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CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM LEAF EXTRACT OF PANDAN, *Pandanus amaryllifolius* ROXB.

(Komposisi Kimia Minyak Pati daripada Ekstrak Daun Pandan, *Pandanus amaryllifolius* Roxb.)

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Abstract

Pandan or *Pandanus amaryllifolius*, an aromatic tropical plant species, has gained much interest among researchers in the quest to develop further use of its essential oils beyond food flavoring, traditional medicines and limited food industries. There has been lack of comprehensive investigations on therapeutic activities of its essential oils (EOs) that may have potential use as therapeutic agents in the treatment of various health issues. The present investigation reports on the chemical composition of EOs from leaf extracts sourced from three different locations in Peninsular Malaysia. Leaf extracts of *P. amaryllifolius* were drawn out from leaves of plants grown in the states of Kedah, Selangor and Johor using Soxhlet extraction method with ethanol as the solvent resulting in extraction yields of 21.08%, 20.54%, and 15.87%, respectively. The leaf extracts were further analyzed by gas-chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared spectroscopy (FTIR). A total of 57 chemical compounds were identified comprising of fatty acids, steroids, aromatic compounds and non-polar components making up 80.49-84.74% of total oils. A total of 11 common peaks were determined consisting of pyranone (0.78-1.74%); coumaran (1.12-5.31%); 1,4-di-tert-butylphenol (2.68-6.10%); pinane (0.80%-1.46%); ethyl palmitate (1.04%-1.66%); 3,6,6-trimethyl-1-(1-phtalazinyl)-1,5,6,7-tetrahydro-4H-indazol-4-one (0.75-1.69%); phytol (1.43-6.19%); purpurogallin (1.34-2.02%); squalene (14.14-33.83%); decamethyltetrasiloxane (0.27-0.52%); and vitamin E (2.58-3.66%) from the three different locations. Stigmasterol was not detected from plants sourced in Selangor but was detected in samples from Kedah and Johor with an amount of 6.73% and 9.05%, respectively. There were 11 common peaks observed in all IR spectra from the three plants' sources exhibiting functional groups. The findings from the study present useful additional information to existing literature on extractable EOs from pandan for potential use in pharmaceutical or nutraceutical applications in the production of functional food.

Keywords: *Pandanus amaryllifolius*, chemical composition, essential oils, functional group

Abstrak

Pandan atau *Pandanus amaryllifolius*, satu spesies tumbuhan aromatik tropika, telah mendapat perhatian di kalangan penyelidik dalam usaha membangunkan penggunaan minyak pati (EO) pandan selanjutnya melebihi kegunaan sebagai perasa makanan, perubatan tradisional dan dalam industri makanan yang terhad. Terdapat kekurangan penyelidikan yang menyeluruh mengenai aktiviti terapeutik EO yang mungkin mempunyai potensi sebagai ejen terapeutik dalam rawatan berbagai isu kesihatan. Penyelidikan ini melaporkan komposisi kimia EO daripada ekstrak daun dari tiga lokasi berbeza di Semenanjung Malaysia. Ekstrak daun *P. amaryllifolius* telah dihasilkan daripada pokok yang ditanam di negeri-negeri Kedah, Selangor dan Johor dengan menggunakan kaedah pengekstrakan Soxhlet bersama etanol sebagai pelarut dan menghasilkan masing-masing 21.08%, 20.54%, dan 15.87%. Selanjutnya, ekstrak daun telah dianalisis dengan menggunakan kromatografi gas-spektrometri jisim (GC-MS) dan spektroskopi inframerah transformasi Fourier (FTIR). Sejumlah 57 sebatian kimia telah dikenal pasti yang terdiri daripada asid

lemak, steroid, sebatian aromatik dan komponen bukan berkutub meliputi 80.49-84.74% minyak pati yang diperolehi. Terdapat sejumlah 11 puncak persamaan dan telah dikenal pasti sebagai mengandungi piranon (0.78-1.74%); koumaran (1.12-5.31%); 1,4-di-tert-butilfenol (2.68-6.10%); pinan (0.80-1.46%); etil palmitat (1.04-1.66%); 3,6,6-trimetil-1-(1-pitalazinill)-1,5,6,7-tetrahydro-4H-indazol-4-on (0.75-1.69%); fitol (1.43-6.19%); purpurogalin (1.34-2.02%); skualin (14.14-33.83%); dekamiltetrasiloxan (0.27-0.52%); dan vitamin E (2.58-3.66%) di kalangan tiga minyak pati pandan dari lokasi berbeza. Stigmasterol tidak dapat dikesan daripada pokok yang diperolehi dari Selangor tetapi di rekod diperolehi dari sampel Kedah dan Johor masing-masing dengan 6.73% dan 9.05%. Sebanyak 11 puncak persamaan diperolehi di semua spektrum IR daripada tiga sumber pokok dan mempamerkan kumpulan berfungsi. Keputusan daripada kajian mengemukakan informasi tambahan yang kepada penulisan sedia ada mengenai potensi EO yang boleh diekstrak dan yang mempunyai potensi dalam kegunaan farmaseutikal dan nutraseutikal dalam pengeluaran makanan.

Kata kunci: *Pandanus amaryllifolius,* komposisi kimia, minyak pati, kumpulan berfungsi

Introduction

Pandanus amaryllifolius or Pandan, belonging to the genus Pandanus, is a tropical plant species commonly used in South and Southeast Asian cooking as a flavoring. Its unique flavor and aroma are contributed by a compound, 2-acetyl-1-pyrroline (2AP) which enhances the sweet flavor of many Asian dishes [1]. The rice-aromatic compound can be also found in *Vallaris glabra* Ktze or Bread Flower, Kerak Nasi or Bunga Kesidang in Malay. Traditionally, pandan leaves have also been used to refresh body, reduce fever and relieves indigestion [2]. A study by Balinado and Chan [3] documented that the decoction of pandan leaves helped to cure urinary tract infection, hypertension, abdominal pain, and kidney problems. Traditionally, the Malays believe that pandan leaves can rid body odor for hygienic purposes as well as ward off mystical forces through post-partum bath among Malay women in the state of Kedah [4].

Pandan leaves have been widely used in medicinal applications using their aqueous extracts. However, the beneficial effects of the extracts containing essential oils (EOs) have not been thoroughly investigated. It is well-known that plants' EOs have been used in therapeutic treatments since the 15th century. EO is a concentrated hydrophobic aroma compound containing major active secondary metabolites including terpenes and oxygenated compounds which are known to be associated with sources of many therapeutic activities [5]. Various studies have reported that monoterpenes and sesquiterpenes are the predominant constituents of EOs from many plants [6-8] such as roses [9], cinnamon [10], lemon [11] and lavender [12]. Various tropical plants in Malaysia have gained the attention of researchers for being rich in these compounds and have prompted interests in the exploration of pharmaceutical properties of plants' EOs.

Extraction of plants' EOs is an essential procedure. The Soxhlet method is a popular extraction technique that has been proposed in 1879 by Franz Von Soxhlet. The method is a standard technique widely used to extract fats and oils to study and analyze bioactive compounds. The Soxhlet method has been reported to be a suitable technique as it takes shorter time for extraction and less solvent use [13] as well as its simplicity to handle and relatively safe [14]. This classical extraction method has proven to yield more phenolic content than the cold-pressed oils technique of Al Juhaimi and Özcan [15].

Gas chromatography-mass spectroscopy (GC-MS) is a tool capable of identifying active principles in plants' extractions. It identifies compounds such as fatty acids, steroids, aromatic compounds, and non-polar components with high separation efficiency and sensitivity to detect components in a mixture especially on volatiles compounds [16]. Gomathi et al. [17] added that GC-MS combines two analytical techniques into one method that analyzes mixtures of chemical compounds where the gas chromatography separates the components while the mass spectroscopy analyzes the components into individual constituent. Another analysis that is traditionally used in bioanalysis is Fourier Transform Infrared Spectroscopy (FT-IR) which is an adsorption spectroscopy that uses light to emit bright rays in the infra-red wavelength range to obtain an infrared spectrum that gives characteristics of the components in a mixture of solid, liquid or gas. Each compound has a specific infrared spectrum depending on its molecular structure.

In a study by Ghasemzadeh and Jaafar [18], leaf extract of *P. amaryllifolius* from Bachok in northern part of Peninsular Malaysia registered the highest total phenolic content, total flavonoid content, antioxidant activity, and anticancer activity compared to extracts from Pontian in southern part of Peninsular Malaysia. Ghasemzadeh et al. [19] recorded that bioactivities of leaf extracts and their bioactive components present from *Etlingera elatior* flower extracts were affected due to different geographical locations. The present study was conducted to evaluate chemical compositions of *P. amaryllifolius* EOs from different locations of north (Kedah), west (Selangor) and south (Johor) Malaysia representing the upper middle and bottom regions of the peninsular.

Materials and Methods

Sample preparation and essential oils extraction

Fresh leaves of *P. amarylliofolius* were obtained from plants originally planted in three states of Kedah, Selangor, and Johor. Harvested leaves were thoroughly washed with tap water to remove soil and dirt. The leaves were cut into small pieces to promote large surface area for rapid drying process. Subsequently, the leaf samples were frozen and dried for 48 hours using a freeze dryer. The cut leaves were ground into powder and kept in dark bottles. Samples of 5.0 g each of the ground leaves were weighted and placed in thimbles before inserting in the Soxhlet extractor chamber. A 250 mL of boiling flask was filled with 180 mL of ethanol as the extraction solvent which was heated to reflux for 10 hours. The product was collected and concentrated using a rotary evaporator at a temperature of 50 ºC. The samples were kept in airtight containers at under 4 ºC.

Gas chromatography-mass spectrometry analysis

GC-MS analysis was conducted using 7890A gas chromatography with 5975 Series mass spectrometer (Agilent, American). A fused silica capillary HP-5 ms column was used for separation. Electron ionization system with ionization energy of 70 eV was used and helium gas as the carrier gas at a constant flow rate of 1 mL/min. The injector and mass transfer line temperatures were set at 250 ºC and 300 ºC, respectively while the oven temperatures were set up from 50 ºC to 200 ºC at 8 ºC/min. Plant samples were kept at isothermal for 20 minutes and finally raised to 300 ºC at 10 ºC/min. Diluted samples (1/100 v/v, in ethanol) of 0.2 µL each were manually injected in split less mode. Identification of compounds of the EOs was based on GC retention times registered on the capillary column, with computer matching of mass spectra with those of standards. Other qualitative analyses was done upon mass spectra fragmentation patterns with similar compounds and principle (major) compounds with peak areas of more than 2% from the data base.

Fourier transform infrared spectroscopy analysis

FTIR spectrum of the EOs was analyzed using FTIR spectrometer (Nicolet 6700 from Thermo Scientific). The samples were run by using attenuated total reflection (ATR) method and measured with a number of 32 scans on the spectrum. The IR spectra were reported in percent transmittance in the range number regions of 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The functional groups were determined by IR correlation charts.

Results and Discussion

Oils extraction from *P. amaryllifolius*

Soxhlet extraction of all freeze-dried samples of *P. amaryllifolius* gave 21.08%, 20.54% and 15.87% of dark green wax oil yields (Figure 1) from Kedah, Selangor, and Johor, respectively, out of total dried leaves used. A study by Ghasemzadeh and Jaafar [18] found that samples of aqueous extracts of pandan leaves collected from state of Kelantan, the upper part of Peninsular Malaysia registered highest bioactivities compared to the lower parts of the Peninsular such as in states of Pahang and Johor. One possible factor affecting yields of EOs is the drying technique used. Freeze-drying of leaves using a freeze-dryer has the ability of maintaining and preserving plants' constituents as proven in a study by Yahya et al. [1] in which the highest extraction yield was obtained by freeze-drying followed by oven-drying.

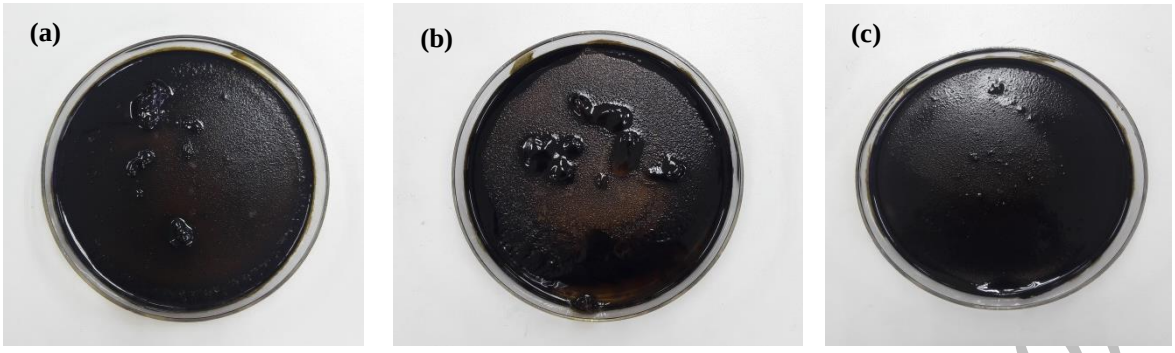

Figure 1. Dark-green-wax oil yields from (a) Kedah (21.08%), (b) Selangor (20.54%), and (c) Johor (15.87%)

Table 1. The main compounds identified in volatile oils extracted from *P. amaryllifolius* samples and the identified components of 11 common peaks from Kedah, Selangor and Johor

No.	Component	Common Peaks	RT*	Relative Peak Area (%)		
				Kedah	Selangor	Johor
1	Methoxyamine	-	4.320	0.17	1.18	ND
2	2-Cyclopentene-1,4-dione	-	5.014	0.11	0.25	ND
3	1,2-Cyclopentanedione	-	5.665	ND	1.50	ND
4	3-methylcyclopentanone	-	6.576	ND	ND	1.06
5	2-methyl-2pentenal	-	6.586	2.05	ND	ND
6	α-hydroxy-γ-butyrolactone	-	6.766	0.75	6.79	ND
7	Dimethylguanidine	-	8.678	ND	1.81	ND
8	Pyranone	1	9.583	1.74	1.06	0.78
8	Pyranone	1	9.583	1.74	1.06	0.78
9	Catechol	-	10.558	0.81	ND	0.33
10	Coumaran	2	10.881	5.31	1.12	2.42
11	Triethylene glycol	-	11.050	2.14	ND	ND
12	2-methoxy-4-vinylphenol	-	12.612	0.44	ND	0.40
13	Dimethyl lauramine	-	15.583	0.56	ND	0.71
14	1,4-di-tert-butylphenol	3	15.742	2.68	6.10	4.55
15	2,3,5,6-Tetrafluoroanisole	-	16.579	0.49	ND	0.51
16	Butyric acid silver	-	17.103	ND	ND	1.02
17	Ethyl (2-methylphenyl)carbamate	-	17.103	2.00	ND	ND
18	N-(Diethyl-λ-sulfanylidene)acetamide	-	17.701	ND	1.60	ND
19	3-(1-Ethoxyethoxy)butyl benzoate	-	17.833	1.99	ND	ND-
20	1-Nonadecene	-	19.676	0.27	ND	0.30
21	Ethyl coumarate	-	20.148	0.29	ND	0.20
22	Pinane	4	20.312	0.94	0.80	1.46
23	Methyl palmitic acid	-	21.678	0.27	ND	0.42
24	Ethyl palmitic acid	5	22.981	1.04	1.07	1.66

25	3,6,6-trimethyl-1-(1-phtalazinyl)-1,5,6,7-tetrahydro-4H-indazol-4-one	6	23.341	0.75	1.69	1.36
26	Phytol	7	26.025	4.35	1.43	6.19
27	Ethyl linolenate	-	27.884	0.92	ND	1.54
28	2,3,5-trimethylphenanthrene	-	44.326	0.30	ND	1.26
29	Purpurogallin	-	44.554	1.76	ND	1.56
30	4,5-diphenylimidazole	-	44.670	1.63	ND	ND
31	Purpurogallin	8	45.051	2.01	1.34	2.02
32	2,5-dipheylfuran	-	45.057	2.16	ND	ND
33	4,5-diphenylimidazole	-	45.126	ND	0.79	1.86
34	Purpurogallin	-	45.131	2.01	ND	ND
35	Hentriacontane	-	45.740	ND	1.76	ND
36	Purpurogallin	-	46.995	ND	ND	2.07
37	Flufylline	-	46.995	4.56	ND	ND
38	Icosane	-	47.090	ND	2.20	0.43
39	Octadecane	-	48.186	ND	1.72	ND
40	Squalene	9	48.488	14.14	33.83	16.92
41	1-Bromoicosane	-	49.124	ND	1.52	ND
42	Crotocin	-	49.452	0.92	2.26	ND
43	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	-	49.452	ND	ND	1.27
44	Hexamethylcyclotrisiloxane	-	49.531	0.39	0.52	ND
45	2-(Adamantan-1-yl)-N-methylactamide	-	50.156	ND	1.15	0.79
46	Decamethyltetrasiloxane	10	50.087	0.27	0.48	0.52
47	β-tocopherol	-	50.474	1.07	ND	0.77
48	Vitamin E (α-tocopherol)	11	51.252	3.66	2.58	3.01
49	5-methyl-2-phenyl-1H-indole	-	52.290	2.37	ND	2.55
50	Stigmasterol	-	52.671	6.73	ND	9.05
51	Methyl trimethicone	-	52.677	ND	3.84	ND
52	2-Ethylacridine	-	53.386	ND	4.35	ND
53	Clionasterol	-	53.391	8.01	ND	9.10
54	4-decocyl 1,2-dimethyl 1,2,4-benzenecarboxylate	-	56.452	ND	ND	1.01
55	Methyl 2,2,6-trimethyl-1-[(1E)-3-oxo-1-buten-1-yl]-7-azabicyclo[4.1.0]heptane-7-carboxylate	-	56.532	1.08	ND	1.41
56	1,4-phenylenebis(trimethylsilane)	-	56.738	1.62	ND	ND
57	N-(4-methoxyphenyl)-2,2-dimethylpropanamide	-	56.749	ND	ND	2.32
			TOTAL	83.11	84.74	80.49

RT = Retention time, ND = Not detected

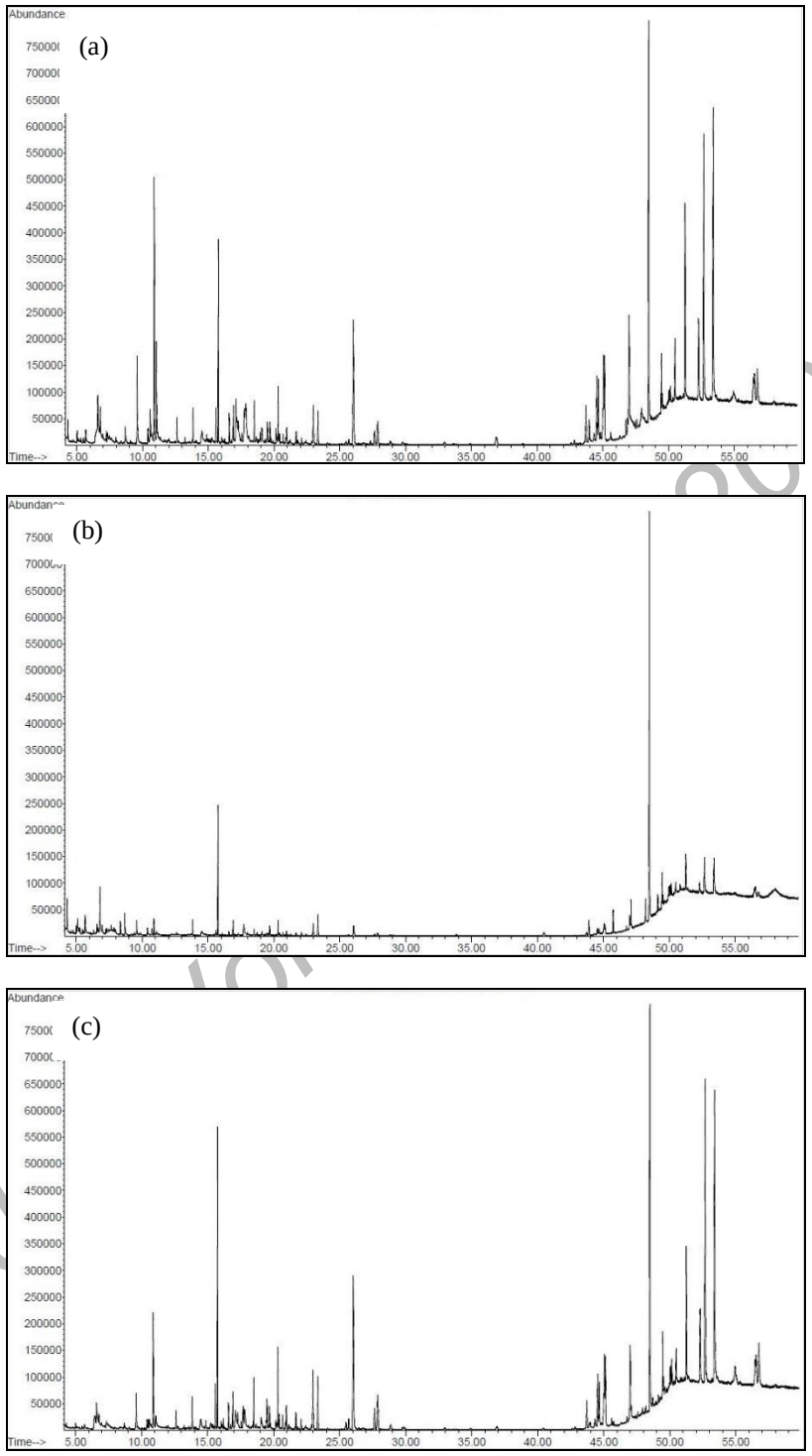

Figure 2. The GC-MS chromatograms of *P. amaryllifolius* essential oils from (a) Kedah, (b) Selangor, and (c) Johor

In the present study, volatile oils extracted from samples were analyzed by GC-MS and are shown in Table 1. A total of 57 components were identified in the samples from Kedah, Selangor, and Johor representing 83.11%, 84.74% and 80.49%, respectively. The total ion chromatograms of the samples are shown in Figure 2. The chromatograms demonstrate the chemical characteristics of the samples. The distributions of the peaks were distributed majority at retention time of 4.00 – 27.00 and 43.00 – 56.00. The EOs of the samples from the three states shared 11 similar peaks. These peaks were chosen as they existed in all chromatograms of all samples and named as "common peaks" which were pyranone (0.78-1.74%); coumaran (1.12-5.31%); 1,4-di-tert-butylphenol (2.68-6.10%); pinane (0.80-1.46%); ethyl palmitate (1.04-1.66%); 3,6,6-trimethyl-1-(1-phtalazinyl)-1,5,6,7-tetrahydro-4H-indazol-4-one (0.75-1.69%); phytol (1.43-6.19%); purpurogallin (1.34-2.02%); squalene (14.14-33.83%); decamethyltetrasiloxane (0.27-0.52%); and vitamin E (2.58-3.66%). Squalene and phytol were two components that were also identified in a study by Chen and Ge [20] with relative percentages of 16.81% and 42.15%, respectively.

The total of principal components of Eos of samples from Kedah, Selangor and Johor were 15, 8 and 11, respectively. In Kedah, the components were 2-methyl-2pentenal (2.05%), coumaran (5.31%), triethylene glycol (2.14%), 1,4-di-tert-butylphenol (2.68%), ethyl (2-methylphenyl)carbamate (2.00%), phytol (4.35%), purpurogallin (2.01%), 2,5-dipheylfuran (2.16%), flufylline (2.01%), purpurogallin (4.56%), squalene (14.14%), vitamin E (α-tocopherol) (3.66%), 5-methyl-2-phenyl-1H-indole (2.37%), stigmasterol (6.73%), and clionasterol (8.01%) while α-hydroxy-γ-butyrolactone (6.79%), 1,4-di-tert-butylphenol (6.10%), icosane (2.20%), squalene (33.83%), crotocin (2.26%), vitamin E (α-tocopherol) (2.58%), 2-ethylacridine (3.84%), methyl trimethicone (4.35%) are in Selangor. Furthermore, in Johor coumaran (2.42%), 1,4-di-tert-butylphenol (4.55%), phytol (6.19%), purpurogallin (2.02%), purpurogallin (2.07%), squalene (16.92%), vitamin E (α-tocopherol) (3.01%), 5-methyl-2-phenyl-1H-indole (2.55%), stigmasterol (9.05%), clionasterol (9.10%), and N-(4-methoxyphenyl)-2,2-dimethylpropanamide (2.32%). These components were mentioned as the principal compounds because of their peaks could be clearly observed from the chromatograms. These principal compounds gave significant difference as the dominant compounds which characterize the different origin of EOs.

Squalene was identified as the major compound of EOs which contributed to the highest percentage area 14.14%, 33.83% and 16.92% of EOs from Kedah, Selangor, and Johor, respectively. Squalene is a compound similar to beta-carotene where it is an intermediate in the synthesis of cholesterol [21]. It is associated with very low density of lipoproteins and distributed in human tissue. At its greatest concentration, it has been documented to protect human skin from lipid peroxidation caused by ultraviolet exposures and other factors from ionizing radiation [21]. Phytol with 4.35%, 1.43% and 6.19% of EOs from Kedah, Selangor, and Johor, respectively was found as one of the components from the analysis done through GC-MS. Phytol ($C_{20}H_{40}O$) with a molecular weight of 296.539 g/mol has been reported by Zeb et al. [22] to have antibacterial, antimicrobial and insecticidal properties. One unique discovery proposed was that this terpene alcohol performs as a potential an anticancer candidate in pharmaceutical application [23]. The study was done on mice with DMBA-induced breast cancer where phytol showed its regulation on pro-carcinogen and apoptosis on cancer cells with the capacity to repair damaged cells. Other studies have shown that phytol is a precursor or limiting factor of Vitamin E ($C_{29}H_{50}O_2$) and Vitamin K1. Vitamin E, also known as tocopherol, was recorded in the EOs extracted from the plants under study with 3.66%, 2.58% and 3.01% content from Kedah, Selangor and Johor, respectively. It has been documented that this fat-soluble vitamin helps in maintaining skin moisture and restores the regular oil stability of the pores and skin [24]. The vitamin has been popular in treating minor sunburns where it heals damage caused by ultraviolet radiation [25]. Numerous brands of skincare products have included vitamin E as part of the ingredients to improve its sun protection properties when applied topically.

Steroids, recorded as stigmasterol and clionasterol, were present in plant samples at 6.73% and 8.01% in EOs from Kedah, and 9.05% and 9.10% in EOs from Johor respectively. Various studies have shown that environmental factors have strong influence on volatile components. Li et al. [16] reported that *Cinnamomum cassia* from nine different locations in China showed differences in bioactive compounds from plants of different geographical locations. *C. cassia* from Guangxi had higher content of trans-cinnamaldehyde by 73.56– 77.20% than Guangdong's by 66.28–71.22%. The study was conducted to identify the quality of cinnamon as part of the Chinese traditional herbal medicine to meet quality control requirements used throughout China. In another study in India,

methanolic extract of *Withania somnifera* from Uttarakhand had higher total phenolic content than in Rajasthan where the influence of environmental conditions, climate, edaphic factors and altitudes were said to be the cause of the differences [26]. The compounds listed in Table were according to their elution order on HP5-MS capillary column.

FT-IR Analysis of the EOs

IR spectroscopy (Figure 3) shows molecules that absorb frequencies which later stretched its characteristics of their structures by producing a wave spectrum. The IR fingerprints of the EOs of *P. amaryllifolius* were observed to be mostly in the range of 400-1700 cm^{-1} and 2800-3500 cm^{-1}. There were 16 common peaks that were observed in all the IR spectra from the samples under study. The peak at 3320 cm^{-1} was assigned to the stretching vibration of O-H. The peaks of 2972 cm^{-1}-2927 cm^{-1} and 2881 cm^{-1} were attributed to C-H stretching vibration and tertiary C-H group (CH_3). The spectra giving the characteristics of alcohol with C-OH deformation within the bending vibration absorption at 1450 cm^{-1} peak and aldehyde carbonyl C=O at 1655 cm^{-1} peak where the aromatic stretching bond occurred to absorb frequency. The peak at 1274 cm^{-1} corresponded to esters C-O stretching. The peaks at 1086 cm^{-1} and 1044 cm^{-1} were recognized for deformation of methoxyl group of C-O-C. In the fingerprint region, at peaks, 801 cm^{-1} and 879 cm^{-1} were assigned to benzene ring=CH vibration absorption with C-H deformation. Alkenes vibration absorption was identified at peak 630 cm^{-1}.

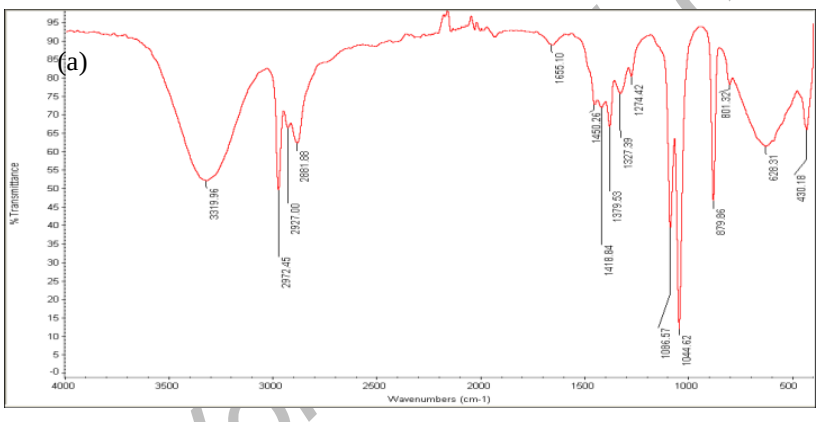

Figure 3. The IR spectrums of *P. amaryllifolius* essential oils from (a) Kedah, (b) Selangor, and (c) Johor.

Conclusion

The present study has presented a collection of important compounds putatively known for their therapeutic properties. Analyzed by gas chromatography-mass spectroscopy (GC-MS) and Fourier transform infrared spectroscopy (FT-IR) ***P. amaryllifolius*** appears to be a good source of compounds squalene, stigmasterol, alpha and beta tocopherol, coumaran and phytol which are known to be very important constituents in skin care products. Further study on genetic improvement of Pandan is suggested for higher concentration of EOs for better utilization of the plant beyond its use in culinary and traditional treatments.

Acknowledgment

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