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CHEMICAL COMPOSITIONS OF TAPA LEAVES (*PYCNARRHENA CAULIFLORA*): A NATURAL FOOD FLAVOR ENHANCER

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ABSTRACT

The leaves of 'Pokok Tapa' or scientifically known as *Pycnarrhena cauliflora* (Menispermaceae) are used by Borneo communities as folkloric medicine and as a natural food flavour enhancer in their cooking. However, a thorough literature search revealed that not much scientific work has been done on the species. Thus, the aim of this study is to determine the chemical compositions of the plant's leaves through proximate analysis, phytochemical screening, and high-performance liquid chromatography (HPLC) profiling. The proximate analysis indicated that the dried leaves contain key nutritional components such as carbohydrate, crude fibre, total fat, and crude protein in the percentage of 78.5, 59.0, 2.7 and 8.2, respectively. The high protein content could contribute to the flavor enhancer potential of the leaves. Meanwhile, the phytochemical screening test on the methanolic leaf extract showed the presence of several classes of compounds which were alkaloids, terpenoids, and tannins. Among the classes of compounds that have been reported in the *Pycnarrhena* species were the alkaloids isoquinoline, bisbenzylisoquinoline and aporphine while none of the terpenoids or tannins. The HPLC profile obtained at 230 and 281 nm UV wavelengths indicate strong $\pi \rightarrow \pi^{\star}$ and $n \rightarrow \pi^{\star}$ electronic transitions of the moderate polarity alkaloids. These alkaloids might also contribute to the umami flavor of the plant's leaves. However, identification the alkaloids and free amino acid composition of the crude protein is necessary to support these findings.

Keywords: Borneo herb, *Pycnarrhena*, phytochemical, proximate analysis, HPLC.

INTRODUCTION

Pycnarrhena cauliflora (Miers.) Diels. (Menispermaceae) is a liana species, thrives in tropical regions, particularly Borneo [1]. Known by various names such as 'pokok ajinomoto,' 'kiamis,' 'tapa,' 'tapa tahambia,' or 'tapa bohuang' in Sabah, and 'sengkubak' and 'kemangi imbo' in Indonesia, its usage varies among local ethnic groups [2]. Extensive literature search reveals that the prevalence traditional applications of *P. cauliflora* are more prominent in Kalimantan, Indonesia, and East Malaysia [3]. Beyond its role as a food enhancer and seasoning, the leaves of *P. cauliflora* have traditionally been employed to address diverse ailments, including eye irritation, headaches, fever, seizures, malaria, stomach bloating, and snakebites [4,5].

Besides that, the plant exhibits several biological activities, including cytotoxicity, antioxidant properties, antiparasitic effects, anticancer potential, and protein tenderizing attributes [6-10]. Despite screening for major phytochemical groups (alkaloids, flavonoids, tannins, terpenoids, steroids, saponins, and phenols), there are lack of reports on the chemistry of the plant. Nevertheless, recent GC-MS analysis of volatile constituents from its leaves revealed compounds such as α-bergamotene, β-sesquiphellandrene, α-cubebene, and sabinene, all belonging to the terpene class [11]. Thus, the aim of this study is to determine the chemical compositions of the plant's leaves through proximate analysis, phytochemical screening, and high-performance liquid chromatography (HPLC) profiling.

MATERIAL AND METHOD

Plant Materials

Dried *P. cauliflora* leaves were purchased online through E-commerce from Indonesian vendor. Then, the leaves were delivered to AuRIns, UiTM Puncak Alam for authentication.

Preparation of Extracts

The sample (500 g) was cut into smaller fragment and pulverized using electrical grinder. The powder was extracted with 100 % MeOH at room temperature for 72 h and then filtered through Whatman No. 3 filter paper (Whatman, England). The filtrate was collected, and excess solvent was evaporated under reduced pressure using rotary evaporator at temperature between 40-45 ºC yielding 20 g of the extract. The extract was stored in -4 ºC prior to analysis.

Proximate Analysis

The proximate analysis were determined on the pulverized sample according to the official analysis methods of the AOAC International [12]: total ash content by igniting a *ca*. 3–5 g test sample in a furnace at 550 ºC until whitish or grayish ash obtained (923.03); moisture content by oven drying a *ca*. 2 g test sample at 100 ºC to a constant weight (950.46); protein content by the Kjeltec Auto Analyzer (923.03); total fat content by petroleum ether extraction using a Soxhlet apparatus (991.36). The carbohydrate content was determined according to [13]: % Carbohydrates = 100 – (% Protein + % Fat + % Ash + % Moisture).

Phytochemical Tests

The extract underwent qualitative phytochemical analyses following the procedures outlined by Azmi et al. [14]. The identification of alkaloids (Mayer's reagent), flavonoids (Shinoda Test), terpenoids (Salkowski Test), tannins (Ferric Chloride Test), and saponins (Froth Test) was carried out.

Chemical Profiling using High Performance Liquid Chromatography (HPLC)

The profile of the extract was analysed by using DIONEX Ultimate 3000 HPLC system (ThermoFisher, USA). Separation was accomplished on a Phenomenex Luna ® 5 µm C18 (2) 100 À (150 X 4.6 mm particle size) column. Mobile phase A was UPW and mobile phase B was HPLC grade MeCN. A constant flow rate of 1 mL/min was used and a mobile phase gradient was applied to achieve a good baseline separation: min 0; 10% B, min 25; 100% B, min 30, 100% B, min 33; 10% B, min 35; 10% B.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize the proximate analysis and phytochemical screening of *P. cauliflora* leaf extract. The proximate analysis (Table 1) revealed that the dehydrated leaves contain essential nutritional components, including carbohydrates (78.5%), crude fiber (59.0%), total fat (2.7%), and crude protein (8.2%). While, the phytochemical screening results (Table 2) indicated the presence of several compound classes, notably alkaloids, terpenoids, and tannins. Most alkaloids are biogenetically derived from amino acids such as glutamic acid, aspartic acid, phenylalanine, and tyrosine [15]. The combination of high protein and moderate alkaloid content suggests the potential presence of umami tastants in the leaves. Previous studies have also noted that the bitter taste of alkaloids may contribute to the umami flavor of certain foods [16].

Alkaloids such as isoquinoline, bisbenzylisoquinoline, and aporphine have been previously reported within the *Pycnarrhena* genus, while terpenoids and tannins remain undocumented [17]. To further investigate the chemical composition and structural complexity of P. cauliflora leaf extract, HPLC chemical profiling was performed. Guided by phytochemical analysis, which revealed a high concentration of alkaloids, two UV maxima wavelengths (230 nm and 281 nm) were selected for detection, focusing on this class of compounds.

The chromatograms (Figures 1 and 2) revealed consistent chemical profiles, with the major constituents eluting between 14 and 20 minutes, indicative of moderate polarity. These HPLC profiles confirmed the presence of alkaloids, with most peaks concentrated in the central region of the chromatograms. This observation aligns with the characteristic electronic transitions of $\pi \rightarrow \pi^{\star}$ and $n \rightarrow \pi^{\star}$ commonly associated with alkaloid compounds at 230 nm and 281 nm [15-18].

Table 1: Proximate Analysis

Components	Content
Total ash	3.0%
*Moisture	7.6%
Protein	8.2%
Total Fat	2.7%
Carbohydrate	78.5%
Crude fiber	59.0%

*Moisture was presented based on wet mass; others were presented based on dry mass

Table 2: Phytochemical Tests

Phytochemical constituents	Qualitative content
Alkaloid	++
Flavonoid	-
Terpenoid	+
Tannins	+
Saponins	-

*Qualitative approximation scale: '+' trace, '++' moderate, '-' absent

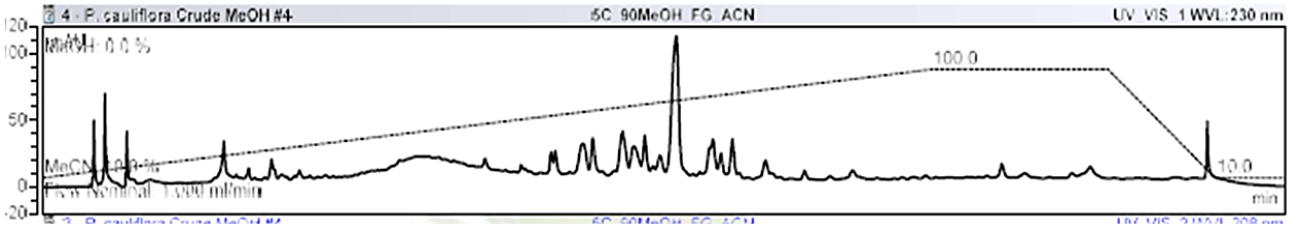

Figure 1: Chromatogram of the extract detected at 230 nm wavelength

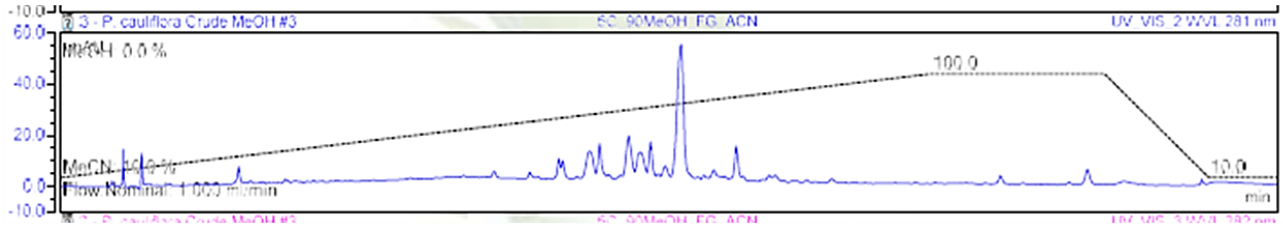

Figure 2: Chromatogram of the extract detected at 281 nm wavelength

CONCLUSION

The chemicals content of *P. cauliflora* leaves were found to be protein, fat, carbohydrate, fiber, alkaloids, terpenoids, and tannins. High protein and moderate alkaloids contents could contribute to the flavor enhancer potential of the plant's leaves. Additional investigations are required to identify the specific alkaloids which give rise to the peaks in the chromatograms and to assess these alkaloids for their potential as flavor enhancers. Furthermore, there is a need for the identification of the free amino acid composition within the crude protein.

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