# A Study on Potential Antioxidant and Larvicidal Properties of from *Tadehagi triquetrum* (L.) H. Ohashi ( Lauk-thay)

Thandar Aung[1], Than Than Oo [2]

## ABSTRACT

This study was done to examine some phytoconstituents, antioxidant and larvicidal activities in the extracts of leaves from *Tadehagi triquetrum* (L.) H. Ohashi. Antioxidant and larvicidal potential of crude pet-ether, ethyl acetate, 95% ethanol and watery extracts from leaves of *T. triquetrum* were compared and assayed by DPPH free radical scavenging assay method and 3rd and 4th instars *Aedes aegypti* larvae according to WHO standard method. According to DPPH free radical scavenging assay, 95 % EtOH extract ($IC_{50}$ = 1.64 μg/mL) was found to possess the highest antioxidant activity compared to the other extracts: EtOAc ($IC_{50}$ = 1.71 μg/mL), $H_2O$ ($IC_{50}$ = 3.64 μg/mL) and PE extract ($IC_{50}$= 6.41 μg/mL). The larvicidal activities of those crude extracts were investigated in the range of 0.0125 to 0.2 g/ 100 mL by *Aedes* larvae method at Department of Medical Research (DMR). The highest knockdown of *Aedes* larvae was found at the concentration of 0.2 g/100 mL of EtOAc extract. The highest mortality rate (99.20 %) of *Aedes* larvae at the concentration of 0.2 g/100 mL was found in EtOAc extract. The lowest mortality rate (13.6 %) of $H_2O$ extract was observed at the concentration of 0.0125 g/100 mL. Among the tested four crude extracts, EtOAc extract showed the highest lethal concentrations activity ($LC_{50}$ = 0.0183 g/100 mL and $LC_{90}$ = 0.0727 g/100 mL). This study suggested that both EtOAc and 95% EtOH fraction from leaves of *T. triquetrum* may have the potential to be further developed into therapeutic option for treating oxidative stress and alternative sources of mosquito control agents.

**Keywords**: *Tadehagi triquetrum* (L.) H. Ohashi, phytoconstituents, antioxidant activity, larvicidal activities

## 1. INTRODUCTION

*Tadehagi triquetrum* is a species of flowering plant in the legume family, Fabaceae. It belongs to the sub family Faboideae. The species has two subspecies with nominate one, but sometimes they give full species status by some authors[1]. The maximum height of this shrub tree is 3m and leaves alternate, linear-oblong, ovate with a tapering tip. Flowers show raceme inflorescence type, which are small, pale purplish in color. The fruit is a hairy legume[2]. It is widespread in all South Asian, East Asian, and Southeast Asian countries[3]. Medicinal uses attributed to *T. triquetrum* ranged from treating urinary problems, stomach ache, and diarrhoea to applications as general restorative and tonic. *T. triquetrum* has a potent repellent effect, and a moderate larvicidal effect on *Chrysomya megacephala* fly larvae. This species is used extensively in the traditional preparation of fermented products. In Laos it is widely used in the preparation of fermented fish by placing it on top of the fish in the mouth of the earthenware

[1] Dr, Associate Professor, Department of Chemistry, Pathein University, Myanmar
[2] Dr, Professor, Department of Chemistry, Pathein University, Myanmar

fermentation jar[4]. In Myanmar, it is reported in the traditional production of fermented fish as an effective additive in producing fly larvae-free nga-pi, and it is reported to have a mainly larvicidal effect[5]In Japan, a closely related species in the subtribe Desmodiinae, *Desmodium caudatum* (Thunb.) DC. is used in the preparation of *miso* to prevent the growth of maggots[6].

**Botanical Aspects of *Tadehagi triquetrum* (L.) H. Ohashi**

Scientific Name - *Tadehagi triquetrum* (L.) H. Ohashi
Synonym *Desmodium caudatum* (Thunb.) DC
Family - Fabaceae
Myanmar Name - Lauk-thay
Plant part used - Leaves

Figure 1. *Tadehagi triquetrum* (L.) H. Ohashi leaves

**Aim and Objectives**

The aim of this study was to investigate some phytoconstituents and screen some bioactivities. To fulfill this aim, the research was carried out according to the following objectives.

- To extract the sample with various solvents
- To determine the phytochemical tests
- To investigate antioxidant, and larvicidal activities of leaves of *T.triquetrum*

## 1. MATERIALS AND METHODS

**Collection and Preparation of *T. triquetrum* Sample**

The leaves of *T. triquetrum* were collected from Pathein University Campus, Pathein Township, Ayeyaewady Region in Myanmar, during January to February 2017. The collected leaf samples were identified as *T. triquetrum* ( Lauk-thay) at Department of Botany, Pathein University. A total of 5 Kg of Lauk-thay fresh leaves samples were collected and cleaned with water. For dry material, the leaves of *T. triquetrum* were left to dry for a week away from direct sunlight. Plant material that was free of insect feeding marks, lesions or other damage was used. They were then air dried at room temperature. The dried material was ground into powder using grinding machine. The powdered leaves material obtained was stored in clean air tight container.

**Preparation of Crude Extracts by Direct Extraction Method for Screening of some Biological Activities**

50 g of dried powdered sample was extracted with 150 mL of PE (60-80 °C) for 6 h by using soxhlet extractor. The filtrate was concentrated by removal of the solvent under reduced pressure to give pet-ether crude extract. Preparation of ethyl acetate extract, 95% ethanol and watery extracts were also prepared by similar manner mentioned in above procedure. Each extract was dried at normal pressure on a water bath and stored under refrigerator for screening some bioactivities.

**Qualitative Screening of the Phytochemicals**

In order to classify the types of organic constituents present in leaves samples, preliminary phytochemical tests on samples were carried out by the series of test tube tests.

**Bioactivities**

**(a) *In vitro* screening of antioxidant activity of some crude extracts of *T. triquetrum***

DPPH radical scavenging activity was determined by UV spectrophotometic method. The control solution was prepared by mixing 1.5 mL of 60 μM DPPH solution and 1.5 mL of 95% EtOH. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 μM DPPH solution and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. The absorbance was measured in triplicate for each solution and then means values obtained were used to calculate percent inhibition of oxidation by the following equation.

$$\%\ \text{Oxidative Inhibition} = \frac{A_{DPPH} - (A_{\text{Test sample}} - A_{\text{Blank}})}{A_{DPPH}} \times 100$$

$A_{DPPH}$ = absorbance of DPPH in 95% EtOH solution

$A_{\text{Test sample}}$ = absorbance of (sample + DPPH) solution

$A_{\text{Blank}}$ = absorbance of (sample + 95% EtOH solution)

$$\text{Average, } \overline{X} = \frac{X_1 + X_2 + X_3 + \cdots + X_n}{n}$$

$$\text{Standard deviation (SD)} = \sqrt{\frac{(\overline{x} - x_1)^2 + (\overline{x} - x_2)^2 + (\overline{x} - x_3)^2 + \cdots + (\overline{x} - x_n)^2}{n-1}}$$

where, $\overline{X}$ = average % inhibition of oxidation

$x_1, x_2, x_3, \ldots, x_n$ = % inhibition of test sample solution

n = number of times

Then, $IC_{50}$ (50% oxidative inhibitory concentration) values were also calculated by linear regressive excel programme.

(b) **Larvicidal testing procedure**

Based on preliminary tests, further dilutions were prepared with same type of test water. Different emulsified concentration as 0.000125- 0.00200 g/mL of dried Lauk-thay leaves of pet ether, ethyl acetate, 95% ethanol and watery extracts were prepared freshly by dissolving in 100 mL each of purified water in 250 mL plastic cups. Laboratory reared third and fourth instar *Aedes aegypti* larvae were inside each 250 mL plastic cups and also negative control test was done simultaneously. Fifty (50) each *Aedesae gypti* larvae were put into different concentrations of pet-ether, ethyl acetate, ethanol, and watery crude extracts of *T. triquetrum*. Detail testing was done according to WHO standard method[7, 8] at Entomology Department, DMR, Yangon. The exposure period of larvae were exposed 24 h for each replication and concentration in laboratory at 26-30ºC and 70 to 90% relative humidity. In the experiments, five replicates were carried out. Knock down was checked and counted after 60 minutes exposure time and mortality was checked and recorded after 24 h *Tadehagi triquetrum* (L.) H. Ohashi of exposure periods. Dead larvae were identified when the larvae failed to move after probing with a needle in the cervical region.

Persistency test of *T.triquetrum* leaves extract was done against 10 each 3rd and 4th instar larvae of *Aedes* mosquitoes in 0.025 g / 100 mL dilutions (one timed diluted solutions) daily for 3 days. Mortality rate was recorded after 24 h daily. The lethal concentrations of $LC_{50}$ and $LC_{90}$ values were calculated after 24 h using dose-effect probit calculations.

## 2. RESULTS AND DISCUSSION

By the preliminary photochemical investigation of the leaf extract of *T. triquetrum*, the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, steroids, and tannins were observed and α-amino acids, starchs, cyanogenic glycosides, and terpenoids were absence.

**(a) *In vitro* antioxidant activity of some crude extracts from the leaves of *T.triquetrum***

The antioxidant activities of PE, 95 % EtOH, EtOAc, and $H_2O$ extracts of the leaves of *T. triquetrum* were studied by DPPH (1, 1-diphenyl-2-pricryhydrazyl) free radical scavenging UV spectrophotometric assay method. The results are shown in Table 1.

Table 1. Percent Oxidative Inhibition and $IC_{50}$ Values of Crude Extracts from the leaves of *T. triquetrum* and Standard Ascorbic Acid

| Extracts | Percent Oxidative Inhibition (%) (mean ±SD) in different concentrations (μg/mL) | | | | | $IC_{50}$ ( μg/mL) |
|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 5 | 10 | |
| PE | 20.49±3.07 | 25.82±3.61 | 33.61±5.79 | 44.70±3.27 | 65.89±2.28 | 6.41 |
| EtOAc | 36.49±0.68 | 49.54±0.24 | 58.25±0.49 | 70.59±0.44 | 79.72±0.12 | 1.71 |
| 95% EtOH | 31.30±0.49 | 49.82±0.12 | 64.21±0.36 | 73.82±0.12 | 83.02±0.32 | 1.64 |
| $H_2O$ | 29.08±0.44 | 33.86±0.69 | 48.48±0.23 | 66.14±0.39 | 78.75±0.23 | 3.64 |
| Ascorbic acid | 14.04±2.09 | 54.83±2.48 | 72.44±3.83 | 77.13±1.47 | 87.40±2.37 | 1.17 |

**(b) Larvicidal activity of leaves of *T. triquetrum***

The larvicidal activities of PE, EtOAc, 95 % EtOH, and $H_2O$ were investigated in the range of 0.002 to 0.000125 g/mL by *Aedes* larvae method at Department of Medical Research (DMR), Yangon.

Table 2 Knockdown effect (within 60 minutes) of different dilutions of *T. triquetrum* leaves extracts against 3rd and 4th instars *Aedes aegypti* larvae

| Concentration ( g / 100 mL) | *T. triquetrum* extracts Number of knockdown and % Knockdown | | | |
|---|---|---|---|---|
| | 95 % EtOH | PE | $H_2O$ | EtOAc |
| 0.20 | 214<br>85.60% | 227<br>90.80% | 196<br>78.40 | 238<br>95.20% |
| 0.10 | 183<br>73.20% | 204<br>81.60% | 152<br>60.80% | 220<br>88.00% |
| 0.05 | 129<br>51.60% | 120<br>48.00% | 98<br>39.20% | 187<br>74.80% |
| 0.025 | 79<br>31.60% | 82<br>32.80% | 53<br>21.20% | 133<br>53.20% |
| 0.0125 | 37<br>14.80% | 43<br>17.20% | 25<br>10.00% | 51<br>20.40% |
| Control | 0<br>0% | 0<br>0% | 0<br>0% | 0<br>0% |

**Total Larvae = 250**

According to the larvicidal activity study, the highest knockdown effect at the concentration of 0.2 g / 100 mL was found to be 95.20 % in EtOAc extract, followed by 90.80 % PE and 85.60 % in 95 % EtOH extracts. The lowest effect was found as 78.40 % in watery

extract shown in Table 2. The lowest knockdown (10.00 %) of *Aedes* larvae was found at the concentration of 0.0125 g / 100 mL of watery extract.

Table 3. Mortality effect (Within 24 h) of different dilutions of *T. triquetrum* leaves (Lauk-thay) extracts against 3rd and 4th instars *Aedes aegypti* larvae

| Concentration (g / 100 mL) | Number of Mortality and % Mortality of Crude Extracts | | | |
|---|---|---|---|---|
| | 95 % EtOH | PE | $H_2O$ | EtOAc |
| 0.2 | 230<br>92.00% | 247<br>98.80% | 205<br>82.00% | 248<br>99.20% |
| 0.1 | 205<br>82.00% | 218<br>87.20% | 177<br>70.80% | 227<br>90.80% |
| 0.05 | 161<br>64.40% | 163<br>65.20% | 118<br>47.20% | 204<br>81.60% |
| 0.025 | 109<br>43.60% | 130<br>52.00% | 67<br>26.80% | 159<br>63.60% |
| 0.0125 | 76<br>30.40% | 83<br>33.20% | 34<br>13.60% | 94<br>37.60% |
| Control | 0<br>0% | 0<br>0% | 0<br>0% | 0<br>0% |

**Total larvae = 250**

Table 3 shows that the highest mortality rate of *Aedes* larvae at the concentration of 0.2 g /100 mL was found 99.20 % in ethyl acetate extract and followed by 98.40 % and 92.20 %, and 82.00 % in pet-ether, 95 % ethanol and water extracts. In 0.1g/100 mL concentrations of all extract were found 82.00 %, 87.20 %, 70.80 % and 90.80 % mortality in ethanol, petroleum ether, watery and ethyl acetate extracts, respectively. The lowest mortality rates of all extracts were observed at the concentration of 0.0125g /100 mL.

Table 4. Lethal concentration (LC) values of extracts of *T. triquetrum* leaves against 3rd and 4th instar *Aedes aegypti* larvae

| Lethal Concentration (LC) | *Tadehagi triquetrum leaves* Extracts (gm / 100mL) | | | |
|---|---|---|---|---|
| | 95%EtOH extract | PE extract | $H_2O$extract | EtOAc extract |
| LC50 | 0.0283 | 0.0241 | 0.0198 | 0.0183 |
| LC90 | 0.1727 | 0.0953 | 0.0925 | 0.0727 |
| Chi Squire $X^2$ | 1.7380 | 23.6830 | 2.4187 | 6.8792 |
| *Df* | 4 | 4 | 4 | 4 |
| P value | 0.05 | 0.05 | 0.05 | 0.05 |

$LC_{50}$= Lethal Concentration dose 50, $LC_{90}$= Lethal Concentration dose 90, *df=degree of freedom*

The doses of 50% mortality ($LC_{50}$) and 90% mortality ($LC_{90}$) values of all extracts against 3th and 4th instar *Aedes* larvae were shown in Table 4. The lowest dose for 50% mortality was found 0.0183 g/mL of ethyl extract concentration followed by 0.0198 g/100 mL of watery extract. Ethanol extract of sample was found the highest amount of dose 0.0283 g / 100 mL concentration for 50% mortality of 3th and 4th instar *Aedes* larvae.

The lowest dose (highest efficacy) for 90% mortality was found 0.0727 g/100 mL of ethyl acetate extract concentration followed by 0.0925g/100 mL of watery extract and 0.0953g /100 mL concentration of PE extract. 95 % ethanol extract found the highest amount of dose 0.1727g / 100 mL concentration for 90% mortality of 3th and 4th instar *Aedes* larvae.

The highest persistence effect of different solvent extracts for 100% mortality of 3rd and 4th instar *Aedes* larvae were found to be only one day for all extracts. And 20-50% mortality was found day 2 in all extracts. Day 3 and after day 3 the persistency was found zero %, therefore degradability of all extracts of *T. triquetrum* leaves was observed within 2-3 days.

## 3. CONCLUSION

From the overall assessments of the present work, the following inferences could be deduced. The leaves of *T. triquetrum* possess various chemical components such as alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins, and showed negative results for α-amino acids, starchs, cyanogenic glycoside and terpenoids.. The constituents such as alkaloids and steroids present in the sample may contribute to possess bioactivities such as antimicrobial, antioxidant, anticancer, antitumor, antipyretic, and antiulcer properties. *In vitro* antioxidant activity of 95% EtOH extract of the leaves of *T. triquetrum* was found to be the highest antioxidant activity ($IC_{50}$= 1.64 μg/mL) than that of other extracts. According to larvicidal activities test, ethyl acetate and 95 % ethanol extracts have the highest larvicidal activity *in vivo* test larvae model, 3rd and 4th instar *Aedes* larvae. Larvicidal activity of *T. triquetrum* is probably due to presence of, saponins, alkaloids and flavonoids. The result obtained from this study indicated that tested crude extracts of *T. triquetrum* may play an important role in medicinal properties used *in vitro* and may be effective.

**ACKNOWLEDGEMENTS**

We would like to thank to the Department of Higher Education, Ministry of Education in Myanmar, for giving us the opportunity to do this research. We would also like to thank to Dagon University, for inviting us to present this research paper. Our deepest gratitude is expressed to Dr Si Si Hla Bu, Rector of Pathein University, for her encouragement, kind guidance, and kind help to do this research. We wish to thank Dr Than Tun and Dr Nilar Myint, Pro-Rectors of Pathein University for their invaluable advices and encouragement. Thanks are also extended to Dr Ye Myint Aung (Professor and Head) and Dr Than Than Oo (Professor), Department of Chemistry, Pathein University, for their helpful advice, precious suggestions and provision of research facilities at the Department of Chemistry, Pathein University, Myanmar.

**REFERENCES**

1. http://www.theplantlist.org/tpl1.1/record/ild-46682

2. http://www.flowersofindia.net/catalog/slides/Trefle%20Gros.html

3. http://indiabiodiversity.org/species/show/225688

4. De Boer H J, Vongsombath C, Pålsson K, Björk L, Jaenson TG. Traditional repellents against haematophagous invertebrates: A comparative study of plants used in 66 villages in Lao PDR. *Journal of Medical Entomology*. 2010;47:400-414.

5. Lwin K.S, Tu M. Effect of *Desmodium triquetrum* extract on some pathogenic bacteria. *Union of Burma Journal of Life Science.* 1968; vol 1: pp 166–170.

6. Liu TS. List of economic plants Taiwan, Section 5. Taipei, Taiwan;1952. Medicinal plants.

7. WHO, Instructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticides. , 1963; 81: WHO/VBC; 1981:807

8. WHO, Report of WHO informal consultation on the evaluation and testing insecticides. 1996; CTD/WHO PES/IC/96;1:69.