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**Original article**

# Study on the analgesic, anti-inflammatory and hypouricemic effects of 50% ethanolic extract from *Jasminum subtriplinerve* Blume

Tuoi Thi Hong Do[a*], Quang Nhat Tu[b], Tinh Quoc Vo[a], Oanh Thi Kim Nguyen[c], Tuyen Le Thanh Nguyen[c]

[a]*Department of Pharmacology, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam;*
[b]*Laboratoire de Biologie et Pharmacologie Appliquée CNRS, Ecole Normale Supérieure Paris Saclay, Université Paris-Saclay, France;*
[c]*Saigon pharmaceutical science and technology center, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam.*

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**Abstract:** Previous studies have reported *in vitro* antioxidant and inhibitory activity on xanthine oxidase of *Jasminum subtriplinerve* Blume, Oleaceae, which suggested the potential prevention of gout and supplementary treatment. This study evaluated analgesic, anti-inflammatory, and hypouricemic effects of the 50% ethanolic extract of *J. subtriplinerve* (EEJS) at the oral doses of 800 and 1200 mg/kg in mice. For acute oral toxicity, after oral administration of single doses of EEJS; mortality and toxic signs in male and female mice were noticed within 72 hours and 14 days. The analgesic effect was observed in acid acetic induced writhing in mouse model within 40 minutes. The anti-inflammatory effect was determined in mice-induced edema by 1% carrageenan. The hypouricemic effect was evaluated in mice with the peritoneal injection of potassium oxonate inducing acute and chronic hyperuricemia. The results showed that there was not any toxic sign in mice given orally at the maximum dose ($D_{max}$) of 20 g EEJS/kg. At the dose of 800 and 1200 mg/kg, EEJS did exhibit analgesic effect until 40$^{th}$ minute. EEJS 1200 mg/kg expressed acute anti-inflammatory effect. EEJS had no acute hypouricemic effect at the oral doses of 800 and 1200 mg/kg. When given to mice with chronic hyperuricemia, 800 mg/kg EEJS reduced 30-44% blood uric acid concentration compared to pathological group. In conclusion, EEJS did not cause any toxic sign in mice at the $D_{max}$ of 20 g/kg. This extract had analgesic and chronic hypouricemic effects at the oral dose of 800 mg/kg in mice.

**Keywords:** *Jasminum subtriplinerve* Blume; acute toxicity; anti-inflammatory effect; analgesic effect; hypouricemic effect.

## 1. INTRODUCTION

In recent years, gout has become common arthritis caused by chronic hyperuricemia (serum uric acid levels of > 6.8 mg/dL) associated with the content of monosodium urate crystals in joints [1]. Intracellular oxidation has been reported to increase uric acid production [1]. The current gout medications have many side effects such as intestinal lesions, kidney problems, neutropenia, and anemia, especially when used for a long time [1]. Therefore, finding medicines of natural origin is extremely necessary due to the potent efficacy with fewer side effects of medicinal plants [2]. Our previous study reported Savigout capsule from a mixture of *Panax vietnamensis* Ha et Grushv. extract and aqueous extract of flower buds of *Cleistocalyx operculatus* (Roxb.)

*Address correspondence to Tuoi Thi Hong Do at the Department of Pharmacology, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Dinh Tien Hoang Street, 70000, Ho Chi Minh City, Vietnam; E-mail: hongtuoi@ump.edu.vn

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Merr and Perry exhibited acute and chronic uric acid-lowering effects at the doses of 185 and 370 mg/kg, in which hypouricemic effects of 185 mg/kg were like those of 10 mg/kg allopurinol [3]. According to Truong Tuyet Mai et al (2013), after 12 weeks, diabetic patients treated with aqueous extract from *C. operculatus* flower buds (25 g of plant materials was added to 1000 ml of water and then boiled for about 10 minutes and used within 1 day) showed a reduction in uric acid compared to that of the placebo group [4]. Moreover, xanthine oxidase relates to metabolic disorders including hyperuricemia and gout because that it catalyzes the hypoxanthine and xanthine to uric acid. Therefore, the recent studies screened for xanthine oxidase inhibitory medicinal plants from Vietnam including *Blumea balsamifera*, *Chrysanthemum sinense*, *Tetracera scandens*, *Caesalpinia sappan*, *Gomphrena celosiode*, *Salvia officinalis*… suggesting a potential source of novel pharmaceuticals [2, 5, 6]. Additionally, a clinical trial in Taiwanese people showed that vegetarian diets reduce the risk of gout [7]. However, some plant-based nutrition with high purine levels (e.g, beans, soy, chickpeas, and pea) could be a risk factor of hyperuricemia [8]; hence, it is necessary to provide scientific evidence for plant utilization in gout patients.

In Vietnam, Vang se (*Jasminum subtriplinerve* Blume, Oleaceae) has been popularly used as a tea and in traditional remedy associated with the chemical compositions of flavonoids, polyphenols, and terpenoids [9-11]. Do N. Dai *et al*. (2016) showed that the oil constituents of the *J. subtriplinerve* leaves include α-terpineol, geraniol, linalool, and cis-linalool oxide [9]. Using the ethyl acetate extract of the aerial parts of *J. subtriplinerve*, Nguyen Thi Hong Huong et al (2008) isolated 6'-O-menthiafoloylverbascoside, isoverbascoside, iso-oleoverbascoside, apiosylverbascoside, rutin, isoquercitrin, astragalin, and verbascoside [10]. Nguyen Chi Bao (2016) isolated oleanolic acid, betulinic acid as well as a mixture of β-sitosterol and stigmasterol from the *J. subtriplinerve* aerial parts [11]. *J. subtriplinerve* has been widely used as a tea in the traditional medicine and a commercial extract for postpartum women to treat infections, metritis, mastitis, agalactia, and arthritis. The ethyl acetate, ethanol, methanol, and water extracts from *J. subtriplinerve* have been reported *in vitro* antioxidants [12]. This plant also exhibited inhibitory activity on xanthine oxidase as well as anti-inflammatory effect *via* nitric oxide-inhibitory assay [2, 13]; which suggested the potential gout prevention and treatment assistance. Therefore, this study evaluated analgesic, anti-inflammatory, and hypouricemic effects of the 50% ethanol extract of *J. subtriplinerve* (EEJS) at the oral doses of 800 and 1200 mg/kg in mice.

## 2. MATERIALS AND METHOD

### 2.1. Preparation of EEJS

Small branches with leaves and flowers of *J. subtriplinerve* were collected in Phu My Town, Ba Ria - Vung Tau Province, Vietnam in September 2019. Materials were air-dried at ambient temperature, ground to powder (100 g with an average humidity of 6.6%) and extracted with 50% ethanol under percolation protocol: dried powdered samples were packed in the percolator, added with 2000 ml of 50% ethanol, and macerated for 24 hours; the outlet of the percolator was opened so that the liquid trickled down slowly (1 ml per minute) until the completed extraction. The EEJS was evaporated almost to dryness on a rotary evaporator and a 50 $^{o}$C water bath [14]. The concentrated extract (34.4 g) had average humidity of 11.73 ± 0.24%; the extraction efficiency was 36.8%.

### 2.2. Animals

Healthy male and female *Swiss albino* mice (154 animals) with an age of 6 - 7 weeks, weighing 28 - 32 g from the Nha Trang Institute of Vaccine and Medical Biology, IVAC (Nha Trang, Vietnam) were used. The animals were fed on free access to food pellets (IVAC, Nha Trang, Vietnam) and water ad libitum, maintained at room temperature (27 ± 3 $^{o}$C), with a 12 hours light/dark cycle. Mice were acclimatized to experimental conditions for 5 days before the experiment. All experimental protocols were carried out under the agreement of the scientific committee, specialty of Biochemistry, Microbiology, Parasitology, and Pharmacology, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam (Decision No. 1500/QĐ-ĐHYD on the 21$^{st}$ May 2019 and Decision No. 4104/QĐ-ĐHYD on the 18$^{th}$ September 2019). The number of animals was minimized to follow 3R rule.

### 2.3. Identification of phenolic compounds in EEJS

EEJS was dissolved in 50% ethanol and used for histochemical tests to identify the presence of phenolic compounds with 2% ferric chloride, 20% sodium hydroxide, and 1% lead acetate (Chemsol Company, Vietnam).

### 2.4. Quantification of total phenolics content [15]

EEJS was dissolved in distilled water at the concentration of 200 µg/ml and filtrated through a paper filter. Then, 0.5 ml of EEJS and the standard pyrogallol (Sigma-Aldrich, USA) solutions at different concentrations (10 - 50 µg/ml) were taken to test tubes before 0.5 ml of Folin - Ciocalteu reagent (10%; w/v, Merck, Germany) was added. After 5 minutes, 1.5 ml of sodium carbonate solution (29%; w/v, Chemsol Company, Vietnam) and 2.5 ml of distilled water were added to the mixtures and well shaken. The mixtures were incubated at room temperature under dark conditions for 30 minutes. The absorbance of solutions was measured at 760 nm on a UV-Visible spectrophotometer. The blank sample including Folin - Ciocalteu reagent, sodium carbonate solution and distilled water were carried out at the same time. The total phenolic content repeated 3 times was expressed as mg of pyrogallol equivalents/ g of EEJS.

### 2.5. Acute toxicity study

The study on the acute toxicity of the EEJS was conducted in mice according to the guideline for preclinical and clinical trials of traditional medicines and natural products of the Vietnamese Ministry of Health [16]. For this study, 10 healthy adult mice of both sexes (5 males, 5 females) were kept without food for at least 12 hours before dosing and access to water ad libitum. Mice were orally administered with a single dose of EEJS at the highest concentration in which EEJS could be easily transferred *via* mouse gavage needle (0.4 g/ml) using oral maximum volume for mice (50 ml/kg). Mice were observed individually for any toxic sign or mortality at

least once during the first 30 minutes after dosing within the first 4 hours, then once a day during the first 72 hours, and daily thereafter for a total of 14 days. At the end of the study, organs were excised after killing mice and pathologically observed.

### 2.6. Evaluation of the peripheral analgesic effect on acetic acid-induced pain model [17]

Healthy male mice (40 animals) were randomly divided into 4 groups (8 animals per group) and given oral administration of 10 ml/kg distilled water (pathology group) or 10 mg/kg diclofenac (Novartis, Swiss) (control group) or EEJS at the doses of 800 mg/kg and 1200 mg/kg. After 30 min, mice were injected intraperitoneally with a single dose of 0.7% (v/v) acetic acid (Prolabo, France) to induce the writhing noticed every 5 minutes, within 40 minutes.

### 2.7. Evaluation of acute anti-inflammation effect [18]

The volumes of mice left paw were measured with a plethysmometer before inducing inflammation ($V_0$). Healthy male mice (32 animals) were randomly divided into 4 groups (8 animals per group) and fasted overnight. Mice were daily given oral administration of 10 ml/kg distilled water (pathology groups) or 10 mg/kg diclofenac (Novartis, Italy) (control group) or EEJS at the doses of 800 mg/kg and 1200 mg/kg. After 1 hour of administration, mice were administered a 25 µl intraplantar injection of 1% carrageenan suspension (w/v, Sigma-Aldrich, USA). The volume of the left paw was measured after 1, 3, 5, 24 and 48 hours of injection of carrageenan ($V_t$). After measurement of paw volume at hour 24, mice were orally administered once distilled water or diclofenac or EEJS. The percentage difference between $V_o$ and $V_t$ was considered as the paw edema percentage ($\rho V_t$).

### 2.8. Evaluation of treatment effect of EEJS on hyperuricemia in mice

The four clinical phases of gout include asymptomatic hyperuricemia, acute attacks, intercritical gout and chronic gout. To test the hypouricemic effect in mice, acute and chronic hyperuricemia models by varying the duration of potassium oxonate were carried out, in which acute hyperuricemia is a phenomenon of a rapid increase in blood uric acid levels with a single intraperitoneal dose of potassium oxonate but not maintained while chronic hyperuricemia can be induced by repeated injection of potassium oxonate for 15 days [19, 20]. In this study, EEJS was evaluated its hypouricemic effect in mouse models of acute and chronic hyperuricemia.

#### *2.8.1. Evaluation of treatment effect of EEJS on acute hyperuricemia in mice [19, 20]*

Healthy male mice (40 animals) were randomly divided into 5 groups (8 animals per group) and fasted overnight. Physiological and pathology groups were administrated orally with normal saline; meanwhile, EEJS and 10 mg/kg allopurinol treated mice were administrated orally with 800 and 1200 mg/kg of EEJS, and 10 mg/kg allopurinol (Stada, Vietnam) (control group). One hour before the normal saline, EEJS and allopurinol gavage, except for physiological mice injected with normal saline, other mice were intraperitoneally (i.p.) injected with 300 mg/kg potassium oxonate (Sigma-Aldrich, USA) dissolved in CMC-Na 0.5%, 10 ml/kg. Mice in group 1 are injected peritoneum NaCl 0.9%, 10 ml/kg. After 2 hours of potassium oxonate injection, blood samples were collected from each mouse and anticoagulated with EDTA, centrifuged 3000 rpm/10 minutes at 25 $^oC$. Plasma was used to quantify uric acid by uric acid kit (Erba, Italy) according to the manufacturer's instructions.

#### *2.8.2. Evaluation of treatment effect of EEJS on chronic hyperuricemia in mice [19, 20]*

Healthy male mice (32 animals) were randomly divided into 4 groups (8 animals per group): 1: normal, 2: pathology, 3: allopurinol, 4: EEJS. Mice in treatment and pathological groups were intraperitoneally injected with 300 mg/kg potassium oxonate (PO) at day 1, 250 mg/kg at day 3, 200 mg/kg at day 5 and 150 mg/kg at days 7, 9, 11, 13, 15. The normal group was injected intraperitoneally with saline. EEJS group were orally treated at the dose of 800 mg/kg while normal and pathological groups received saline (10 ml/kg), once daily from day 1 to day 15. The allopurinol group (control group) was given medicine 10 mg/kg on days 7 and 15. On days 7 and 15, mice fasted for at least 12h before oral administration and intravenous blood from the tail was taken 2h after the oral administration to quantify uric acid.

### 2.9. Statistical analysis

The data were presented as mean ± SD (standard deviation) and statistically analyzed by Kruskall-Wallis and Mann-Whitney tests, using SPSS version 22. The difference between groups was considered statistically significant at a p-value < 0.05.

## 3. RESULTS

### 3.1. Identification and quantification of phenolic compounds

The positive result of histochemical tests with 2% ferric chloride, 20% sodium hydroxide and 1% lead acetate (Table 1) showed the presence of phenolic compounds in the EEJS.

The absorbance at 760 nm of the reaction mixture from standard pyrogallol was presented in Table 2. The calibration curve of absorbance versus concentration of pyrogallol (µg/ml) was expressed in Figure 1.

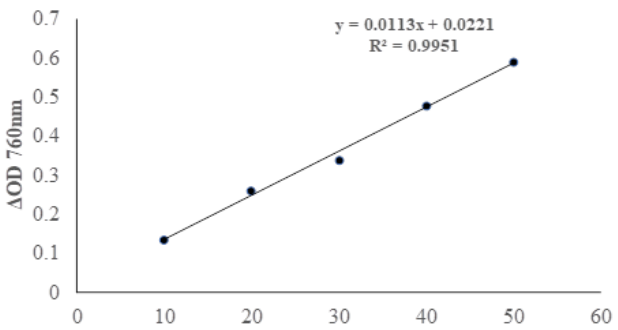


**Figure 1.** Calibration curve of absorbance versus concentration of pyrogallol (µg/ml)

Therefore, the content of total phenolics (X) was expressed as pyrogallol equivalent per gram of the extract (mg pyrogallol/g extract) and calculated from the regression equation of the calibration curve: C (µg/ml) = ($\rho OD_{760nm}$ - 0.0221)/0.0113 and the equation: X (mg pyrogallol/g) = (C x V x k)/[m x (1 – h)]; in which: C (µg/ml): total polyphenol concentration; V: EEJS volume (V = 1 ml); k: dilution factor (k = 500); m: EEJS weight (m = 100.1 mg); and h: EEJS humidity (h = 11.93%).

Based on $\rho OD_{760nm}$ of repeated 3 times (0,192; 0,195 and 0,194), the total phenolic content in EEJS was 86.11 ± 0.75 mg/g, corresponding to about 8.6% (w/w).

**Table 1.** Histochemical tests for identification of phenolic compounds in EEJS

| Reaction | Positive reaction | Result |
|---|---|---|
| With NaOH | Dark coloured solution compared to control | ++ |
| With $FeCl_3$ | Dark green solution | +++ |
| With $(CH_3COO)_2Pb$ | Precipitation | +++ |

**Table 2.** Absorbance at 760 nm of reaction mixture from standard pyrogallol at different concentrations

| Concentration (µg/ml) | 0 (blank) | 10 | 20 | 30 | 40 | 50 |
|---|---|---|---|---|---|---|
| $OD_{760\ nm}$ | 0,068 | 0,202 | 0,328 | 0,407 | 0,545 | 0,656 |
| ΔOD760 nm | | 0,134 | 0,260 | 0,339 | 0,477 | 0,588 |

**Table 3.** Analgesic effect on acetic acid-induced writhing response in mice

| Group (n = 8) | Writhing response (time) (Mean ± SD) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | 36-40 |
| **Pathology** | 10.6 ± 7.0 | 18.1 ± 4.5 | 18.0 ± 4.2 | 13.1 ± 3.5 | 11.3 ± 4.0 | 9.6 ± 4.9 | 8.0 ± 3.0 | 7.6 ± 5.0 |
| **Diclofenac 10 mg/kg** | 4.1 ± 4.3 | 8.9 ± 8.3$^{\#}$ | 7.9 ± 8.2$^{\#}$ | 6.5 ± 6.2$^{\#}$ | 5.5 ± 5.7$^{\#}$ | 4.5 ± 3.7$^{\#}$ | 2.9 ± 3.2$^{\#\#}$ | 2.5 ± 3.0$^{\#\#}$ |
| **EEJS 800 mg/kg** | 2.4 ± 1.9$^{\#\#}$ | 8.5 ± 5.2$^{\#\#}$ | 6.1 ± 2.5$^{\#\#}$ | 4.3 ± 2.5$^{\#\#}$ | 3.1 ± 3.0$^{\#\#}$ | 3.0 ± 2.8$^{\#\#}$ | 1.9 ± 1.1$^{\#\#}$ | 1.1 ± 0.6$^{\#\#}$ |
| **EEJS 1200 mg/kg** | 4.0 ± 5.2$^{\#}$ | 10.8 ± 9.6 | 9.9 ± 6.3$^{\#}$ | 6.8 ± 4.8$^{\#}$ | 5.8 ± 3.1$^{\#}$ | 5.3 ± 4.3 | 3.4 ± 2.6$^{\#}$ | 2.4 ± 2.1$^{\#}$ |

*$^{\#}p < 0.05$, $^{\#\#}p < 0.01$: compared to the pathology group*

**Table 3 (continue).** Paw edema percentage in mice induced acute inflammation by carrageenan

| Group (n = 8) | Paw edema percentage (%) (△Vt) (Mean ± SD) | | | | |
|---|---|---|---|---|---|
| | $\triangle V_{1h}$ | $\triangle V_{3h}$ | $\triangle V_{5h}$ | $\triangle V_{24h}$ | $\triangle V_{48h}$ |
| **Pathology** | 65.00 ± 35.91 | 89.58 ± 20.74 | 78.54 ± 18.37 | 55.84 ± 15.42 | 51.67 ± 13.92 |
| **Diclofenac 10 mg/kg** | 60.83 ± 17.89 | **45.00 ± 12.74$^{\#\#}$** | **24.17 ± 18.06$^{\#\#}$** | **9.58 ± 10.31$^{\#\#}$** | **9.17 ± 9.89$^{\#\#}$** |
| **EEJS 800 mg/kg** | 60.00 ± 27.14 | 82.50 ± 25.93$^{@@}$ | 81.88 ± 23.77$^{@@}$ | 57.92 ± 17.55$^{@@}$ | 33.75 ± 20.96$^{@}$ |
| **EEJS 1200 mg/kg** | 62.50 ± 25.38 | 88.34 ± 13.21$^{@@}$ | 79.38 ± 13.90$^{@@}$ | 55.83 ± 15.57$^{@@}$ | 36.24 ± 15.49$^{\#@@}$ |

*$^{\#}p < 0.05$, $^{\#\#}p < 0.01$: compared to the pathology group*
*$^{@}p < 0.05$, $^{@@}p < 0.01$: compared to the diclofenac group*

### 3.2. Acute oral toxicity in mice

The acute toxicity test in mice indicated that oral administration of a single 20 g/kg dose of EEJS did not cause any visual signs of toxicity or mortality within the first 72 hours and during the entire 14 days observation period. The gross examination of the internal organs showed no abnormality of the color or texture in EEJS-treated animals.

### 3.3. Analgesic effect of EEJS in mice

The number of abdominal writhing in acetic acid-induced mice was presented in Table 2. Diclofenac treatment (10 mg/kg) significantly inhibited the number of writhing in comparison with the pathology group ($p < 0.05$). EEJS at the oral doses of 800 mg/kg and 1200 mg/kg reduced the number of writhing ($p < 0.05$). There was no difference in writhing amount between 2 EEJS groups as well as between 2 EEJS groups and diclofenac one ($p > 0.05$). This result demonstrated the analgesic effect of both oral doses of EEJS.

### 3.4. Anti-inflammatory effect of EEJS in mice

As shown in Table 3, carrageenan 1% induced paw edema (50 - 90%) compared to normal conditions. The 10 mg/kg diclofenac significantly inhibited paw edema after 3 hours of carrageenan injection ($p < 0.01$). Compared to the pathology group, 800 mg/kg EEJS did not decrease the carrageenan-induced paw edema for 48 hours observation ($p > 0.05$) while 1200 mg/kg EEJS significantly decreased the paw edema only at hour 48 ($p < 0.05$).

### 3.5. Effect of EEJS on acute hyperuricemia

After 2 hours of 300 mg/kg potassium oxonate injection, the uric acid concentration in the control group increased 1.8 times compared to the physiological group (Table 4, $p < 0.01$).

In the group of allopurinol 10 mg/kg, the uric acid concentration decreased significantly by 60.31% compared to the control group ($p < 0.01$). Thus, the model of acute hyperuricemia with 300 mg/kg potassium oxonate shows the response to allopurinol. Therefore, this model is used to investigate the uric acid-lowering effects of the extraction.

In two EEJS groups, the concentration of uric acid lower than the control group was not statistically significant ($p > 0.05$). Therefore, the extract at doses of 800 and 1200 mg/kg did not show the treatment effect for acute hyperuricemia. The concentration of uric acid in the 800 mg/kg group was lower than that in the 1200 mg/kg group; hence, the study evaluated the treatment effect for chronic hyperuricemia of EEJS at the oral dose of 800 mg/kg.

**Table 4.** Acid uric concentration of tested groups in model of acute hyperuricemia

| Group (n = 8) | Physiology | Pathology | Allopurinol 10 mg/kg | EEJS 800 mg/kg | EEJS 1200 mg/kg |
|---|---|---|---|---|---|
| **Uric acid oncentration (mg/dl)** | 2,12 ± 0,53 | 3,83 ± 1,00** | 1,52 ± 0,57## | 3,48 ± 0,22**@@ | 3,77 ± 0,47**@@ |

***p < 0,01: compared to the physiology group; ##p < 0,01: compared to the pathology group; @@p < 0,01: compared to the control group*

**Table 5.** Acid uric concentration after 7 days and 15 days

| Group (n = 8) | Uric acid concentration (mg/dl) | |
|---|---|---|
| | After 7 days | After 15 days |
| **Physiology** | 2,48 ± 0,76 | 2,65 ± 0,70 |
| **Pathology** | 4,57 ± 1,00** | 4,34 ± 0,83** |
| **Allopurinol 10 mg/kg** | 2,12 ± 0,69## | 2,71 ± 0,41## |
| **EEJS 800 mg/kg** | 2,55 ± 0,74## | 2,66 ± 0,71## |

***p < 0,01: compared to the physiology group; ##p < 0,01: compared to the pathology group; @@p < 0,01: compared to the control group*

### 3.6. Effect of EEJS on chronic hyperuricemia

In the pathology group, the uric acid concentration increased 1.84 times (day 7) and 1.64 times (day 15) compared to the physiological group at the same tested time (Table 5, $p < 0.01$). The allopurinol group had a statistically significant decrease in uric acid concentration ($p < 0.01$) compared to the pathology group at the same time (-53.6% on day 7; -37.6% on day 15). In the EEJS group, the uric acid concentration decreased by 44.2% and 38.7% after treatment for 7 and 15 days, respectively, compared to the pathology group ($p < 0.01$). Compared with 10 mg/kg allopurinol, 800 mg/kg EEJS showed a similar uric acid-lowering effect ($p > 0.05$).

## 4. DISCUSSION

For the gout treatment, there are two types of medications used to reduce the inflammation, pain associated with gout attacks and to prevent gout complications by lowering blood uric acid levels. Recently, some Vietnamese medicinal plants have been known as a potential source for the prevention and treatment of gout. This work determined whether *J. subtriplinerve* could be used for gout prevention and treatment assistance due to analgesic, anti-inflammatory, and hypouricemic effects in mice.

The present study showed that phenolic compounds are present in the EEJS with a total content of 86.11 ± 0.75 mg/g as pyrogallol equivalent corresponding to about 8.6% (w/w). This result is consistent with the reported chemical composition of *J. subtriplinerve*. From the twigs and leaves of this plant, previous research groups isolated some phenolic and flavonoid compounds such as rutin, astragalin, isoquercitrin, verbascoside, isoverbascoside, isoleoverbascoside, apioverbascoside, 6'-O-menthiafoloylverbascoside [10].

The previous study of Vietnamese scientists reported about *in vitro* antioxidant and anti-inflammatory effects of *J. subtriplinerve*. The present work has been the first report about *in vivo* safety and pharmacological activities of *J. subtriplinerve* in mice; this provided more preclinical data of *J. subtriplinerve*. For oral administration, EEJS did not cause any toxic sign in mice given oral $D_{max}$ of 20 g/kg, corresponding to 54.35 g plant powder/kg or about 254.18 g material plant per day in adults. This result contributes to affirming the safety of products from *J. subtriplinerve* because the oral doses in adults have been 20 - 30 g of plant/day (about 1/10 $D_{max}$). The present study showed that EEJS expressed *in vivo* analgesic and chronic hypouricemic effects at the oral dose of 800 mg/kg in mice corresponding to about 10.18 g material plant/day in adults. This oral dose in mice could be chosen as a therapeutic dose in human because of its safety and efficacy. The obtained results could help to reduce the oral administration of *J. subtriplinerve*, leading to a decrease in the cost of gout treatment.

The pharmacological activity of EEJS could relate to its chemical composition of flavonoids, terpenoids, polyphenols, and terpenoid glycosides. In fact, phenolics are responsible for a lot of pharmacological properties such as antioxidant, inhibitory activity on xanthine oxidase, anti-inflammatory effect; thus, these compositions are present in most drugs used in phytotherapy. In fact, rutin decreased serum uric acid levels and attenuated fructose-induced metabolic abnormalities in rats. Rutin retrograded dysregulations of renal-specific transporter, organic anion and cation transporters responsible for fructose-induced hyperuricemia; leading to prostaglandin E2 reduction and nitric oxide elevation in fructose-fed rat kidneys [21]. *J. subtriplinerve* composes of the isoquercitrin with *in vitro* and *in vivo* antioxidative activity [22]. Moreover, the triterpenoids oleanolic acid, betulinic acid, β-sitosterol, stigmasterol in the *J. subtriplinerve* could be contributing to their anti-inflammatory and immunomodulatory effects [23]. The obtained results in the present study suggest that EEJS could be used for further studies on developing novel functional foods or new drugs.

For the limitations of the present paper, there was no study on the total chemical compositions of *J. subtriplinerve* besides the phenolic content. The study did not report on the relative correlation between chemical compositions of *J. subtriplinerve* and its pharmacological effects including analgesic and chronic hypouricemic effects. The present work has not investigated yet the effect of *J. subtriplinerve* on the cytokine release in the initiation of the gout disease as well as on the different pathways of inflammation and hyperuricemia. For future research, it is necessary to study on chemical compositions of *J. subtriplinerve* in the relationship to the mechanism of its pharmacological effects to valorize this potential natural source in the prevention and/or treatment of gout disease.

## Conclusion

The *in vitro* previous results suggested that *J. subtriplinerve* could be a potential material for gout treatment due to its phenolic constituents; however, the *in vivo* evidence has been limited. The present examined the phenolic content, safety, and anti-gout effect in mice of 50% ethanol extract from *J. subtriplinerve*. This plant also exhibited inhibitory activity on xanthine oxidase as well as anti-inflammatory effect via nitric oxide-inhibitory assay. The total phenol content of 50% ethanolic extract from *J. subtriplinerve* corresponded to about 8.6% (w/w). In mice, this extract did not cause any toxic signs at the oral dose of 20 g/kg, exhibited analgesic and chronic hypouricemic effects at the oral dose of 800 mg/kg. This suggests that 50% ethanolic extract from *J. subtriplinerve* could be used for further studies to develop natural products for gout treatment.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ORCID ID

Tuoi Thi Hong Do https://orcid.org/0000-0002-5445-0694
Quang Nhat Tu https://orcid.org/0000-0001-7451-9671
Tinh Quoc Vo https://orcid.org/0000-0002-6078-8157
Oanh Thi Kim Nguyen https://orcid.org/0000-0001-9425-2396
Tuyen Le Thanh Nguyen https://orcid.org/0000-0002-0073-7171

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