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 glucononitol. 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 lemini. 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 lemini 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 lemini 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.](image)

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$_3$OD) before they were subjected to NMR analysis on a Bruker 500 Ultrashield$^\text{TM}$ 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$_3$Cl 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 F254 (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 scraper. 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 CHCl3 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 (Rf) 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 CHCl3 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 (Rf 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.
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 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$_3$OD), the structure of a compound was proposed (Table 2, Figure 3).

Table 2. The $^1$H-NMR data (500 MHz, CD$_3$OD) for the coloured band (R$_f$ = 0.7).

<table>
<thead>
<tr>
<th>$^1$H-NMR chemical shift, $\delta_H$ (ppm, parts per million)</th>
<th>Multiplicity (J, Hz)</th>
<th>Spectral interpretation / suggested functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.92</td>
<td>t ($J = 7.0$)</td>
<td>a terminal methyl, -CH$_3$</td>
</tr>
<tr>
<td>1.59-1.68</td>
<td>s</td>
<td>methylene protons, -CH$_2$CH$_3$</td>
</tr>
<tr>
<td>3.46 – 3.55</td>
<td>m</td>
<td>a hydroxylated methine, HOC-H</td>
</tr>
<tr>
<td>3.65</td>
<td>m</td>
<td>a hydroxylated methine, HOC-H</td>
</tr>
<tr>
<td>3.75</td>
<td>t ($J = 5.0$)</td>
<td>a hydroxylated methine, HOC-H</td>
</tr>
<tr>
<td>4.00</td>
<td>t ($J = 6.5$)</td>
<td>a hydroxylated methine, HOC-H</td>
</tr>
<tr>
<td>4.13</td>
<td>m</td>
<td>a terminal hydroxylated methylene, -CH$_2$OH</td>
</tr>
</tbody>
</table>

Based on the data, the chemical shift of CD$_3$OD or residual protons corresponding to CHD$_2$OD was observed at $\delta_H$ 3.31 ppm. Meanwhile, the chemical shift of H$_2$O (or HOD) was recorded at $\delta_H$ 4.92 ppm. Several peaks were seen between $\delta_H$ 0.92 until $\delta_H$ 4.13 ppm, possibly due to hydroxylated hydrocarbon. The peak showed at $\delta_H$ 0.92 and 1.67 ppm indicated an aliphatic hydrocarbon. The peak appeared in the region $\delta_H$ 3.65 ppm displayed alcoholic protons. No peaks were observed after $\delta_H$ 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$_9$H$_{20}$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.
Acknowledgement
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