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THE EXTRACTION OF *VITEX* POUCH

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Abstract

In this study, the unprecedented extraction of the *Vitex* pouch was performed. The compounds from methanolic and chloroform extracts were isolated by using thin layer chromatography (TLC). The compound of interest was investigated by using ^{1}H-Nuclear Magnetic Resonance (NMR, 500 MHz) spectroscopy. From the NMR spectral examination, the compound from the methanolic extract was suggested as gluconolitol. Indeed, there are some parameters that could enhance the attainment of this research, which include high performance liquid chromatographic supplies. Nevertheless, more information and understanding on the pharmaceutical and chemical analysis of the *Vitex* species were obtained. To sum up, it is anticipated that incoming research with advanced technology for this natural product could be explored in the future.

Keywords: chromatography, extraction, pouch, spectroscopy, *Vitex.*

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Introduction

The *Vitex* species is locally known as lemuni. It showed numerous biological activities (Akaniro-Ejim *et al*., 2016; Yao *et al*., 2016; Ali *et al*., 2017) and contains a variety of constituents (Hu *et al*., 2016; Mishra *et al*., 2014; Abdul Rasyid *et al*., 2017). Literature reviews were conducted (Vishwanathan *et al*., 2010; Basri *et al*., 2014; Zahid *et al*., 2016) and revealed the effectiveness of *Vitex* in treating female reproductive problems, for example premenstrual syndrome and menopause (Rafieian-Kopaei *et al*., 2017). This medicinal plant is also involved in the traditional practices, as reported by Wakhidah *et al*. (2017). In addition, it is utilised as a food colorant, for example, in preparing the lemuni rice. Nevertheless, the process of making this herbal rice is quite tedious. The leaves and flowers were picked and washed, before they were ground with water and sieved. The bluish extract was obtained, mixed with rice and boiled to cook.

Special attention is given in the separation and identification of chemical composition from various *Vitex* species by using standard chromatographic techniques (Abdul Wahab *et al*., 2011; Kamal *et al*., 2016). Recently, Lagurin *et al*., (2017) noted on the chemical profiling of *Vitex* via ^{13}C-Nuclear Magnetic Resonance (NMR) spectroscopy. Apart from the phytochemical analysis, the *Vitex* pouch is proposed (Abdul Hakeem *et al*., 2016) to provide convenient means to make lemuni rice. It is aimed to provide an alternative for the preparation of the rice (Mohd Ali *et al*., 2017). In a continuous study of this plant species, the *Vitex* pouch is subjected to the organic extraction. The isolation of *Vitex* constituent was performed, and the spectroscopic data of the pure component was examined.

Materials and Methods

Plant materials and chemicals

The crude drug, which is the flowering stems of *V. trifolia*, was collected from Subang Jaya, Selangor Darul Ehsan, Malaysia, in February 2015. A voucher specimen (no. 0215USJ) was deposited in

Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam, Selangor Darul Ehsan, Malaysia. Meanwhile, the polyethylene pouch (Figure 1) of two different sizes were obtained from the retail. Two types of pouches were used in the experiment which were small (90 cm x 70 cm) and medium (110 cm x 105 cm) sizes. Dried leaves, fruits and twig parts of the *Vitex* were placed inside those pouches.

Figure 1. An example of the *Vitex* pouch and the dried leaves.

The solvents that were used include chloroform ($CHCl_3$), methanol (MeOH), acetic acid and butanol. Freshly prepared ferric chloride and anisaldehyde were used as the reagent to stain and visualize the inactive compounds under ultraviolet (UV) lamp. The isolated compounds were dissolved in the deuterated methanol (CD_3OD) before they were subjected to NMR analysis on a Bruker 500 Ultrashield™ spectrometer.

Extraction of *Vitex* pouch

The small *Vitex* pouches were placed in the glass container containing 100 ml of $CHCl_3$. Meanwhile, the medium *Vitex* pouches were placed in the glass container containing 200 ml of $CHCl_3$. This step was repeated by using MeOH. These extractions were retained in a fume hood for 4 days. The pouches were removed from the mixture and extracts were concentrated by using rotary evaporator in order to obtain the crude residue.

Chromatography of the *Vitex* extract

The MeOH extracts were prepared for thin layer chromatography (TLC) by using MeOH and $CHCl_3$ as the solvents. The TLC plate (aluminium sheet coated, Merck silica gel 60 F_{254}) was resized with a common household scissor, approximately about 2.5 cm width and 10 cm length. The TLC plate was used to detect the presence of the compound from the extracts. The base line of the plate was marked approximately 1 cm from the bottom. The TLC mobile phases were tested to obtain a good solvent system that show an effective separation on the TLC plate. The solvent system was optimized by adjusting the polarity of the solvents if the compounds were tailed and did not display satisfactory separation on the TLC plate. The efficient solvent that showed upstanding separation without tailing was chosen after TLC plate was developed.
The best mobile phase that was used for TLC was butanol: acetic acid: water (5:1:4) for MeOH extract and chloroform: toluene (10:0) for CH_3CI extract. During the development of the TLC plate, the mobile phase in the chamber was below the base line of the TLC plate. The extracts were drawn up into a glass capillary tube and were spotted on a dry TLC plate at a base line that was previously marked. The TLC plate was removed from the solvent after the solvent reach the solvent front. The plate was dried by using a dryer and was examined under both short (254 nm) and long (365 nm) wavelength of the ultraviolet (UV) light. The spots were observed and marked. The plate was sprayed with anisaldehyde to detect inactive compound under UV lamp (Figure 2). The plate was heated for 3 minutes until the coloured spots could be visualized.

Purification of the extracts

The isolated compound was purified by using preparative TLC [20 cm x 20 cm glass plates, coated with silica gel 60 F_{254} (Merck)]. The plate was marked with 2 cm as a base line from the bottom and 1.5 cm from both left and right side of the plate. The extracts were deposited on the plate by using glass capillary tube as one continuous line. The line was repeated 3 to 4 times. The plate was placed in the glass chamber containing butanol: acetic acid: water (5:1:4), similarly as in the analytical procedure. After the development process was completed, the plate was taken out from the chamber and was allowed to dry.

The dried plate was observed under short and long wavelengths of UV light. Any band of interest was marked by using soft pencil. The plate was stained by using anisaldehyde. The plate was dried by using a dryer for 3 minutes until the coloured bands appeared. The coloured bands were marked and scrapped off by using scrapper. The compound was re-dissolved with the MeOH and was filtered by using cotton wool to remove the silica.

Result and Discussion

In the experiment, the pouch was soaked in the organic solvent. The extract was concentrated by utilising the rotary evaporator, without undergo filtration process. It was convenient and not time-consuming, because the infusion did not leave loose leaf behind. However, since the pouch was filled with various parts of the plants, which may include both leaves and stem, the colour of the infusion was vary. For instance, the pouch that was filled with mostly with the *Vitex* leaves, gave concentrated extract, than those packed with stem. The polarity of the solvents used was also different. The MeOH extract showed more intense greenish colour, as compared to the $CHCl_3$ extract.

The purification process was done prior to NMR analysis. It was carried out to obtain the pure compound. The glass TLC plate was used by applying one continuous streak of sample using capillary tube. It is important to take into consideration that the sample must not too concentrated or too dilute. This was done by viewing the TLC plate under UV lamp before developing the plate in the chamber. Only one TLC plate was used during this step, which was for MeOH extract of the medium pouch.

Analytical and preparative TLC

The TLC plate of the MeOH extract from the small pouch, showed six (6) spots when it was observed under short wavelength UV lamp (Figure 2). Similarly, six (6) spots were observed in the crude extract from the medium pouch. The retention factor (R_f) or the distance travelled of the solute from the base line to the solvent front, was calculated and recorded, to express the movement of the compound. The TLC plate of the $CHCl_3$ extract from the small pouch gave five (5) spots under short wavelength of the UV light. Three (3) spots were observed under short UV ray from the extracted medium pouch (Table 1).

The preparative TLC is a useful technique to purify small quantities of the MeOH extract. The plate was developed in a big chamber with butanol: acetic acid: water (5:1:4), as the mobile phase. It was visualized under long and short wavelengths of the UV light. The sorbents of the TLC mostly contain fluorescent powder that brightly glows when exposed to long wavelength. Under short wavelength, the spots appeared dark in colour. Three (3) bands were observed on the glass plate, under the short wavelength UV light and one (1) band was observed under long wavelength UV light. The TLC plate was stained with anisaldehyde and no coloured bands were observed. After some time, a band (R_f value = 0.7) was observed. This desired band was scrapped off and dissolved in the MeOH. The solution was filtered to obtain the purified compound for NMR analysis.

Figure 2. The reagent spraying (left) and the MeOH extract under UV lamp (254 nm, right).

Table 1. Retention factor (R_f) for the spots from the methanol and chloroform extracts for both small and medium pouches.

Spots	MeOH extract		$CHCl_3$ extract	
	Small	Medium	Small	Medium
1	0.5	0.5	0.3	0.3
2	0.5	0.5	0.5	0.4
3	0.6	0.6	0.7	0.9
4	0.7	0.7	0.8	
5	0.7	0.8	0.9	
6	0.7	0.9		

The R_f values (Table 1) can be influenced by the polarity of the solvent. In MeOH extract, the mobile phase was butanol: acetic acid: water (5:1:4). The elution was not efficient because the mobile phase is too polar since water was added into it. However, the separation of the solutes was efficient since there was no tailing observed. Since the silica gel TLC plate was used in this experiment, it has a strong interaction with the most polar compound. Thus, the more polar compound migrates slower on the plate than the less polar compound. In $CHCl_3$ extract, the solvent system shows efficient separation when using 100% chloroform.

According to Malaysian Herbal Monograph (2009), the compound's profile from the MeOH extract of *Vitex* dried leaves was screened by using High Performance TLC (HPTLC). The HPTLC is in fact, the innovative form of TLC and could provide better resolution than TLC. For example, HPTLC gives efficient compound separation than TLC. It was noted that, the mobile phase used in the monograph was ethyl acetate: methanol: water (100: 13.5: 10) instead of acetic acid. The acidity of the mobile phase has a significant influence to the compound separation. Addition of acetic acid and water will increase the polarity of the mobile phase. Furthermore, reverse phase chromatography engages polar mobile phase and non-polar stationary phase. Hence, the best stationary phase that could also be recommended in this study is C18 silica gel. This is because hydrophobic compound could adsorb to the hydrophobic stationary phase and therefore, hydrophilic molecule could elute first.

Nuclear Magnetic Resonance (NMR) spectroscopy

The TLC plate was wrapped up with aluminium foil for two days before it was scrapped off. In theory, the preparative TLC should be performed within the same day because the compound can be

easily oxidized. Unfortunately, there is a possibility that some compounds are degraded prior to NMR analysis. From the ^{1}H-NMR spectrum (500 MHz, CD_3OD), the structure of a compound was proposed (Table 2, Figure 3).

Table 2. The ^{1}H-NMR data (500 MHz, CD_3OD) for the coloured band (R_f = 0.7).

^{1}H-NMR chemical shift, δ_H (ppm, parts per million)	Multiplicity (J, Hz)	Spectral interpretation / suggested functional groups
0.92	t (J = 7.0)	a terminal methyl, -CH_3
1.59-1.68	s	methylene protons, -CH_2CH_3
3.46 – 3.55	m	a hydroxylated methine, HOC-H
3.65	m	a hydroxylated methine, HOC-H
3.75	t (J = 5.0)	a hydroxylated methine, HOC-H
4.00	t (J = 6.5)	a hydroxylated methine, HOC-H
4.13	m	a terminal hydroxylated methylene, -CH_2OH

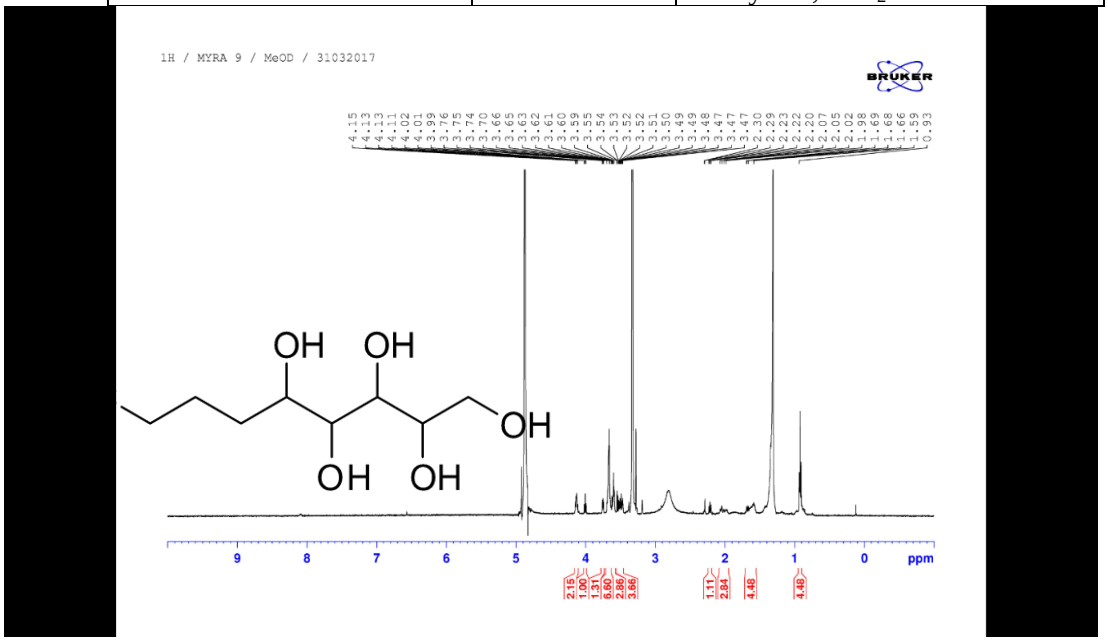

Figure 3. The ^{1}H-NMR (500 MHz, CD_3OD) spectrum and the chemical structure of glucononitol.

Based on the data, the chemical shift of CD_3OD or residual protons corresponding to CHD_2OD was observed at δ_H 3.31 ppm. Meanwhile, the chemical shift of H_2O (or HOD) was recorded at δ_H 4.92 ppm. Several peaks were seen between δ_H 0.92 until δ_H 4.13 ppm, possibly due to hydroxylated hydrocarbon. The peak showed at δ_H 0.92 and 1.67 ppm indicated an aliphatic hydrocarbon. The peak appeared in the region δ_H 3.65 ppm displayed alcoholic protons. No peaks were observed after δ_H 6 ppm indicating the absence of olefinic, aromatic and aldehyde protons. The presence of the aliphatic carbon chain and alcoholic protons, could illustrate the presence of glucononitol (Banerji *et al*., 1969), which is a polyhydroxylalcohol, also known as 2,3,4,5–tetrahydroxynonanol, $C_9H_{20}O_5$ (Figure 3).

Conclusion

The TLC was simple and convenient, in order to obtain the chromatographic profile of the natural compounds. It is an easy tool to screen and separate the *Vitex* components from the crude mixture. The TLC method can provide a primary identification of the plant's component, prior to the analytical proton NMR method. In this research, glucononitol was probably extracted from the methanolic extract of the *Vitex* pouch. It is hoped that more information on this underutilised herbal plant could be gathered.

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References

Abdul Hakeem, N. R. S., Md Yusof, N., Jahidin, A. H., H. H. Mizaton, Mohsin, H. F. and Abdul Wahab, I. "*Vitex* Species: Review on Phytochemistry and Pouch Design for Nutritional Benefits," *Scientific Research Journal*, **13**(2), 1 – 8 (2016).

Abdul Rasyid, F., Fukuyoshi, S., Ando, H., Miyake, K., Atsumi, T., Fujie, T., Saito, Y., Goto, M., Shinya, T., Mikage, M., Sasaki,Y. and Nakagawa-Goto, K. "A Novel Clerodane Diterpene from *Vitex cofassus*," *Chem. Pharm. Bull*., **65**, 116–120 (2017).

Abdul Wahab, I., Mizaton, H. H., Jahidin A. H. and Mohsin, H. F. "The Identification of Natural Compounds: Methoxylated Phenolics from *Vitex* and *Pandanus* Species" in C. A. Brebbia & S. S. Zubir (Eds.). Management of Natural Resources, Sustainable Development and Ecological Hazards III (WIT Transactions on Ecology and the Environment), Selangor, Malaysia, 153 - 158 (2011).

Akaniro-Ejim, N., Ubani, C., Nubila, N., Nzei, A., Nwodo, U. and Okoh, A. "Evaluation of Saponin Extract from *Vitex doniana* and *Pentaclethra macrophylla* for Antibacterial Activity," *Applied Sciences*, **6**(6), 180 - 189 (2016).

Ali, M., Aminu, F. and Ibrahim, I. S. "In-Vitro Assessment of Antibacterial Activity and Phytochemical Screening of *Vitex Doniana* on Clinical Isolate of *Salmonella Typhi*," *International Journal of Advanced Academic Research, Science, Technology & Engineering*," **3** (1), 9 – 16 (2017).

Basri, F., Sharma, H. P., Firdaus, S., Jain, P. and Ranjan, A. "A Review of Ethnomedicinal Plant-*Vitex negundo* Linn.," *International Journal of Advanced Research*, **2**(3), 882-894 (2014).

Banerji, A., Chadha, M. S. and Malshet, V. G. "Isolation of 5-hydroxy-3,6,7,3-pentamethoxyflavone from *Vitex* negundo," *Phytochemistry*, **8** (2), 511–512 (1969).

Hu, P., Li, D. H., Hu, X., Li, S. G., Sai, C. M., Sun, X. C., Su, T., Bai, J., Wang, Z. H., Li, Z. L. and Hua, H. M. "Lignans and triterpenoids from *Vitex negundo* var. *heterophylla* and their biological evaluation," *Fitoterapia*, **111**, 147–153 (2016).

Kamal, N., Clements, C., Gray, A. and Edrada-Ebel, R. "Anti-infective Activities of Secondary Metabolites from *Vitex pinnata*," *Journal of Applied Pharmaceutical Science*, **6**(1), 102–106 (2016).

Lagurin, L. G., Magsalin, J. D. J., Zosa A. R. and Dayrit, F. M. "Chemical profiling and chemical standardization of *Vitex negundo* using ^{13}C NMR," *Journal of Medicinal Plants Research*, **11**(1), 11-21 (2017).

Malaysian Herbal Monograph, Malaysian Herbal Monograph Committee (Editor), Forest Research Institute Malaysia, Kepong, Malaysia, Volume 2 (2009).

Mishra, S., Pani, S. R., Rout, K. K., Nayak, S. K. and Sahoo, S. Bioassay Guided Fractionation and Hepatoprotective Activity of Oleanolic Acid Acetate Isolated from *Vitex negundo* Linn. *Journal of Biologically Active Products from Nature*, **4**(2), 89-100 (2014).

Mohd Ali, A., Jahidin, A. H., Abdul Wahab, I., Mohsin, H. F. and Mizaton, H. H. "Uncang Lemuni Sebagai Produk Herba Warisan Tradisional," *Journal of Tourism, Hospitality & Culinary Arts*, **9** (2017), accepted for publication.

Rafieian-Kopaei, M. and Movahedi, M. "Systematic Review of Premenstrual, Postmenstrual and Infertility Disorders of *Vitex Agnus Castus*," *Electronic Physician*, **9**(1), 3685-3689 (2017).

Vishwanathan, A. S. and Basavaraju, R. A. "Review on *Vitex negundo* L. – A Medicinally Important Plant. *EJBS*, **3**(1), 30-42 (2010).

Wakhidah, A. Z., Silalahi, M. and Pradana, D. H. "Inventory and conservation plant of oke sou traditional ceremony; A welcoming tradition of maturity girl on the community of Lako Akediri Village, West Halmahera, Indonesia," *Biodiversitas*, **18**, 65-72 (2017).

Yao, J.-L., Fang, S.-M., Liu, R., Oppong, M. B., Liu, E-W., Fan G.-W. and Zhang, H. A. "Review on the Terpenes from Genus *Vitex*," *Molecules*, **21**, 1179 – 1199 (2016).

Zahid, H., Rizwani, G. H. and Ishaqe, S. "Phytopharmacological review on *Vitex agnus-castus*: a potential medicinal plant," *Chinese Herbal Medicines*, **8**(1), 24–29 (2016).