INVESTIGATION OF MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS, PRELIMINARY PHYTOCHEMICAL EXAMINATION AND ANTIMICROBIAL ACTIVITYON LEAVES OF *MUNTINGIA CALABURA* L.

Htay Htay Lwin[1], Yadana[2], Aye Aye Aung[3]

Abstract

The plant *Muntingia calabura* L., Myanmar name "Hnget-thagya" belongs to the family Muntingiaceae. The plants are widely distributed in Myanmar. It was collected from Hpa-an township, Kayin State from October to December (2019). In this study, morphological and histological characters, phytochemical properties and antimicrobial activity of *Muntingia calabura* L. were investigated. In morphological study, the plant is small trees, leaves alternate and simple. Inflorescences solidary cymes. Flower white. In histological study, anisocytic stomata present on lower surface of lamina. Unicellular simple, stellate and multicellular head glandular trichomes are also present. Type of Vascular bundles in lamina, midrib and petiole are collateral type. The powdered sample has been investigated and presented as diagnostic characters for the standardization of powdered drugs. Phytochemical tests were done at Department of Botany, Hpa-an University. Phytochemical tests of *Muntingia calabura* L. leaves showed the presence of alkaloid, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid. Antimicrobial activity of leaves of *Muntingia calabura* L. was carried out at Botany Department, University of Yangon by using different solvent extracts (petroleum ether, ethyl acetate, acetone, ethanol, methanol and water). The extracts of *Muntingia calabura* L. leaves indicated antimicrobial activity against *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Echerichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, methonolic extracts showed the most significant antimicrobial activity against *Aspergillus flavus* and *Candida albicans* as well as ethanolic extract on *Candida albicans*. Peteroleum ether and aqueous extract showed antimicrobial activity on all microorganisms. *Muntingia calabura L.* leaves are effective on protection of diseases which caused by microorganisms.

Keyword: *Muntingia calabura* L., morphological and histological characters, phytochemical test and antimicrobial activity.

Introduction

The plant *Muntingia calabura* L.belongs to the family Muntingiaceae (Zakaria, *et al*., 2016). This family is small recently recognized neotropical family with genera formerly included in Tiliaceae (Heywood, *et al*., 2007). In Myanmar, it is locally known as Hnget-thagya and calabur; cherry tree; cotton candy berry; Jamaican cherry; Panama berry; Panama cherry; strawberry tree in English. It is native to the American continent and is widely cultivated in warm areas of Asian region (Chin, 1989). It is widely cultivated in warm areas in India and Southeast Asia such as Malaysia, Indonesia, and the Philippines (Morton, 1987; Zakaria, *et al*., 2010; Sani, *et al*., 2012 and Yusof, *et al*., 2013). In Philippines Islands, the flowers are made into infusion, used after the manner of lime flower in Europe for head-aches (Burkill, 1935). Medicinal plants are known for their rich sources of secondary metabolites such as triterpene glycosides, flavonoids, tannins alkaloids and other aromatic compounds (Sindhan, *et al.,* 1999).

Muntingia calabura L. leaves extracts also possesses antibacterial activity (Zakaria, *et al*., 2006). In *Muntingia calabura* L. leaves, flowers, barks and roots have been used as a folk remedy

[1] Dr, Associate Professor, Department of Botany, Hpa-an University
[2] Dr, Associate Professor, Department of Botany, Hpa-an University
[3] Dr, Lecturer, Department of Botany, Hpa-an University

to treat headaches, fever and incipient cold. According to Peruvian folklore, the leaves are used to provide relief from gastric ulcers and to reduce swelling of the prostate gland. Besides, they are also employed as antiseptic, antispasmodic and antidyspeptic agent (Zakaria, *et al*., 2006), antitumor (Kaneda, *et al*., 1991 and Su B-N, *et al*., 2003), antibacterial and antinociception (Zakaria, *et al*., 2006), anti-inflammatory, antipyretic (Zakaria, *et al*., 2007), antioxidant and antiproliferative activities exhibited by the leaves of *Muntingia calabura* (Zakaria, *et al*., 2011).

Many plant extracts show their antimicrobial traits, which is due to the presence of compounds synthesized in the secondary metabolism of the plant. These secondary metabolites mainly consists of phenolics including polyphenols, flavonoids, tannins and quinones known for their potent antioxidant, cytotoxic and antimicrobial activities (Dai, *et al*., 2010, Pahari, *et al*., 2012 and Wong, *et al*., 2014). In this paper, morphological characters, phytochemical test and antimicrobial activity of *Muntingia calabura* L. were carried out.

The aim and objectives are to determine the morphological and histological characters, preliminary phytochemical tests and to examine the antimicrobial activities from the different solvent extracts by using on six types of microorganisms.

Materials and Methods

Collection and identification of *Muntingia calabura* L.

The plant *Muntingia calabura* L. was collected from Hpa-an township, Kayin State, from October to December (2019). The collected plant was identified with the available literatures of Hundley and Chit Ko Ko, (1961), Kress, *et al*., (2003), Heywood, *et al*., (2007) and Mahmood, *et al*., (2014).

Histological study of *Muntingia calabura* L.

In histological studies, free hand section of leaves (lamina, midribs, petioles) from the fresh specimens were prepared by using chloral hydrate solution for clearing reagents, safranin for testing lignin. These characters were determined according to the literatures of Metcalfe and Chalk (1950); Wallis (1967) and Pandey (1996).

Preparation of powdered samples of *Muntingia calabura* L. leaves

The collected samples were washed with water to remove impurities. After washing the samples cut into small pieces then air dried at room temperature when constant weight was obtained the dried plant material were homogenized by blender to get powder and stored in air tight containers to prevent moisture changes and contamination. Diagnostic characters of powder were examined to get standardization of powdered drug in traditional medicine.

Phytochemical investigation of *Muntingia calabura* L. leaves

In this investigation, the powdered *Muntingia calabura* L. leaves were tested to find out the presence or absence of chemical constituents such as alkaloid, α-amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid compounds. Preliminary phytochemical tests were carried out according to the methods of Marini Bettolo, *et al*., (1981), Central Council for Research in Unani Medicine (1987) and Sasikala and Sundaraganapathy (2017).

Antimicrobial activities of different solvent extracts from *Muntingia calabura* L. leaves

Antimicrobial activities of different solvent extracts of *Muntingia calabura* L. leaves were tested on six pathogenic microorganisms by using paper disc diffusion method at the Department of botany, University of Yangon.

Preparation of crude extracts

The powdered of *Muntingia calabura* L. leaves were extracted with various solvents such as petroleum-ether, ethyl-acetate, acetone, ethanol, methanol and water. The filtrates were evaporated by using water bath.

Preparation of sample for testing antimicrobial activity

Screening of Antimicrobial activity of crude extracts had been done by paper disc diffusion method. Paper disc having six millimeter diameter were utilized for antimicrobial test. Assay medium was prepared according to the method described by Cruickshank (1975). Assay medium was boiled and 20- 25 ml of the medium was poured into each conical flask, plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the conical flasks were cooled down to 40- 45°C and each of 0.1- 0.2 ml of test organisms were also added into the flask and then, poured into sterilized petridishes. After solidification, paper disc impregnated with sample were applied on the agar plates and incubated at 37°C for 24 hours. Then the diameter of inhibitory zone was measured with the help of a transparent ruler.

Results

Morphological characters of *Muntingia calabura* L.

Small trees, with spreading branches. The leaves are simple, alternate, distichous, oblong or lanceolate, long pointed at the apex, oblique at the base with dark green color and stellate, simple and glandular hair on the both surfaces. Inflorescences solidary or two to three flowers. The flowers are ebracteate, ebracteolate, pedicellate, complete, bisexual, actinomorphic, regular, 5-merous, hypogynous. Calyx (5), fuse at the base, sepaloid, lanceolate, hairy, persistent. Petals 5, white, caducous. Stamens numerous, apostamenous, filament filiform, anther yellow, dithecous, dorsifixed, longitudinal dehiscence. Carpel (5), pentacarpellary, syncarpous, pendulous placentation, numerous ovules, style stout and thick, stigma capitate. The fruits are abundant, in round shape, with red or yellow, smooth, soft, juicy pulp, with very sweet and filled with exceedingly tiny, yellowish seeds.

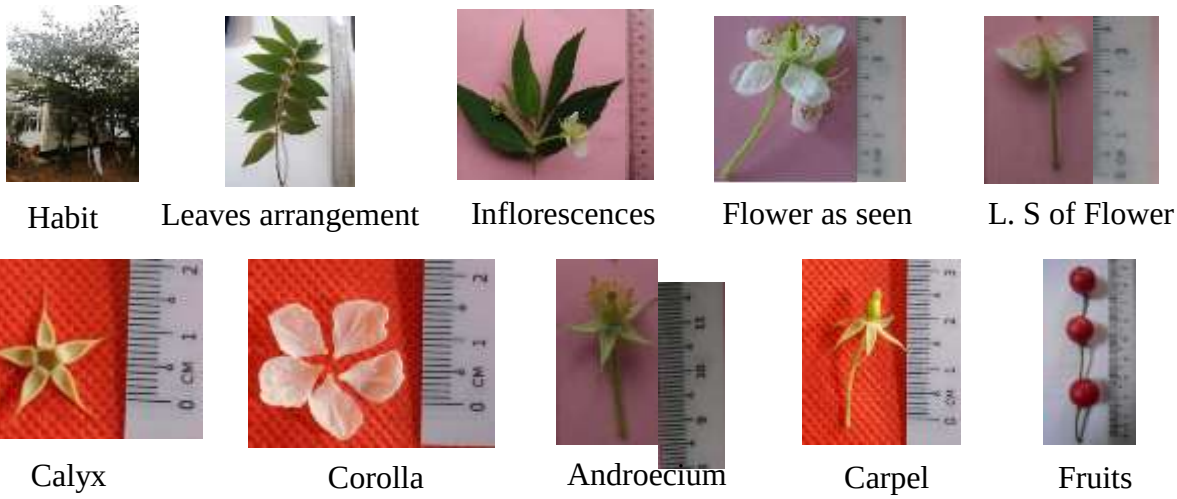


Figure 1 Morphological characters of *Muntingia calabura* L.

Histological characters of leaves on *Muntingia calabura* L.

Lamina

In surface view, the cuticle is thin, the epidermal cells both surfaces are thin- walled parenchymatous and the anticlinal wall of the upper surface is straight and lower surface is wavy. Anisocytic stomata are present only on the lower surface. The stomata are oval in outline with two-reniform shaped guard cells and contain abundant chloroplasts. Cluster of calcium oxalate crystals are present on both surfaces. Unicellular stellate, simple with multicellular head glandular trichomes are present on the both surfaces.

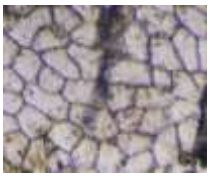
In transverse section, cuticle layer is thin on both surfaces. Both upper and lower epidermal cells are barrel shaped, thin-walled, parenchymatous cells. Palisade parenchyma found beneath the upper epidermis is 1 – 2 layers. These cells are vertically elongated and compactly arranged, with abundant chloroplast. The spongy mesophyll cells are 1 – 2 layers, loosely arranged, irregular in shape, with many intercellular spaces. Vascular bundles are embedded in the mesophyll cells. They are collateral type. Cluster of calcium oxalate crystal are abundantly present among the mesophyll cells.

Midrib

In transverse section, the cuticle layer is thin. Both upper and lower epidermal cells are more or less barrel shaped, thin-walled, parenchymatous cells. Below the epidermis, 2 – 4 layers of collenchymatous cells are present towards the upper surface and 2 – 3 layers the lower surface. Inner to the upper and lower collenchymatous layers consists of parenchymatous cells are 4 – 6 layers, rounded to oval in shape. Collateral type vascular bundles are embedded in the parenchymatous layers. Cluster of calcium oxalate crystals are present in cortex layers and vascular bundles. Unicellular simple, stellate and multicellular head glandular trichomes are present on the both surfaces.

Petiole

In transverse section, the cuticle layer is thin. The epidermal cells of both surfaces are more or less barrel shaped, with thin-walled, parenchymatous cells. The collenchymatous cells are 2 – 3 layers, rounded to polygonal shaped. The parenchymatous cells between two collenchymatous layers are 5 – 7 layers and are polygonal to isodiametric in shape. Vascular bundles are collateral type and embedded in the parenchymatous layers. Cluster of calcium oxalate crystals are present in cortex layers and vascular bundles. Unicellular simple, stellate and multicellular head glandular trichomesare present.



Upper surface view of lamina(X400)

Upper surface view of lamina showing stellate trichomes (X100)

Lower surface view of lamina (X400)

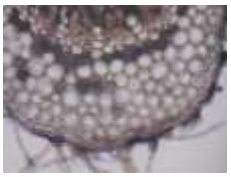
Transverse section of lamina (X100)

Transverse section of lamina showing trichomes (X100)

Transverse section of midrib (X100)

Transverse section of midrib showing upper cortex (X200)

Transverse section of midrib showing vascular bundle (X200)



Transverse section of midrib showing lower cortex (X200)

Transverse section of petiole in outline (X40)

Transverse section of petiole showing cortex layer (X400)

Transverse section of petiole showing vascular bundle (X100)

Figure 2 Microscopical characters of leaves of *Muntingia calabura* L.

Diagnostic characters of powdered plant of *Muntingia calabura* L.

The powdered *Muntingia calabura* L. was green coloured and odourless. It was slightly bitter taste. It consists of fragment of lamina, unicellular simple trichomes, stellate and glandular trichomes, fragment of upper epidermal cells with chloroplast, stomata, spiral vessels, annular vessels, fibres, tracheids and cluster of calcium oxalate crystals.

Table 1 Sensory characters of powder leaves of *Muntingia calabura* L.

Characters	**Powdered leaves of *Muntingia calabura* L.**
Colour	Green
Odour	Odourless
Taste	Slightly bitter
Texture	Fibrous with sticky

Fragment of lamina (X 400)

Various types of trichomes (X 400)

Fragment of epidermal cells (X 400)

Fragment of stomata (X 400)

Annular vessel (X 200)

Spiral vessel (X 200)

Glandular trichome (X 400)

Simple trichome (X 100)

Multicellular glandular trichomes (X 200)

Figure 3 Diagnostic characters of powdered leaves of *Muntingia calabura* L.

Phytochemical investigation of *Muntingia calabura* L. leaves

Preliminary phytochemical tests indicated the presence of alkaloid, α-amino acids, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid of *Muntingia calabura* L. leaves. The experimental results were shown in Table (2).

Table 2 Phytochemical test of *Muntingia calabura* L. leaves

No.	Test	Extract	Test reagents	Observation	Results
1.	Alkaloid	EtOH	1.Dragendorff's reagent 2. Mayer's reagent 3.Wagner's reagent 4. Hager's reagent	Orange brown ppt White ppt Reddish brown ppt Yellow ppt	+ + + +
2.	α-amino acids	H_2O	Ninhydrin reagent	Pink spot	+
3.	Carbohydrate	H_2O	α-napthol+conc:H_2SO_4	Red ring	+
4.	Flavonoid	EtOH	HCl / Mg	Pink color	+
5.	Glycoside	EtOH	H_2O + NaOH	Yellow color	+
6.	Phenolic compound	EtOH	H_2O + 10% $FeCl_3$	Greenish blue color	+
7.	Protein	H_2O	Millon's reagent	White ppt turns red on heating	+
8.	Reducing sugar	H_2O	Fehling's A and B	Brick red ppt	+
9.	Saponin	H_2O	H_2O	Frothing	+
10.	Starch	H_2O	Iodine solution	Blue black	+
11.	Steroid	EtOH	$CHCl_3$ + conc:H_2SO_4	Green color	+
12.	Tannin	H_2O	5% $FeCl_3$ + H_2SO_4	Yellow brown ppt	+
13.	Terpenoid	EtOH	$CHCl_3$ + conc:H_2SO_4	Pink color	+

(+) = Present

Antimicrobial activities of different solvent extracts of *Muntingia calabura* L. leaves by using paper disc diffusion method

Table 3 Antimicrobial activities of different solvent extracts from *Muntingia calabura* L. leaves against (6) tested organism

No	Solvents	*A. flavus*	*B. subtilis*	*C. albicans*	*E. coli*	*P.fluorescen*	*X. oryzae*
1.	Acetone	16 mm	14 mm	14mm	8mm	8 mm	10mm
2.	Ethyl acetate	14 mm	10mm	14mm	8 mm	8 mm	8 mm
3.	Ethanol	14mm	12mm	20mm	8 mm	16 mm	14 mm
4.	Methanol	20 mm	14mm	20mm	8mm	16 mm	14 mm
5.	Pet- ether	8mm	8mm	10mm	8 mm	8 mm	8 mm
6.	Aqueous	12mm	10 mm	10mm	8 mm	10 mm	10 mm

Paper disc size = 6 mm

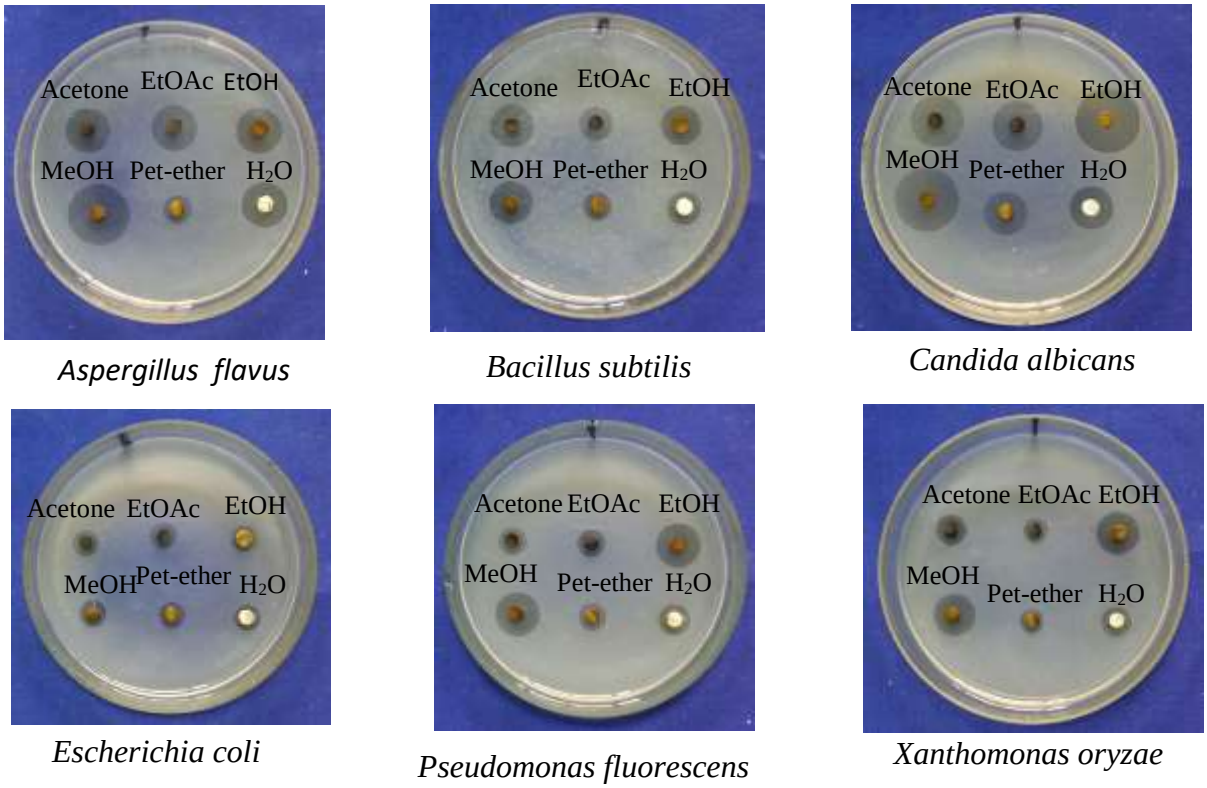


Figure 4 Antimicrobial activities of *Muntingia calabura* L. leaves

Discussion

In this investigation, morphological and histological characters, phytochemical test, and antimicrobial activity of *Muntingia calabura* L. leaves were carried out.

In morphological study, this plant is small trees, with spreading branches. The leaves are simple, alternate, distichous, stellate, simple and glandular hair on the both surfaces. Inflorescences solidary or two to three flowers. The flowers are bisexual, actinomorphic. Calyx (5), fuse at the base. Petals 5, white, caducous. Stamens numerous. Carpel (5), pentacarpellary, syncarpous, pendulous placentation, numerous ovules, style stout and thick, stigma capitate. The fruits are abundant, in round shape, with red or yellow with very sweet. These characters were agreed with those described by Heywood, *et al*., (2007) and Mahmood, *et al*., (2014).

In histological study, the epidermal cells the upper surface is straight and lower surface is wavy. Anisocytic stomata present only on the lower surface. Vascular bundles of lamina, midrib and petiole are collateral type. Cluster of calcium oxalate crystals present in mesophyll layer of lamina and cortex layer and vascular bundle of midrib and petiole. Unicellular simple, stellate, and glandular hair with multicellular head present in lamina, midrib and petiole. These characters were agreed with those described by Metcalfe and Chalk (1950) Wallis (1967) and Pandey (1996).

Singh, *et al*., (2017) reported that phytochemical screening of *Muntingia calabura* L. extracts revealed the presence of sterols, flavonoids, alkaloids, and tannins.

Minh, *et al*., (2019) reported that *Muntingia calabura* L. leaf contains different phytochemicals that include terpenoids, reducing sugars, flavonoids, saponins, tannins, phenols and carbohydrates.

Zakaria, (2007) mentioned that cherry leaf contains flavonoid, tannin, triterpene, saponin and polifenol as antioxidants.

In this research, the powdered sample of *M. calabura* L. contained alkaloid, α- amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid.

Ramasamy, *et al*., (2017) stated that the methanol leaf extract of *Muntingia calabura* L. inhibited the growth against *Xanthomonas oryzae, Erwinia amylovora* and *Agrobacterium tumefaciens.*

Buhian, *et al*., (2016) mentioned that methanolic and acetate fractions of the leaf crude extracts were shown to inhibit the growth of *Staphylococcus aureus*.

Pujaningsih, *et al*., (2018) reported that the extract from the cherry leaf produced higher inhibitory resistance to *S. aureus* and *E. coli*.

In this research, the different solvent extracts (petroleum ether, acetone, ethyl-acetate, ethanol, methanol and distilled water) of *Muntingia calabura* L. leaves were tested on six pathogenic microorganisms such as *Aspergillus flavus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas fluorescens, Xanthomonas oryzae* by using paper disc diffusion method. According to this experiment, all extracts (petroleum ether, acetone, ethyl-acetate, ethanol, methanol and aqueous) showed antimicrobial activity all test organisms. Among them, methanolic extract showed the most significant antimicrobial activity against *Aspergillus flavus* (20mm), acetone, ethyl acetate, ethanolic extracts showed the more significant antimicrobial activity (14 – 16 mm) and petroleum ether and aqueous extracts showed antimicrobial activity against *Aspergillus flavus* (8 –12 mm). Acetone and methanolic extract showed the more significant antimicrobial activity on *Bacillus subtilis* (14 mm), ethyl acetate, ethanol, petroleum ether and aqueous extracts showed antimicrobial activity against *Bacillus subtilis* (8 –12 mm). Ethanolic and methanolic extracts showed the most significant antimicrobial activity against *Candida albicans* (20mm), acetone and ethyl acetate showed more significant antimicrobial activity (14 mm) and petroleum ether and aqueous extracts showed antimicrobial activity against *Candida albicans* (10 mm). Ethanolic and methanolic extracts showed more significant antimicrobial activity against *Pseudomonas fluorescens* (16 mm) and *Xanthomonas oryzae* (14 mm), acetone, ethyl acetate, petroleum ether and aqueous extracts showed antimicrobial activity against *Pseudomonas fluorescens* and *Xanthomonas oryzae* (8 –10 mm). All extracts showed antimicrobial activity against *Escherichia coli* (8 mm).

Conclusion

The plant *Muntingia calabura* L. belongs to family Muntingiaceae. It is a small tree, with spreading branches. The leaves are simple, alternate, distichous. In histological characters, anisocytic stomata present on lower surface. Simple, stellate, and glandular trichomes present on lamina, midrib and petiole.

Due to the presence of active constituents such as alkaloid, α-amino acid, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, steroid, tannin and terpenoid these documents were highlighted to know effective medicinal bioactivities of *Muntingia calabura* L. leaves.

The extracts of *Muntingia calabura* L. leaves indicated antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Candida albicans, Echerichia coli, Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, acetone extract showed more significant antimicrobial activity against *Aspergillus flavus, Bacillus subtilis* and *Candida albicans* (14 – 16 mm), antimicrobial activity against *Escherichia coli, Pseudomonas fluorescens and Xanthomonas oryzae* (8 –10 mm). Ethyl acetate extract showed more significant antimicrobial activity on *Aspergillus flavus* and *Candida albicans* (14 mm) and *Bacillus subtilis, Escherichia coli,*

Pseudomonas fluorescens and Xanthomonas oryzae on antimicrobial activity (8 – 10 mm). Ethanolic extract showed the most significant antimicrobial activity on *Candida albicans* (20 mm), more significant activity on *Aspergillus flavus, Pseudomonas fluorescens and Xanthomonas oryzae* (14 – 16 mm) and antimicrobial activity on *Bacillus subtilis, Escherichia coli* (8 – 10 mm). Methanolic extract showed the most antimicrobial activity against *Aspergillus flavus* and *Candida albicans* (20 mm) and more significant activity on *Bacillus subtilis, Pseudomonas fluorescens and Xanthomonas oryzae* (14 – 16 mm) and antimicrobial activity on *Escherichia coli* (8 mm). Petroleum ether and aqueous extracts showed antimicrobial antimicrobial activity against *all microorganisms.*

Therefore, extracts of *Muntingia calabura* L. leaves is effective in protecting against bronchitis caused by *Aspergillus flavus*, endocarditis, meningitis, infection of wounds, ears, eyes, respiratory tract, urinary tract and gastrointestinal tract caused by *Bacillus subtilis* alimentary tract infection, cardiac infection, sores and inflammation by *Candida albicans*, diarrhoea, dysentery by *Echerichia coli*. Fever, nausea and vomiting and rapid heart rate in human and leaf blight caused by *Pseudomonas fluorescens*. Extracts of *Muntingia calabura* L. leaves can prevent rice blight caused by *Xanthomonas oryzae.* So, *Muntingia calabura* L. leaves is effective on protection of diseases which caused by microorganisms.

Acknowledgement

I would like to express my special thanks to Dr Than Than Myint Pro-Rector, Hpa-an University for their allowing me to do this research paper. I also wish to extend my gratitude to Dr Khin San Nu, Professor and Head Dr Ohnmar Thein, Professor, Department of Botany, Hpa-an University for their advice and encouragement in preparing this paper.

References

Burkill, L. H. (1935). "A Dictionary of The Economic Products of the Malay Peninsula". Vol I .Governments of the Straits Settlements and Federated Malay States by the Crown Agents for the Colonies, Millbank, London, S. W. I.

Buhian, W. P. C., Rubio, R. O., Martin-Puzon, J. J. (2016). "Chromatographic fingerprinting and free-radical scavenging activity of ethanol extracts of Muntingia calabura L. leaves and stems". *Asian Pacific Journal of Tropical Biomedicine.*

Central Council for Research in Unani Medicine. (1987). "Phytochemical Standards of Unani Formulation"**.** Ministry of Health, New Delhi.

Chin WY. (1989). "A guide to the wayside trees of Singapore". BP Singapore Science Centre.

Cruickshank, R. J. P. (1975). "Medicinal Microbiology". Living Stone Ltd., London.

Dai, J., Mumper, R. J. (2010). "Plant phenolics: extraction, analysis and their antioxidant and anticancer properties". *Molecules*, 15, (10), 7313-52.

Heywood, V. H., Brumitt, R. K., Culhan, A. and Seberg, O. (2007). "Flowering Plants of The World"**.** London: Oxford University Press.

Hundley, H.G and Chit Ko Ko. (1961). "List of Trees, Shrubs, Herbs and Principle Climbers of Burma". (3rd ed.), Government printing press, Burma. Rangoon.

Kaneda, N., Pezzuto, J. M., Soejarto, D. D., Kinghorn, A. D., Farnsworth, N. R., Santisuk, T. (1991). "Plant Anticancer Agents", XLVIII. New Cytotoxic Flavonoids from Muntingia calabura Roots. J. Nat Prod. 54(1):196–206.

Kress, W. J., Farr, R. A. D. E., and Yin Yin Kyi. (2003). "A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar"**.** Department of Systematic Biology, Botany, National Museum of Natural History, Washington, DC.

Mahmood, N. D., Nasir, N. L. M., Rofiee, M. S., Tohid, S. F. M., Ching, S. M., The, L. K., Salleh, M. Z., Zakaria, Z. A. (2014). "Muntingia calabura: A review of its traditional uses, chemical properties and pharmacological observations". Vol, 52, Issue, 12, Pages, 1598 – 1623.

Marini Bettolo, G. B., Nicolet tic, M., and Patmia, M. (1981). "Plant Screening by Chemical Chromatographic Procedure Under Field Conditions. Journal of Chromatogram.

Metcalfe, C. R. and Chalk, L.(1950). "Anatomy of the Dicotyledons"**.** Vol. II. Oxford at the Clarendon Press.

Morton, J. F. 1987. "Jamaica cherry. In: Morton JF, ed. *Fruits of Warm Climates*. Miami, Florida 2: 65-69.

Minh, N. P., Nhan, N. P. T., Khoa, N. N. A., Thao, N. N., Tram, N. T., Sang, V. P.(2019). "Different Parameters of Herbal Tea Production from Straw Berry (Muntingia calabura) Leaf". J. Pharm. Sci. & Res. Vol. 11(4), 1451-1454.

Pahari, B., Chakraborty, S., Chaudhuri, S., Sengupta, B., Sengupta, P. K. (2012). "Binding and antioxidant properties of therapeutically important plant flavonoids in biomembranes": insights from spectroscopic and quantum chemical studies. *Chem Phys Lipids*, 165, (4), 488-96.

Pendy, S. N and Chadha, A. C. (1996). "Plant Anatomy and Embryology". Vikas Publishing House Pvt. Ltd., New Delhi.

Ramasamy,R., Jaivel, N., Smitha, K. P. and Marimuthu, P. (2017). "Screening of antibacterial activity of *Muntingia calabura* leaves extracts against bacterial pathogens". International Journal of Chemical Studies 2017; 5(5): 313-316.

Sani M. H, Zakaria, Z. A., Balan, T., The, L.K., Salleh, M.Z. (2012). "Antinociceptive activity of 1481 methanol extract of *Muntingia calabura* leaves and the mechanisms of action involved". 1482 *Evid Based Comp Altern Med* 2012: Article ID 890361. doi:10.1155/2012/890361.

Sindhan, G.S., Hooda, I ., Parashar, R.D. (1999). "Evaluation of plant extracts for the control of powdery mildew of pea". J. Mycol. Pl. Pathol. 29(2):257-258.

Singh R, Iye S, Prasad S, Deshmukh N, Gupta U, Zanje A, Patil S, Joshi S. (2017). "Phytochemical Analysis of *Muntingia calabura* Extracts Possessing Anti-Microbial and Anti-Fouling Activities". International Journal of Pharmacognosy and Phytochemical Research 9 (6); 826-832. ISSN: 0975-4873.

Su B-N, Jung Park, E., Vigo, J. S., Graham, J. G., Cabieses, F., Fong, H. H. S. (2003). "Activity-guided isolation of the chemical constituents of Muntingia calabura using a quinone reductase induction assay. Phytochemistry". 63(3):335–41.

Wallis, T. E. (1967). "Textbook of Pharmarcognosy". Fifth edition. J. & A. Churchill LTD. 104 Gloucester place, London.

Wong, I. L., Chan, K. F., Chen, Y. F., Lun, Z. R., Chan, T. H., Chow, L. M. (2014). "In vitro and in vivo efficacy of novel flavonoid dimers against cutaneous leishmaniasis". *Antimicrob Agents Chemother*, 58, (6), 3379-88.

Yusof, M. M., The, L. K., Zakaria, Z. A., Ahmat, N. (2013). "Antinociceptive activity of the 1533 fractionated extracts of *Muntingia calabura*". *Planta Med* 77: PF21. doi: 10.1055/s 0031-1534 1282409.

Zakaria, Z. A., Fatimah, C. A., Jais, A. M. M., Zaiton, H., Henie, E. F. P., Sulaiman, M. R. (2006). "The in vitro Antibacterial Activity of *Muntingia calabura* Extracts". Int J Pharmacology. 2(4):439–42.

Zakaria, Z.A., C.A. Fatimah, A. M.M. Jais, H. Zaiton, E.F.P. Henie, M.R. Sulaiman, M.N. Somchit, M .Thenamutha and D .Kasthuri. (2006). "The *in vitro* antibacterial activity of *Muntingia calabura* extracts". Int. J. Pharmacol. 2(4):439-442.

Zakaria, Z. A., Sulaiman, M. R., Jais, A. M., Somchit, M.N,, Jayaraman, K.V., Balakhrisnan, G. (2006). "The antinociceptive activity of *Muntingia calabura* aqueous extract and the involvement of L-arginine/nitric oxide/cyclic guanosine monophosphate pathway in its observed activity in mice". Fundam Clin Pharmacol. 20(4):365–72.

Zakaria, Z. A., Mustapha, S., Sulaiman, M. R., Mat Jais, A. M., Somchit, M. N., Abdullah, F.C. (2007). "The antinociceptive action of aqueous extract from *Muntingia calabura* leaves: the role of opioid receptors". Medical Principles Practice. 16(2):130–6.

Zakaria, Z. A., Hazalin, N. A., Zaid, S. N. H., Ghani, M. A., Hassan, M. H., Gopalan, H. K.(2007). "Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models". J.Nat Med. 61(4):443–8.

Zakaria Z. A. (2007). " Free radical scavenging activity of some plants available in Malaysia". IJPT.6: 87-91.

Zakaria, Z. A., Sufian, A. S., Ramasamy, K., Ahmat, N., Sulaiman, M. R., Arifah, A. K., Zuraini, A. and Somchit, M. N. (2010). "*In vitro* antimicrobial activity of *Muntigia calabura* extracts and 1575 fractions". *Afr. J Microbiol Res* 4: 304-308.

Zakaria, Z. A., Mohamed, A. M., Jamil, N. S. M., Rofiee, M. S., Hussain, M. K., Sulaiman, M. R. (2011). "In VitroAntiproliferative and Antioxidant Activities of the Extracts of *Muntingia calabura* Leaves". Am J Chin Med.;39(01):183–200.

Zakaria, Z. A., Balan, T., Azemi, A. K., Omar, M. H., Mohtarrudin, N., Ahmad, Z., Abdullah, M. N. H., Desa, M.N. M., The, L. K. and Salleh, M. Z. (2016). "Mechanism(s) of action underlying the gastroprotective effect of ethyl acetate fraction obtained from the crude methanolic leaves extract of *Muntingia calabura*". Zakaria et al. BMC Complementary and Alternative Medicine (2016) 16:78. DOI 10.1186/s12906-016-1041-0