Vitex Species: Review on Phytochemistry and Pouch Design for Nutritional Benefits

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ABSTRACT

Vitex trifolia or locally known as lemuni, is well documented for its medicinal properties including anticancer, antibacterial and wound healing. For years, the leaves and flowers were consumed for anti-aging and general well-being. It is also commonly served as nutraceuticals to post-partum women during their confinement period. Previous study showed that V. trifolia possesses high antioxidant properties that could prevent oxidative stress related diseases such as cancer, atherosclerosis, diabetes and many more. From the literature, V. trifolia leaves are used as poultice for rheumatic pains, sprains, antithelmintics and inflammations. Meanwhile, the fruits are utilised in amenorrhea. The roots, on the other hand, are used for the treatment of cough and fever. The plant is known to possess pharmacologically-active constituents such as the essential oil, halimane-type diterpenes, flavonoids, triterpenes, lignans, iridoids and ecdysteroids. Review papers were consistently published to justify the non-exhaustive biological exploration of this genus. Nevertheless, only 11 per cent of the total Vitex species were phytochemically examined. Apart from being eaten raw or blanched, Vitex is also taken as a food colourant in preparing lemuni rice. The process of making lemuni rice is quite tedious. First, the leaves and flowers are picked and washed. Both parts are then ground with water and sieved to give a bluish extract which is then mixed with rice and boiled to cook. Hence, the Vitex pouch is introduced to provide an easy way to prepare lemuni rice. This packaging is designed to enclose Vitex samples in a pouch that simply can be placed together with rice and boiled to cook.

Such instant preparation will hopefully promote the consumption of lemuni rice among busy locals. Future plans include international marketing and production of other Vitex nutritional products.

Keywords: *nutrition, phytochemistry, pouch, spectroscopy, vitex*

INTRODUCTION

Vitex species, locally known as lemuni, contain a variety of potentially bioactive molecules, such as iridoids, flavonoids, diterpenoids, derivatives and phytosteroids. These plants were used in traditional medicine to cure a wide range of ailments, for example, depression, allergy, wounds, diarrhoea and also gastrointestinal diseases [1]. A number of biological activities of this species were reported. Besides the antioxidant and antileptic activities of *V. agnus-castus*, *V. trifolia* also possesses several pharmacological properties such as hepatoprotective, wound healing activity and reducing allergy disease [2]. Meanwhile, *V. negundo* was proven to have analgesic and anti-inflammatory actions. In India, *V. penduncularis* and *V. pinnata* were found to have insecticidal properties [2]. Among 250 species in the *Vitex* genus, *V. trifolia* is one of the very well-known species and is commonly being used in *Vitex* studies. Based on research, this species is believed to have various therapeutic benefits. The plant is known to possess biologically-active constituents such as the essential oil, halimane-type diterpenes, flavonoids, triterpenes, lignans, iridoides and ecdysteroids [1]. It is widely used in India as the remedies in their Ayuverdic and Unani systems of medicines [3].

Most parts of *Vitex* plants (the leaves, fruits, roots and stems) are known to have many medicinal values. However, the specific chemical constituents that present in each part are still unclear. The importance of understanding which chemical constituents in each plant part can help to determine the compounds that are responsible for the therapeutic benefits. Thus, by extracting these *Vitex* species based on individual parts, the detailed information on the chemical compound could be understood. In Asian countries, *V. trifolia* leaves are consumed to improve memory, remove bad taste in mouth, cure fever and as a pain reliever [4]. The leaves are used internally or externally in baths to cure Ciguatera fish poisoning-related pruritus. Besides its antipyretic activity, it can also increase body weight

and being reported to have anti-tumour activity [2]. It can also being used as nematicidal and antithelmintic agents. The Asians choose *V. trifolia* as their remedy due to its ability to cure rheumatic pains, sprains, leucoderma and bronchitis. It is also utilised for hair growth and eye vision improvement [5]. By monitoring its effects on the modulation of cytokines, mediators of inflammation, the leaves part is believed to have anti-inflammatory property [2].

Regarding the other plant parts, the roots are used to treat febrifuge, cough and fever. The stems of *V. trifolia* was proven to be very toxic against cultures of several cell lines [2]. The aerial parts of this plant are useful in diabetes treatment. Meanwhile, the leaf extract is anti-cancerous. The fruits, in the other hand, are good for amenorrhoea. When the flowers of this *Vitex* species were mixed with honey, they were used in fevers, accompanied with severe thirst [4]. Besides *V. trifolia*, *V. negundo* is also a well-known species. In addition to this species being easily found and collected, *V. negundo* also possesses a wide variety of compounds. Although it does not have therapeutic aids as much as *V. trifolia*, studies of *V. negundo* have afforded numbers of phytochemicals, such as volatile oils, lignans, flavonoids, terpenes, and steroids [2]. The plant analysis showed that its leaves contains alkaloid (nishundine), luteolin-7-glucoside, vitexicarpin 1 (or casticin, 3',5-dihydroxy-3,4',6,7-tetramethoxyflavone, Figure 1), iridoid glycosides, an essential oil and other constituents like vitamin C, carotene, gluco-nonital, benzoic acid, β-sitosterol and C-glycoside. The seeds contain hydrocarbons, β-sitosterol, benzoic acid and phthalic acid, diterpenoids, flavonoids, artemetin and triterpenoids. Meanwhile, the bark contains fatty acids, valinic acid, p-hydroxybenzoic acid and luteolin [6].

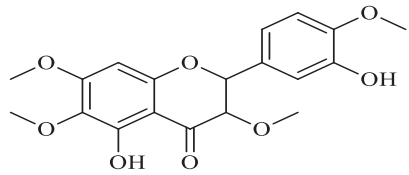

Figure 1: The chemical structure of vitexicarpin 1

Studies demonstrated that vitexicarpin 1 could be isolated from the flowers of Viticis Fructus (*Vitex rotundifolia Linne fil.*). It has long been used as an anti-inflammatory herb in traditional Chinese medicine [7].

A number of recent pharmacological reports indicated that 1 inhibits the proliferation of lymphocytes and thus, inhibit the growth of cancer cells in various cell lines [7]. Previously, a study on the leaves of *V. trifolia* yielded three compounds, viteosin A, 1 and vitetrifolin E. Vitexicarpin 1 was the most active among the three compounds. The mechanism of activity seems to be non-competitive antagonism to histamine and stabilisation of mast cells membrane function. The leaves extract gave an essential oil. Meanwhile, the fruits contain an acid resin, an astringent organic acid, malic acid, traces of an alkaloid and colouring matter [8].

In this study, the focus is given in the separation and identification of the chemical composition in the leaves extract of a *Vitex* species by using standard chromatographic technique. Apart from the phytochemical analysis, the *Vitex* pouch is also introduced, in order to provide an alternative for the preparation of lemuni rice. Traditionally, *Vitex* is eaten raw or blanched. It is also consumed as a food colourant in preparing lemuni rice. Nevertheless, the process of making lemuni rice is quite tedious. The leaves and flowers were picked and washed, before they were ground with water and sieved to give a bluish extract. This colorant is then mixed with rice and boiled to cook. Hence, the *Vitex* pouch is proposed to provide convenient means to prepare lemuni rice.

METHODOLOGY

Raw material

The crude drug, which is the flowering stems of *V. trifolia* was collected from Klang, Selangor Darul Ehsan, Malaysia, in July 2011. A voucher specimen (no.0711VKLG) was deposited in Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam, Selangor Darul Ehsan, Malaysia. The plant material was later divided into leaves, fruits and twig parts.

Chemicals

The chemicals that were used include the solvents; chloroform ($CHCl_3$), ethanol (EtOH) and methanol (MeOH). Cerric sulphate was used as the spray reagent to visualise ultraviolet (UV) inactive compound.

Extraction, Isolation and Purification of the *Vitex* Extract

The plants parts were cut before they were extracted. The leaves were introduced to EtOH extraction, silica column and preparative thin layer chromatography (TLC). Plates coated with silica gel (silica 60 F254) was used as the stationary phase. A pure compound 1 was purified and subjected to Nuclear Magnetic Resonance (NMR) spectroscopy (500 MHz, CD3OD).

Designing *Vitex* Pouch for Fresh Herbal Packaging

Vitex is cherished in local rice preparation; however, this herb is seen as having relatively low commercial value in food industry. Therefore, there is a call to incorporate *Vitex* natural materials into new food products. For travellers or tourists, the difficulty of getting *Vitex* fresh material could be minimised with the provision of a novel form of this crop. Therefore, a packaging is designed to consist of ten grams of *Vitex's* dried, shredded leaves, flowers and stem in a pouch that simply could be placed together with rice and boiled to cook (Figure 2). A pouch (11.0 cm x 10.5 cm) was utilised to enclose the powdered *Vitex* samples. For presentation purposes, two smaller pouches (9.5 cm x 7 cm) could be placed in a zip bag, having the dimension of 14 cm x 10 cm. An example of a complete product could consist of an outer case or box, the above pouch to dispense *Vitex's* nutrient into the rice and a packet of rice, for example, dedicated for two travelling persons. Appropriate labelling could be designed for the boxes or packaging, in order to attract customers. In addition to *Vitex* pouch, other herbs such as the fragrant *Pandanus* leaves, a few cinnamon stick, clove, star anise and low-fat coconut milk could be added. Such instant preparation will hopefully promote the consumption of lemuni rice among busy locals. The shelf life of 12 months could be expected for the above food materials.

Figure 2: An example of the *Vitex* pouch and the dried leaves (source by author)

A preliminary sensory evaluation of the lemuni rice sample was carried out, four hours after its preparation [9]. A panel was randomly selected among the university staff for the evaluation. Nevertheless, a standard scale [10] was not utilised to evaluate the appearance, aroma, taste, mouth feel and acceptability of the sample. None of the necessary precautions were taken to prevent carrying over the taste perception. This evaluation is only meant as a general introduction to the *Vitex* product. Therefore, a more standardised sensory assessment will be performed in near future.

RESULT AND DISCUSSION

Isolation and Purification of the *Vitex* Extract

The samples were extracted and monitored via TLC analysis, in order to obtain their chromatographic profiles. A preliminary spectroscopic evaluation of an iridoid glycoside 2 is shown in the 1H-NMR spectrum (500 MHz, CD3OD, Figure 3). The signals at were assignable to aromatic protons, originated from a p-hydroxybenzoyl group (δH 6.8 - 8.0 ppm), three olefinic protons (δH 5.0 - 6.5 ppm), aliphatic protons and glucose residue (δH 3.2 - 5.0 ppm), another two aliphatic protons (δH 2.7 - 3.0 ppm), and finally -CH_2 protons, possibly adjacent to a carbonyl group; -CH_2-C=O at δH 1.91 ppm.

The resonances attributed to a p-hydroxybenzoyl group [δH 6.85 and 7.93, respectively H-2' (2H) and H-3' (AB system, doublets, J = 7.5 Hz) was observed. This characteristic moiety was frequently encountered in *Vitex* species. The acyl group was attached to C-10, and the C-10 methylene protons' resonances were found to be shifted downfield at δH 1.91 (singlet, 2H). The signals of one doublet at δH 6.37 (J = 5.5 Hz) and one double doublets at δH 5.14 (J = 3.5 and 5.0 Hz) were due to the olefinic protons at C-3 and C-4, respectively. The proton at C-4 was believed to couple to a proton at δH 2.72 ppm (1H, multiplet, H-5), which could couple to a proton at δH 4.49 (1H, multiplet, H-6). The chemical shift of H-6 suggested the presence of one hydroxyl group at C-6. The proton at C-6 might also correlate to an olefinic proton at δH 5.82 (1H, broad singlet, H-7). In addition, the proton at C-5 could correspond with a proton at δH 3.01 (1H, triplet, H-9).

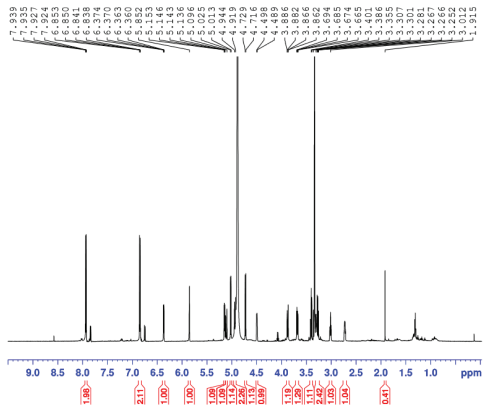

Figure 3: 1H-NMR (500 MHz, CD3OD, δH 0 - 9 ppm) spectrum of the suggested iridoid glycoside 1

From these findings, the data for compound 2 was recommended to be similar to those for agnuside, C22H26O11 (Figure 4). This suggestion was also supported by comparison of the 1H-NMR data [11-15]. The IUPAC name of 2 was established as [5-hydroxy-1-[3,4,5-trihydroxy-6-(hydroxymethyl)-oxan-2-yl]oxy-1,4a,5,7a-tetrahydrocyclopenta[c]pyran-7-yl]methyl 4-hydroxy-benzoate. Hence, compound 2, was proposed as a known composition of *V. trifolia* and it is commercially available [16]. The occurrences of 2 were also reported in other *Vitex*, including *V. cannabifolia, V. rotundifolia*, *V. cymosa*, *V. agnus-castus* and *V. negundo*. This compound could be seen to compose of aucubin 3 (Figure 5) and p-hydroxybenzoic

acid, another natural component of *Vitex* species. The presence of the both compounds 2 and 3 are quite common in almost *Vitex* species. Hence, the above compounds may be considered as chemotaxonomic markers of the genus *Vitex* [17]. The quantification of 2 via high performance liquid chromatography (HPLC) proposed that the leaves contained relatively high amount of 2. Therefore, the bark part should not be disrupted, thus setting the efforts in conserving the plant [16].

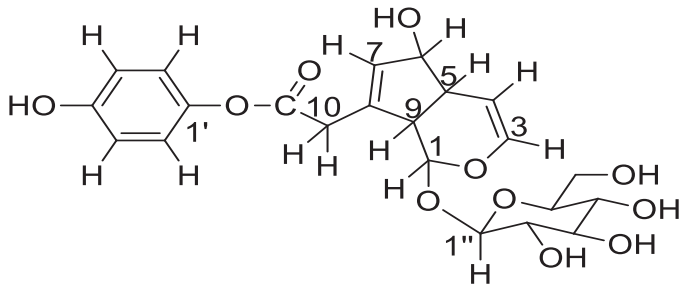

Figure 4: Compound 2 was proposed as agnuside, a known composition of *V. trifolia*

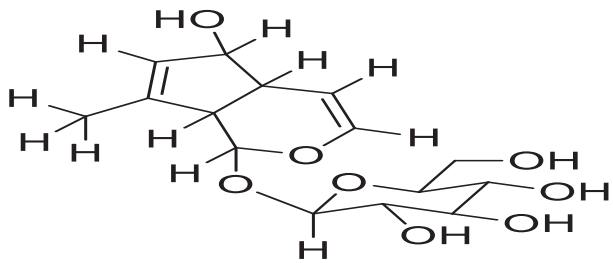

Figure 5: The chemical structure of aucubin 3

Panel Suggestion of the *Vitex* Pouch and Lemuni Rice

The plant materials were stored to dry, naturally, before they were used for the pouch. Bigger, industrial scale could apply an oven drying, instead. Other methods of preparation would include; either freeze drying of *Vitex's* aqueous extract; or by using liquid nitrogen. However, these two

approaches would not assure the freshness of the colourant and its antifungal properties. On the other hand, the pouch design could be considered as a type of traditional packaging, due to its basis of prior drying of the plant materials as the primary preservation method [18]. A more modern approach would be to convert the concentrated Vitex extract into cubes, as seen for chicken/beef stock cubes, possibly with added chemical preservatives or artificial colours. The cubes would later be broken up or dissolved with boiling water while the lemuni rice is cooked.

The introductory assessment of the pouch and lemuni rice were carried out by a team of 13 members. It was apparent that the rice taste is contributed by the added spice in the lemuni rice, such as cinnamon and star anise. The volatile and non-volatile components of *Vitex* and the spices give the overall flavour and aroma. Specifically, the volatile composition of the *Vitex* extractives, which is also referred as the essential oils, could benefit the consumer, since the essential oils of *V. trifolia* was shown to possess insecticidal properties [2]. In addition, the dark-blue characteristic colouring of the rice provide a distinct, vivid impact to the rice, as compared to the plain, white rice. The panel also suggested that the unique taste and rich aroma of *Vitex* leaves was well blended with the rice. It was summarised that the panels altogether liked the lemuni rice, prepared with the designed pouch.

CONCLUSION

Iridoids and terpenoids are frequently discovered from this traditional plant. Extensive experiments, including two-dimensional NMR, will be carried out to fully characterise this plant's natural metabolite. Review papers were consistently published to justify the non-exhaustive exploration of this genus, both phytochemically and biologically [19-21]. On another note, such instant lemuni rice preparation will hopefully promote *Vitex* consumption among busy locals. The design and concept of the *Vitex* pouch could be realised and disseminated to the public. With this proposal, the herbal product could be introduced and acquired for a quick, convenient and effective preparation of lemuni rice. The temperature range of about 80-100^{o}C will occur during the rice cooking process, thereby infusing

the *Vitex's* nutrients into the rice. Generally, most food products can be constantly reformulated, therefore, the natural ingredients and its nutrition content may change. Therefore, further invitation should be extended to food engineers, biotechnologists and nutritionists, in order to investigate this point. The future plans would include international marketing and production of other *Vitex* nutritional products.

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