The identification of natural compounds: methoxylated phenolics from *Vitex* and *Pandanus* species

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Abstract

Plants produce various types of natural compounds, including polyphenols. These compounds are also found with methoxyl groups attached to the oxygenated carbon atoms. Nevertheless, the occurrence of methoxylated phenolics in two unrelated plants, *Vitex* and *Pandanus* species, could be considered unusual. From the literature review, most of the published phytochemicals from both plants include triterpenes and alkaloids. In this study, silica gel chromatographic separation of their leaves extracts yielded a quercetin pentamethyl ether **1** and a novel lignan, [4,8-bis(2,4-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane, **2,** from *Vitex trifolia* (family: Lamiaceae) and *Pandanus pygmaues* (family: Pandanaceae), respectively. The identification of both compounds is described from the results of the spectroscopic experiments. The chemosystematic analysis of these plants are however, would not be initiated by the discovery of these minor constituents. The *Vitex*'s terpenoids and pandan alkaloids, which are the principal metabolites of these plants, are in the anticipation of chemistry learning from nature.
Keywords: Vitex, Pandanus, phenolics, chromatography, spectroscopy, nature.

1 Introduction

Plants produce various types of natural compounds, including polyphenols. These compounds are also found with methoxyl groups (-OCH_3) attached to the oxygenated carbons. Nevertheless, the occurrence of methoxylated phenolics in two unrelated plants, *Vitex* and *Pandanus* species (respectively from Verbenaceae and Pandanaceae plant species), could be considered unusual. From the literature review, most of the published phytochemicals from both plants

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include alkaloids, terpenoids and phenolics. Phenylnaphthalene-type lignan alkaloid with antioxidative properties [1], labdane-type [2] and glucoside diterpenoids [3] were separated from *Vitex trifolia* and *Vitex agnus-castus*. Meanwhile, two phenolic compounds, lignan [4] and flavanoid [5, 6], were respectively isolated from *Vitex negundo* and *Vitex rotundifolia*. In the case of *Pandanus*, both pyrrolidine- [7–9] and piperidine-type [10] alkaloids were established as the *Pandanus'* biomolecule. Tirucall-dien-3-one [11] and cyclolanostanes [12] were found as the major phytosterol of this plant genus. Simple phenolics such as hydroxybenzoic acid [13] plus benzofuran and lignan [14] were included as its natural chemical component. In this paper, the result from the chromatographic technique of the leaves of *Vitex trifolia* (*V. trifolia*) and *Pandanus pygmaues* (*P. pygmaues*) is presented.

2 Methodology

The chloroform ($CHCl_3$) extracts of the leaves of *V. trifolia* and *P. pygmaues* were fractionated and purified. A Thin Layer Chromatography (TLC) of *V. trifolia* $CHCl_3$ extract was developed in the solvent system of EtOAc : $CHCl_3$ = 30:70. The components of the mixture were successfully separated. There were four bands observed under the ultraviolet (UV) light. Since the results showed more than one fraction, the extract was subjected to a preparative TLC for purification. After preparative TLC was performed, four different compounds were purified. The compounds obtained from this purification were subjected to the TLC again, in order to confirm its purity. Compound **1** (5 mg, orange florescence) showed one spot and this indicated that it was pure. Meanwhile, one compound **2** (5 mg) was obtained from the $CHCl_3$ extract of the leaves of *P. pygmaues* by using preparative TLC. It gave a purple spot after the TLC plate was sprayed with sulphuric acid (R_f = 0.61, hexane:acetone = 56:44). The molecular structures of both compounds were later, determined by Nuclear Magnetic Resonance (NMR) experiments. The silica chromatographic separation of their leaves extracts yielded a pentamethoxyl quercetin (or quercetin pentamethyl ether) **1** and a lignan **2**, respectively from *V. trifolia* and *P. pygmaues*.

3 Result and discussion

The identification of both compounds is described from the analysis of their spectroscopic data. Figure 1 shows the ^{1}H-NMR spectra (500 MHz, $CDCl_3$) of compound **1** from *V. trifolia*. It was found that, the most deshielded signal at δ_H 12.65 ppm (*s*, 1 H) could be assigned to a phenolic having the intramolecular hydrogen bonding with a keto group at its *β*-position [6]. Therefore, it is suggested that **1** could bear the carbon skeleton of a flavonoid and be identified as quercetin pentamethyl ether (Figure 2).

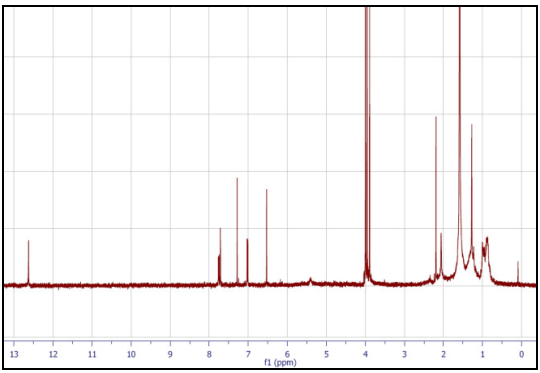

Figure 1: ^{1}H-NMR spectra (500 MHz, $CDCl_3$) of compound **1** from *V. trifolia* chloroform extract.

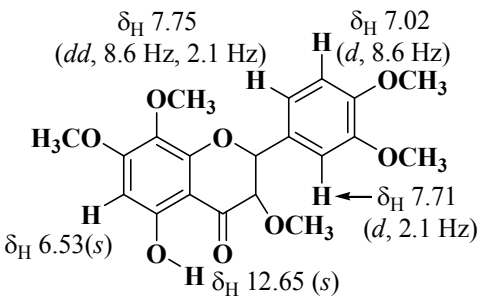

Figure 2: The structure of pentamethoxyl quercetin **1** from *V. trifolia*.

^{1}H-NMR (500 MHz, $CDCl_3$) of **1**: δ_H 3.892 (s,3H, OCH_3), 3.950 (s,3H, OCH_3), 3.990 (s,3H, OCH_3), 3.996 (s,3H, OCH_3), 3.998 (s,3H, OCH_3), δ_H 6.53 (*s*, 1H), δ_H 7.02 (*d*, *J* = 8.6 Hz,1H), δ_H 7.71 (*d*, *J* = 2.0 Hz,1H), δ_H 7.75 (*dd*, *J* = 8.6, 2.1 Hz, 1H), δ_H 12.65 (s, 1H, O*H*).

The chemical shifts of the ^{13}C-NMR spectrum (125 MHz, $CDCl_3$) of **2** ranges from δ_C 29 – 147 ppm (Figure 3). This showed the absence of a carbonyl group. In addition, two possible methoxy groups appeared at δ_C 55.99 and 54.20 ppm. Meanwhile, the ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) showed signals below δ_H 7.0 ppm only (Figure 4). Yet, it is believed that **2** could be an aromatic compound. Three aromatic protons appeared at δ_H 6.91 (*s*), 6.90 (*d*, J = 10 Hz) and 6.84 (dd, J = 8 Hz, 2 Hz). A broad singlet at δ_H 5.63 (*br s*) would correspond to a proton involving in an H-C-O- bond. Four aliphatic multiplets (δ_H 3 – 5 ppm) were also detected as a doublet at δ_H 4.75 (*d*, J = 4.2 Hz), two double doublets respectively at δ_H 4.26 (*dd*, J = 6.7, 9.5 Hz) and δ_H 3.89 (*dd*, J = 3.2, 9.5 Hz), and finally a multiplet at δ_H 3.12 (*dd*, J = 4.6, 6.7 Hz).

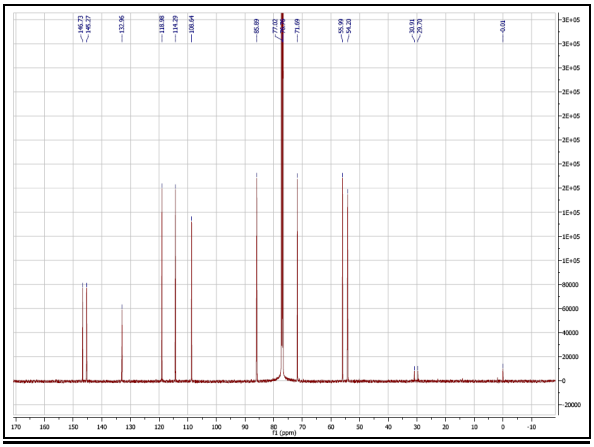

Figure 3: The ^{13}C-NMR spectra (125 MHz, $CDCl_3$) of **2** from *P. pygmaues*.

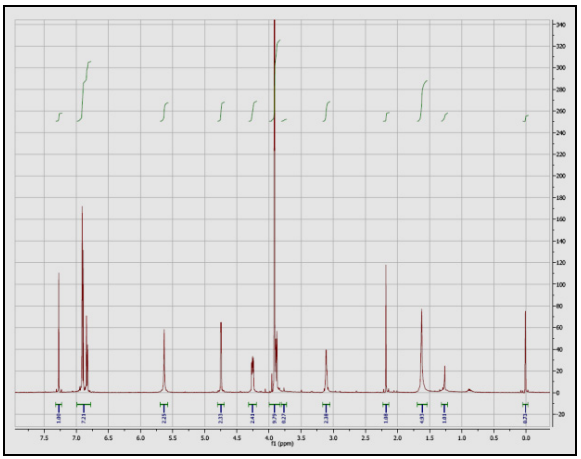

Figure 4: The ^{1}H-NMR spectra (500 MHz, $CDCl_3$) of **2** from *P. pygmaues*.

The ^{1}H-NMR spectrum also displayed signals at δ_H 3.91 ppm, supporting the hypothesis for the methoxy groups. It is suggested that respectively one dimethoxyphenyl and oxygenated five-membered ring could be constructed for compound **2**. A novel lignan, exclusively as [4,8-bis(2,4-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]octane] (Figure 5), was concluded for **2**, which is also an isomer of eudesmin, previously isolated from *P. odoratissimus* [14].

In summary, the identification of both compounds is described from the results of the spectroscopic experiments. The chemosystematic analysis of these plants are however, would not be initiated by the discovery of these minor constituents.

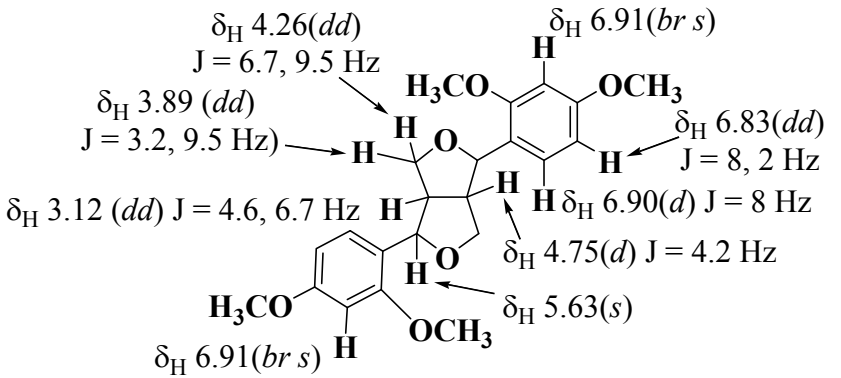

Figure 5: The structure of compound **2** from *P. pygmaues*.

4 Conclusion

In conclusion, the *Vitex*'s and *Pandanus*' natural constituent could be characterized as polymethoxylated compounds. These principal metabolites are in the anticipation of chemistry learning from natural resources.

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