

# Mutagenicity Effect of *Centella asiatica* in Aqueous Extract by Using Ames Test

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**Abstract:** *Centella asiatica* L. or locally known as Pegaga nyonya is a weekly aromatic smelling herb that belongs to a genus of the plant family Apiaceae (Umbelliferare). It has been used widely in folk medicine for hundreds of years to treat a wide range of illness. In this study, the mutagenic potential of *C. asiatica* aerial parts (leaves and stems) and roots in aqueous extracts were determined using the Ames test. The method involved was pre-incubation on *Salmonella typhimurium* TA 98 and TA 100 bacterial strains in the presence and absence of metabolic activator S9 system. The extracts were evaluated using two-fold value of the number of revertant's colony in negative control plate as cut-off point, to determine the mutagenicity effects. The results showed that all aqueous extracts of the aerial parts and roots of *C. asiatica* were non mutagenic because it shown no significance difference ( $p < 0.05$ ) when compared to negative control towards TA98 and TA100 strain with and without S9 metabolic activation for all concentration studied. In conclusion, *C. asiatica* aerial parts and roots aqueous extract were non mutagenic on both *S. typhimurium* strains and can be used as part of traditional medicine.

**Keywords:** *Centella asiatica*, *Salmonella typhimurium*, T A9 8, TA100, Mutagenicity effect, Ames assay.

## INTRODUCTION

Plants have been employed for medical treatments for more than 60,000 years. Nevertheless, they are frequently employed without scientific knowledge or its biological and therapeutic properties. So, scientific studies of the chemical properties, biological activities or evaluation of its genotoxic properties have been emerging as a health priority [1-4]. Reference [5] stated that even though plant extracts have been used in the treatment of diseases but in nature many plants synthesize toxic substances in order to defence itself against infections, insects and herbivores and these compounds could have potentially deleterious effects in humans. Nowadays, majority of the world's population in developing countries are still rely on herbal medicines to meet their health needs in cases where synthetic medicine could not relieve patients who suffer from hard-to-cure illnesses [6]. It has been estimated that more than 80% of the world's population utilizes plants as their primary source of medicinal agents [6]. People in Malaysia itself are commonly consumed various type of vegetable in their diet intake as it has diverse medicinal properties and some of it may also exert anticarcinogenic and antimutagenic activities. But, no one realize that those herbs may potentially posses mutagenic effect. Therefore, it is very important to evaluate

the safe use of the herbs before used as part of herbal medicinal.

Therefore, in this research green leafy vegetables that is widely consumed as herb in different parts of the world namely *Centella asiatica* or locally known as Pegaga Nyonya was selected in attempt to elucidate the mutagenic effect of the plant. Pegaga (*Centella asiatica* Linn.) is a perennial creeping plant with cup-shaped of leaves, glabrous stems and rooting at nodes. The leaves are thin, soft and green in colour. The whole plant including leaves, stem and root are consumed as vegetable or 'ulam', juice and therapeutic agents among the Malays and as a cooling drink by the Chinese [7-10]. Pegaga is generally used in health food and cosmetic products and also is associated with wound healing agents [11]. The health benefit of pegaga is thought to be due to several saponin constituents including triterpene acids (asiatic acid and madecassic acid) and their respective glycosides (asiaticoside and madecassoside). Previous study also showed that the total triterpenoids namely asiatic acid, madecassic acid, asiaticoside and madecassoside have been shown to significantly influence the synthesis of collagen, improve wound healing and fibronectin in human skin fibroblasts culture [7, 11]. Besides that, Pegaga extract also has anti-ulcer effects especially with reference to its Asiatic acid and asiaticoside content [12-14]. The asiaticoside of this plant has been reported to possess strong antioxidant properties [15], act as antimicrobial [16] and anti-inflammatory [17].

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Assessment of mutagenicity is very important as an initial test for more complex mixture because there is a possibility that one or more components can be positive. To evaluate mutagenic activity, many techniques are used and the primary technique is the Ames test which is also known as the *Salmonella*/microsome test, is one of the primary test systems used in investigating the mutagenic effect of chemicals or extracts. It is one of the most reliable short-term bacterial test systems, inexpensive and provides results very quickly [18, 19]. Although there were many research on this herb before, there are still little report on its mutagenicity effect. Therefore, the aim of this study was to evaluate the mutagenic effect of *C.asiatica* in aqueous extract in the absence and presence of the metabolic activation (S9).

## METHODOLOGY

### Plant Preparation

The whole plant of *C.asiatica* was originally collected from Melaka which is one of the main supplier of this herb in Malaysia. Then, the sample was washed three times before cut into different parts which are aerial parts (leaves and stems) and root. The samples were then dried at room temperature. Voucher specimens (UKMB 30014) were deposited at the Herbarium University National Malaysia, Bangi, Faculty of Science and Technology.

### Extraction Method

The aqueous extract was obtained by employing the method of [20] with some modification. The fresh aerial parts and roots of *C.asiatica* were dried at room temperature and reduced to ground powder by using the microfiner grinder. One hundred fifty grams (150 g) of the powdered aerial parts (leaves and stems) and roots were soaked separately in 1.5 L of distilled water and stored overnight at 4 °C to prevent microbial activity. The samples were then filtered out using Whatman filter paper under vacuum before freeze dried to obtain crude extract. Then, the dry extract was kept at 4 °C in a Schott bottle prior to the bioassays.

### Bacterial Strains

The *S. typhimurium* strains TA 98 and TA 100 was obtained from Molecular Toxicology (Moltox) Inc. are histidine requiring mutant as previously described by [18, 19]. The genotypes of test strains were checked routinely for their histidine and biotin requirement, deep rough (rfa) character, UV sensitivity (uvrB mutation) and presence of the R factor (pKM101) plasmid. They were stored at -80°C. *S. typhimurium* TA 98 is frame shift strain which contain the his3052 mutation and *S. typhimurium* TA100 contain the base-pair substitution mutation hisG46.

### Mutagens

2-Nitrofluorene (2-NF), sodium azide (NaN<sub>3</sub>) and 2-aminoanthracene (2-AA) were purchased from Sigma-Aldrich and dissolved in dimethyl-sulfoxide (DMSO).

## Mutagenicity Assay

The mutagenicity assay with *S. typhimurium* was performed as described by [18]. The test is based on the plate incorporation method, using *S. typhimurium* test strains (TA100, TA98) with and without an exogenous metabolic system: S9 fraction in S9 mix. The S9 microsome fraction was purchased from Molecular Toxicology (Moltox) Inc. and stored at -80 °C. The test strains from frozen cultures were grown overnight for 12–16h at 370 °C in the Oxoid Nutrient Broth No. 2. All concentrations studied of aqueous extracts were added to 2 ml of top agar, supplemented with 0.5 mM L-histidine and 0.5 mM D-biotin, mixed with 100 µ l of bacteria culture and then poured onto a plate containing minimum agar. The plates were incubated at 37°C for 48 h and his<sup>+</sup> revertant colonies were counted after 2 days of incubation. The influence of metabolic activation was tested by adding 500 µ l of S9 mixture. Negative and positive control cultures gave number of revertants per plate that were within the normal limits found in the laboratory. Data were collected with a mean ± SEM of three plates (*n* = 3).

## STATISTICAL ANALYSIS

Data were collected and expressed as the mean ± SEM of three independent experiments (*n*=3) and analyzed for statistical significance from control. The data were tested for statistical differences by one-way ANOVA and the criterion for significance was set at *p*<0.05.

## RESULT AND DISCUSSION

The aqueous extract of *C. asiatica* aerial parts (leaves and stems) and roots was evaluated for its mutagenic inducing ability in Ames test using two *S. typhimurium* strains namely TA98 and TA100 in the absence and presence of S9-mix, respectively as shown in Table 1 and 2. According to [18], compound tested with Ames test have mutagenic effect when there is more than 2-fold increase in the number of revertant colonies over negative control. The result showed that both aerial parts and roots were not mutagenic on the tested strains even in the presence of metabolic activation for all concentration used as it showed no significance difference (*p*<0.05) when compared with negative control. Since the test without metabolic activation showed no mutagenic effect, for the test with metabolic activation (S9), only the lowest (3.125 mg/ml), middle 12.5 mg/ml) and highest (50 mg/ml) concentration was tested. Clearly, the mean number of revertant following treatment with the aqueous samples extracts in both tester strains were at the background level similar to the negative control.

Overall, the highest numbers of mean revertant/plate were observed at the highest dose which is 50 mg/ml with and without the metabolic activation. It also showed that the metabolic activation (S9) induces the mean number of revertant colony on both strains but showed no mutagenic effect. Reference [21] stated that, the absence of the mutagenic effect was caused by the presence of chlorophyll and chlorophyllin that could inhibit selectively the activity of mutagens having polycyclic structures by forming complexes

**Table 1. Mutagenic Effects of Aqueous Extract of *C.asiatica* Aerial Parts without Metabolic Activation S9 (-S9) and with Metabolic Activation S9 (+S9)**

Strain	Concentration	No. of Revertant Colony Mean±SEM(-S9)	No. of Revertant Colony Mean±SEM(+S9)
TA98	Positive control	339 ± 15.7*	420 ± 14.0*
	Negative control	11 ± 0.8	14 ± 1.0
	3.125 mg/ml	12 ± 1.2	11 ± 0.3
	6.25 mg/ml	11 ± 1.2	NT
	12.5 mg/ml	10 ± 1.2	15 ± 2.5
	25 mg/ml	13 ± 1.3	NT
	50 mg/ml	12 ± 1.2	16 ± 1.9
TA100	Positive control	803 ± 41.8*	1352 ± 28.0*
	Negative control	163 ± 7.5	208 ± 3.0
	3.125 mg/ml	141 ± 11.3	208 ± 9.3
	6.25 mg/ml	147 ± 11.3	NT
	12.5 mg/ml	126 ± 9.8	211 ± 5.2
	25 mg/ml	148 ± 7.2	NT
	50 mg/ml	150 ± 9.7	234 ± 4.9

Negative control = sterile distilled water, positive control; strain TA 98 (-S9 = 2-NF, +S9 = 2-AA), strain TA 100 (-S9 = NaN<sub>3</sub>, +S9 = 2-AA), NT=Not tested

The results are the means ± SEM of three separate experiments (n=3).

\*p < 0.05 vs. negative control.

**Table 2. Mutagenic Effects of Aqueous Extract of *C.asiatica* Roots without Metabolic Activation S9 (-S9) and with Metabolic Activation S9 (+S9)**

Strain	Concentration	No. of Revertant Colony Mean±SEM(-S9)	No. of Revertant Colony Mean±SEM(+S9)
TA98	Positive control	339 ± 15.7*	420 ± 14.0*
	Negative control	11 ± 0.8	14 ± 1.0
	3.125 mg/ml	13 ± 2.4	11 ± 0.8
	6.25 mg/ml	11 ± 0.3	NT
	12.5 mg/ml	12 ± 1.6	12 ± 0.6
	25 mg/ml	13 ± 2.7	NT
	50 mg/ml	15 ± 2.3	13 ± 0.8
TA100	Positive control	803 ± 41.8*	1352 ± 28.0*
	Negative control	163 ± 7.5	208 ± 3.0
	3.125 mg/ml	142 ± 8.3	213 ± 8.0
	6.25 mg/ml	142 ± 8.3	NT
	12.5 mg/ml	132 ± 3.9	215 ± 12.1

Table 2. contd....

Strain	Concentration	No. of Revertant Colony Mean±SEM(-S9)	No. of Revertant Colony Mean±SEM(+S9)
	25 mg/ml	119 ± 5.3	NT
	50 mg/ml	115 ± 14.7	224 ± 30.2

Negative control = sterile distilled water, positive control; strain TA 98 (-S9 = 2-NF, +S9 = 2-AA), strain TA 100 (S9 = NaN<sub>3</sub>, +S9 = 2-AA). NT=Not tested

The results are the means ± SEM of three separate experiments (n=3).

\*p < 0.05 vs. negative control.

with them. Therefore, the precursors that could interact with sodium nitrite of this model such as 1- aminopyrene, a polycyclic aromatic hydrocarbons compound, might be trap by chlorophyll to forming complexes. This may describe the mechanism how the aqueous extracts of the sample in this study inhibited the mutagenesis of both bacterial tester strains. Besides that, the presence of antioxidant and phytochemicals in this herb also might cause the absence of the mutagenic activity [21]. The last few years, it has seen that a major increase in the use of herbal medicine in the developed world, therefore knowledge of the mutagenic and toxic effects of plants has also become very important [22]. It is because plants are used in many areas for a number of purposes and that is why many studies on plant extract that examine their mutagenic properties for safe consumption was also done [22]. Although the different parts of the aqueous extract of this herb showed non mutagenic effect but further investigation should be done in different active crude extracts such as methanolic extract because there are a number of studies done on other plant extracts showed mutagenic effects in different solvent. For instances, positive results have been reported from the Ames test using extracts of *Crinum macowanii*, *Catharanthus roseus*, *Combretum mkhzense* *Diospyros whyteana*, *Plumbago auriculata*, *Ziziphus mucronata*, and *Chaetacme aristata* [23], as well as the genus *Helichrysum* Mill. [24]. A hydroalcoholic extract of *Ocotea duckei* leaves was found to be mutagenic for the *Salmonella typhimurium* TA97a, TA100, and TA102 strains, with or without S9 mix [25]. Reference [3] found that *Gnaphalium* sp. and *Valeriana procera* extracts induced mutations of *S. typhimurium* TA98 with or without S9 mix and of TA100 with S9 mix, respectively. The tubers of *Gloriosa superba* were found to contain potent mutagenic properties in an Ames mutagenicity test on *S. typhimurium* [26].

It was also shown that compounds present in the methanolic extracts of the leaves of *Alchornea castaneaeifolia* and *A. glandulosa* were mutagenic in an Ames test [27]. Since plants are primary food sources, identifying and examining their mutagenic, toxicity, or carcinogenic properties are very important to ascertain its safety level.

## CONCLUSION

In conclusion, the aqueous extracts showed non mutagenic effect on both strains with and without metabolic activation and are safely consumed and also can be used as part of traditional medicine. However, further investigation should be carried out in different active crude extracts such

as methanolic extract since previous study showed a positive result in other plants extracts.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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## REFERENCES

- [1] Basaran, A. A.; Yu, T.W.; Plewa, M.J.; Anderson, D. An investigation of some Turkish herbal medicines in *Salmonella typhimurium* and the Comet assay in human lymphocytes. *Tetrag. Carcinog. Mutagen.*, **1996**, *16*, 125-138.
- [2] De Sa Ferreira, I.C.F; Vargas, V.M. Mutagenicity of medicinal plant extracts in *Salmonella*/microsome assay. *Phytother. Res.*, **1999**, *13*, 397-400.
- [3] Deciga-Campos, M.; Rivero-Cruz, I.; Arriaga-Alba, M.; Castañeda-Corral, G.; Angeles-López, G.E.; Navarrete, A.; Mata, R. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. *J. Ethnopharmacol.*, **2009**, *110*(2), 334-342.
- [4] Mata, R.; Morales, I.; Pe rez, O.; Rivero- Cruz, I.; Acevedo, L.; Enriquez, M. Antimycobacterial compounds from *Piper sanctus*. *J. Nat. Prod.*, **2004**, *67*(12), 1961-1968.
- [5] Demma, J.; Engidawork, E.; Hellman, B. Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. *J. Ethnopharmacol.*, **2009**, *122*, 136-142.
- [6] Yu, F; McGuire, P.M.; Li, R.; Wang, R. Effects of *Hydrocotyle sibthorpioides* extract on transplanted tumors and immune function in mice. *Phytomedicine*, **2007**, *14*, 166-171.
- [7] Brinkhaus, B.; Lindner, M.; Schuppan, D.; Hahn, E.G. Chemical, pharmacological and clinical profile of the East Asian medical plant *Centella asiatica*: Review Article. *Phytomedicine*, **2000**, *7*(5), 427-448.
- [8] Tiek, N.L. Pegaga (*Centella asiatica*) – More about its Healing Properties. FRIM in Focus, **1997**, *2*(2), 10-11.
- [9] Turton S. Australian Journal of Medicinal Herbalism. New South Wales., **1993**, *5*(3), 57-61.
- [10] Zakaria, M.; Mohd, M.A. Traditional Malaysia Medicinal Plants. Kuala Lumpur: Fajar Ba kti Sdn Bhd. **1994**.
- [11] Vogel, H.G.; De Souza, N.; D' Sa, A. Effect of Terpenoids Isolated from *Centella asiatica* on Granuloma Tissues. *Acta Ther.*, **1990**, *16*, 285-298.
- [12] Chatterjee, T.K., Chakraborty, A.; Pathak, M. Effect of Plant Extract *Centella asiatica* (Linn.) on cold resistant Stress Ulcer in Rats. *Ind. J. Exp. Biol.*, **1992**, *30*, 889- 891.
- [13] Cheng, C.L.; Koo, M.W.L. Effect of *Centella asiatica* on ethanol induced gastric mucosal lesions in rats. *Life Sci.*, **2000**, *67*(21), 2647-2653.
- [14] Somchit, M.N.; Halijah, H.; Wan Kartini, W.H. Antiulcer effect of *Centella asiatica* and *Piper betle* extracts: A comparative study. *J. Trop Med. Plants.*, **2002**, *3*(1), 18-22

- [15] Shukla, A.; Rasik, A.M.; Dhawan, B.N. Asiaticoside-induced elevation of antioxidant levels in healing wounds. *Phytother. Res.*, **1999**, 3(1), 50-54.
- [16] WHO. *Medicinal Plants in South Pacific Manila*. Geneva WHO Regional Office for the Western Pacific, **1998**, pp. 42-43.
- [17] Chen, Y.; Dai, Y.S.; Chen, B.F. The effect of tetrandrine and extracts of *Centella asiatica* on acute radiation dermatitis in rats. *Biol. Pharm. Bull.*, **1999**, 22(7), 703-706.
- [18] Maron, D.H.; Ames, B.N. Revised methods for the *Salmonella typhimurium* mutagenicity test. *Mutat. Res.*, **1983**, 113, 173-215.
- [19] Mortelmans, K.; Zeiger, E. The Ames *Salmonella*/microsome mutagenicity assay. *Mutat. Res.*, **2000**, 445, 29-60.
- [20] Wong, S.P.; Lai, P.L.; Jen, H.W.K. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.*, **2006**, 99, 775-783.
- [21] Hayatsu, H.; Negishi, T.; Arimoto, S.; Hayatsu, T. Porphyrins as potential inhibitors against exposure to carcinogens and mutagens. *Mutat. Res.*, **1993**, 290, 79-85.
- [22] Parekh, J.; Jadeja, D.; Chand, S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk. J. Biol.*, **2005**, 29, 203-210.
- [23] Elgorashi, E.E.; Taylor, J.L.S.; Maes, A. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicol. Lett.*, **2003**, 143, 195-207.
- [24] Reid, K.A.; Maes, J.; Maes, A. Evaluation of the mutagenic and antimutagenic effects of South African plants. *J. Ethnopharmacol.*, **2006**, 106, 44-50.
- [25] Marques, R.C.P.; Batistuzzo de Medeiros, S.R.; Da Silva Dias, C. Evaluation of the mutagenic potential of yangambin and of the hydroalcoholic extract of *Ocotea duckei* by the Ames test. *Mutat. Res.*, **2003**, 536, 117-120.
- [26] Hemaiswarya, S.; Raja, R.; Anbazhagan, C. Antimicrobial and mutagenic properties of the root tubers of *Gloriosa superba* Linn. (Kalihari). *Pak. J. Bot.*, **2009**, 41, 293-299.
- [27] Dos, S.F.; Calvo, T.R.; Colus, I.M.S. Mutagenicity of two species of the genus *Alchornea* measured by *Salmonella* microsome assay and micronucleus test. *Rev. Bras. Farmacogn.*, **2010**, 20, 382-389.

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