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Solvents Extraction Effects on Bioactive Compounds of Ajwa Date (Phoenix Dactylifera L.) Flesh using Mixture Design

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Date fruits (Phoenix Dactylifera L.) are considered as an ideal food because it consists of high nutrients and provide beneficial effect to human health. Bioactive compounds in Ajwa dates have different polarities thus require different polarities of solvents for the extraction. In this study, we investigated the effects of selective extracting solvents and their combination on extraction of bioactive compounds in Ajwa flesh using mixture design. Methanol, chloroform and hexane were used in the design. Identification of bioactive compounds using GCMS shows Ajwa date flesh contain antioxidants, antifungal, anticholesterol, antimicrobial, anticancer and anti-inflammatory. Most bioactive compounds were identified in methanolic extract (D1) except β-Sitosterol and Vitamin E were identified in hexane extract (D3). Meanwhile, Longifolenaldehyde and Nonadecyl pentafluoropropionate were identified in hexane layer (D5H) from the combination of methanol: hexane (1:1).

1. Introductıon

Solvent extraction is a selective separation procedure for isolating and concentrating valuables metals from aqueous solutions with the aid of an organic solution. Solvent extractions are the most typically procedures used for extraction preparation of plant materials due to their ease of use, efficiency, and wide applicability. It is known that the yield of chemical extraction depends on the type of solvents with varying polarities (Kchaou et al., 2013), extraction time and temperature (Gironi et al., 2011), sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the sample (Handa et al., 2016). Rezaie et al (2015) stated that the efficiency of the different solvent extraction strongly depends on the matrix of plant materials and the type of extractable compounds. Therefore, there is no universal extracting solvent for individual plant materials (Wijekoon et al., 2011). Bioactive compounds from plant materials will be extracted depending on the solvent system used during extraction. Polar solvents will dissolve polar compounds while non-polar solvents commonly dissolve non-polar compounds. The polarity can be measured as the dielectric constant. The greater the constant, the more polar the molecule is. The term “mixture” to a chemist is any combination of several substances. Three mixture design or also known as simplex-centroid design is a type of mixture design used to analyse the relationship involved in a process that contains several variables (Cornell, 2002). Simplex-centroid design is constructed to form a triangle with the three corners correspond to single components, the three midpoints of each side correspond to binary mixtures and the centre of the triangle correspond to ternary mixture which equal mixture of all three components (Brereton, 2003). In other studies, date fruits were reported to have flavonoids (Farag et al., 2014), phenolics (Benmeddour et al., 2013), sterols, anthocyanins, carotenoids and procyanidins (Baliga et al., 2011) compounds which identified using liquid chromatography technique. The purposes of this study were to evaluate the effect of various polarities of solvents (methanol, chloroform and hexane) on the extractability of bioactive compounds in Ajwa date flesh using GCMS.

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2. Methods

2.1 Chemicals and sample preparation

Solvents were of GCMS grade and purchased from Merck (Methanol, Chloroform and Hexane) and Sigma-Aldrich (Supelco-33027: N, O-bis(trimethylsylil) trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS). Depitted ajwa dates (packed in 250 g) batch no (6000/D002) was purchased from Syarikat Abdul Ghafar (SAG), Butterworth, Penang. Samples were cleaned and rinsed using distilled water to remove adherent dust particles. The sample cut uniformly into 5 - 7 mm size and stored at 4 ^{0}C until further analysis.

2.2 Identification of bioactive compounds

2.2.1. Mixture design

To select the best solvent or mixture of solvent for extraction of bioactive compounds in Ajwa, three solvents were selected namely methanol, chloroform and hexane. Then, these three selected solvents were mixed according to the mixture design concept to produce other design which is the mixture of polar, semi- or non-polar solvents. The three mixture designs are illustrated in Figure 1. Based on this concept, seven designs were formed and labeled as D1 (100 % methanol), D2 100 % chloroform D3 (100 % hexane), D4 methanol: chloroform (1:1), D5 methanol:hexane (1:1), D6 chloroform:hexane (1:1) and D7 methanol:chloroform:hexane (1:1:1). Since methanol and hexane are immiscible, both layers were collected and labeled D5M and D5H for the methanol and hexane layer.

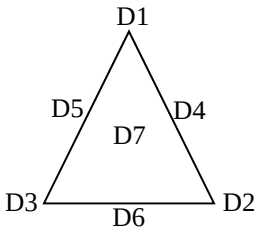

Figure 1: Illustration of three mixture design concept.

2.2.2. Extraction

A total of 30 mL solvent was added to one gram (1 g) of date sample in a 50 mL of conical flask and vortexed at room temperature over a 3 h period. The mixture was filtered using Whatman No.1 filter paper and the filtrate was concentrated to 1 mL using rotary evaporator at temperature 60 $^{\circ}$C (BUCHI, Switzerland).

2.2.3. Derivatization

For derivatization step, 80 µL of derivatization reagent (BSTFA) and 20 µL of TMS were added to 1,000 µL of extract in a 2 mL vial and vortexed to mix thoroughly. The vial was covered with aluminum foil and placed in an oven at 65 °C for an hour. The samples were later analysed for bioactive compounds using GCMS.

2.2.4. Instrumentation

Gas chromatograph mass spectrometer (GCMS) analysis were performed on an Agilent 7890A gas chromatograph (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with triple-axis detector using a 30 m (length) and 0.25 mm (diameter) column (model DB-5MS UI). The film thickness of the column was 0.25 µm and 5 % phenyl methyl-polysiloxane was used as the stationary phase. The column temperature was set to 80 °C for 3 min, then increased to 250 °C at the rate 7° C/min and then held for 15 min. The injector temperature was 250 °C; splitless mode was used and the injection volume was 10 µL. The flow rate of the carrier gas was set to 20 mL/min after 2 min. Total runing time was 42.286 min. Mass spectra were obtained from the range m/e 45 to 600 and the electron ionization at 70 eV. The chromatograms of the sample were identified by comparing their mass spectra with NIST/EPA/NIH version 2.0.

3. Results and discussion

3.1 Bioactive compounds in Ajwa dates

Date fruits are considered as staple food because it consists of high nutrients and provides beneficial effect to human health. Generally, previous studies mostly investigated the antioxidants activity in date fruits due to the

presence of phenols. Apart from that, previous studies reported that date fruits contain anthocyanins, carotenoids and other nutritional composition such as fiber and minerals content. Most previous studies used the combination of polar solvents for the extraction. To our knowledge, only few has reported using the combination of solvents with various polarities (strong polar and strong non-polar) as the extraction solvents. Thus, we studied the effects of selective extracting solvents and their combination on extraction of bioactive compounds in Ajwa dates using three mixture design. Differ in polarities of extracting solvents give vary of bioactive compounds extracted. Summary of bioactive compounds extracted using three mixture design were tabulated in Table 1. The percentage area (% Area) and probability of each bioactive compounds were stated as well in Table 1.

Table 1: Bioactive compounds in Ajwa dates extracted using the three-mixture design

Bioactive Compounds	D1	D2	D3	D4	D5M	D5H	D6	D7
2, 3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	(6.4125, 62)	*ND	*ND	(0.3329, 64)	(1.4522, 72)	*ND	*ND	*ND
3,5-Dihydroxy-2-methyl-4H-pyran-4-one	(0.2232, 86)	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Lauric acid	(6.8498, 99)	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Capric acid	(1.1803, 96)	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Caprylic acid	(1.1064, 81)	*ND	*ND	*ND	*ND	*ND	*ND	*ND
Palmitic acid	(1.4083, 99)	*ND	*ND	(2.1681, 83)	(1.7317, 99)	*ND	*ND	(3.2519, 92)
Stearic acid	(0.3863, 99)	*ND	*ND	(0.8969, 98)	(0.3642, 99)	*ND	*ND	(1.4571, 81)
β-Sitosterol	*ND	*ND	(0.228, 96)	*ND	*ND	*ND	*ND	*ND
Longifolenalde-hyde	*ND	*ND	*ND	*ND	*ND	(1.8783, 62)	*ND	*ND
Nonadecyl pentafluoropro-pionate	*ND	*ND	*ND	*ND	*ND	(1.5469, 64)	*ND	*ND
Vitamin E	*ND	*ND	(7.6968, 97)	*ND	*ND	*ND	*ND	*ND

*ND = Not Detected

Some studies reported that 2, 3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 3,5-Dihydroxy-2-methyl-4H-pyran-4-one have antioxidant activity (Yu et al., 2013). These two compounds were identified in D1, D4 and D5M which is polar design. Other antioxidants identified in D3 and D5H are β-Sitosterol (Mahaddalkar et al., 2015) and Nonadecyl pentafluoropropionate (Renukadevi et al., 2011). Both designs are nonpolar design. Therefore, these results are in agreement with those studies who reported that date fruits as a good source of natural antioxidant compounds (Harthi et al., 2015).

Furthermore, three fatty acids; capric (n-Decanoic acid) (Huang et al., 2014), lauric (Dodecanoic acid) (Nitbani et al., 2016) and caprylic acid (Octanoic acid) (Kim and Rhee, 2016) have been reported as potential antimicrobial compounds where in the case of Caprylic acid has been reported that the antimicrobial or antibacterial activity is due to the ability of these medium chain saturated fatty acids to act against several Gram- negative and Gram- positive bacteria. Huang et al. (2014) reported that lauric and capric acids have anti-inflammatory activities while other functions of caprylic and capric acids include anticonvulsant properties in seizures (Wlaz et al., 2015), by decreasing blood glucose and alternatively increasing β-hydroxybutyrate concentrations. Palmitic acid (Hexadecanoic acid) and stearic acid (Octadecanoic acid) are reported as anticholesterol (Andrea and Scott, 1988). Other benefits of fatty acids include useful effects on human health

(Nai-Sheng et al., 2014) when myristic (Tetradecanoic acid) and myristoleic acids accumulate in the muscle. Seemingly methanolic extract is the best design to extract fatty acids and all designs with combination of methanol still could extract the fatty acids which mean that these extracted fatty acids are polar fatty acids. These fatty acids also can be detected using UHPLC (Wabaidur et al., 2016).

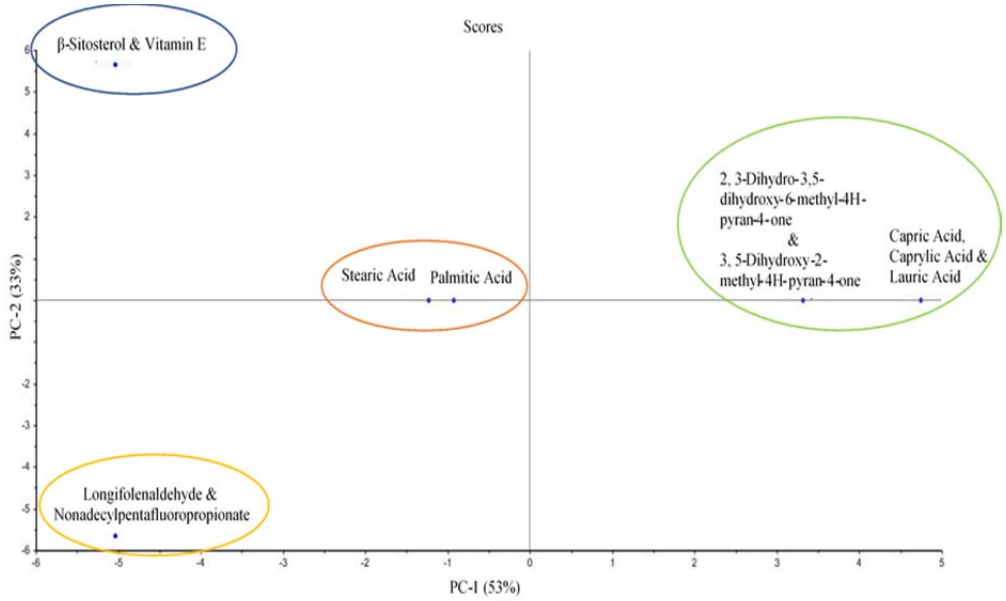

Figure 1: PCA of Bioactive compounds (Scores plot)

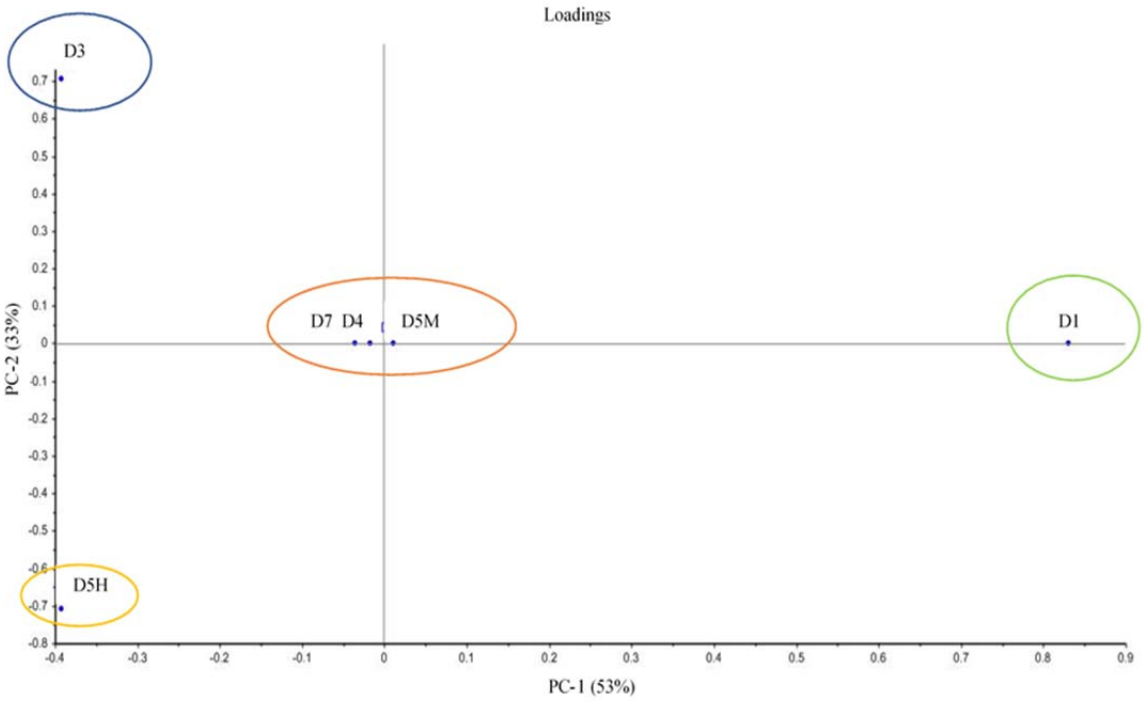

Figure 2: PCA of Mixture designs (Loadings plot)

4. Statistical analysis

Multivariate statistical analysis often used for examining relationships among multiple variables at a time. Principal Component Analysis (PCA) is one of unsupervised data analysis approaches which used to obtain

an overview of the data sets. It provides a visual representation of the relationships between samples and variables (CAMO Software India). Typically, multivariate data in term of table (samples against variables) were analysed simultaneously to obtain an overview pattern of the data sets in term of plots (scores and loadings plot). PCA overview of bioactive compounds and mixture design was shown in Figure 1 and Figure 2. Apparently, the data set was clustered into four groups (Figure 1 and Figure 2). The relationships between Scores plot and Loadings plot were labelled as group I, II, II and IV on both plots. Scores plot represent the bioactive compounds while Loadings plot represent the mixture design. Group I in Scores plot is belongs to group I in Loadings plot. The same goes to group II, III and IV in Scores plot is belongs to group II, III and IV in Loadings plot, respectively. This is due to the position of these groups are identical in both plots. The pattern of PCA plot could be seen clearly as the samples and variables were distributed far from each other. Group IV located far from group I and II because of the difference polarity of the solvents (Loadings plot) and the bioactive compounds (Scores plot) that merely detected in non-polar design (D3 and D5H). Nonetheless, group III located near group I since bioactive compounds in group III similarly identified in group I. Other bioactive compounds identified in Ajwa flesh are N-Methoxy-N-methylacetamide which only identified in D1 meanwhile Acetyl-19,21-epoxy-15,16-dimethoxy- Aspidospermidin-17-ol could be identified in D7. 9-Octadecenoic acid, cis-13-Octadecenoic acid, 9, 17-Octadecadienal, 9,12-Octadecadienoic acid, Methyl-β-d-ribofuranoside, α-Methyl-l-sorboside and 5-Hydroxymethylfurfural (5-HMF) were identified mostly in all designs, except D2 and D6. 5-HMFcontent known to vary in various foods such as honey, jam, biscuits and apple since this compound is a result of sugar degradation via the Maillard reaction when carbohydrate-rich foods are heated (Gurkan and Altunay, 2015). Another bioactive compound function will be studied.

5. Conclusions

Overall, date flesh contains a number of potential pharmacological functions due to the presence of bioactive compounds in it. Many studies use the mixture of polar solvents extraction to extract targeted group of compounds, but in this study, by following the three-mixture design concept, with the mixture of solvent extraction with varying polarities, other bioactive compounds could be extracted and identified using GCMS. Combination of strong polar solvent and strong non-polar solvent gives antifungal and antimicrobial potent compounds, which cannot be extracted using single polar or non-polar solvent. Compounds with antifungal activity as well as anti-inflammatory, anti-diabetic and anti-asthmatic can be identified only in non-polar or mixture solvents. No anticancer potent compounds were detected in polar solvent. β-Sitosterol and Vitamin E can be identified merely in non-polar solvent. Most phytochemical activities were extracted using polar or combination with polar solvent while anticancer potent compounds were detected in non-polar solvent. Therefore, we can assume that anticancer compounds only present in semi- or non- polar solvent extraction. However, medium chain fatty acids normally have antimicrobial activity can be identified in all mixture design except D2 and D3. Other than antimicrobial activity, medium chain fatty acids tend to have anticonvulsant (caprylic and capric acid) and anti-inflammatory (capric and lauric acid) activity. To conclude, extraction solvent system of varying polarities differs significantly.

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