

ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY ACTIVITY OF *DURIO ZIBETHINUS* FLOWER

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Abstract

Durio zibethinus, a well-known plant has garnered scientific interest due to its reported medicinal properties. This study investigated the antioxidant effects of flavonoid-enriched ethanolic extract derived from *D. zibethinus* flowers, specifically focusing on its ability to inhibit xanthine oxidase *in vitro*. The *D. zibethinus* flower ethanolic extract exhibits significant antioxidant effects and xanthine oxidase inhibitory activity, suggesting its promise as a natural therapeutic agent for Gout treatment.

Introduction

The plant *Durio zibethinus* is a rich source of diverse secondary metabolites, including various classes of flavonoids, polyphenols, carotene, tannins, ascorbic acid, saponins, and alkaloids. Furthermore, its composition includes steroids, mucilage, glycosides, carbohydrates, reducing sugars (Zhan *et al.* 2021, Tran *et al.* 2024). Historically, *D. zibethinus* leaves have been employed in traditional medicine for the treatment of abdominal pain, bacterial infection, infertility (Ho and Bhat 2015, Ansari 2016, Tran *et al.* 2024). The fruit, conversely, has demonstrated notable antioxidant, antimutagenic, antihypercarcinogenic, anti-inflammatory, and antimicrobial properties (Charoenphun and Klangbud 2022, Tran *et al.* 2024). Given the escalating interest in herbal medicines supported by rigorous clinical trials and characterized by their bioactive pharmacological content, *D. zibethinus* stands out as a plant with significant therapeutic promise due to its complex array of beneficial compounds. The knowledge regarding the efficacy of *D. zibethinus* provides a compelling foundation for comprehensive investigations aimed at the discovery of novel therapeutic compounds and a deeper elucidation of its pharmacological constituents. Therefore, further research on *D. zibethinus* is warranted to fully explore its extensive therapeutic activities.

Therefore, bioactive compounds could be extracted to transform those wasted thined flower value-added products and reduce agricultural waste. This study evaluate the potential of ethanolic extract of *D. zibethinus* flower in the treatment of Gout via antioxidant property and xanthine oxidase inhibitory activity.

Materials and Methods

Ethanol (99%) (EtOH), quercetin, ascorbic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and other reagents were analytical grade (HPLC grade) was provided by Sigma-Aldrich, Singapore.

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The flower of *Durio zibethinus* were collected from Di Linh district, Lam Dong province, Vietnam. The plants were taxonomically identified by the Research group in Pharmaceutical and Biomedical Sciences. After harvesting, the flower was decontaminated under running tap water, then was dried at 60°C for 72 hrs (Tran *et al.* 2024).

Flavonoids were isolated from *D. zibethinus* flowers utilizing a maceration technique. One gram flower samples were steeped in 70% ethanol at a 1 : 20 (w/v) sample-to-solvent ratio for 24 hrs. To assess the influence of extraction parameters, experiments were conducted across a temperature range of 40-80°C. The mixtures underwent homogenization via constant shaking for 4 hrs using an IKA homogenizer (Germany). The resulting extract was filtered using Whatman No. 1 filter paper. The combined filtrates were then subjected to solvent evaporation at 60°C using a rotary evaporator. The resulting flavonoid-enriched samples were subsequently dried under vacuum at room temperature for 3 hrs to yield solvent-free material, which was then stored at 4°C for subsequent analyses (Tran *et al.* 2024).

The total flavonoid content (TFC) of the extracts was determined using a modified aluminum chloride colorimetric assay described by Tran *et al.* (2024). 1 gm dry sample was reconstituted in ethanol. After that, 1 mL of sample or quercetin standard solution (20-100 mg QE/g) was combined with 4 ml of distilled water. A reagent blank was prepared using distilled water. Subsequently, 0.3 ml of 5% NaNO₂, 0.3 ml of 10% AlCl₃, and 0.5 ml of 1 M potassium acetate were sequentially added. The reaction mixture was incubated at room temperature for 10 minutes, and absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Quantification of TFC was performed using a calibration curve generated with quercetin as the standard ($y = 0.002x - 0.0024$, $R^2 = 0.9991$).

DPPH radical scavenging activity of the extract was evaluated according to Tran *et al.* (2024). Briefly, serial dilutions of the extract (20, 40, 60, and 80 µg/ml) were prepared. 1 ml of each diluted extract was then mixed with 1 mL of 0.004% DPPH solution (in 50% aqueous ethanol) and incubated at 37°C for 30 min. Following incubation, the absorbance of the solution was measured at 517 nm using a UV-Vis spectrophotometer (V-730, Jasco, USA).

DPPH scavenging activity (% inhibition) was calculated using the following equation:

$$\% \text{ inhibition} = \frac{A_0 - A}{A_0} \times 100$$

Ascorbic acid served as a positive control. The IC₅₀ value was determined by plotting the sample concentration against the percentage of residual DPPH.

The ABTS assay was conducted following a previously established protocol (Hussen and Endalew 2023). 5 µl of *D. zibethinus* flower ethanolic extract, prepared at concentrations ranging from 50 to 300 µg/ml, were mixed with 4 ml of the prepared ABTS^{•+} solution. These mixtures were incubated in the dark at room temperature for 2 hrs, after which the absorbance was measured at 734 nm using a UV-Vis spectrophotometer. A mixtures of 10 ml of 7 mM ABTS, 2.45 mM K₂S₂O₈ and 20 ml of ethanol was used as control. The percent scavenging of ABTS^{•+} radical was determined using the following equation:

$$\text{ABTS \% Scavenging} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The antioxidant activity of *Durio zibethinus* flower ethanolic extract against ABTS^{•+} was expressed as IC₅₀.

XO inhibitory activity of the extract was determined by enzymatic-colorimetric method, using a standard kit (MAK078, Sigma-Aldrich, USA). XO activity of standard XO (X4376, Sigma-Aldrich, USA) was measured in condition with or without extracts (50, 100, 200 and 400 µg/ml)

or positive control (Allopurinol). The XO inhibitory activity of the extracts/control was calculated using the following equation:

$$\% \text{ inhibition} = 1 - \frac{\text{XO activity of the samples/control}}{\text{XO activity of blank}}$$

The IC_{50} , half maximal inhibitory concentration of the extract, was calculated from the concentration-response curve.

Data were analyzed using IBM SPSS ver. 20.0 (IBM, Chicago, IL, USA). ANOVA was employed for statistical analyses. Post-hoc Fisher's least significant difference (LSD) pairwise comparison tests were then conducted. p values < 0.05 were considered to be significant.

Results and Discussion

Effect of temperature on the total flavonoid content (TFC) of ethanolic extract from *D. zibethinus* is presented in Table 1. A significant positive correlation between increasing temperature and TFC was observed, with a maximum yield of 358.67 ± 7.64 mg QE g^{-1} extract achieved at 60 °C. However, further elevation of the extraction temperature to 70 °C to 80 °C resulted in a reduction in TFC. This phenomenon is attributed to the dual effect of temperature: while higher temperatures initially enhance extraction efficiency, prolonged exposure to elevated temperatures can lead to the degradation of heat-sensitive phytochemicals. Consequently, 60°C was identified as the optimal temperature for maximizing flavonoid extraction from durian flower under the specified ethanolic conditions.

Table 1. Effects of extraction temperature on TFC of durian flower ethanolic extracts.

Temperature (°C)	TFC (mg QE/g)
40	$197,00 \pm 10,00^c$
50	$273,67 \pm 2,89^b$
60	$358,67 \pm 7,64^a$
70	$287,00 \pm 5,00^b$
80	$162,00 \pm 5,00^c$

Data represent the Mean \pm SD (n = 3). Values with different superscript lowercase letters (a-c) within a column of each extraction factor indicate statistically significant differences ($P < 0.05$). TFC: total flavonoid content, mg QE/g extract, mg of quercetin equivalents per gram of dry matter.

The antioxidant activities of *D. zibethinus* flavonoid-enriched ethanolic extracts by DPPH and ABTS assay is demonstrated in Table 2. The antioxidant effect of *D. zibethinus* flavonoid-enriched ethanolic flower extract was relatively low ($IC_{50} = 146.94 \pm 2.81$ $\mu\text{g/ml}$ and $178,51 \pm 0,99$ $\mu\text{g/ml}$) compared to ascorbic acid. Additional research should be conducted to evaluate the antioxidant activity of *D. zibethinus* flavonoid-enriched ethanolic flower extract *in vivo*.

The xanthine oxidase inhibitory effect of *D. zibethinus* flavonoid-enriched extracts is presented in Table 3. The flavonoid-rich ethanolic extract from *D. zibethinus* may be beneficial in preventing or treating conditions associated with high uric acid levels. Result indicated that *D. zibethinus* flavonoid-enriched ethanolic extracts exerted good XO inhibitory effects. Therefore, further isolation and structural elucidation of compounds from *D. zibethinus* extract is feasible to achieve promising active compounds

Table 2. Antioxidant effect of *D. zibethinus* flavonoid-enriched ethanolic extract evaluated by DPPH assay.

Samples	IC ₅₀ values (µg/ml)	
	DPPH assay	ABTS assay
<i>D. zibethinus</i> flower flavonoid-enriched ethanolic extracts	146.94 ± 2.81 ^a	178,51 ± 0,99 ^a
Ascorbic acid	31.72 ± 3.14 ^b	14,98 ± 0,37 ^b

Data represent the Mean ± SD (n = 3). Values with different superscript lowercase letters (a–b) within a column indicate statistically significant differences ($P < 0.05$).

Table 3. Xanthine oxidase inhibitory effect of *D. zibethinus* flower flavonoid-enriched ethanolic extract.

Samples	IC ₅₀ values (µg/ml)
<i>D. zibethinus</i> flower flavonoid-enriched ethanolic extracts	135,32 ± 4,66 ^a
Allopurinol	7.52 ± 1.50 ^b

Data represent the Mean ± SD (n = 3). Values with different superscript lowercase letters (a–b) within a column indicate statistically significant differences ($P < 0.05$).

It is concluded that an extraction temperature of 60°C optimizes the total flavonoid content (TFC) in *D. zibethinus* ethanolic extract. While the observed antioxidant activity was comparatively modest, prior research indicates that subsequent fractionation processes could enhance this activity. The flavonoid-enriched *D. zibethinus* extract demonstrated promising xanthine oxidase inhibitory effects. These findings suggest that future research should conduct the isolation and structural elucidation of compounds together with *in vivo* animal studies to further evaluate the extract's anti-gout potential.

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