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Phytochemical Constituents Of Leaves And Stems Of *Pandanus Odoratissimus* In Vietnam

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Abstract:
Background*: Pandanus odoratissimus is traditionally used across Southeast Asia, yet comparative profiling of key secondary metabolites between organs remains limited. This study addressed that gap by comparing phenolics, flavonoids, and saponins in leaves and stems collected from coastal regions of central Vietnam.*
Materials and Methods*: Six leaf and six stem samples were ethanolic-extracted (90%, sonication), and total phenolic content (TPC) was quantified by the Folin–Ciocalteu method (mg GAE/g). Total flavonoid content (TFC) was determined using the $AlCl_3$ colorimetric assay (mg QE/g). Total saponin content (TSC) was determined using the vanillin–sulfuric acid method with aescin as standard (mg AE/g). Alkaloids were qualitatively assessed with Dragendorff's reagent. Data were processed in R; leaf–stem differences were evaluated by permutation tests (10,000 resamplings).*
Results*: Leaves and stems contained comparable TPC on average (58.99 vs 51.65 mg GAE/g), and the difference was not statistically significant (permutation test $p = 0.2902$). Leaves showed markedly higher TFC than stems (22.91 vs 6.74 mg QE/g; $p = 0.0021$). In contrast, stems exhibited substantially higher TSC than leaves (27.87 vs 18.14 mg AE/g; $p = 0.0014$). Alkaloids were not detected in any sample by Dragendorff's test.*
Conclusion: *P. odoratissimus exhibits organ-specific allocation of secondary metabolites: flavonoids are enriched in leaves, saponins in stems, while phenolics occur at comparable levels across organs. The absence of alkaloids aligns with prior reports for this species and highlights reliance on non-alkaloid defenses. These findings refine the phytochemical profile of P. odoratissimus and support targeted utilization of different organs for prospective pharmacological or functional-food applications.*
Keywords: *Pandanus odoratissimus; phenolics; flavonoids; saponin; alkaloids.*

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I. Introduction

Pandanus odoratissimus Linn., commonly known as screw pine, is a species of the genus *Pandanus* (family Pandanaceae) widely distributed across tropical and subtropical regions such as Vietnam, India, Thailand, and several other Southeast Asian countries [1]. Various parts of *P. odoratissimus* have long been utilized in daily life as well as in traditional medicine. The essential oil extracted from its leaves has been used to treat headaches and earaches, and as a topical remedy for musculoskeletal pain and stiffness. In addition, the leaves can be employed as natural breath fresheners or as a preservative for rice [1]. Extracts of *P. odoratissimus* have been reported to exhibit antiviral [2], antibacterial [3], analgesic [4], anti-inflammatory [5], antioxidant [6], and hepatoprotective activities [7]. Phytochemical investigations have revealed that the leaves and stems of *P. odoratissimus* contain diverse bioactive compounds, including phenolics, tannins, terpenoids, saponins, and glycosides [8, 9]. Several compounds previously isolated from this species include eudesmin, pinoresinol, epipinoresinol, de-4′-O-methyleudesmin, and 3,4-bis(4-hydroxy-3-methoxy-benzyl)-tetrahydrofuran [9].

Although *P. odoratissimus* has been reported to possess diverse biological activities and a wide range of phytoconstituents, comparative studies on the distribution of key secondary metabolites between different plant parts remain limited. Therefore, the objective of this study was to assess and compare the levels of phenolics, flavonoids, alkaloids, and saponins in stems and leaves of *P. odoratissimus*. For this purpose, six stem samples and six leaf samples were collected from coastal areas in central Vietnam. This investigation aims to provide insights into the phytochemical composition of *P. odoratissimus* and contribute to the understanding of its potential applications in pharmacology and functional food development.

II. Materials And Methods

Materials

Six leaf samples and six stem samples of *P. odoratissimus* were collected in Nghe An province and Khanh Hoa province, Vietnam, from May 2021 to August 2024. Common solvents such as ethanol, DMSO, and inorganic salts were obtained from Daihan Scientific (South Korea), while reference standards and other chemicals were supplied by Merck (Germany).

Extracts preparation

To prepare the extract for chemical analysis, 50 g of each sample was powdered, then extracted in triplicate with 1 L of ethanol 90% in a sonication bath at 70°C. The solution was filtered and evaporated to yield the total extract.

Total phenolic assay

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method with gallic acid as the standard [10]. Absorbance was measured at 760 nm, and results were expressed as mg gallic acid equivalents per gram of dry sample (mg GAE/g).

Total flavonoid assay

The total flavonoid content was quantified by the aluminum chloride colorimetric method [10] using quercetin as a standard, with absorbance measured at 510 nm and results expressed as mg QE/g.

Alkaloid assay

The total alkaloid content was qualitatively assessed using Dragendorff's reagent, which produces an orange–brown precipitate in the presence of alkaloids [11].

Total saponin assay

The total saponin content was determined by the vanillin–sulfuric acid method [12] using aescin as the reference standard, with absorbance measured at 560 nm and results expressed relative to the blank.

Data processing

All analytical data were compiled and subjected to statistical analysis using R software (version 4.5.1, R Foundation for Statistical Computing, Vienna, Austria). Before analysis, data were checked for completeness and consistency. Group comparisons between leaves and stems samples were performed using the permutation test with 10,000 resamplings to assess differences in total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC). Results are presented as mean ± standard deviation (SD), and a p-value < 0.05 was considered statistically significant.

III. Results And Discussion

Total phenolic contents of the *P. odoratissimus* stems and leaves

The total phenolic content (TPC) of *P. odoratissimus* exhibited variation between leaves and stems (Table X; Figure X). In leaf samples, TPC values ranged from 51.21 ± 7.72 to 71.68 ± 9.58 mg GAE/g, with a mean value of 58.99 mg GAE/g. The distribution of values was relatively consistent, with most samples falling within a narrow range of 55–62 mg GAE/g, suggesting a stable accumulation of phenolic compounds in leaves. In stems, however, TPC values were more dispersed, spanning from 37.01 ± 8.41 to 76.78 ± 8.31 mg GAE/g, with a mean value of 51.65 mg GAE/g. Notably, while one stem sample reached the highest observed TPC across all samples (76.78 ± 8.31 mg GAE/g), several others showed markedly lower values compared to leaves, resulting in higher overall variability in this group. The boxplot representation further illustrates these patterns: leaves displayed a higher and more uniform median TPC, whereas stems showed greater variability, with values spanning both higher and lower than the range observed in leaves. This suggests that phenolic accumulation in stems is less consistent and may be influenced by environmental or developmental factors.

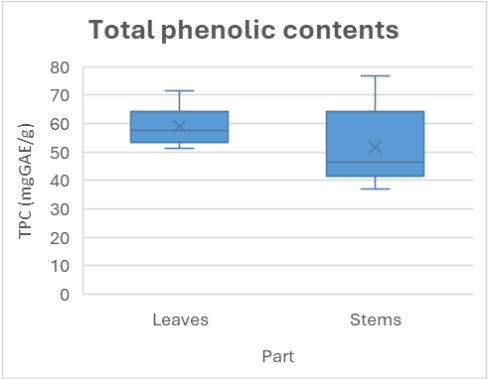

Figure 1. Total phenolic contents (TPC, expressed as mg GAE/g) in leaves and stems of *Pandanus odoratissimus*. Boxplots represent median, interquartile range, minimum, and maximum values, with "×" indicating the mean.

Statistical analysis was performed using a permutation test with 10,000 resamplings to evaluate the difference between leaves and stems. The test revealed that although the mean TPC of leaves (58.99 mg GAE/g) was higher than that of stems (51.65 mg GAE/g), the difference (7.34 mg GAE/g) was not statistically significant ($p = 0.2902$). This indicates that the apparent differences in TPC between the two organs may largely reflect sample-to-sample variability, particularly within the stem group, rather than a consistent tissue-specific pattern.

Overall, these results suggest that both leaves and stems of *P. odoratissimus* contain substantial amounts of phenolic compounds, though leaves tend to accumulate them more consistently. The absence of a statistically significant difference highlights the considerable variability in stems, which may mask potential organ-specific trends.

Total flavonoid contents of the *P. odoratissimus* stems and leaves

The total flavonoid content (TFC) of *P. odoratissimus* showed a pronounced difference between leaves and stems (Figure 2). In leaf samples, TFC values were consistently high, ranging from 18.69 ± 3.58 to 29.27 ± 3.02 mg QE/g, with a mean value of 22.91 mg QE/g. These results indicate that leaves are a rich source of flavonoids, with relatively narrow variation across the six samples analyzed. By contrast, stem samples contained substantially lower amounts of flavonoids, with TFC values ranging from 3.21 ± 0.19 to 9.99 ± 1.45 mg QE/g and a mean of 6.74 mg QE/g. The variability among stem samples was also greater in relative terms, with some stems containing less than one-third of the lowest flavonoid value observed in leaves.

The boxplot further illustrates this stark contrast: leaf samples clustered tightly around higher TFC values, whereas stem samples were distributed within a much lower range. This consistent pattern across replicates suggests a clear tissue-specific difference in flavonoid accumulation. Statistical evaluation using a permutation test with 10,000 resamplings confirmed that the observed difference in TFC between leaves and stems was highly significant ($p = 0.0021$). The magnitude of the mean difference (16.18 mg QE/g) further underscores that leaves of *P. odoratissimus* accumulate markedly higher flavonoid levels than stems.

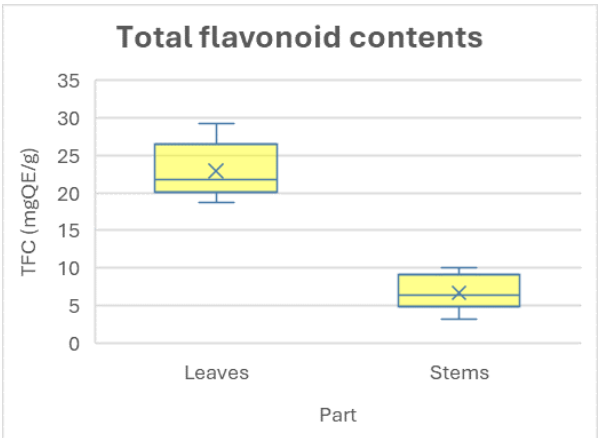

Figure 2. Total flavonoid contents (TFC, expressed as mg QE/g) in leaves and stems of *Pandanus odoratissimus*. Boxplots represent median, interquartile range, minimum, and maximum values, with "×" indicating the mean.

Taken together, these findings demonstrate that flavonoids are predominantly concentrated in the leaves of *P. odoratissimus*. This is consistent with the role of flavonoids as protective secondary metabolites involved in shielding photosynthetic tissues from UV radiation, mitigating oxidative stress, and contributing to defense mechanisms against biotic and abiotic challenges. The relatively low flavonoid content in stems suggests that this organ is less involved in such protective functions compared to leaves.

Total saponin contents of the *P. odoratissimus* stems and leaves

The total saponin content (TSC) of *P. odoratissimus* displayed a clear distinction between leaves and stems (Figure 3). In leaf samples, TSC values ranged from 14.59 ± 1.54 to 20.42 ± 1.83 mg AE/g, with a mean of 18.14 mg AE/g. These results indicate that leaves contain relatively uniform and moderate levels of saponins across samples. In contrast, stem samples exhibited substantially higher TSC, ranging from 21.61 ± 1.08 to 39.25 ± 0.96 mg AE/g, with a mean of 27.87 mg AE/g. Considerable variation was observed among stems, with some samples exceeding twice the lowest value found in leaves. The boxplot highlights this organ-specific distribution: leaves showed a narrow interquartile range and stable TSC values around 18 mg AE/g, while stems exhibited both higher median and wider variability, with several samples reaching values above 30 mg AE/g.

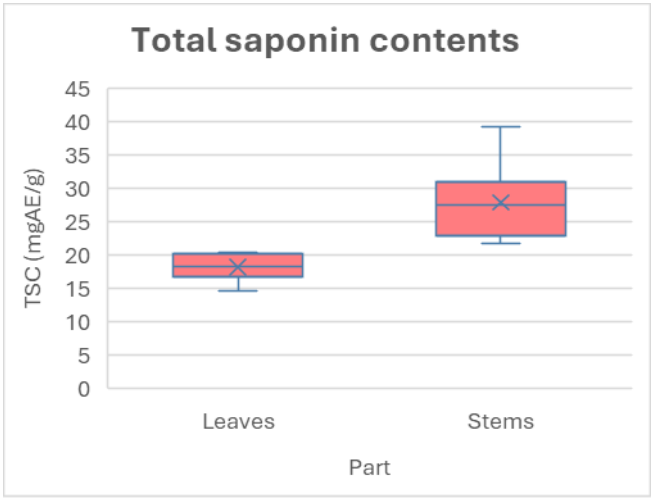

Figure 3. Total saponin contents (TSC, expressed as mg AE/g) in leaves and stems of *Pandanus odoratissimus*. Boxplots represent median, interquartile range, minimum, and maximum values, with "×" indicating the mean.

Statistical analysis using a permutation test with 10,000 resamplings confirmed that the difference in TSC between leaves and stems was highly significant ($p = 0.0014$). The mean difference of –9.73 mg AE/g (leaves – stems) indicates that saponins accumulate predominantly in stems rather than in leaves. These findings suggest that *P. odoratissimus* stems are richer in saponins compared to leaves, consistent with the role of saponins as structural and defensive metabolites often concentrated in supportive and protective tissues. The higher variability among stems may reflect differences in developmental stages or environmental conditions influencing secondary metabolite accumulation.

Discussion

The comparative analysis of secondary metabolites in *P. odoratissimus* revealed distinct distribution patterns across leaves and stems. Phenolic compounds were present in both organs at substantial levels, with leaves showing relatively stable values and stems displaying greater variability. Although the mean phenolic content was slightly higher in leaves than in stems, statistical analysis did not demonstrate a significant difference between the two parts. This suggests that phenolics are broadly distributed in both tissues, but their accumulation in stems may be more heterogeneous, possibly influenced by developmental stage or environmental stress factors. In contrast, flavonoid accumulation was strongly organ-specific. Leaves consistently exhibited markedly higher flavonoid contents than stems, with a statistically significant difference confirmed by permutation testing ($p < 0.01$). The predominance of flavonoids in leaves aligns with their biological role in photoprotection, antioxidant defense, and UV absorption, which are especially critical in photosynthetic tissues exposed to direct sunlight. Saponins displayed an inverse pattern compared to flavonoids: stems contained significantly higher saponin levels than leaves ($p < 0.01$). Saponins are often involved in plant defense, structural integrity, and interactions with herbivores or pathogens. Their higher abundance in stems suggests a protective function in supportive tissues, contributing to structural reinforcement and deterrence of

biotic stressors. The observed variability among stem samples may be associated with differences in physiological maturity or environmental growth conditions.

In addition to these quantitative assays, a qualitative test for alkaloids using Dragendorff's reagent indicated that alkaloids were absent in all analyzed samples. This finding is consistent with previous phytochemical studies reporting that *P. odoratissimus* contains phenolics, flavonoids, and saponins, but not alkaloids. Alkaloid production appears to be limited within the genus, having been reported only in a few species, such as *Pandanus amaryllifolius* [13-16], *Pandanus utilis* [17] and *Pandanus dubius* [18, 19]. The absence of alkaloids in *P. odoratissimus* suggests that its chemical defense strategy relies primarily on non-alkaloid metabolites such as phenolics, flavonoids, and saponins. Taken together, these results provide new insights into the phytochemical composition of *P. odoratissimus*. The distinct distribution patterns of phenolics, flavonoids, and saponins across plant organs highlight the functional specialization of secondary metabolites in response to ecological and physiological demands. The absence of alkaloids further supports the notion that *P. odoratissimus* relies on alternative classes of bioactive compounds for protection and adaptation, distinguishing it chemically from other members of the genus.

IV. Conclusion

This study provides a comparative evaluation of secondary metabolites in the leaves and stems of P. odoratissimus collected from coastal areas of central Vietnam. Phenolic compounds were detected in both tissues at comparable levels, although stems exhibited greater variability. Flavonoids were significantly more abundant in leaves, consistent with their role in photoprotection and antioxidative defense, whereas saponins accumulated predominantly in stems, highlighting their potential contribution to structural reinforcement and defense against biotic stressors. In contrast, alkaloids were not detected, corroborating previous findings that this species does not produce alkaloids, unlike some other members of the genus.

Overall, these results enhance the current understanding of the phytochemical composition of P. odoratissimus and provide a scientific basis for its potential utilization in pharmacological and functional food applications. Further studies focusing on the isolation, characterization, and bioactivity evaluation of individual compounds are warranted to explore the therapeutic potential of this species.

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