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Original Article

Effect of Plant Stage and Solvent Extraction on Catechin Contents in *Borrasus flabellifer* L. Male Flower

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ABSTRACT

Borrasus flabellifer L. is a plant in the Arecaceae (Palmae) family with numerous bioactivities such as diuretic promotion, antioxidant, and antimicrobial effects. In this study, we are interested in the phytochemical compounds present in *B. flabellifer* L. male flowers. We investigated the effects of plant stage and solvent used for extraction. Initially, both the young and mature male flowers of this plant were extracted using water or ethanol. Several standard compounds, including gallic acid, catechin, caffeic acid, and quercetin, were utilized to screen the phytochemicals in these plant flowers. After analyzing the samples using High-Performance Liquid Chromatography (HPLC) method, only catechin was detected in all extracts, while the others were not detected (did not match the peak spectrum pattern compared with standard). Using a detection wavelength and curve parameters with an R^2 value of 0.9999, catechin detection via HPLC was performed at 280 nm within the range of 13.12 to 210.00 µg/mL detection. Regarding the stage of the male flower, the young ones extracted with water provided the highest catechin content at 6.65 µg/mg extract, while the others fell within the range of 4.02 to 4.44 µg/mg extract. Therefore, it has been confirmed that catechin can be found in *B. flabellifer* L., and water should be used as the solvent for extraction for further applications of this plant.

Key words: *Borrasus flabellifer* L., Male flower, Catechin, HPLC, Young, Mature.

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INTRODUCTION

B. flabellifer L., belonging to the Arecaceae (Palmae) family, is commonly found in tropical regions, especially in Asian countries such as India, along the Cambodian-Vietnamese border, Malaysia, and Thailand. It is also occasionally found in Hawaii and South Florida.[1] The plant is dioecious, with male and female flowers present on separate plants. The male palm tree's inflorescence has a spadix (flower spike) and does not bear any fruit. *B. flabellifer* L. is a rich source of nutrients and has been used to treat malnutrition.[2] In East Asian countries, including India, Pakistan,[3] and Thailand, the male flowers of *B. flabellifer* L. are traditionally used to treat patients with diuretic problems.[4] Previous studies have shown that the methanolic extract from the male flowers of *B. flabellifer* L. inhibits the increase of serum glucose levels in sucrose-loaded rats. This effect could potentially be attributed to the presence of spirostane-type steroid saponins.[5] Additionally, diuretic activity was demonstrated in albino rats exposed to both ethanolic and aqueous seedling extracts of *B. flabellifer* L., showing a significant increase in urinary levels of Na^+, K^+, and Cl^-.[6] Previous studies have reported the presence of phytochemical compounds and their corresponding bioactivities in various organs of *B. flabellifer* L. For instance, the root contains alkaloids, flavonoids, and tannins with antimicrobial, antioxidant, and anti-inflammatory activities,[7] while the flowers contain flavonoids, saponins, tannins, and phenolics with antioxidant and anti-inflammatory activities.[8,9] However, it is difficult to find studies on other specific compounds in this plant. Therefore, an attempt to screen the phytochemicals in this plant using the High-Performance Liquid Chromatography (HPLC) method was conducted. Moreover, it is well-known that the metabolites in the plant vary due to various internal factors (such as genetic factors) and external factors (such as location, climate, and harvesting period). Our goal is to investigate the phytochemical compounds in this plant at various stages of flower development and determine the suitable solvent for phytochemical extraction.

MATERIALS AND METHODS

Plants collection and identification

Male flowers and leaves of *B. flabellifer* L. (Figure 1) were collected from Chinat province, Thailand. Both plant organs were used for identification and reference by Miss Tapewalee Kananthong, Expert in Taxonomy, Forestry Officer, the Forest Herbarium, Royal Forest Department, Thailand (Reference BKF No.193907).

Extracts preparation

B. flabellifer L. young and mature male flowers were cut and dried at 50°C before being ground to a powder. The extract was prepared by boiling the dried powder in water for 30 minutes, followed by filtration through Whatman paper (no. 1). The spray drying technique was then applied to remove the water from the extract. Ethanol extracts were prepared by macerating the young and mature flowers with ethanol (1 g plant: 10 ml ethanol). The mixture was sonicated for 30 minutes before undergoing maceration with agitation for 7 days. Sonication was chosen for extraction due to its ability to use high-energy sound waves to facilitate solvent penetration beyond the cell walls into the plant cells. This process increases surface contact between solvents and samples, enhances mass

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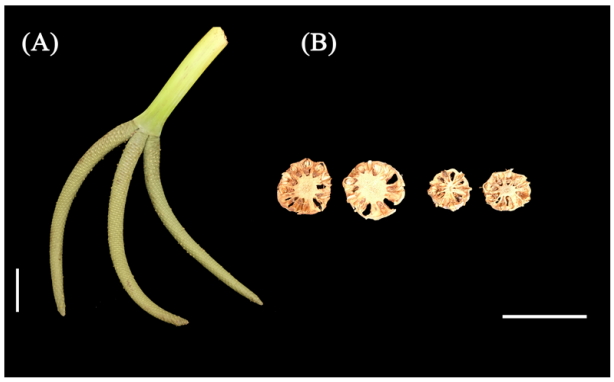

Figure 1: Fresh (A) and dry cut-male flowers (B) of *B. flabellifer* L. the bar indicates 10 cm

transport of the solvents into the plant cells, and facilitates the release of compounds. After macerating for 7 days, ethanol was removed using a vacuum rotary evaporator. Subsequently, the crude extracts, including young-water (YW), mature-water (MW), young-ethanol (YE), and mature-ethanol (ME) extracts, were stored at -20°C before analysis of phytochemical compounds using HPLC.

Standard solution and calibration curve establishment

Standard stock solutions of four flavonoids were prepared in methanol, at concentration of 2.1 mg mL^{-1} for catechin, 1.9 mg mL^{-1} for quercetin, 1.2 mg m^{L-1} for gallic acid, and 2.8 mg mL^{-1} for caffeic acid. To determine the linear relationship between peak areas and concentration of each component, a range of 13.12 - 210 µg mL^{-1} for catechin, 11.88 - 190 µg mL^{-1} for quercetin, 17.50 - 280 µg mL^{-1} for caffeic acid and 15.00 - 240 µg mL^{-1} for gallic acid were tested. Five concentrations for each standard were analyzed. The linearity equations were calculated by linear regression analysis.

Chromatographic analysis

HPLC analysis was operated using HPLC system Shimadzu (LC-20A pump, SPD-M20A diode array detector, CTO-20 A column temperature box, SIL-20A auto sample injector, DGU-20A degassing device). The chromatography was performed on Inertsil® ODS-3 C18 reversed-phase column (4.6 X 250 mm) pack with 5 µm diameter particles. The mobile phase was a gradient prepared from 0.5% acetic acid in water (A) and 0.5% acetic acid in acetonitrile (B). The elution program was design as follows: 0 min (5% B), 0 - 21 min (5 - 20% B), 21 -25 min (20 - 25% B), 25 - 26 min (25 - 50% B), 26 - 30 min (50 - 50% B), 30 -31 min (50 - 100% B), 31 - 40 min (100 - 100% B), 40 - 41 min (100 - 5% B) and 41 - 45 min (5 % B). The flow rate was 1 mL min^{-1}. The column temperature was set at 30°C and the sample injection volume was 10 µL.

RESULTS AND DISCUSSION

Effect of detection wavelength

The choice of the proper detection mode is crucial to ensure that all the components are detected. This problem can be overcome by using a multiple-wavelength scanning mode with a diode array detector (DAD), which is capable of monitoring several wavelengths simultaneously. The UV-vis spectrum of gallic acid, catechin, caffeic acid, and quercetin standards dissolved in the mobile phase was obtained by the DAD. UV-vis absorbing spectrograms of the four flavonoids are shown in Figure 2. The one-peak spectrum of gallic acid, caffeic acid, and catechin appeared at 270 nm, 323 nm, and 279 nm, respectively, while two peaks of quercetin were observed at 255 nm and 369 nm. The peaks at 255 nm of gallic acid, caffeic acid, and quercetin are lower than at 280 nm. Therefore, 280 nm was confirmed as the determination wavelength for all of the standards.

The method for simultaneously determining the four components in extracts showed no interferences when demonstrated using HPLC, as shown in Figure 3. It can be seen that good separation can be achieved using the given conditions. Symmetrical, sharp, and well-resolved peaks were observed for gallic acid, catechin, caffeic acid, and quercetin. The retention times were 8.3 min, 19.5 min, 22.5 min, and 33.6 min, respectively. Then, the peak spectra of extracts were investigated and compared with those of the standards. The results showed that, although the detected compounds in extracts and all four standards occurred at the same retention time, only the patterns of catechin peak spectra were the same, while the others were not. This might be the result of the plant material, or impure compounds in the extract interfering with the absorbance during detection. It may be necessary to modify the extraction method to increase the ability to obtain a higher content, do partition to get higher amount of specific compound, along with utilizing a detection method of higher sensitivity than this. Therefore, the identification of compounds in *B. flabellifer* L. focused solely on catechin.

Calibration curves

The calibration curves were generated by plotting the peak area of the analyte against its concentration for five different levels of concentration (Figure 4). Each concentration of the mixed standard solution was injected in triplicate, and regression parameters were computed. These results highlight the applicability of external standard calibration for quantitative analysis. The coefficient of determination (R^2) is 0.9999, as indicated in Table 1, along with detailed regression equation data.

Catechin contents in the extracts

The extracts were injected into HPLC system and separated under the condition that were explained earlier. The chromatograms of each extract were shown in Figure 5.

Each sample was analyzed in triplicate and identified based on the retention times and peak spectra in comparison with standards. The contents of the extracts were shown in Table 2.

The different letters within the column refer to the significant differences derived from statistical analyses. The test values are presented as Mean ± SD, and were analyzed by ANOVA with Duncan's Multiple Range Test at $p < 0.05$.

The results of the experiment, when compared in terms of young flowers, reveal that the extract obtained from water extraction contains a higher amount of catechin (6.65 µg/mg extract) compared to extraction using ethanol (4.02 µg/mg extract), which is significantly higher by a

Table 1: Calibration curve parameters.

Parameter	Value
Compound	Catechin
Range (µg. mL^{-1})	13.12 - 210.00
Equation	y = 6,833.17x + 866.21
R^2	0.9999

Table 2: Determination of the catechin contents (µg $mgExtract^{-1}$) in *B. flabellifer L.* extracts.

Extract	Catechin (µg/mg Extract)
Young-Water	6.65 ± 0.03 a
Young-Ethanol	4.02 ± 0.05 c
Mature-Water	4.41 ± 0.05 b
Mature-Ethanol	4.44 ± 0.04 b

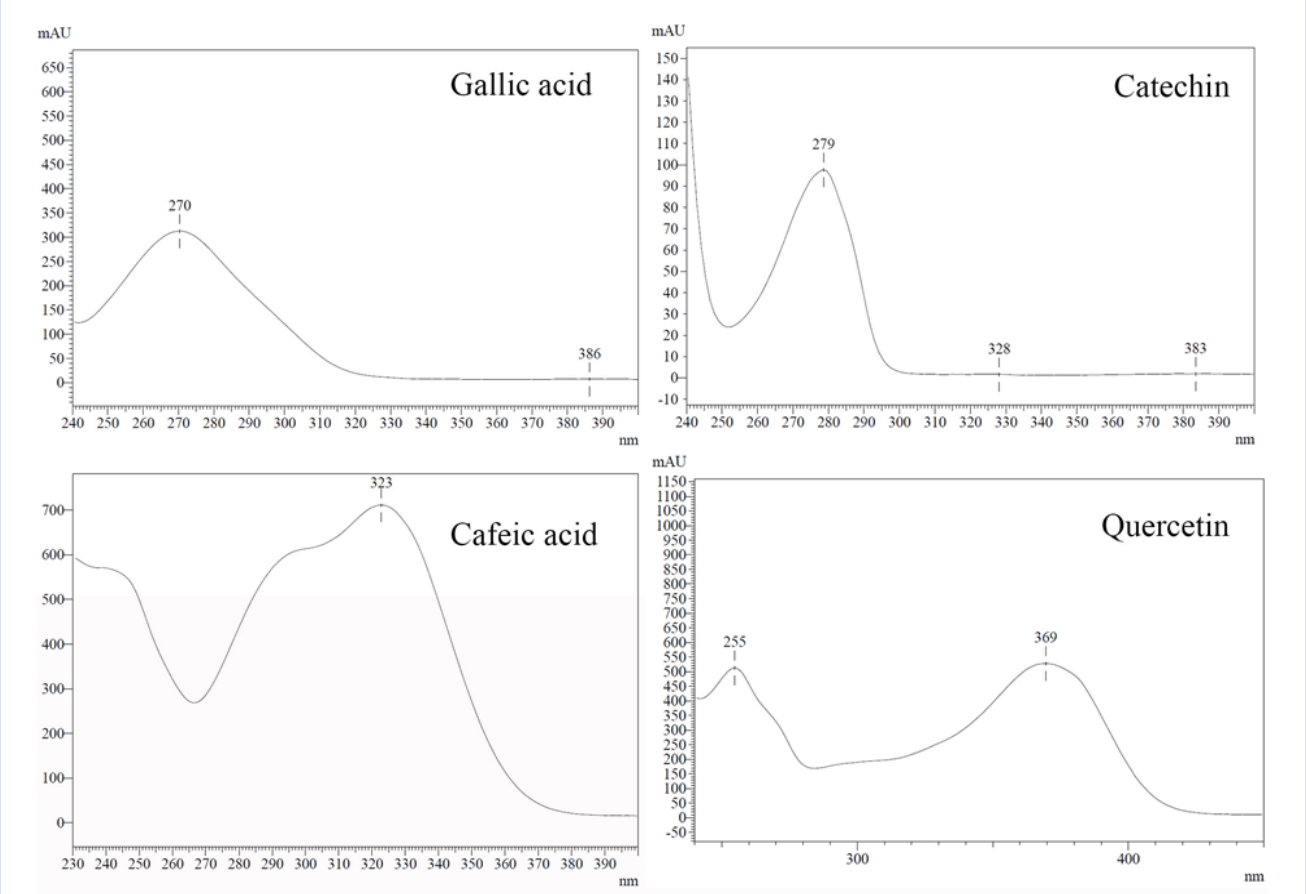

Figure 2: UV-vis absorbing spectrograms of gallic acid, catechin, caffeic acid, and quercetin

factor of 1.65 times. As for the extraction from mature male flowers, the detected catechin quantity does not exhibit a statistically significant difference. Upon investigating the factors related to the stage of male flower development concerning catechin content, it was found that when comparing the water extracts of young and mature male flowers of *B. flabellifer* L., the young material provides higher catechin content than the mature one. However, when comparing ethanol extracts, it is observed that the mature extract yields higher catechin content.

Catechins can be found in various plants, such as apples, cherries, grapes, strawberries, and tea.[10-13] The catechin content in these plants varies within a range of 2-80 mg/100 g sample. Particularly, green tea (*Camellia sinensis*) is a major source of this compound, containing 10-80 mg/100 g sample.[13,14] In this study, young male flowers were found to provide the highest content of catechin at 6.65 µg/mg of extract (equivalent to 106 mg/100 g sample), a quantity that tends to be greater than in green tea. This finding is interesting because catechins have been reported in various research works to promote diuretic activity at both preclinical and clinical levels. The diuretic effect of the aqueous extract of green tea leaves was investigated in Swiss male mice using doses ranging from 14-70 mg/kg of body weight. The study found that green tea led to an increase in glomerular filtration rates, which in turn affected its diuretic activity.[15] Another study explored the interaction of green tea extract with hydrochlorothiazide on diuretic activity in rats, using high (500 mg/kg, p.o) and low (100 mg/kg, p.o) doses of green tea extract.[16] The results revealed that the combination increased the diuretic potential. Furthermore, the evaluation of (-)-epicatechin on diuretic activity and saluretic effects, combined with standard diuretics, was conducted in female Wistar normotensive and spontaneously hypertensive rats at doses of 0.3, 1, and 3 mg/kg.[17] The study demonstrated that (-)-epicatechin was able to stimulate both diuresis and saluresis (Na^+, K^+, and Cl^-), without interfering with plasma electrolyte content, urinary pH, and uric acid values. In traditional medicine in certain countries, including Thailand, *B. flabellifer* L. has been mentioned for its use as a diuretic agent to promote urine excretion. The presence of catechin in this plant might be the reason behind its ability to enhance diuretic properties. The extraction of catechin from tea is always mentioned in the way of using water as a solvent for extraction.[18] Various solvents, including citric acid water, ethanol, and water, were tested for the isolation of catechins and caffeine from Turkish green tea.[19] The results confirm that hot water extraction (at 80°C) yields higher catechin content compared to other solvents.

In this study, we not only examine the solvent used for extraction but also focus on another important factor: the stage of male flower. The results of this study show that when water is used as the solvent for extraction, the young material presents a significantly higher content of catechin (1.5 times) compared to the mature material. This finding aligns with a previous study that extracted tea from *Camellia sinensis* var. sinensis.[20] Additionally, the results indicate that the content of all catechins, except for catechin gallate, in the shoots is higher than what is detected in the mature leaves, with some catechins showing an almost 3.6-fold increase. However, in any case, within the *B. flabellifer* L., there are still several other important substances that require further study. Apart from catechin, which is one compound with notable diuretic properties, along with other compounds in this plant. Therefore, it is necessary to continue screening for additional biomarkers.

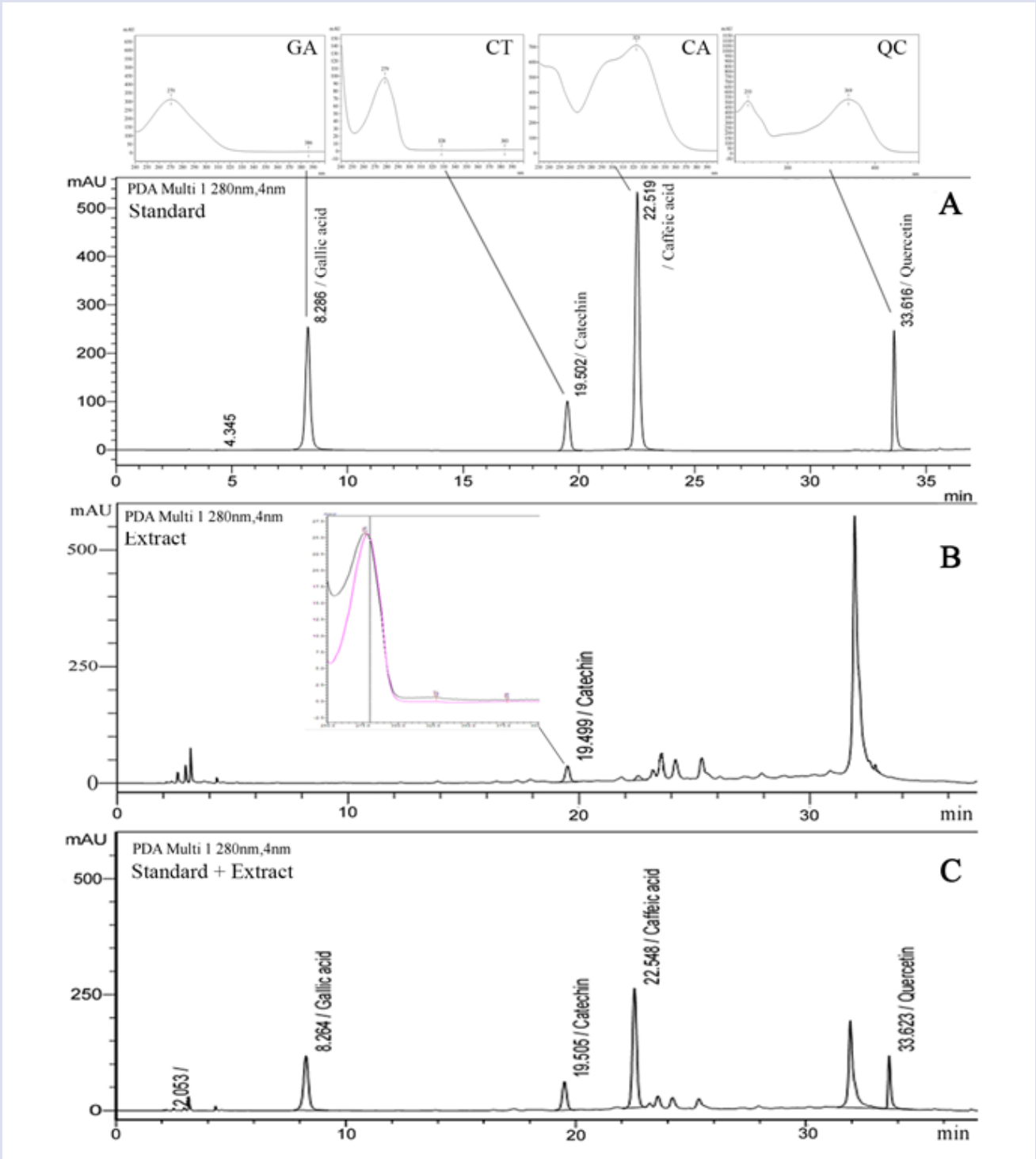

Figure 3: Chromatogram of standards (A), *B. flabellifer* L. extract (B) and Mixuture of standards and extract (C)

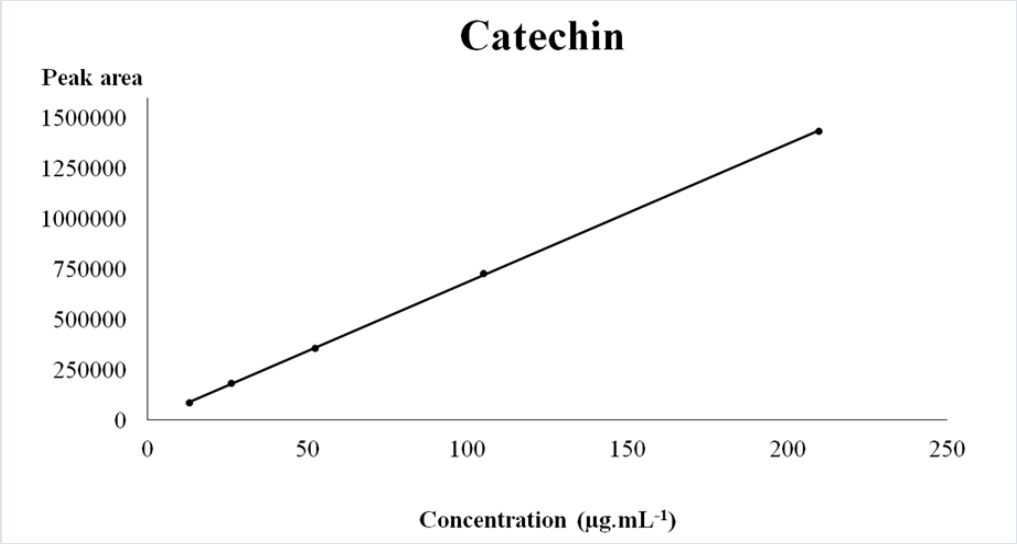

Figure 4: Calibration curves of catechin standard

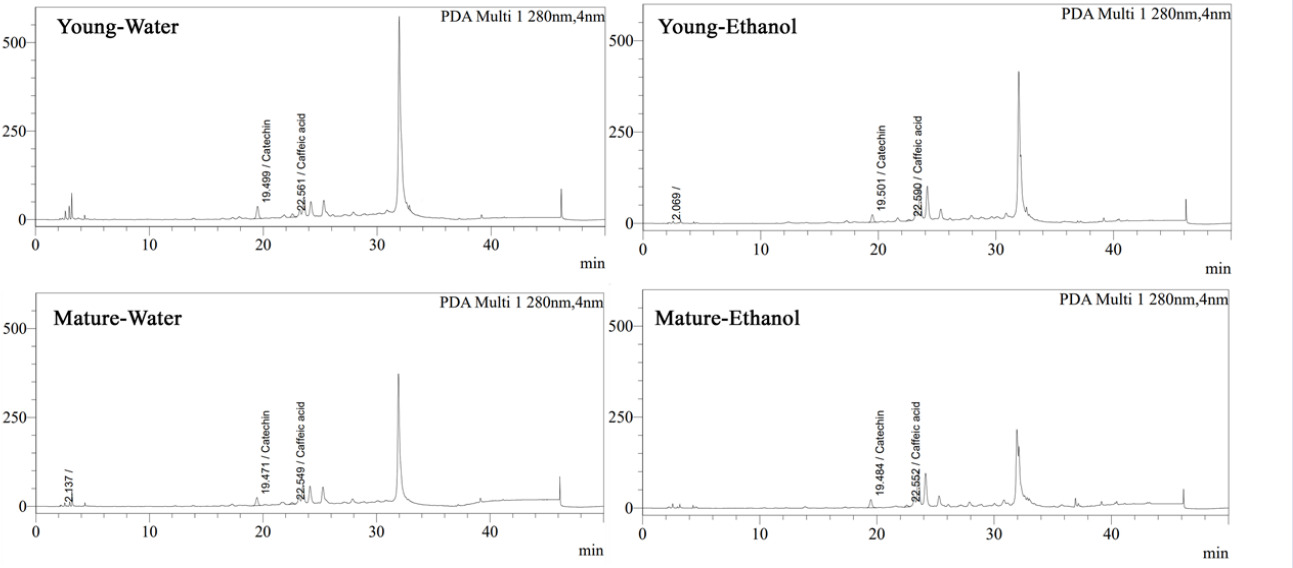

Figure 5: HPLC chromatograms of *B. flabellifer* L. extracts

CONCLUSION

After studying the important compounds in *B. flabellifer* L. using the HPLC technique under the stated conditions, it was found that catechin is present, but gallic acid, caffeic acid, and quercetin were not detected. Based on this experiment, if a significant amount of catechin is desired, it is recommended to utilize young male flowers and extracted with water.

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CREDIT AUTHORS STATEMENT

Patnaree Wongmanit collected plant and prepared extracts. Chayut Fongsuk operated the methodology of phytochemical analysis, experiments of HPLC, data collections and analysis. Kanoktip Pansuksan conducted the conceptualization, funding acquisition, resources, plant extracts preparation, interpret the data, writing the original draft and finalization the manuscript, project administration.

DECLARATION OF COMPETING INTEREST

All the authors declare that they have no conflicts of interest.

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