MORPHOLOGY, MICROSCOPICAL CHARACTERS, PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITYOF *TADEHAGI TRIQUETRUM* (L.) OHASHI

Khin Min MinThwe[1]

Abstract

Tadehagi triquetrum (L.) Ohashi is one of the medicinal plants under the family Fabaceae and it is well known as "laukthay" in Myanmar. This plant was collected from Myitkyina Township, Kachin State. Morphological and microscopical characters, phytochemical constituents and antimicrobial activities were investigated so as to certain their correct identification. The plant was perennial erect shrub, woody and reddish green angular branches with hairs on the angles. The corolla were papilionaceous and the fruits were jointed. In microscopical study, paracytic stomata were found on both surfaces. The vascular bundle was collateral type and endarch. The powdered leaves were tested for the phytochemical constituents and observed that alkaloids, flavonoids, phenolic compounds, steroids, saponins, tannins, glycosides and carbohydrates were present. The alkaloids, flavonoids, terpenoids, phenolic compounds, steroids, saponins, tannins, glycosides and carbohydrates were present in powdered root. In antimicrobial activity, the various solvent extracts of leaves and roots were tested by agar well diffusion method. The ethyl acetate extract of leaves and the methanol extract of roots showed the highest activity.

Key words: *Tadehagi triquetrum*, morphology, anatomy, antimicrobial activity

Introduction

Herbal medicine has a great tradition of maintaining human health for centuries. A majority of the world's population living in the developing countries still relies on herbal medicine to meet its health care needs (WHO, 1999). Medicinal plants are abundant in Myanmar. Eighty five percent of the population lives in rural areas. Most of people use the traditional medicinal plants for the treatment of diseases (Medicinal plants of Myanmar, 2000). *Tadehagi triquetrum* (L.) Ohashi,a species of Fabaceae, was distributed in Chin, Kachin, Kayin, Mandalay, Sagaing, Shan, Yangon areas of Myanmar (Kress *et al*., 2003). Allen, (1981) stated that it is widespread in all South Asian, East Asian, and Southeast Asian countries. The maximum height of this shrub tree is 3m. Leaves alternate,linear-oblong, ovate with a tapering tip. Flowers are small and pale purplish in color. Fruit is hairy and distinctly jointed.

The whole plant is used medicinally as an antipyretic, as a diuretic, for invigorating the spleen, and for promoting digestion. Chemical constituents from *Tadehagi triquetrum* (L.) Ohashi and their antihyperlipidemic activities was studied by Allen(1981). The traditional clinics have been using this medicinal plant practically in the past several decades. However, the systematic and comprehensives investigation is found to be lacking. Therefore, this research was conducted for safety and efficacy in treating lungs tonic and tuberculosis.

Aim of the present research work is to examine the medicinal plant scientifically which have effective medicinal values. The objectives are to identify and study the morphological and microscopical characters of *Tadehagi triquetrum* (L.) Ohashi, to perform the preliminary phytochemical test, and to evaluate the antimicrobial activity.

[1] Dr, Lecturer, Department of Botany, University of Myitkyina

Materials and Methods

The leaves and roots of *Tadehagi triquetrum* (L.) Ohashi were collected from the Myitkyina Township, Kachin State, from December 2017 to December 2018. Fresh specimens of vegetative and floral parts were used for identification. The leaves and roots were dried in shade for several days. When completely dried, these were pulverized by grinding machine to get the powder and stored in an air tight container for the microbiological studies.

For the microscopical studies, the lamina, midrib, petiole, stem and root from the fresh specimens were observed according to methods of Esau (1953) and Pandey(1993).

For phytochemical investigation, the leaves and roots were air dried at room temperature for two weeks. After the samples were completely dried, these were pulverized by grinding machine to get the powder and stored in an air tight container to prevent moisture changes and contamination.

Preliminary phytochemical examination for the leaves was carried out to detect the organic compounds qualitatively according to British Pharmacopoeia, (1965). Alkaloids test, Flavonoids test, Terpenoids test, Phenolic compounds test, Steroids test, Saponins test, Tannins test, Glycosides test, and Carbohydrates test were according to Marini-bettolo(1981), Unani Medicine (1987) and Trease and Evans (1980).

Antimicrobial activity of crude extracts of *Tadehagi triquetrum* (L.) Ohashi by Agar well diffusion method. Antimicrobial activity of crude extracts from the leaves and roots of *Tadehagi triquetrum* (L.) Ohashi have been investigated.

Antimicrobial activity of the leaves and roots of *Tadehagi triquetrum* (L.) Ohashi were conducted by using chloroform, methanol, ethyl acetate, 95% ethanol extracts. In this study, agar well diffusion method was used to screen the antimicrobial activity. These experiments were conducted in the Laboratory of Development Center for Pharmaceutical Technology (DCPT).

The powders of leaves and roots were extracted by using chloroform, methanol, ethyl acetate, 95% ethanol for about 2 weeks and then filtered. The solvents were then evaporated by using water bath to obtain a paste.The different solvents extracts were tested against six pathogenic microorganisms by using agar well diffusion method described by Cruickshank (1975). The strains of six pathogenic microorganisms were *Bacillus subtilis* (JAP-0221215), *Bacillus pumilus* (IFO-12102), *Staphylococcus aureus* (ATCC-12277), *Pseudomonas aeruginosa* (IFO-3080), *Candida albicans* (IFO-1060), and *Escherichia coli* (ATCC-25922).

Results

Morphological characters

Tadehagi triquetrum (L.) Ohashi, Ginkgoana 1:290.1973. (Figure 1)

Myanmar name: Laukthay

English name : Trefle Gross

Family : Fabaceae

Flowering period: October to December

Perennial erect or ascending shrub, about 1.5 cm high; branches angular, hairs on the angles, woody and reddish green.Leaves unifoliate compound, alternate and stipulate; stipules lanceolate; petioles distinctly winged, glabrous and green; leaflets oblong-lanceolate. Inflorescences terminal and many-flowered; peduncles triquetrous, tomentose and green. Flowers bisexual, zygomorphic; bracts lanceolate, pubescent, reddish green; bracteoles acute and minute, pale red.Calyx 5-lobed; tube campanulate, hairy and reddish green; lobes deltoid to linear. Corolla papilionaceous, exserted; standard orbicular; keel lanceolate, glabrous and pale purple. Stamens 10, diadelphous; staminal tube, pale green and glabrous; anthers uniform, dorsified, dithecous longitudinal slit and pale brown. Ovary oblongoid, densely villous and reddish green, unilocular, 6-9 ovuled on the marginal plancentae; style curved, glabrous and pale green; stigma simple. Pods flattened, linear, indehiscent, seeded softy pubescent with hairs and green. Seeds elliptic, small, glabrous and brown.

Figure1 Morphological characters of *Tadehagitriquetrum* (L.) Ohashi

A. Habit B. Inflorescence

C. Fruits D. Roots

Microscopical Characters

Lamina

In surface view, the cuticle is smooth and the epidermal cells of both surfaces are thin-walled parenchymatous, irregular wavy. The upper epidermal cells are slightly wavy and lower epidermal cells are wavier. Paracytic stomata are pressed on both surfaces and more abundant on the lower surface. The stomata are oval in outline with two reniform shaped guard cells and contain abundant chloroplasts. In transverse section, the cuticle was present on both surfaces. The epidermal cells were thin-walled and barrel shaped parenchymatous cells. The mesophyll was composed of palisade and spongy parenchymatous cells. Two layered thick palisade cells were right angle to the upper epidermis, elongated and compactly arranged with numerous

chloroplasts. The spongy mesophyll cells were placed beneath the palisade parenchyma consisting of 2-3 layers thick. They were rounded to elongated cells and contained numerous chloroplast (Figure 2 C).

Midrib

The epidermal cell of both surfaces are parenchymatous and rectangular in shaped along the length of the midrib. Unicellular uniseriate trichomes are present similar to those of the lamina.

In transverse section, concave in the lower surface and convex in the upper surface were covered with thick cuticle (Figure 2 D). The epidermal cells were thin-walled, barrel-shaped parenchymatous cells and compactly arranged. The upper epidermal cells were larger than the lower epidermal cells. The angular collenchymatous cells were present 1-2 layers in thickness below the upper epidermis. They were rounded to isodiametric in shape. The parenchymatous cells were 3-5 layers in thickness above the vascular bundle and 4-6 layers in thickness below the vascular bundle. The vascular bundle was crescent shaped in outline and collateral type. The four bundles was surrounded by a sheath of sclerenchymatous cells.

Petiole

In transverse section, the petiole was oval in outline (Figure 2 E). The cuticle layer was thick. The epidermal cells were barrel-shaped parenchymatous cells and compactly arranged. The cortex was made up of 6-7 layered parenchymatous cells. They were thin-walled and oval to rounded cells. The vascular bundles are semi-circular shaped four bundles and embedded in the parenchtmatous tissues. The arrangement was collateral type. A large pith region located in the center.

Stem

In transverse section, the young stem was circular in outline (Figure 2 F). The epidermal cells were barrel-shaped parenchymatous and one layer thick. The cortex was made up of collenchymatous cells towards the peripheral region. The collenchymatous cells were angular type and consisted of 4-6 layers. Endodermis located below the cortex. The cells were thin-walled, barrel-shaped parenchymatous cells and one layer thick. The pericycle fibre formed as a ring around the four vascular bundles, ectopholic siphonostele type. The vascular bundle were rounded.

Root

In transverse section, the roots are circular in outline (Figure 2 G). Cortex is made up of several layers of parenchymatous cells and vascular bundles are evenly distributed. At maturity, the epidermal cells are thin-walled and rectangular in shape. The cortex is composed of three layers. Phellem or cork cells are three to four layers with thin-walled and compact rectangular cells, phellogen or cork cambium consists of two to four layers and phelloderm or secondary cortex composed of five to seven layers, thin-walled parenchymatous and irregular arranged. Xylem towards the inner and phloem outside the xylem. Alternating layers of vascular bundles are collateral type. Storage parenchymatous cells are present betweeen the vascular bundles.

Preliminary phytochemical investigation

The preliminary phytochemical investigation the leaves of *Tadehagi triquetrum* (L.) Ohashi indicated the presence of alkaloids, flavonoids, phenolic compounds, steroids, saponins, tannins, glycosides, carbohydrates. The terpenoids was absent. The preliminary phytochemical investigation was shown in Table 1.

The preliminary phytochemical investigation of the roots of *Tadehagi triquetrum* (L.) Ohashi indicated the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, steroids, saponins, tannins, glycosides and carbohydrates. The preliminary phytochemical investigation was shown in Table 2.

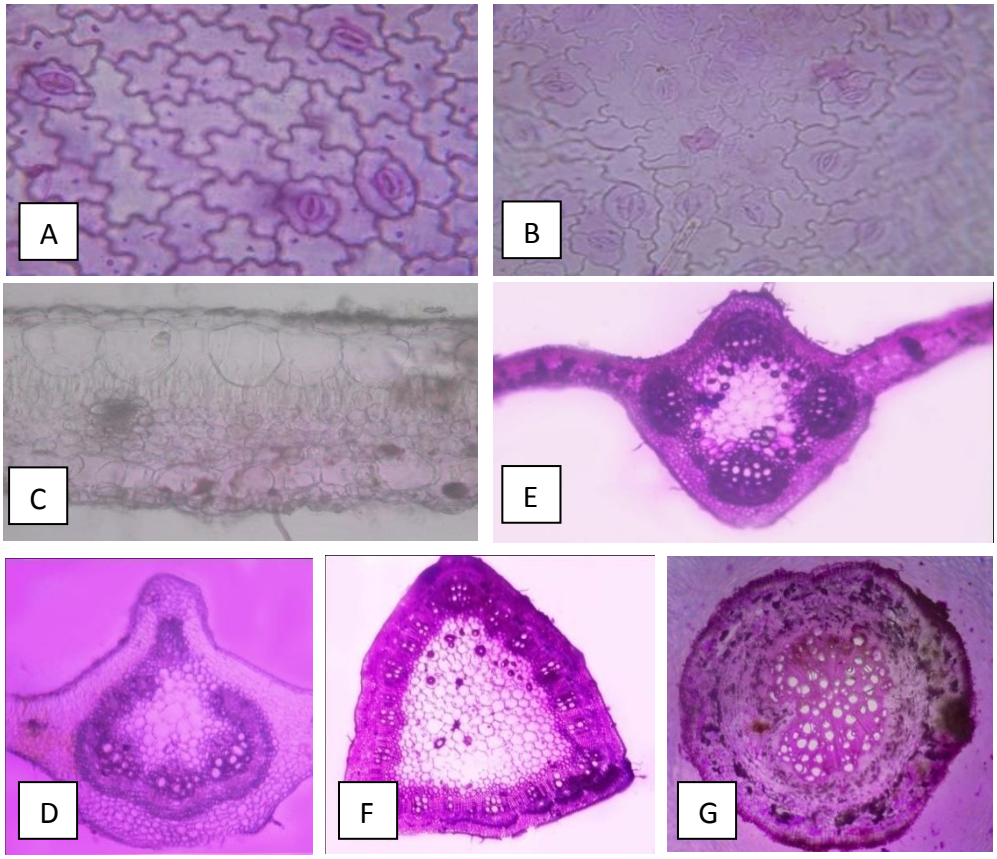

Figure 2 Microscopical characters of *Tadehagi triquetrum* (L.) Ohashi

A. Surface view of lamina showing upper epidermal cells

B. Surface view of lamina showing lower epidermal cells

C. Transverse section of lamina

D. Transverse section of midrib showing trichomes

E. Transverse section of petiole

F. Transverse section of stem

G. Transverse section of mature root

Table 1 Preliminary Phytochemical test on the leaves

No	Tests	Extracts	Test reagents	Observations	Results
1	Alkaloids	1%HCl	Dragendroff's reagent	Yellow ppt.	+
			Wagner's reagent	Orange ppt.	+
			Mayer's reagent	White ppt.	+
2	Flavonoids	EtoH	Mg turning, conc:HCL	Yellow brown	+
3	Terpenoids	EtoH	$CHCL_3$,Conc:H_2SO_4	No colour	-
4	Phenolic compounds	EtoH	5% $FeCL_3$	Reddish browncolour	+
5	Steroids	$CHCl_3$	Acetic anhydride, Conc:H_2SO_4	Green colour	+
6	Saponins	H_2O	Distilled water	Marked frothing	+
7	Tannins	H_2O	5% $FeCL_3$	Dark brown	+
8	Glycosides	H_2O	10% Lead acetate	White colour	+
9	Carbohy-drates	H_2O	10% α naphthol, Conc:H_2SO_4	Violet color ring	+

Table 2 Preliminary Phytochemical test on the roots

No	Tests	Extracts	Test reagents	Observations	Results
1	Alkaloids	1% HCL	Dragendroff's reagent	Yellow ppt.	+
			Mayer's reagent	Orange ppt.	+
			Wagner's reagent	White ppt.	+
2	Flavonoids	EtOH	Mg turning,Conc:HCL	Yellow coloration	+
3	Terpenoids	EtOH	$CHCL_3$,Conc:H_2SO_4	Reddish brown colour	+
4	Phenolic compounds	EtOH	5% $FeCL_3$	Black colour	+
5	Steroids	$CHCL_3$	Acetic anhydride, Conc:H_2SO_4	Green colour	+
6	Saponins	H_2O	Distilled water	Marked frothing	+
7	Tannins	H_2O	5% $FeCL_3$	Dark brown	+
8	Glycosides	H_2O	10% Lead acetate	White pp	+
9	Carbohy-drates	H_2O	10% α naphthol, Conc:H_2SO_4	Violet color ring	+

Antimicrobial activity of different solvent extracts from the leaves and root of *Tadehagi triquetrum* (L.) Ohashi

Antimicrobial activity of different solvent extracts from the leaves and roots of *Tadehagi triquetrum* (L.) Ohashi by using agar well diffusion method. The diameter of inhibition zones that appeared were given in Table3,4 and Figure 3,4.

According to this experiment the antimicrobial activities of *Tadehagi triquetrum*(L.) Ohashi were investigated. In the present study, the extracts of all these plants displayed different activity on the tested microorganisms. The results were showed that ethyl acetate extracts of *Tadehagi triquetrum*(L.) Ohashi leaves and methanol extracts of *Tadehagi triquetrum* (L.) Ohashi roots were highly effective extract against all tested microorganisms causing maximum inhibition zone.

Chloroform extract of leaves showed the minimum inhibition zone 11-13 mm against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* Methanol extract showed the minimum inhibition zone 13-14 mm against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Methanol extract showed the medium activities 15 mm zone against *Bacillus pumalis, Candida albicans* and *Escherichia coli.* Ethyl acetate extract of *Tadehagi triquetrum* (L.) Ohashi were highly effective extract 23-40 mm against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escheriachia coli.* Ethanol extract was showed the minimum inhibition zone 13-14 mm against *Staphlococcus aureus* and *Pseudomonas aeruginosa.* Ethanol extract was showed medium activities 15-17 mm zone against *Bacillus subtilis, Bacillus pumilus, Candida albicans* and *Escherichia coli* as shown in Table 3 and Figure 3.

Choloform extract of root showed minimum activities 12-13 mm zone against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* Methanol extracts of *Tadehagi triquetrum* (L.) Ohashi was showed highly effective extract against maxium inhibition zone 28-45 mm against *Bacillus subtilis, Staphyloccus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* Ethyl acetate extract was showed medium activities 16-18 mm zone against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli.* Ethanol extract showed medium activities 16-18 mm zone against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli* as shown in Table 4 and Figure 4.

Table 3 Antimicrobial activity of different solvent extracts from the leaves

Solvent	**Test Organisms**					
	Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aureginosa***	***Bacillus pumilus***	***Candida albicans***	***Escherichia coli***
Chloro-form	12 mm (+)	12 mm (+)	11 mm (+)	11 mm (+)	13 mm (+)	12 mm (+)
Methanol	14 mm (+)	14 mm (+)	13 mm (+)	15 mm (++)	15 mm (++)	15 mm (++)
Ethyl acetate	25 mm (+++)	23 mm (+++)	40 mm (+++)	33 mm (+++)	35 mm (+++)	40 mm (+++)
Ethanol	15 mm (++)	14 mm (+)	13 mm (+)	16 mm (++)	17 mm (++)	16 mm (++)

Agar well – 10 mm; 10 mm ~ 14 mm (+);

15 mm ~ 19 mm (++); 20 mm above (+++)

Table 4 Antimicrobial activity of different solvent extracts from the roots

Solvent	Test Organisms					
	Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Bacillus pumilus***	***Candida albicans***	***Escherichia coli***
Chloro-form	12 mm (+)	12 mm (+)	12 mm (+)	12 mm (+)	12 mm (+)	13 mm (+)
Methonol	28 mm (+++)	33 mm (+++)	45 mm (+++)	35 mm (+++)	45 mm (+++)	30 mm (+++)
Ethyl acetate	17 mm (++)	17 mm (++)	17 mm (++)	18 mm (++)	16 mm (++)	17 mm (++)
Ethanol	17 mm (++)	17 mm (++)	17mm (++)	18 mm (++)	16 m (++)	17 mm (++)

Agar well – 10 mm; 10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++); 20 mm above (+++)

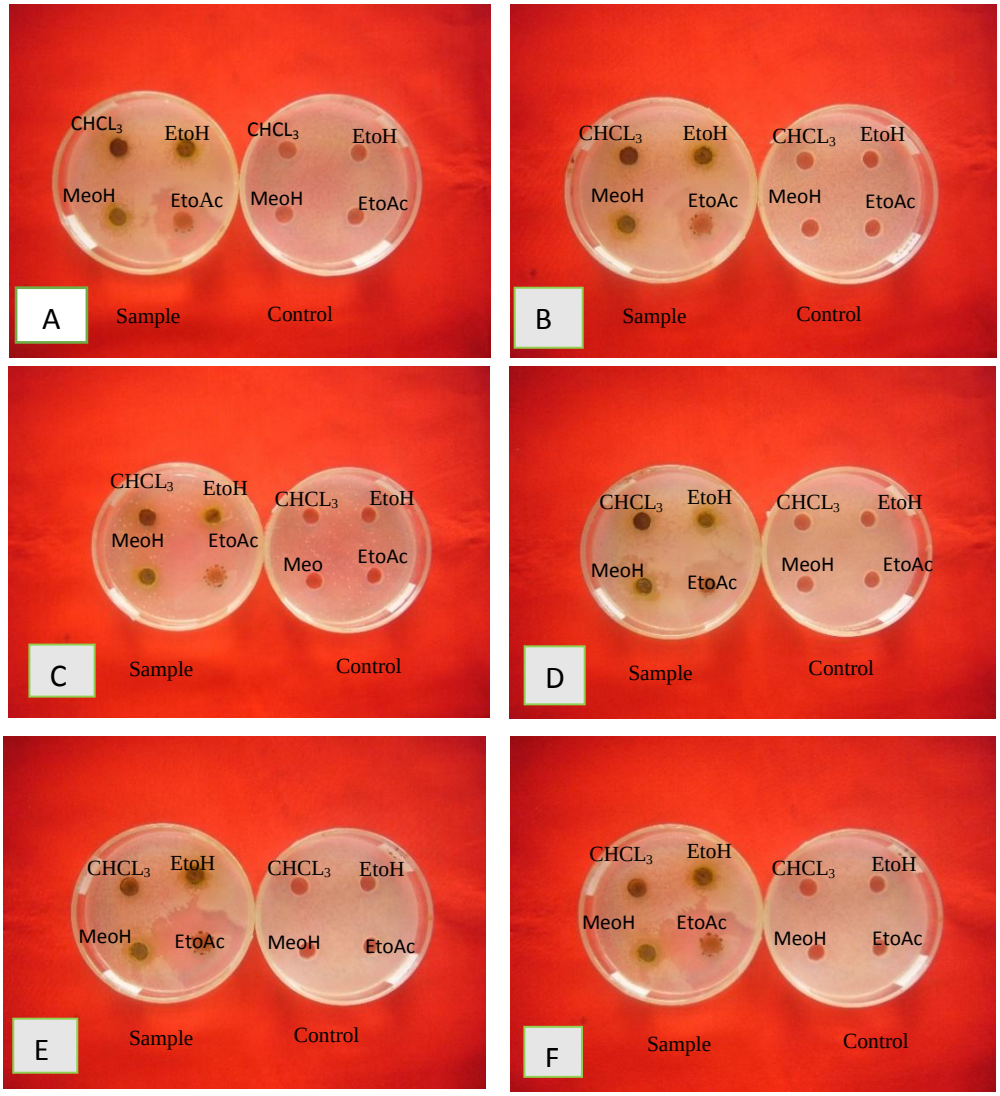

Figure 3 Antimicrobial screening of the leaves extracts from *Tadehagi triquetrum* (L.) Ohashi
A. *Bacillus subtilis*; B. *Staphylococcus aureus*
C. *Pseudomonas aeruginosa*; D. *Bacillus pumilus*
E. *Candida albicans*; F. *Escherichia coli*

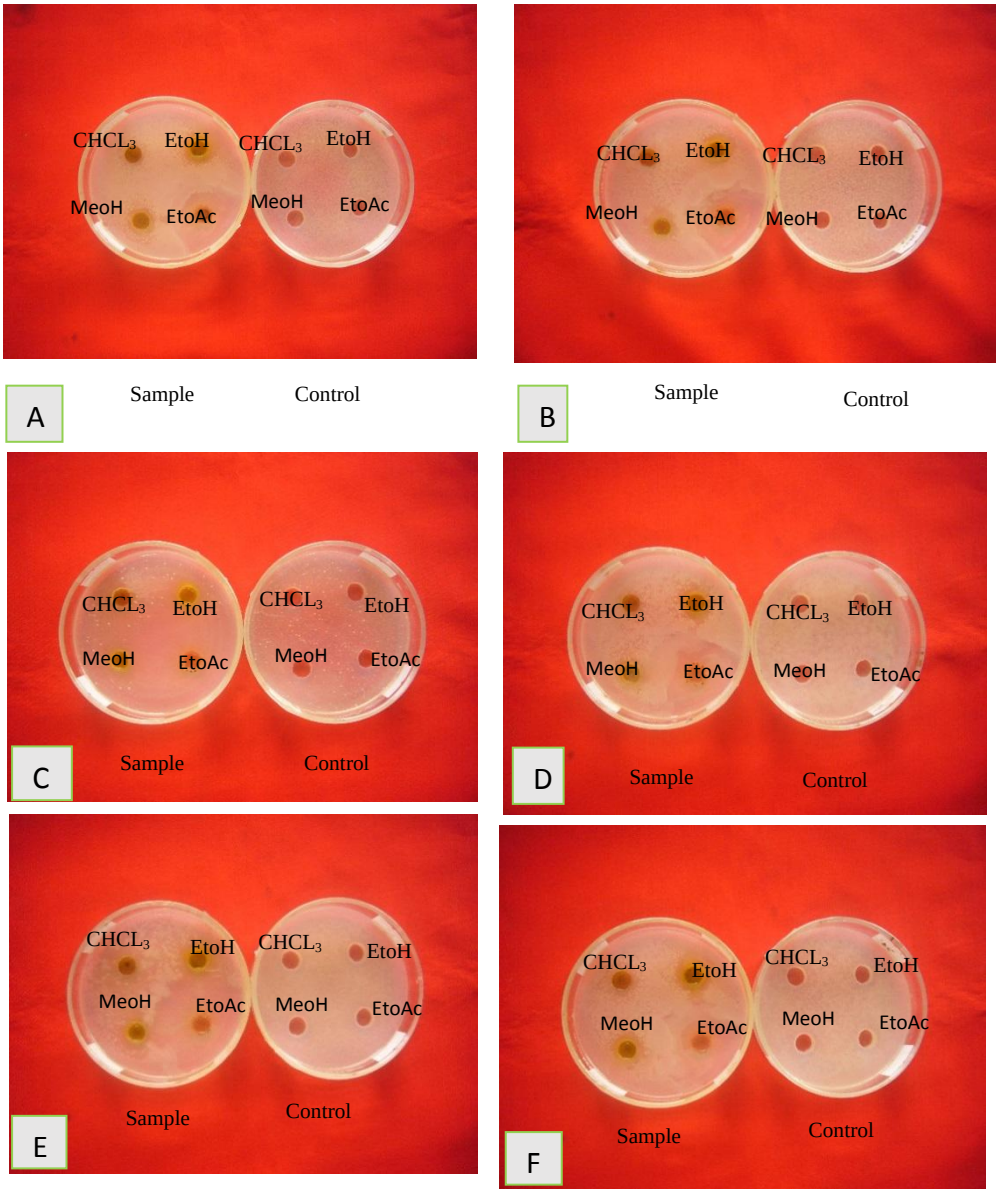

Figure 4 Antimicrobial screening of the roots extracts from *Tadehagi triquetrum* (L.) Ohashi
A. *Bacillus subtilis*; B.*Staphylococcusaureus*
C. *Pseudomonas aeruginosa*; D. *Bacillus pumilus*
E. *Candida albicans*; F. *Escherichia coli*

Discussion and Conclusion

Tadehagi triquetrum (L.) Ohashi is wildely distributed in China, Kachin, Kayin, Mandalay, Sagaing, Shan, Yangon areas of Myanmar (Kress *et al*., 2003). The morphological and microscopical study of the plant revealed that it is a herbaceous shrublet. The leaves are unifoliate with winged petioes, broadly lanceolate flowers are small with slender pedicel, stamens are diadelphouswith uniform anther; stigma are capitates, and pods are flat. These characters are in agreement with Allen (1981) and Dassanyake (1980). In microscopical study, the epidermal cells were wavy and trichomes were presentin leaflets. Paracytic type stomata were present, more in upper surface than lower surface. These characters are in agreement with Esau (1953).

Medicinal uses to expel worms treats spasms in infants, indigestion, piles, and absccesses digestion (decoction of whole plant); for hemorrhoids Leaves, as a poultice on bruises and drunk daily for chronic coughs and tuberculosis (decoction of roots); to treat kidney complaints (infusion of roots); eaten or used in baths for gastro-intestinal and urinary problems ranging from an upset stomach to hepatitis infusion or decoction of roots (Anmin *et al*., 2003).

The prelimary phytochemical test indicated that alkaloids, phenolic, saponin, glycoside, tannin, carbohydrates, flavonoids and steroid were present in the leaves and alkaloids, phenolic, saponin, glycoside, tannin, carbohydrate, flavonoid, terpenoid and steroid were present in the roots. These characters are in agreement with Kimura (1996).

Antimicrobial activities of aqueous and organic solvents extracts of *Tadehagi triquetrum* (L.) Ohashi are tested in against six different microorganisms. However, the leaves showed antimicrobial activity on Ethyl acetate extract *Pseudomonas aeruginosa* and *Escherichia coli* were the highest activity. The roots showed antimicrobial activity on Methanol extract *Pseudomonas aureginosa* and *Candida albicans* were the highest activity.This results will provide to clinician because the determination of the antimicrobial susceptibility of pathogen is important in aiding the clinician to select the most appropriate agent for the treating that disease. From the findings the extracts of this plant could be possible the therapeutic agent for the treating infectious diseases caused by six tested microorganisms.

The *Tadehagi triquetrum* (L.) Ohashi is a usual Chinese herbal medicine. This paper studied on the antimicrobial activity of *Tadehagi triquetrum* (L.) Ohashiextract and its preservative function on cut carnation. The results showed that *Tadehagi triquetrum* (L.) Ohashi extract had some effects both on bacilli and fungi (Chen, 2007).

At present, the government of Myanmar also encourage for development of scientific research on herbal and traditional medicine. Thus, further investigation on propagation of this plant and application of its medicinal uses should upgrade to confirm its activities.

Acknowledgements

First of all, I would like to mention, my deepest gratitude to Dr. Khin Win Naing, Professor and Head of Botany Department, University of Myitkyina for her kind permission to carry out the research in the department.

I would like to thank Dr. Seine Nyoe Nyoe Ko, Professor, Botany Department, University of Myitkyina for their valuable suggestions and encouragements.

I thanks also extented to Dr. Soe Myint Aye, Pro Rector, University of Myitkyina for his kind guidance and encouragements.

My deep appreciation to Daw Cherry Aung, Assistant Manager, Pharmaceutical Research Deputy, for her encouragements and invaluable help in testing the bacterial activity of my sample.

I would like to thank Dr. Tu Tu Wai, Lecturer, Chemistry Department, University of Myitkyina for her kind help and instruction of chemistry portion for this research.

References

Allen EK, Allen ON. (1981). **The leguminosae: A source Book of characteristics, Uses and Nodulation.** University of Wisconsin press, Madison pg.568.

Anminc C, Yingfu M, Yuan G, Zhemin G. (2003). **Encyclopedic Reference of Traditional Chinese Medicine**. Springer Berlin pg-564.

Bristh Pharmacopoeia (1965). **The pharmaceutical press,London and bradford.**17B 100 msbury square, London W.C.I.

Central Council for Research in Unani Medicine (1987). **Phytochemical Standard of Unani.**

Chen, J, X. (2007). **Potential of agricultural residues and hay for bioethanol production**. Appl Biochem Biotechnol 142 (3):276-90.

Cruick Shank, R., j.P. Duguid, B.P Marmion, and R.H.A. Swan (1970). **Medicinal Microbiology.**Churchill living stone Ltd; London.

Dassanyake, M.D. (1996). **Flora of ceylonVol.X.**Amerind Publishing Co.Rt. Ltd., New Delhi.

Esau, K. (1953). **Plant Anatomy.** John Wiley and Sons, Inc. New York. London.

Kress, W.J.R.A Defilipps. E. Earr& Yin YinKyi (2003). **A Checklist of the trees,shrubs, herbsandclimbers of Myanmar.** Department of Systematic Biology Botany, National, Museum of Natural History Washington DC. USA.

Kimura,T. (1996). **International Collation of Traditional and Folk Medicine**. Northeast Asia Part 1, World Scientific publishing Co. Singapore.Pg.62.

Marini Bettolo,G.B. (1981). **Plant screening by chemical and chromatographic procedure under field condition.** Journal Chromatography.

Medicinal Plants of Myanmar (2000). **Ministry of Health of China Department of Traditional Medicine.**

Meskin, Mark S.(2002). **Phytochemicals in Nutrition and Health.** CRC Press.p.123.ISBN 978-1-58716-083-7

Pandey, S.N & A.C chanda. (1993). **Plant Anatomy and Embroyology**.Vikas Publishing House (Put, Ltd)

Trease and Evans (1980). **Pharmacognosy.** 11th Edition, Balliere London.

World Health Organization (1999). **W.H.O Monographs on Selected Medicinal Plants.**Geneva,1.