Vietnam Journal of Community Medicine, Vol. 66, English version No. 2, 292-296

ANTI-INFLAMMATORY ACTIVITY OF NGHE BO CAP (CURCUMA RANGJUED) RHIZOME EXTRACT AND MOLECULAR DOCKING ANALYSIS OF ITS MAJOR BIOACTIVE COMPOUNDS

Phan Anh Thu[1,7], Le Tri Vien[1], Nguyen Nhat Long[1,7], Pham The Hai[2], Do Thi Thao[3], Tran Van Loc[4], Le Nhat Minh[1,6], Phan Nu Yen Chi[8], Le Dung Sy[1], Tran Tuan Anh[2], Nguyen Thi Duong[5], Tran Thi Quynh Ngan[7], Nguyen Le Bao Ngoc[7], Phung Thi Kim Hue[1,7*]

[1]*Institute of Health Research and Educational Development in Central Highlands - Gia Lai, Vietnam*
[2]*University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology - Hanoi City, Vietnam*
[3]*Institute of Biotechnology, Vietnam Academy of Science and Technology - Hanoi City, Vietnam*
[4]*Institute of Chemistry, Vietnam Academy of Science and Technology - Hanoi City, Vietnam*
[5]*Thao Nguyen Duong Traditional Family Medicine Clinic - Bac Gia Nghia Ward, Lam Dong, Vietnam*
[6]*Foreign Trade University - Hanoi City, Vietnam*
[7]*Hung Vuong High School for the Gifted - Vietnam*
[8]*Soka University of America, Life Sciences Concentration - 1 University Drive, Aliso Viejo City, Orange County, California, USA*

Received: 28/11/2025
Revised: 18/12/2025; Accepted: 19/12/2025

ABSTRACT

"Nghe bo cap" is the local name for a plant characterized by yellow, scorpion-shaped rhizomes. Taxonomic identification classified this species as Curcuma rangjued (Hue et al., 2024), distributed primarily in mountainous regions of Dak Glong Commune, Dak Glong District, Lam Dong Province, Vietnam. Traditional medicine practitioners have employed herbal preparations from this species to treat bronchitis, asthma, gastric disorders, and infections, attributing these applications to purported anti-inflammatory properties. However, no scientific evidence has substantiated these medicinal claims.

This study extracted essential oil from C. rangjued rhizomes and evaluated its inhibitory effects on pro-inflammatory mediators (TNF-α and PGE-2) through in vitro assays. Molecular docking analysis investigated the mechanisms of action for key bioactive compounds identified in the essential oil and assessed potential anti-inflammatory activities.

The essential oil of C. rangjued (GEC) inhibited TNF-α expression at 11.38 μM ($p < 0.05$) and PGE-2 expression at 22.36 μM ($p < 0.01$). Molecular docking revealed that germacrone and curcumenol exhibited binding affinities toward PGE-2 of 0.96 μM and 0.2 μM, respectively, and toward TNF-α of 1.39 μM and 0.10 μM, respectively. These compounds formed stable interactions with TNF-α, consistent with the observed anti-inflammatory activity.

These findings suggest that C. rangjued rhizome possesses therapeutic potential for anti-inflammatory through the bioactive compounds germacrone and curcumenol. Further in vivo studies are warranted to validate these preliminary results and establish clinical applications.

Keywords: Curcuma rangjued, Cytokine, Inflammation, Nghe Bo Cap, TNF-α, PGE-2.

1. INTRODUCTION

Nghệ bọ cạp is the Vietnamese vernacular name for a Curcuma species widely cultivated in the Central Highlands of Vietnam as a medicinal plant. Its rhizomes possess a scorpion-like shape, which explains the common name “Nghe Bo Cap”. Although leaves and flowers resemble those of Curcuma longa, several discontinuous morphological traits distinguish it from other Curcuma taxa known in the region (Hue et al., 2024). Comparative morphological and molecular analyses have identified this plant in Vietnam as Curcuma rangjued (Hue et al., 2024), a species whose characteristics match the taxon originally described from

*Corresponding author
Email: whitelily109@gmail.com **Phone**: (+84) 914730099 **DOI:** 10.52163/yhc.v66i8.4076

Northern Thailand (Surapon Saensouk, 2021).

Curcuma rangjued is phylogenetically close to C. longa, a species recognized for managing chronic diseases through anti-inflammatory, antioxidant, and antiproliferative activities (Sharifi-Rad et al., 2020). However, the principal bioactive compounds of C. longa, curcumin and its derivatives, exhibit poor water solubility, thereby restricting clinical efficacy (Aggarwal et al., 2013). Accordingly, C. rangjued shows greater therapeutic promise than other Curcuma species currently used in Vietnam (Hue et al., 2024).

In type 2 diabetes mellitus, inflammatory mediators contribute to neurodegeneration and elevate the risk of Alzheimer's disease (Twarowski et al., 2023). Hyperactivated immune activity in diabetes releases substances that damage neurons, producing cerebral inflammation that links the two conditions and increases Alzheimer's-related memory impairment (Mushtaq et al., 2015).

Given its pronounced anti-inflammatory activity, C. rangjued represents a promising medicinal plant for the concurrent management of interrelated conditions including diabetes, Alzheimer's disease, atherosclerosis and cancer.

2. METHODS

2.1. Study subjects and locations

Extract (Nb) from rhizomes of Curcuma rangjued (commonly known as nghệ bọ cạp); pro-inflammatory mediators TNF-α and PGE2; RAW264.7 murine macrophage cell line.

In silico molecular docking targets: TNF-α (PDB ID: 2AZ5), PGE2 synthase (PDB ID: 7CX2)

2.2. Materials and methods

Plant material and chemicals

All chemicals and reagents were purchased from Sigma-Aldrich (USA). Mouse insulin ELISA kits were obtained from FineTest (Wuhan, China). Fresh rhizomes of C. rangjued were collected in November 2024 from natural populations in Đăk Hà district, Lam Dong province,

2.3. Experimental procedures

2.3.1. Extract extraction and compositional analysis

Nb was extracted according to Abubakar et al. (2020) with modifications. Fresh rhizomes (400 g) were extracted three times by refluxing with 2 L n-hexane at room temperature for 16 h each. Combined extracts were concentrated under reduced pressure using a rotary evaporator. The residue underwent steam distillation to yield volatile compounds, which were dried over anhydrous Na_2SO_4.

Chemical composition was determined by Gas chromatography-mass spectrometry (GC-MS) using a Thermo Scientific Trace 1310 system coupled to an ITQ 900 ion trap detector. Separation was performed on a TG-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with helium as carrier gas. Injector and detector temperatures were set at 260 °C and 240 °C, respectively. The temperature program was: 60 °C (held 2 min), increased at 4 °C/min to 220 °C (held 10 min). Compounds were identified by comparison of mass spectra with libraries W09N08 and HPCH1607 and by retention indices using MassFinder 4.0 software.

2.3.2. Inhibition of TNF-α and PGE2 production in LPS-stimulated RAW264.7 cells

The assay was performed according to Jianjun et al. (2017) and Li et al. (2018) with minor modifications.

Cytokine expression assay

RAW264.7 cells were seeded in 96-well plates at 2×10^5 cells/mL and incubated overnight at 37 °C under 5% CO_2. Test samples dissolved in 10% DMSO were added at various concentrations. Dexamethasone (final concentration 10 µM) served as positive control. Negative control wells contained cells treated with LPS only, while vehicle controls contained 10% DMSO without test compound. After 2 h pre-incubation, LPS (1 µg/mL) was added to all wells except the physiological control (-LPS).

After 24 h, supernatants were collected and analyzed for cytokine levels. TNF-α and PGE2 concentrations were quantified using commercial ELISA kits (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. Supernatants were diluted 4-fold for TNF-α and 2-fold for PGE2 assays. Standard curves were constructed for each cytokine, and concentrations were calculated from optical density values, where Y-values represented optical density (OD) and X-values represented cytokine concentrations.

Cell viability assay

Cell viability was assessed by MTT assay. After supernatant removal, fresh medium (190 µL) and MTT (10 µL, 5 mg/mL) were added to each well and incubated at 37°C with 5% CO_2 for 4 hours. After medium removal, formazan crystals were dissolved in 100% DMSO (50 µL). Absorbance was measured at 540 nm using a BioTek microplate spectrophotometer. Cell viability was calculated as:

$$\%\ \text{Viability} = [(OD(\text{sample}) - OD(\text{blank})) / (OD(\text{DMSO}) - OD(\text{blank}))] \times 100$$

2.3.3. Molecular Docking Analysis

Molecular docking simulations were conducted to assess the mechanisms of action for active compounds from Nb against therapeutic target (anti-inflammatory), following methodologies described by Tran et al. (2024)

Simulations employed the Triangle Matcher algorithm in Molecular Operating Environment (MOE) software version 10.09, performed in triplicate. Targets and positive controls are as follows:

- Anti-inflammatory activity was evaluated through inhibition of TNF-α (PDB ID: 2AZ5, positive control: dihydrobiopterin) and mPGES-1 (PDB ID: 7CX2, positive control: rofecoxib). The analysis examined compound interactions with target binding sites and protein

interaction networks through two steps:

(i) Redocking reference ligands into target proteins to validate the docking protocol by evaluating root mean square deviation (RMSD) values and comparing predicted interactions with published experimental data.

(ii) Docking active compounds into validated binding sites of target proteins.

Inhibitory effects were quantified through binding interactions and the predicted enzyme inhibition constant (Ki, μM), calculated as:

$$Ki = \exp(\Delta G/RT)$$

Where, R represents the universal gas constant (1.985 × 10^{-3} kcal mol^{-1} K^{-1}),

+ T denotes absolute temperature (298.15 K),

+ ΔG represents binding free energy (kcal mol-1), estimated using the London dG scoring function refined by the Generalized Born/Volume Integral/Weighted Surface Area (GBVI/WSA) solvation method (Hieu et al., 2018).

2.4. Statistical analysis

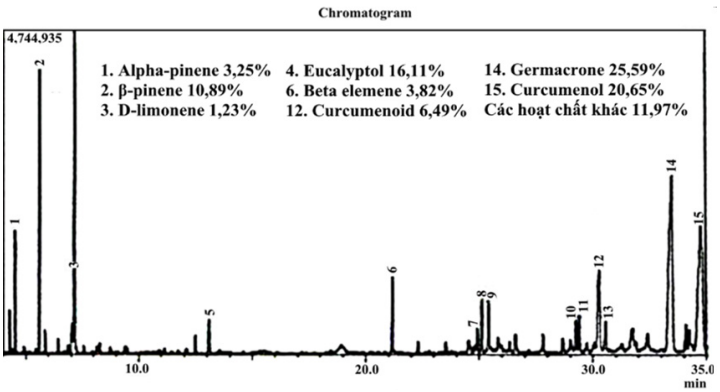
Data are expressed as mean ± standard deviation (SD). Significant differences between treatment groups and controls were evaluated by Student's t-test, F-test, and one-way analysis of variance (ANOVA). Value $p < 0.05$ were considered statistically significant (Zhang et al., 2010).

3. RESULTS

3.1. Recovery and Active Compound Quantification

Extraction of Curcuma rangjued rhizomes with n-hexane yielded a viscous yellow liquid (1.09 ± 0.18% w/w relative to fresh rhizome weight). Subsequent essential oil recovery (Nb) from this fraction achieved a yield of 4.5 ± 0.48%.

GC-MS) analysis identified 15 active compounds in Nb. Unlike Curcuma longa essential oil, Nb contained germacrone (~25.59%), curcumenol (~20.65%), β-pinene (~10.89%), and eucalyptol (~16.11%) as major constituents (Figure 1).

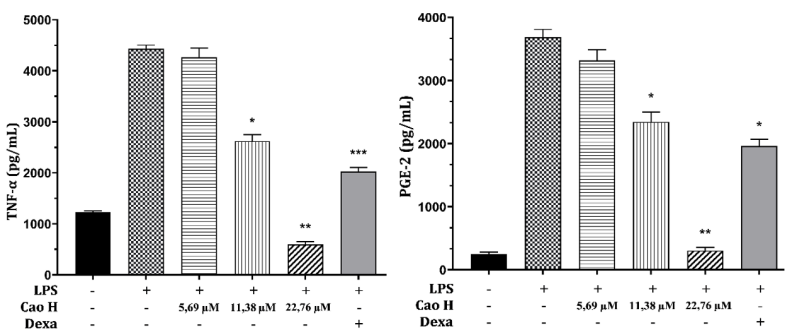


GC-MS analysis was performed using a TG-5MS column (30 m × 0.25 mm × 0.25 μm film thickness) with injector and detector temperatures of 260°C and 240°C, respectively. Compounds were identified by comparing retention indices (RI) and mass spectra with NIST 14 and Wiley 8 library databases (Adam, 2017).

Figure 3.1. GC-MS chromatogram of active constituents in the n-hexane fraction (Nb)

3.2. Effect of Nb on Cytokine Expression

Nb inhibited TNF-α and PGE-2 expression at 11.38 μM ($p < 0.05$) and 22.36 μM ($p < 0.01$) (Figure 3.2).

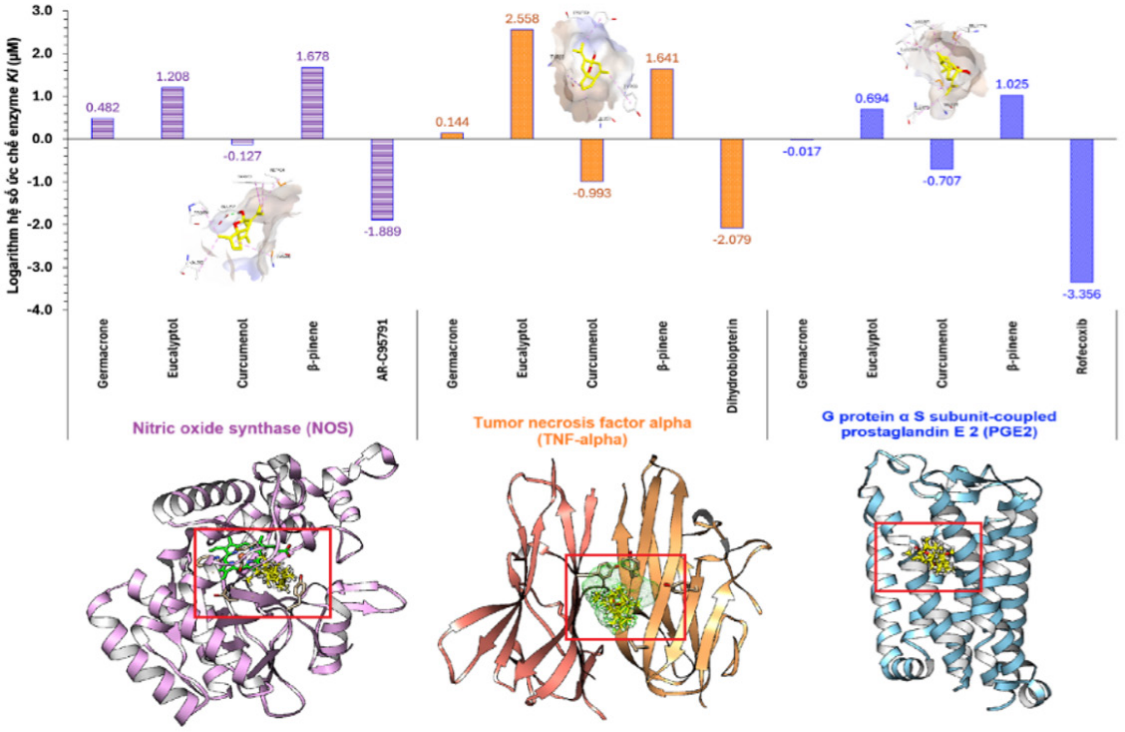


*Tested GEC concentrations corresponded to 2×IC_{50}, 1×IC_{50}, and 0.5×IC_{50}, where IC_{50} was derived from a prior nitric oxide (NO) inhibition assay. Significance levels: *p < 0.05; **p < 0.01 compared to vehicle control (+LPS). Dexamethasone (Dexa, 10 μM) used as the positive control. Wells containing 10% DMSO with lipopolysaccharide (LPS) stimulation served as vehicle control (+LPS). Blank wells contained only culture medium without LPS served as the negative physiological control (−LPS)*

Figure 2. Effect of GEC on pro-inflammatory cytokine expression (TNF-α, PGE-2)

3.3. Anti-inflammatory Mechanisms of Nb Active Compounds

To obtain more definitive evidence, molecular docking was carried out to investigate the anti-inflammatory mechanisms of major active compounds (Figure 3.3).



Data represent triplicate independent molecular docking simulations evaluating germacrone, β-pinene, eucalyptol, and curcumenol for inhibitory activity against targets associated with nitric oxide (NO) production, TNF-α, and PGE-2 synthesis.

Figure 3. Comparison of inhibition constants (Ki) and binding affinities (ΔG) of selected compounds (germacrone, β-pinene, eucalyptol, curcumenol) against inflammatory targets (NO production, TNF-α, PGE-2).

Germacrone and curcumenol demonstrated the strongest predicted inhibition of PGE-2 synthesis (mPGES-1), with Ki values of 0.96 μM and 0.2 μM, respectively. Common interactions included hydrogen bonds (H-bonds) with Thr82, Ser120, and Ser308, and alkyl/π-alkyl interactions with Ile85, Met116, Leu301 and Leu304. Curcumenol displayed significant inhibitory activity against TNF-α, with Ki values of 1.39 μM and 0.10 μM, with hydrogen bonds to Arg24 and Arg254 and van der Waals (vdW) contacts with Ala27 and Met258. Concurrently, germacrone and curcumenol inhibited NO production with Ki values of 3.03 μM and 0.75 μM, respectively.

4. DISCUSSION

Unlike Curcuma longa essential oil, C. rangjued essential oil (Nb) contains 15 active compounds, including germacrone (~25.59%), curcumenol (~20.65%), β-pinene (~10.89%), and eucalyptol (~16.11%) (Figure 1). Many studies demonstrated that germacrone modulates T lymphocyte balance to attenuate inflammation progression (Tan et al., 2022) and reduces oxidative stress (Fang et al., 2023). Curcumenol alleviates inflammation (Yang et al., 2021), whereas eucalyptol exhibits anti-inflammatory and anti-diabetic properties (Kim et al., 2020). Inflammatory cells release mediators that contribute to neurodegeneration (Twarowski et al., 2023) and insulin resistance (Bonam et al., 2022). These pieces of evidence suggest that Nb possesses anti-inflammatory potential with therapeutic applications that may extend to inflammation-associated conditions such as diabetes and Alzheimer's disease, atherosclerosis.

As shown in Figure 2., Nb significantly inhibited TNF-α and PGE-2 expression at 11.38 μM ($p < 0.05$) and 22.36 μM ($p < 0.01$). In our previous work, C. rangjued extract was shown to inhibit nitric oxide (NO) production (Hue et al., 2024), while the current findings demonstrated that Nb reduced PGE-2 and TNF-α expression, both of which are key pro-inflammatory cytokines that play major roles in inflammatory process (Guastadisegni et al., 2002). These results provide clear experimental evidence of Nb's anti-inflammatory activity. Therefore, an important question arises whether the presence of germacrone and curcumenol, the major constituents in Nb, drive this effect.

To address that, we compared with many recent studies showing that germacrone, a compound holding anti-inflammatory properties (Fang et al., 2023), can modulate T lymphocyte balance to slow the progression

of inflammation (Tan et al., 2022) through mechanisms involving T-helper 1/T-helper 2 (Th1/Th2) balance restoration, which enhances nerve impulse transmission (Wang et al., 2019) and provides neuroprotection (Riaz et al., 2020). Curcumenol inhibits TNF-α-induced inflammation by deactivating NF-κB and MAPK signaling pathways (Yang et al., 2021). Given that GEC contains 25.59% germacrone and 20.65% curcumenol, this raises the question of whether these two compounds is responsible for Nb's anti-inflammatory activity.

To clarify the contribution of the major constituents, molecular docking analysis was performed (Figure 3) and the results revealed that germacrone and curcumenol exhibited the strongest inhibition against PGE-2 synthesis, through H-bonds with Thr82, Ser120, and Ser308, and alkyl/π-alkyl interactions with Ile85, Met116, Leu301 and Leu304. Moreover, curcumenol effectively inhibited TNF-α via H-bonds at Arg24 and Arg254 and vdW contacts at Ala27 and Met258, while both compounds inhibited NO production with Ki values of 3.03 μM and 0.75 μM, respectively. Notably, stable interactions occurred at a non-catalytic phosphotyrosine binding region on TNF-α. This is a critical region for selective binding, leading to strong pro-inflammatory cytokine inhibition. In contrast, β-pinene and eucalyptol also affect NO production and inhibit the expression of TNF-α and PGE-2, but showed substantially weaker potency. Thereby, the anti-inflammatory activity of Nb can be attributed primarily to germacrone and curcumenol. These findings indicate that C. rangjued rhizome has the potential to treat many inflammatory diseases through these compounds.

5. CONCLUSION

The rhizome of Curcuma rangjued represents a potential medicinal source. Essential oil extraction yielded 4.5 ± 0.48% (Nb), containing 15 active compounds, including germacrone (~25.59%), curcumenol (~20.65%), β-pinene (~10.89%), and eucalyptol (~16.11%).

Nb inhibited TNF-α and PGE-2 expression at 11.38 μM ($p < 0.05$) and 22.36 μM ($p < 0.01$), respectively. Molecular docking revealed that germacrone and curcumenol inhibited PGE-2 with Ki values of 0.96 μM and 0.2 μM, and inhibited TNF-α with Ki values of 1.39 μM and 0.10 μM, respectively. More importantly, stable interactions of these compounds with TNF-α contribute to Nb's potent anti-inflammatory properties.

These findings indicate that Curcuma rangjued is a highly promising medicinal candidate for the development of next-generation anti-inflammatory agents, owing to the strong bioactivities of germacrone and curcumenol.

REFERENCES

[1] Hue, P. T. K., Van Loc, T., Duong, N. T., Ngan, T. T. Q., Ngoc, N. L. B., & Vien, L. T. (2024). Effects of glycemic regulation and acetylcholinesterase inhibition of scorpion turmeric (Curcuma rangjued) in Central Highlands, Vietnam, Vietnam Journal of Community Medicine, Vol. 65, English version, pp. 41-46.

[2] Bonam, V. R., Srinivasan, A. R., & Manoj, D. D. (2022). Linking inflammatory mediators and indicators of insulin resistance in anthropometry specified type 2 diabetic males. Bioinformation, 18(10), 998.

[3] Twarowski, B., & Herbet, M. (2023). Inflammatory processes in alzheimer's disease—pathomechanism, diagnosis and treatment: a review. International journal of molecular sciences, 24(7), 6518.

[4] Hue, P. T. K., Binh, N. Q., Hanh, N. P., Duong, N. T., Ngan, T. T. Q., Ngoc, N. L. B., ... & Vien, L. T. (2024). Taxonomic identification of an additional species, Curcuma rangjued, in the Central Highlands of Vietnam and evaluation of its inhibitory activities against cancer cell lines, Vietnam Journal of Community Medicine, Vol. 65, English version, 2024, pp. 25-30.

[5] Saensouk, S., Boonma, T., & Saensouk, P. (2021). A new species and a new record of Curcuma subgen. Curcuma (Zingiberaceae) from Northern Thailand. Biodiversitas Journal of Biological Diversity, 22(9).

[6] Fenske, R. J., Weeks, A. M., Daniels, M., Nall, R., Pabich, S., Brill, A. L., ... & Kimple, M. E. (2022). Plasma prostaglandin E2 metabolite levels predict type 2 diabetes status and one-year therapeutic response independent of clinical markers of inflammation. Metabolites, 12(12), 1234.

[7] Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), 1-10.

[8] T. T. P. Tran, N. H. Dang, X. N. Nguyen, H. Pham-The, U. T. T. Phan, T. H. Le. (2024). Alkaloids from Piper longum L and Their Anti-inflammatory Properties. Chemistry and Biodiversity Vol 21, No. 12. e202401224.

[9] Tan, T., Huang, Q., Chu, W., Li, B., Wu, J., Xia, Q., & Cao, X. (2022). Delivery of germacrone (GER) using macrophages-targeted polymeric nanoparticles and its application in rheumatoid arthritis. Drug Delivery, 29(1), 692-701.