Determination of Antimicrobial Activities of Essential Oil Extracted from Flowers of *Calophyllum amoenum* Wall.

Tu Tu Wai[1], Kalaya Lu[2]

Abstract

Calophyllum amoenum Wall. is a tree belonging to the family Hypericaceae (Guttiferae). The qualitative phytochemical screening of flowers of *Calophyllum amoenum* Wall. was performed by using standard methods. The antimicrobial activities of crude extract of flowers in various solvent systems were tested by agar well diffusion method on six selected organisms, such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli*. And the essential oil of flowers of *Calophyllum amoenum* Wall. was extracted by steam distillation method. The percentage yield of essential oil was found to be 0.7622%. The antimicrobial activities of this essential oil was evaluated by agar well diffusion method on six selected organisms. This essential oil responded high activities on four tested organisms but showed medium activities on *Bacillus subtilis* and *Candida albicans*. The different chemical components of essential oil of these flowers were identified by Gas chromatography-Mass spectrometry, GC-MS. According to GC-MS data, n-pentadecanol was found to be the main component with the highest area ratio (20.00 %).

Keywords : flowers of *Calophyllum amoenum* Wall., antimicrobial activities, steam distillation method, essential oil, Gas chromatography-Mass spectrometry, GC-MS

Introduction

The medicinal value of plants is local heritage with global importance because of phytochemicals, the natural bioactive compounds found in plants. These phytochemicals are basically divided into primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise of sugars, amino acid, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenes, saponins, phenolic compounds, flavonoids, tannins and so on (Tang W, Eisenbrand G, 1992).

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds extracted from flowers, seeds, bark, stems, roots and other parts of plants. The extraction methods of essential oil are solvent-extraction, maceration, cold pressing, water distillation and steam distillation. The steam distillation is a special type of distillation. The advantage of steam distillation is that it is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are not altered. An essential oil is a complex mixture of polar and non-polar compounds. In general, the essential oil can be subdivided into two distinct groups of chemical constituents; the hydrocarbons which are made up almost exclusively of terpenes and the oxygenated compounds which are mainly esters, aldehydes, ketones, alcohols, phenols, and oxides (Oyedeji OA, AFolayan AJ, 2005).

Among the medicinal plants, *Calophyllum amoenum* Wall. belonging to family Hypericaceae is well known for its aromatic properties. They are mainly distributed in Asia, with some species in Africa, America, Australasia and the Pacific Islands. *Calophyllum amoenum* Wall. is also a kind of tree bearing white fragrant flowers. Flowering period of *Calophyllum amoenum* Wall. is March and fruiting period is April to May. The flowers of *Calophyllum amoenum* Wall. are used for rheumatoid arthritis, headache and burning sensation. In Myanmar, these

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plants occur in Sagaing, Mandalay, Shwebo, Taninthayi and Yangon. In Myanmar culture and tradition, the golden flowers of *Calophyllum amoenum* Wall. are usually important and valuable specimens with regard to Buddhisms (Dassanayake, MD.,1980-2001 and Hundley, H.G. & Chit Ko Ko., 1987).

Aim

The main aim of this research work is to determine the antimicrobial activities of essential oil extracted from flowers of *Calophyllum amoenum* Wall.

Botanical Description of *Calophyllum amoenum* Wall.

Scientific name : *Calophyllum amoenum* Wall.
Family : Hypericaceae
Myanmar name : Tha ra phi
Common Name : Tharapi
Odour : Pleasant and characteristic
Locality : Sintgaing Township, Mandalay Region
Growth Habit : Tree
Parts used : Flowers

Plant, fruits, leaves and flowers of *Calophyllum amoenum* Wall. are shown in Figure 1.

Figure 1. Plant, fruits, leaves and flowers of *Calophyllum amoenum* Wall.

Materials and Methods

Sample Collection and Preparation

The fresh flowers of *Calophyllum amoenum* Wall. were collected from Sintgaing Township, Mandalay Region in the month of March 2019. These flowers were air dried for five days.

The fresh and air dried flowers of *Calophyllum amoenum* Wall. are shown in Figure 2.

Figure 2. Fresh and air dried flowers of *Calophyllum amoenum* Wall.

Preliminary Phytochemical Screening of Flowers of *Calophyllum amoenum* Wall.

Screening for various phytochemical constituents of *Calophyllum amoenum*Wall. flowers was carried out using standard methods (Harborne JB.,1973).

Determination of Antimicrobial Activities of *Calophyllum amoenum* Wall. Flowers

The antimicrobial activities of flowers of *Calophyllum amoenum* Wall. were tested at Pharmaceutical Research Department, Yangon by agar well diffusion method on six selected organisms that include three gram positive bacteria, *Bacillus*

subtilis, *Bacillus pumilus* and *Staphylococcus aureus*, two gram negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*, and fungi, *Candida albicans*.

Extraction of Essential Oil from Flowers of *Calophyllum amoenum* Wall. by Steam Distillation Method

A 300 g of air dried flowers of *Calophyllum amoenum* Wall. was placed in a still and 2 L of distilled water was added to it. After one hour, steam was produced in the boiling by heating distilled water with the hot plate. This vapor steam which contained essential oils and water-soluble plant compounds, was condensed in the water-cooled condenser and collected in a receiver (flat-bottomed flask). It was continued to collect in the receiver, and this extraction was carried out for 4 hours. And it was separated by using separation funnel with n-hexane solvent. After that, the resulting n–hexane extract was dried over anhydrous sodium sulphate. And then this solvent was evaporated under vacuum to get the yellowish crude extract. The crude extracted oil was checked by Thin-Layer Chromatography (TLC) to prove that it contains the crude essential oil or not. As stated above, this distillation procedure was carried out for three times using 300 g of air dried these flowers per distillation. The average yield percent of essentials oil was calculated on the basis on air dried weight 300 g of flowers of *Calophyllum amoenum* Wall. The colour of this essential oil was determined as yellow oil.

Figure 3. Steam distillation apparatus

Figure 4. Essential oil

Figure 5. TLC check

Determination of Antimicrobial Activities of Essential Oil

Antimicrobial activities of essential oil extracted from flowers of *Calophyllum amoenum* Wall. were rechecked at Pharmaceutical Research Department, Yangon by agar well diffusion method on six selected organisms, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*.

Determination of Different Chemical Components of Essential Oil by GC-MS

Chemical components of essential oil were identified by Gas chromatography-Mass spectrometry (GC-MS) at Department of Chemistry, University of Mandalay.

Figure 6. Gas chromatography-Mass spectrometry (GC-MS)

Results and Discussion

In this research work, the qualitative phytochemical screening and antimicrobial activities were carried out in flowers of *Calophyllum amoenum* Wall. And the percentage yield, antimicrobial activities and chemical constituents of essential oil were also determined.

Phytochemical Screening of Flowers of *Calophyllum amoenum* Wall.

Phytochemical screening of flowers of *Calophyllum amoenum* Wall. is shown in Figure 7 and Table 1.

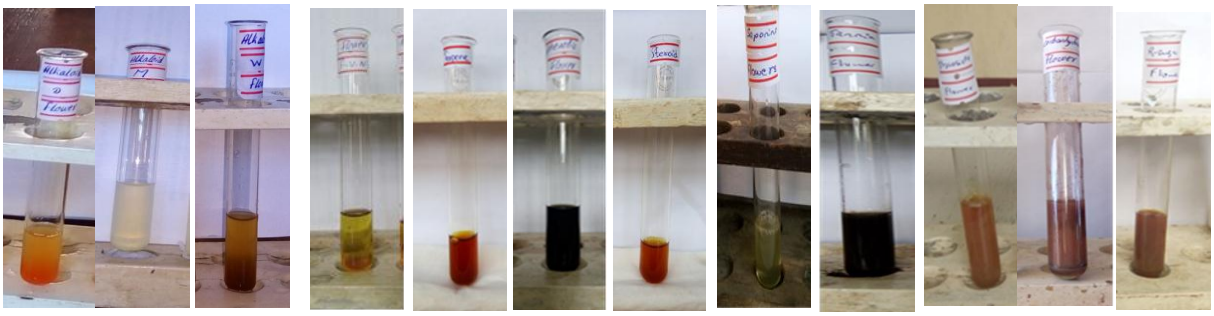


Figure 7. Phytochemical analysis of flowers of *Calophyllum amoenum* Wall.

Table 1. Results of Phytochemical Test on Flowers of *Calophyllum amoenum* Wall.

No.	Tests	Extracts	Test reagents	Observation	Results
1.	Alkaloids	1 % HCl	Dragendroff's reagent	Orange ppt	+
			Wagner's reagent	Reddish brown ppt	+
			Mayer's reagent	White ppt	+
2.	Flavonoids	EtOH	Mg turning, conc: HCl	Greenish yellow color solution	+
3.	Terpenes	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	Red color solution	+
4.	Phenolic compounds	EtOH	10 % $FeCl_3$	Black color solution	+
5.	Steroids	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	No Green coloration	–
6.	Saponins	H_2O	Distilled water	Frothing	+
7.	Tannins	H_2O	10 % $FeCl_3$	Dark brown color solution	+
8.	Glycosides	H_2O	10 % lead acetate	White ppt	+
9.	Carbohydrates	H_2O	10 % α -naphthol, conc: H_2SO_4	Violet color ring of the interface of the two layers	+
10.	Reducing sugar	H_2O	Benedict's solution	Brick-red ppt	+

(+) = the presence of constituents, (–) = the absence of constituents

According to these results, the flowers of *Calophyllum amoenum* Wall. contained alkaloids, flavonoids, terpenes, phenolic compounds, saponins, tannins, glycosides, carbohydrates and reducing sugars. However, steroids were absent. It has been reported that these phytochemicals can prove the presence of medicinally active compounds in flowers of *Calophyllum amoenum* Wall.

Antimicrobial Activities of Flowers of *Calophyllum amoenum* Wall.

The crude extracts of flowers of *Calophyllum amoenum* Wall. were prepared by using n-hexane, ethyl acetate and ethanol solvents. The results of antimicrobial activities of these flowers are shown in Figure 8 and Table 2.

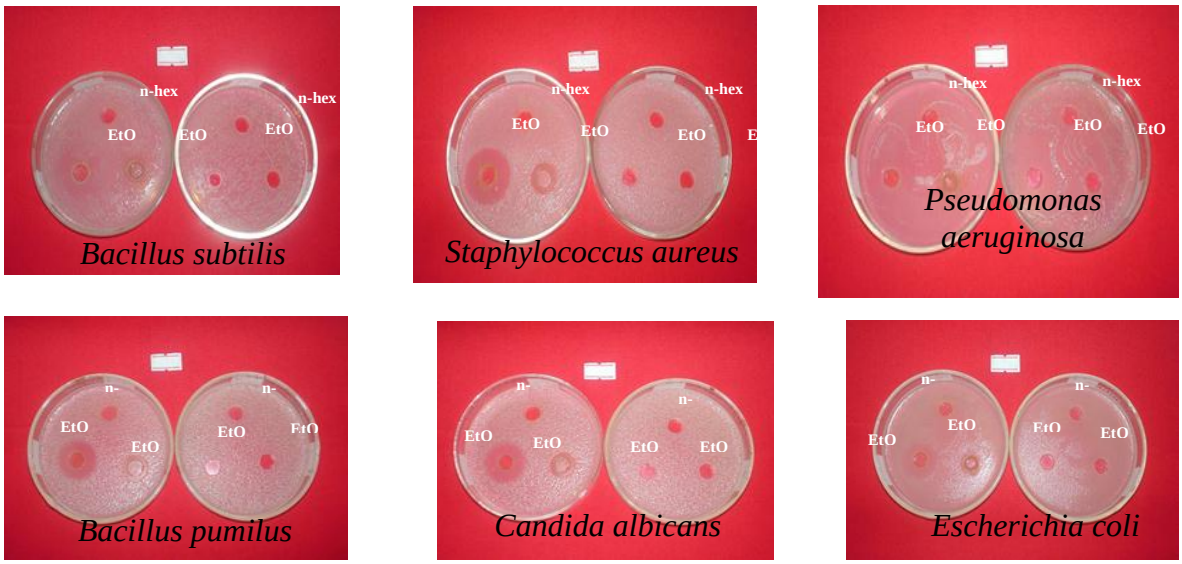


Figure 8. Antimicrobial activities of flowers of *Calophyllum amoenum* Wall.

Table 2. Antimicrobial Activities of Flowers of *Calophyllum amoenum* Wall.

Sample	Solvents	Inhibition zone diameters of different extracts against six microorganisms (mm)					
		I	II	III	IV	V	VI
Flowers of *Calophyllum amoenum* Wall.	n-hexane	–	–	–	–	–	–
	EtOAc	28 (+++)	29 (+++)	28 (+++)	25 (+++)	25 (+++)	25 (+++)
	EtOH	–	18 (++)	–	17 (++)	16 mm (++)	–

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
I. *Bacillus subtilis*
II. *Staphylococcus aureus*
III. *Pseudomonas aeruginosa*
IV. *Bacillus pumilus*
V. *Candida albicans*
VI. *Escherichia coli*

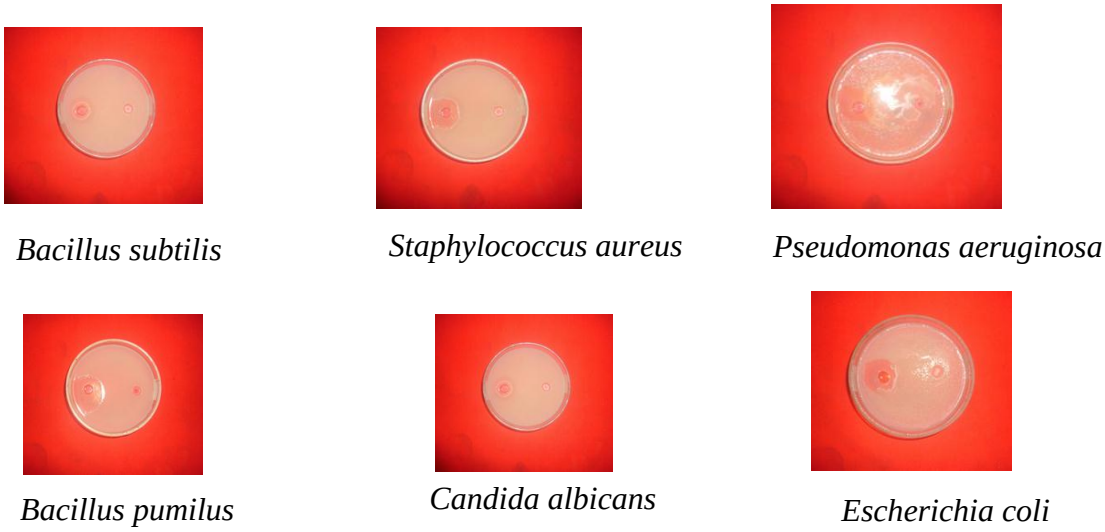
According to these results, the antimicrobial activities of n-hexane crude extract of *Calophyllum amoenum* Wall. flowers showed no activities on all tested organisms. The ethyl acetate crude extract responded high activities on all tested organisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* and ethanol crude extract responded medium activities on *Staphylococcus aureus*, *Bacillus pumilus* and *Candida albicans*, and no activities on *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Percent Yield of Essential Oil

2.2866 g (0.7622 %) of essential oil was extracted from steam distillation of 300 g air dried flowers of *Calophyllum amoenum* Wall. It indicates that the flowers of *Calophyllum amoenum* Wall. could be a source of essential oil.

Antimicrobial Activities of Essential Oil

The antimicrobial activities of essential oil of *Calophyllum amoenum* Wall. flowers are given in Figure 9 and Table 3.



Bacillus subtilis *Staphylococcus aureus* *Pseudomonas aeruginosa*

Bacillus pumilus *Candida albicans* *Escherichia coli*

Figure 9. Antimicrobial activities of essential oil extracted from flowers of *Calophyllum amoenum* Wall.

Table 3. Results of Antimicrobial Activities of Essential Oil

Sample	Solvent	Inhibition zone diameters of essential oil against six microorganisms (mm)					
		I	II	III	IV	V	VI
Essential Oil	n-hexane	19 (++)	25 (+++)	30 (+++)	30 (+++)	18 (++)	25 (+++)

Agar well – 10 mm
10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms
I. *Bacillus subtilis*
II. *Staphylococcus aureus*
III. *Pseudomonas aeruginosa*
IV. *Bacillus pumilus*
V. *Candida albicans*
VI. *Escherichia coli*

According to these results, the essential oil of flowers of *Calophyllum amoenum* Wall. showed high activities on four tested organisms but responded the medium activity on *Bacillus subtilis* and *Candida albicans*. It can prove that this essential oil could be a source of antimicrobial drugs.

GC-MS Analysis of Essential Oil Extracted from Flowers of *Calophyllum amoenum* Wall.

The gas chromatogram and the spectra of eight components of the essential oil are displayed in Figures 10 and 11. In GC-MS analysis, the eight components were identified in flowers oil, which represented about 92.78% of the total composition of the oil. The identification and quantification of eight compounds are shown in Table 5. It was found that n-pentadecanol (20.00 %) is a major component. The components of significant occurrence in this essential oil were heptadecane (18.74 %), cis-13,16-docasadienoic acid (15.58 %), cis-beta-farnesene (12.21 %), beta-sesquiphellandrene (10.50 %), heneicosane (9.21 %), octadecane (3.53 %) and alpha-ionone (3.01 %). These components are useful for fragrance ingredients in perfumery and cosmetics, and personal care products in household

cleaners and detergents. These are also flavoring agents in beverages, baked goods, and candies. Thus, this essential oil could play many different therapeutic roles.

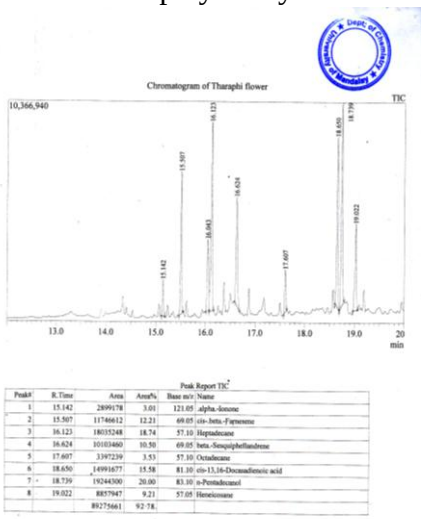


Figure 10. Chromatogram of essential oil

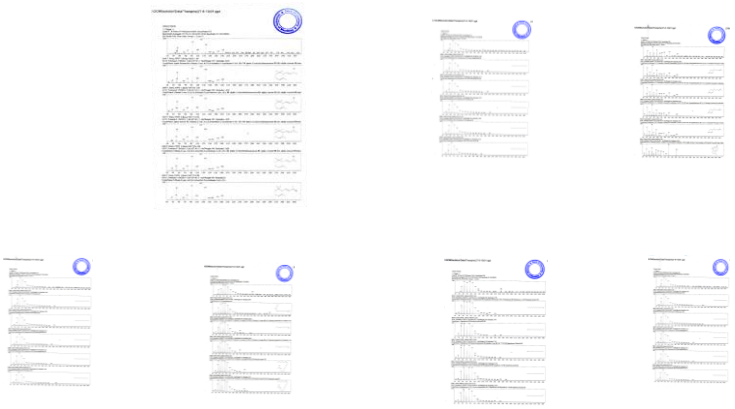
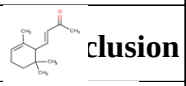
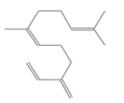
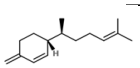
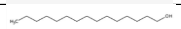
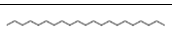


Figure 11. The Spectra of eight chemical components of essential oil

Table 4. Different Chemical Constituents of Essential Oil

No.	Retention time	Compound Name	Structure	Molecular Formula	M W	Area %
1	15.142	alpha-ionone		$C_{13}H_{20}O$	192	3.01
2	15.507	cis-beta-farnesene		$C_{15}H_{24}$	204	12.21
3	16.123	heptadecane		$C_{17}H_{36}$	240	18.74
4	16.624	beta-sesquiphellandrene		$C_{15}H_{24}$	204	10.50
5	17.607	octadecane		$C_{18}H_{38}$	254	3.53
6	18.650	cis-13, 16-docasadienoic acid		$C_{22}H_{40}O_2$	336	15.58
7	18.739	n-pentadecanol		$C_{15}H_{32}O$	228	20.00
8	19.022	heneicosane		$C_{21}H_{44}$	296	9.21

In this research work, the flowers of *Calophyllum amoenum* Wall. showed the presence of alkaloids, flavonoids, phenolic compounds, glycosides, terpenes, saponins, tannins, carbohydrates and reducing sugars, and showed the absence of steroids. These phytochemicals can work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. The antimicrobial activities of n-hexane crude extract of these flowers showed no activities on all tested organisms and that of ethyl acetate crude extract showed high activities on all tested organisms. And ethanol crude extract responded medium activities on *Staphylococcus aureus*, *Bacillus pumilus* and *Candida albicans*. The percentage yield, 0.7622 % of essential oil indicates that the flowers of *Calophyllum amoenum* Wall. are a source of essential oil. And the antimicrobial activities of this essential oil showed high activities on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Escherichia coli*, and medium activities on *Bacillus subtilis* and *Candida albicans*. It shows that the essential oil of these flowers has a great potential of antimicrobial agents. According to Gas Chromatography-Mass Spectrometry (GC-MS) data, the eight components were found in this essential oil. These components were n-pentadecanol (20.00 %) as a main component, and heptadecane (18.74 %), cis-13,16-docasadienoic acid (15.58 %), cis-beta-farnesene (12.21 %), beta-sesquiphellandrene (10.50 %), heneicosane (9.21 %), octadecane (3.53 %) and alpha-ionone (3.3.01 %) as significant components. These components are a potential source to produce antimicroabial drugs. Therefore, flowers of *Calophyllum amoenum* Wall. are not only important in role of Buddha religion applications but also great valuable items in Myanmar traditional medicine system. And these flowers can be an interesting raw material for the application of aromatherapy and flavor. Moreover, the present study brings detailed knowledge about the antimicrobial activity of the individual compounds present in this essential oil that could provide a greater understanding for the future researches. It is recommended that the seasonal flowers in Myanmar should be collected at the time of flowering to do scientific research for AROMA therapy.

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