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# ALLELOPATHIC EFFECT OF *Piper aduncum* L. LEAF EXTRACT ON THE SEED GERMINATION OF *Vigna radiata* L. AS MODEL ORGANISM

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**ABSTRACT.** The presented study was conducted to evaluate the allelopathic effect of the aqueous extract and ethanolic extract of *Piper aduncum* L. (spiked peper) on the seed germination of *Vigna radiata* L. (mung bean). Seed germination percentage (GP) was monitored at 8 hours after treatment (HAT), 24 HAT and 48 HAT. Further, radicle length was examined 48 HAT. The results indicated that after treatment with 1:10 aqueous extract GP was 40%, while after 1:100 ethanolic extract GP was 11.11%, showing significant decrease in seed germination of *V. radiata* at 24 HAT. At 48 HAT, all concentrations showed no significant difference from the control, except 1:10 ethanolic extract, which completely inhibited radicle growth, preventing seed germination. Moreover, at 48 HAT, same treatments induced significant decrease in the radicle length compared to the control. Consequently, *P. aduncum* extracts demonstrated a dose-dependent phytotoxic effect on *V. radiata* which negatively impacted *V. radiata* seeds at higher concentrations in both aqueous and ethanolic extracts.

**Keywords:** radicle length, invasive plant, allelopathy.

## INTRODUCTION

The allelopathic ability of some plants may inhibit the growth of neighboring plants, however, this notion is not entirely accurate because accordingly, allelopathy encompasses both inhibitory and stimulatory effects given within a certain concentration range (WILLIS, 2007). Additionally, plant allelopathy can yield positive effects on weed control, environmental and

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agricultural management, crop protection, and crop re-establishment (SINGH *et al.*, 2019). However, it can also contribute to negative outcomes such as plant invasion, toxicity, and soil sickness. KATO-NOGUCHI (2022) demonstrated that invasive plant species exert allelopathic effects on native plants. The study observed that allelochemicals by invasive plants are toxic to native plants.

One such invasive plant species is *Piper aduncum*, belonging to the Piperaceae family (MORAIS *et al.*, 2023) and it is known as the "spiked pepper" (STARR *et al.*, 2003;WUNDERLIN and HANSEN 2002) or locally known as "buyo-buyo" (Celeste et al., 2023). Several studies indicate that *P. aduncum* possesses essential oil with antifungal, antimicrobial, insecticidal, larvicidal, molluscicidal, and parasiticidal properties (MORAIS *et al.*, 2023; OLIVEIRA *et al.*, 2013; BRAZÃO *et al.*, 2014;VOLPE *et al.*, 2016; VALADARES *et al.*, 2018). It also exhibits potential herbicidal properties against invasive weeds in field conditions (MENDOZA *et al.*, 2014). Further, some studies have assessed the allelopathic effects of certain *Piper* species on weed and crop seed germination (e.g., *Piper betle*) (WORANOOT *et al.*, 2015; CHOOPAYAK *et al.*, 2022). However, there are a number of review papers that deal with the allelopathic potential of *P. aduncum* on crop seed germination (DE ASSIS ALVES *et al.*, 2023). It is along this line that this study will focus on evaluating the allelopathic ability of *P. aduncum* leaf extract on seed germination and radicle growth, specifically targeting *Vigna radiata*. Moreover, *V. radiata* is chosen as a model due to its accessibility, commercial availability, and rapid germination period (2-5 days) (FONG *et al.*, 2021).

This research intends to investigate whether *P. aduncum* benefits or adversely affects the germination and early growth of *V. radiata* seeds by evaluating which concentrations of aqueous and ethanolic extracts exhibits the allelopathic effect on the seed germination and radicle growth of *V. radiata*. Moreover, it is hypothesized that higher concentrations of *P. aduncum* extracts will have a significant inhibitory effect on both the germination and radicle growth of *V. radiata*. Thus, insights gained from this study can aid researchers and vegetable farmers in understanding the potential benefits or drawbacks of *P. aduncum* allelopathic effects. Furthermore, this study will offer guidance to researchers interested in conducting experiments on the allelopathic potential of *P. aduncum*.

## MATERIALS AND METHODS

Spiked pepper (*P. aduncum*) leaves were collected from Mt. Musuan at Central Mindanao University, Musuan, Bukidnon. The leaves were washed with tap water and then rinsed twice with distilled water. Following the washing, the leaves were oven-dried at 60 ˚C for 48 hours (DE ASSIS ALVES *et al.*, 2023). For aqueous or ethanolic extract, 10 grams (g) of the dried powdered *P. aduncum* leaves were soaked in 100 mL of distilled water/ethanol (95%) for 72 hours at room temperature, then filtered using Whatman filter paper, and stored in a small glass container (HASTUTI *et al.*, 2017). Three different concentrations (1:10, 1:100, 1:1000), with three replicates each, of aqueous and ethanolic extracts were prepared. Furthermore, distilled water was served as the control. Table 1 shows a list of treatments.

Table 1. List of Treatments

| Treatment Code | Treatments |
|---|---|
| T1 | Aqueous extract (1:10) |
| T2 | Aqueous extract (1:100) |
| T3 | Aqueous extract (1:1,000) |
| T4 | Ethanolic extract (1:10) |
| T5 | Ethanolic extract (1:100) |
| T6 | Ethanolic extract (1:1,000) |
| T7 | Distilled water (Control) |

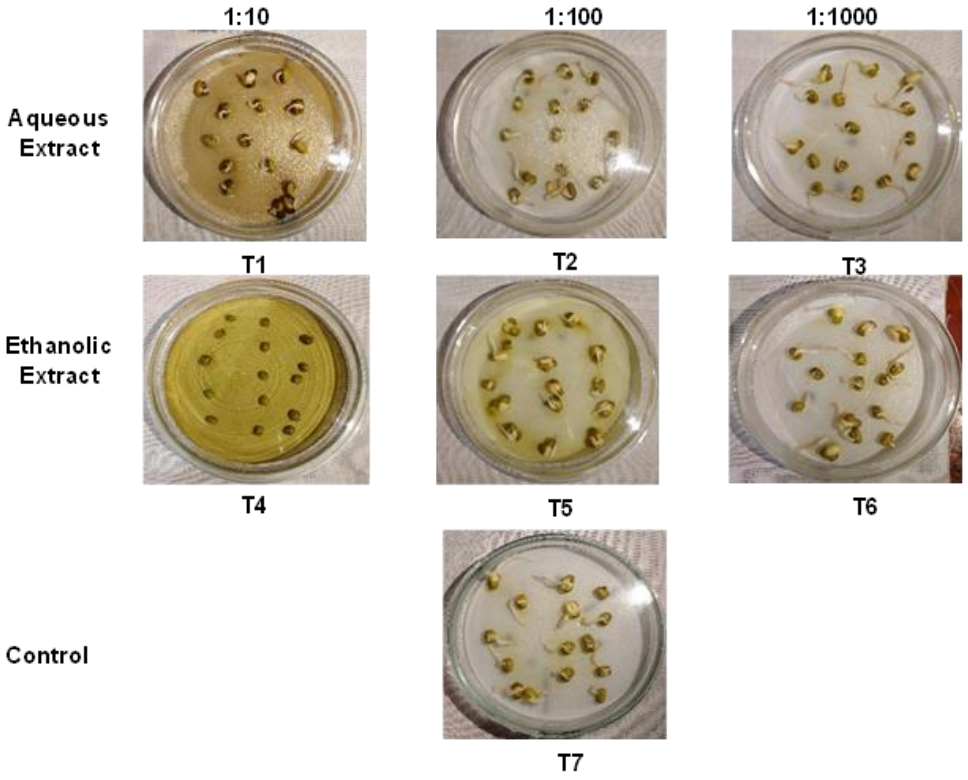


Figure 1. *V. radiata* seeds 48 hours after treatment with aqueous and ethanolic extract of *P. aduncum* on different concentrations (aqueous extract treatments - T1 (1:10), T2 (1:100), and T3 (1:1000); ethanolic extract treatments - T4 (1:10), T5 (1:100), and T6 (1:1000); control - T7).

Seed germination was carried out using the "Petri Dish Method" (ANONYMUS, 1974; WORANOOT *et al.*, 2015) in a completely randomized design, where each treatment was replicated 3 times with 21 petri dishes and a total of 315 *V. radiata* seeds**.**

Three (3) mL of leaf extract at appropriate concentration was added to each petri dish, followed by the placement of Whatman filter paper no. 1 in the dish with extract. Fifteen (15) randomly selected seeds of *V. radiata* were then placed on the filter paper and was replicated three (3) times. The samples were randomly arranged in the laboratory room and stored at room temperature for 48 hours. Additionally, after 48 hours, the radicle lengths were measured in centimeters using the Proview software. The seedlings were photographed with a ruler next to them for calibration, and the radicle lengths were then measured and analyzed using the software (Fig. 1). Germinated seeds were counted at 8, 24 and 48 hours after treatment (HAT) and germination percentage (GP) was calculated.

$$Germination\ \%\ (GP) = \frac{Total\ number\ \ of\ seeds\ germinated}{Total\ number\ \ of\ seeds\ sown}\ x\ 100$$

The Analysis of Variance (ANOVA) was employed to determine the level of significance of GP and radicle length of *V. radiata* seeds treated with aqueous and ethanolic extracts of *P. aduncum* at various concentrations. Additionally, Tukey's HSD test was utilized to assess significant differences among treatment means.

## RESULTS AND DISCUSSION

The *P. aduncum* extract exhibits a phytotoxic effect on *V. radiata* at different concentrations in a dose-dependent manner. The overall results revealed that T1 and T5 showed a significant decrease in seed germination (Fig. 2, Tab. 2) and radicle length of *V. radiata* (Fig.

3, Tab. 3) as compared to the control but does not statistically differ from each other. In contrast T4 is significantly different from the control (Figs. 2, 3 and Tabs. 2, 3), which imply that the leaf ethanolic extract at this concentration, significantly inhibit the seed germination and radicle growth of *V. radiata.*

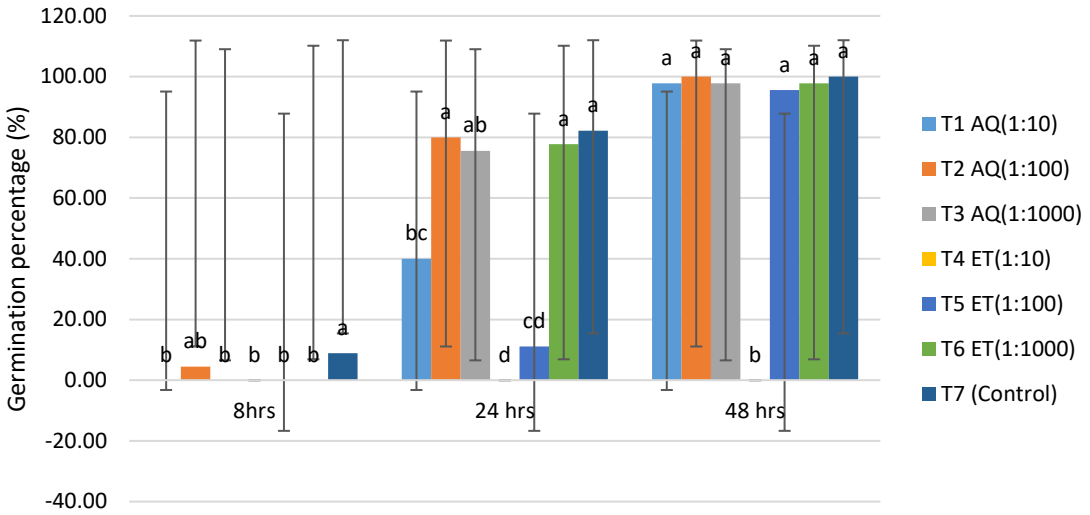


Figure 2. The germination of *V. radiata* seeds at 8, 24, and 48 hours after treatment with aqueous and ethanolic extract of *P. aduncum* on different concentrations (means with the same letter are not significantly different at $p<0.05$ and represented with standard deviation lines).

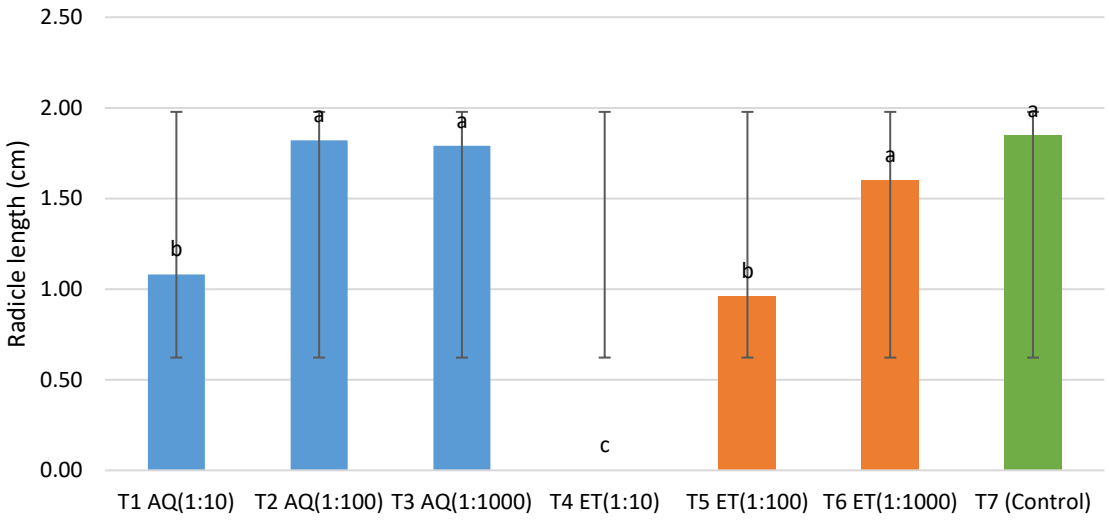


Figure 3. Radicle length of *V. radiata* at 48 hours after treatment with aqueous and ethanolic extract of *P. aduncum* on different concentrations (means with the same letter are not significantly different at $p<0.05$ and represented with standard deviation lines).

As shown in the Fig. 2 and Tab. 2, at 8 HAT, all concentrations (except T2) suppressed seed germination, which was significantly ($\alpha = 0.01$) different from the control. However, at 24 HAT, T1 achieved a 40% GP, while T5 achieved 11.11% GP, though both had lower GP and significantly ($\alpha= 0.01$) differed from the control. There was no signs of germination after T4 treatment throughout the duration of the experiment. Furthermore, at 48 HAT, all samples except T4 showed no significant differences from each other. For radicle growth, as shown in the Fig. 3 and Tab. 3, at 48 HAT, T1 (1.08 cm) and T5 (0.96 cm) caused significant ($\alpha= 0.01$) decrease of radicle growth compared to the control.

SIDDIQUI *et al.* (2018) studied the seed germination and revealed that at 24 HAT the aqueous extract (50 g/L) of *Sapindus mukorossi* and *Leucaena leucocephala* inhibits seed

germination of *Pisum sativum* seeds. This coincides with the result of this study in which an ethanolic extract with a 1:10 concentration showed no signs of radicle growth in *V. radiata*. However, at 48 HAT, results became similar among all concentrations in this study (except T4), showing no significant reduction compared to the control. Hence, these seeds obtained a high percentage of the germination at 48 HAT. The study of DE ASSIS ALVES *et al.* (2023) has also the same result with the effect of the ethanolic extract (T4) in this study, in which, after 48 HAT, the higher concentration of *P. aduncum* aqueous extract exhibited an inhibiting effect on the seeds of *L. sativa*, wherein, at higher concentration (100 mg/mL), the seeds of *L. sativa* were completely inhibited.

**Table 2.** Germination percentage (%) of *V. radiata* seeds at 8, 24, and 48 hours after treatment with aqueous and ethanolic leaf extracts of *P. aduncum*, representing mean, standard deviation (SD), p-value, and confidence interval.

| Treatments | Hours After Treatment (HAT) | | | | | |
|---|---|---|---|---|---|---|
| | 8 HAT** | | 24 HAT** | | 48 HAT** | |
| | Mean | SD | Mean | SD | Mean | SD |
| **T1 AQ(1:10)** | $0.00^{b}$ | 0.00 | $40.00^{bc}$ | 24.04 | $97.78^{a}$ | 3.85 |
| **T2 AQ(1:100)** | $4.44^{ab}$ | 3.85 | $80.00^{a}$ | 17.64 | $100.00^{a}$ | 0.00 |
| **T3 AQ(1:1000)** | $0.00^{b}$ | 0.00 | $75.56^{ab}$ | 10.18 | $97.78^{a}$ | 3.85 |
| **T4 ET(1:10)** | $0.00^{b}$ | 0.00 | $0.00^{d}$ | 0.00 | $0.00^{b}$ | 0.00 |
| **T5 ET(1:100)** | $0.00^{b}$ | 0.00 | $11.11^{cd}$ | 10.18 | $95.56^{a}$ | 3.85 |
| **T6 ET(1:1000)** | $0.00^{b}$ | 0.00 | $77.78^{a}$ | 3.85 | $97.78^{a}$ | 3.85 |
| **T7 (Control)** | $8.89^{a}$ | 3.85 | $82.22^{a}$ | 7.70 | $100.00^{a}$ | 0.00 |
| **p-value** | 0.0005 | | 0.0000 | | 0.0000 | |
| **Confidence Interval** | 95% | | 95% | | 95% | |

Means with the same letter are not significantly different at $p < 0.05$
** Means are significantly different based on $\alpha = 0.01$

**Table 3.** Radicle length (cm) of *V. radiata* at 48 hours after treatment with aqueous and ethanolic extract of *P. aduncum*, representing mean, standard deviation (SD), p-value, and confidence interval.

| Treatments | 48 Hours After Treatment (HAT) ** | |
|---|---|---|
| | Mean | SD |
| **T1 AQ(1:10)** | $1.08^{b}$ | 1.04 |
| **T2 AQ(1:100)** | $1.82^{a}$ | 1.35 |
| **T3 AQ(1:1000)** | $1.79^{a}$ | 1.34 |
| **T4 ET(1:10)** | $0.00^{c}$ | 0.00 |
| **T5 ET(1:100)** | $0.96^{b}$ | 0.98 |
| **T6 ET(1:1000)** | $1.60^{a}$ | 1.26 |
| **T7 (Control)** | $1.85^{a}$ | 1.36 |
| **p-value** | 0.0000 | |
| **Confidence Interval** | 95% | |

Means with the same letter are not significantly different at $p < 0.05$
** Means are significantly different based on $\alpha = 0.01$

However, DE ASSIS ALVES *et al.* (2023) used the aqueous extract of *P. aduncum*, contradicting the result of the aqueous extract (T1) in this study. Nevertheless, T1 and T5 have a similar result with the second concentration found in the result of the study of DE ASSIS ALVES *et al.* (2023), but obtained lesser percentage of seed germination as compared to the control and other treatments, having only 28.8% germination of seeds at 50 mg/mL of concentration.

Since the group of seeds in T4 is completely inhibited, it is impossible to assess and measure the radicle growth. For radicle growth, T1 and T5 did not differ from each other; however, they are significantly *(α = 0.01)* different from the control, showing lesser radicle growth compared to the control. These results are confirmed by the study of CHOOPAYAK *et al.*, (2022), which showed that the ethanolic extract of *Piper betle* (BE – betel oil) caused a negative effect on the root growth of rice. The R/S ratio dropped due to strong radical growth inhibition, with values dropping from 63% at 0.5 mg/mL BE to 16% at mg/mL of BE treatment. SIDDIQUI *et al.,* (2018) also confirmed that the radicle length of *P. sativum* significantly decreased 48 HAT from the aqueous extract of *S. mukorossi* and *L. leucocephala* in all concentrations from 12.5 $gL^{-1}$ to 50 $gL^{-1}$, as compared to the control. However, the phytotoxic effect of *P. aduncum* is a dose-dependent.

Moreover, a study from WORANOOT *et al.*, (2015) also revealed that ethyl acetate and ethanol are the most effective solvents against invasive weeds as they completely inhibited *Chloris barbata* at 0.5 mg/mL concentration, as these solvents yielded high recovery extracts of *P. betle* leaves. This explains that T4 (1:10, ethanolic extract) exhibits more inhibition effect on *V. radiata* seed germination and radicle growth, as compared with T1 (1:10, water-soluble extract). However, T1 still induces a negative effect on the seed germination and radicle growth of *V. radiata*.

*P. aduncum* contains chemical compounds that are mutagenic and toxic to some plants and animals (OLIVEIRA *et al.*, 2013; MORAIS *et al.*, 2023; DE ASSIS ALVES *et al.*, 2023). It comprises monoterpenes, sesquiterpenes (OLIVEIRA *et al.*, 2013; MORAIS *et al.*, 2023), prenylated benzoic acid derivatives, chromenes, flavonoids, alkaloids, amides, and phenylpropanoids (MORAIS *et al.*, 2023). Several studies have shown that aqueous extracts of medicinal plant species have alkaloid and phenolic compounds that prevent seed germination and growth of the target plant species (SIDDIQUI *et al.*, 2018), and phenolic compounds specifically phenylpropanoids were also found in the ethanolic extract of *P. betle* which yields 79.84% (CHOOPAYAK *et al.*, 2022). According to SIDDIQUI *et al.*, (2018) citing the works of EL-KHATIB *et al.*, (2004), alkaloids and phenolic compounds in medicinal plants have negative effects on the target plant species, and these chemical compounds can reduce the absorption of water and minerals, reduce the translocation of vitamins and minerals from roots and to other parts of the plant, and inhibit some enzymatic functions. Other chemical compounds in *P. aduncum* that contribute to a negative effect on the growth of seeds are monoterpenes and sesquiterpenes, which have been shown to have inhibitory effects on seed germination and growth of weeds (MACÍAS *et al.*, 2019).

Furthermore, other factors were also suggested by CHOOPAYAK *et al.*, (2022) that various extraction methods yield different types and quantities of chemical compounds in plant extract which results in influencing its allelopathic activity. According to CHOOPAYAK *et al.*, (2022), a comparison between extraction methods was observed, wherein a method through an ethanolic extract from *P. betle* contains 79.48% of phenylpropanoid compounds, whereas, in the hydrodistillation process of fresh leaves from *P. betle,* it was found out that it contains the same compound (phenylpropanoid), differing only in the amount of extracted compound which only contained 66.1%.

## CONCLUSION

Based on the experiment conducted, *P. aduncum* exhibited a negative allelopathic effect on seed germination and radicle length of *V. radiata* seeds at higher concentrations in both aqueous extract (T1-1:10) and ethanolic extract (T4-1:10 and T5-1:100) of *P. aduncum*. At 8 HAT, all concentrations except T2 (1:10-aqueous extract) showed no germination, significantly different from the control. However, at 24 HAT, T1 (1:10-aqueous extract) obtained 40% GP, and T5 (1:100-ethanolic extract) obtained 11.11% GP but had lower GP compared to the control, while T4 (1:10-ethanolic extract) remained to have no signs of germination throughout

the experiment. Further, at 48 HAT all treatments except T4 (1:10-ethanolic extract) have no significant difference from each other. In terms of radicle growth, T1 (1:10-aqueous extract) and T5 (1:100-ethanolic extract) exhibited significantly less radicle growth compared to the control at 48 HAT.

Therefore, this study proves that *P. aduncum* negatively affects the seed germination of *V. radiata* in certain concentrations, the type of solvent used for extracting secondary metabolites, and allelochemicals found in *P. aduncum*. The results of this study imply the potential of *P. aduncum*, an invasive species, to develop into a biopesticide and for weed management. Furthermore, based on several studies, factors that negatively affect the growth of *V. radiata* seeds are the phytochemicals present in the extract of *P. aduncum*, such as monoterpenes, sesquiterpenes, alkaloids, and phenolic compounds. Hence, *P. aduncum* extract exhibited a phytotoxic effect in a dose-dependent manner in concentrations T1 (1:10-aqueous extract), T4 (1:10-ethanolic extract), and T5 (1:100-ethanolic extract).

This study recommends isolating and elucidating the chemical compounds of *P. aduncum* extracts found in Bukidnon, Philippines. Future studies should use crude extracts to obtain a definitive conclusion regarding secondary metabolites, as this study focused solely on solvent extraction and did not obtain crude extract. Further research should be conducted, specifically focusing on other germination characteristics such as mean germination time, rate of germination, germination uniformity, seedlings dry matter, and vigor test, as these are important parameters in seedling experimentation. Moreover, the research should also focus on the cellular aspects of the affected sample, which will cover the identification of abnormalities in the cell.

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