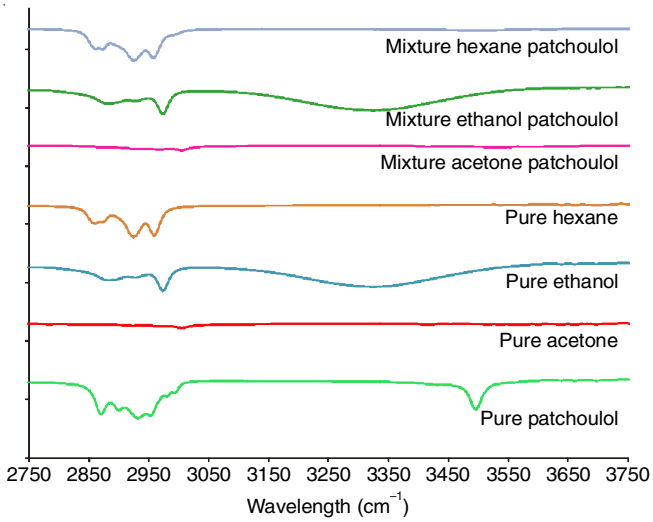

Fig. 7. Infrared spectroscopy of pure patchoulol, pure solvent and mixture of solvent and patchoulol with acetone, ethanol and hexane as the solvent

nevertheless the work did not discuss the detailed analysis of the chemical constituents from the mass spectra data. Table-2 summarizes the main chemical constituents which were detected using GCMS in three different solvents. Eight similar chemical constituents were detected from the three solvents. The common and major constituents were patchoulol or patchouli alcohol, β-patchoulene and α-patchoulene. Meanwhile, the presence of caryophyllene oxide, longifolenaldehyde, ledol, aristolone and *n*-hexadecanoic acid were also detected in the three extracted samples. Patchoulol, ledol and *n*-hexadecanoic acid capable to act both as a hydrogen bond acceptor and donor and their yield trend is in similar to the trend of the reported work [3], ethanol > hexane > acetone. The other constituents such as caryophyllene oxide, longifolenaldehyde and aristolone are hydrogen bond acceptor except for β-patchoulene and α-patchoulene is neither acceptor nor donor. However, the chemical composition in patchouli oil varies. An early work [20] identified patchouli alcohol, α-bulnesene, α-guaiene, norpatchoulenol and seychellene in patchouli oil using GC-FTIR as the most intense aroma compounds. Yahya and Yunus [21] and Dung *et al.* [22] reported the five major chemical constituents identified using steam distillation process were β-patchoulene, α-guaiene, α-patchoulene, patchouli alcohol and α-bulnesene which represent the patchouli oil quality. Meanwhile using hydrodistillation technique, the oil of *Pogostemon cablin* was rich in α-patchoulene (3.3 %), β-patchoulene (4.2 %), patchouli alcohol (23.2 %) and α-guaiene (14.6 %). Donelian *et al.* [23] identified α-patchoulene, α-guaiene, patchouli alcohol and seychellene constituents during extraction using supercritical CO_2 and steam distillation. Supercritical CO_2 extract a higher content of the α-patchoulene, α-guaiene and seychellene. Oil extracted using pressurised liquid extraction exhibit the richest patchouli alcohol content (61.2 %) as well as other constituents such as α-guaiene (5.51 %), β-patchoulene (0.42 %), caryophyllene (0.72 %) and seychellene (3.6 %) [24]. Chemical constituents in patchouli oil can be resolved comprehensively using powerful and two-dimensional gas chromatography (2D-GC) as demonstrated by Wu *et al.* [25]. The 2D-GC spectra is better resolved compared to the GC-MS spectra. Patchouli oil is reported to contain several hundred chemical components [26].

Conclusion

Ewald summation method has improved the g(r) intensities pattern obtained for polar compounds compared to the g(r) from simulation work [3] with the atom based summation method to describe the long range interactions in pure ethanol and water solvents. A stronger hydrogen bonding network formed in ethanol solution compared to water when the patchoulol solute was added into the simulation system. This would suggest a higher patchoulol composition or extraction yield in ethanol compared with water. When the patchoulol solute is added in the ethanol and acetone (polar) and hexane (non-polar) solvents, the hydrogen bonding strength can only be recognized in ethanol solution based on infrared spectroscopy. This would suggest that complex molecular interaction particularly hydrogen bonding can control the solubility of solute in a solvent. However, since the composition percentages of chemical constituents (Table-2) in the three different solvents are lower relative to patchoulol the role of hydrogen bonding in those systems cannot be ascertained.

Nomenclatures

r	Radial
rdf	Radial distribution function
ps	Pico second
ns	Nano second
wt/wt %	weight over weight per cent

COMPASS	Condensed-phase optimized molecular potentials for atomistic simulation studies
FTIR	Fourier transform infrared
GCMS	Gas chromatography and mass spectroscopy
NVE	Constant number of moles, volume and energy
NPT	Constant number of moles, pressure and temperature
OPLS	Optimized potentials for liquid simulations
TIP3P	Transferable intermolecular potential, three-position model

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