INVESTIGATION ON THE ESSENTIAL OIL OF LEAVES OF *Cinnamomum tamala* F. Nees. (Karaway)

Tu Tu Wai*

Abstract

Cinnamomum tamala F. Nees. (Karaway) is a small tree within the Laurace ae family. The qualitative phytochemical screening of Karaway leaves was performed by using standard methods. Karaway leaves contained alkaloids, flavonoids, terpenes, phenolic compounds, sapponins, tannins, glycosides and carbohydrates but showed the absence of steroids and reducing sugars. The mineral elements of Karaway leaves were analyzed by using Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometer. Thirteen minerals were found in Karaway leaves. Antimicrobial activities of various crude extracts of karaway leaves were tested by agar well diffusion method on six selected organisms, such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli.* All of the crude extracts showed low activities on all tested organisms except methanol crude extract. And the essential oil of Karaway leaves was extracted by steam distillation method. The yield percent of essential oil was found to be 1.07 %. Antimicrobial activities of essential oil were also tested by agar well diffusion method on the six selected organisms. The essential oil of Karaway leaves responded high activities on five tested organisms but showed medium activities on *Bacillus pumilus*. The chemical constituents of essential oil were determined by Gas chromatography-Mass spectrometry, GC-MS. In total, twenty components (99.99 % of essential oil) were identified with different area ratios. Linalool was the main component, the highest area ratio (35.04 %). The components of significant occurrence in the oil were trans-cinnamaldehyde (28.88 %), benzaldehyde (10.76 %), cinnamyl acetate (7.81%), D-limonene (3.70 %), eucalyptol (2.81 %), hydrocinnamaldehyde (1.36 %), 2-methylcumarone and geraniol (1.11 %) and estragole (1.08 %).

Keywords: Karaway leaves, *Cinnamomum tamala* F. Nees., steam distillation, essential oil, GC-MS

Introduction

Medicinal and aromatic plants play a vital role in pharmaceutical research and medicine production because they contain bioactive compounds.

* Dr, Lecturer, Department of Chemistry, Myitkyina University

About 80% of the world's population relies on natural substances of plant origin for the primary health care.

Essential oil is a mixture of hydrophobic volatile secondary metabolites, containing from a dozen to several hundred components. Essential oil is extracted from flowers, barks, stems, leaves, roots, fruits, and other parts of the plant by various methods. The great majority of components identified in essential oil includes terpenes (monoterpenes, sesquiterpenes and diterpenes), and the oxygenated compounds which are mainly esters, aldehydes, ketones, alcohols, phenols, and oxides (Adams, 1995).

Essential oil is used in food, cosmetic and pharmaceutical industries. Current medical applications of essential oil range from skin treatment to remedies for cancer. Moreover, essential oil could also act as antimicrobials, antivirals, insecticides and herbicides. The antioxidant activity of essential oils is another biological property of great interest because they can preserve foods from the toxic effects of oxidants (Sood *et al*, 1979).

Among the medicinal plants, *Cinnamomum tamal* F. Nees. (karaway) is well known for its medicinal and aromatic properties widely used in traditional medicine and spice sector (Cragg and Newman, 2005). The Lauraceae family contains about 50 general and approximately 2500-3500 species mostly distributed in tropical and subtropical low land forest of Africa, South America, Southeast Asia and Australia. The leaves and bark of karaway are very commonly used as spice in Indian food. Leaves and barks have aromatic, astringent, stimulant and carminative qualities and are used in rheumatism, colic, diarrhea, nausea, sore eyes and vomiting. Ancient literature has revealed that dried leaves and bark of this plant were prescribed for fever, anemia, antidote for snake, asthma and cough (Gulati, 1982; Showkar *et al*., 2004).

Aim

Aim : The main aim of this research work is to investigate the chemical constituents of essential oil of Karaway leaves.

Botanical Description of *Cinnamomum tamala* F. Nees.

Scientific name	:	*Cinnamomum tamala* F. Nees.
Family	:	Lauraceae
English name	:	Cassia cinnamon
Myanmar name	:	Karaway
Locality	:	Kachin, Mandalay, Shan and Rakhine
Parts used	:	Leaves

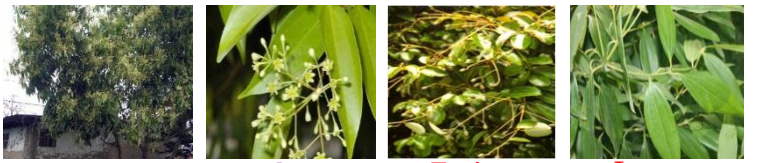


Figure 1: Plant, Flowers, Fruits and Leaves of Karaway

Materials and Methods

Sample Collection and Preparation

Karaway leaves were collected from Myitkyina University campus, Kachin State in September 2017. Karaway leaves were thoroughly washed with tap water, cut into small pieces and air dried for one month, and then stored in airtight glass bottles.

Preliminary Phytochemical Screening of Karaway Leaves

Screening for various phytochemical constituents of Karaway Leaves was carried out using standard methods.

Determination of Mineral Contents in Karaway Leaves

Elemental compositions of Karaway leaves were measured at West Yangon University by Energy Dispersive X-ray Fluorescence Spectrometer (EDX-700), Shimadzu, Japan.

Determination of Antimicrobial Activities of Karaway Leaves

The antimicrobial activities of Karaway leaves were tested at Pharmaceutical Research Department, Yangon by using agar well diffusion method on six selected organisms

such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli.*

Extraction of Essential Oil from Karaway Leaves by using Steam Distillation Method

A 300 g of dried sample was placed in a still and 2L of distilled water was added to it. After 30 min, steam was produced from in the boiling by heating distilled water with the hot plate. This steam which contained essential oil and water-soluble plant compounds, was condensed in the water-cooled condenser and was collected in a receiver (flat-bottomed flask). It was continued to collect in the receiver, and this extraction was carried out for 5 hr. 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 crude extract. The crude extracted oil was checked by TLC. As stated above, this distillation procedure was carried out for three times using (300) g of dried sample per distillation.

Figure 2: Steam Distillation Apparatus

Figure 3: Essential oil of Karaway Leaves

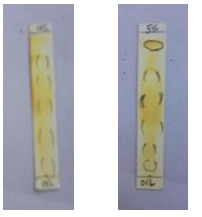


Figure 4: TLC for essential oil to prove that essential oil is a mixture

Determination of Antimicrobial Activities of Essential Oil

Antimicrobial activities of essential oil of Karaway leaves were rechecked by using agar well diffusion method on six selected organisms.

Determination of Chemical Compositions of Essential Oil from Karaway Leaves by GC-MS

Chemical compositions of essential oil were identified by using GC-MS at Department of Chemistry, University of Mandalay.

Figure 5: Gas Chromatography - Mass Spectrometry (GC-MS)

Results and Discussion

In this research work, the qualitative phytochemical screening, elemental analysis and antimicrobial activities of Karaway leaves were carried out. And percentage yield, antimicrobial activities, antioxidant activity and chemical constituents of essential oil were also determined.

Phytochemical Screening of Karaway Leaves

Phytochemical screening of Karaway leaves are shown in Figure 6 and Table 1.

Alkaloids

Flavonoids

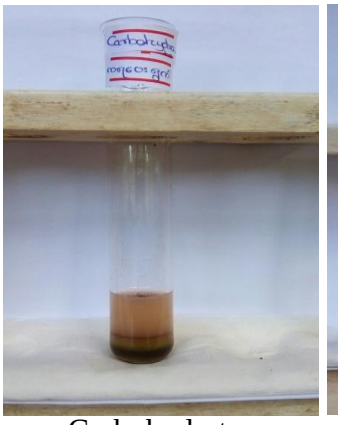
Terpenes

Phenolic Compounds

Saponins

Tannins

Glycosides



Carbohydrates

Reducing Sugars
(Negative Test)

Figure 6: Phytochemical Analysis of Karaway Leaves

Table 1: Results of Phytochemical Test on Karaway Leaves

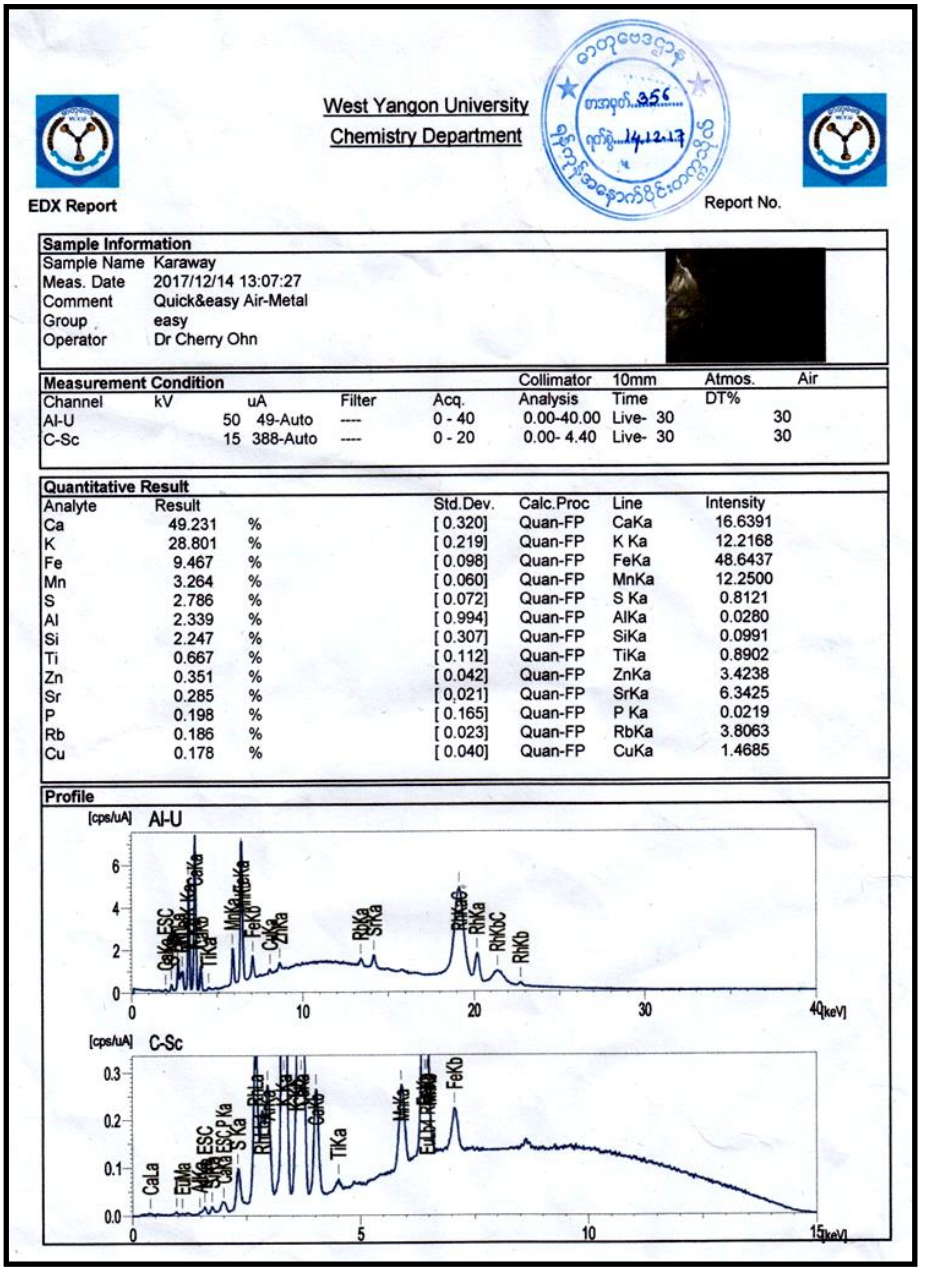
No.	Tests	Extracts	Test Reagents	Observation	Results
1	Alkaloids	1 % HCl	Dragendroff's reagent	Orange ppt	+
			Wagner's reagent	Reddish brown ppt	+
			Mayer's reagent	White ppt	+
2	Flavonoids	EtOH	Mg turning, conc: HCl	Yellow colour solution	+
3	Terpenes	$CHCl_3$	Acetic anhydride, conc: H_2SO_4	Red colour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

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

According to Table 1, the tests on Karaway leaves showed the presence of alkaloids, flavonoids, terpenes, phenolic compounds, saponins, tannins, glycosides and carbohydrates, and the absence of steroids and reducing sugars. These secondary metabolites, alkaloids, flavonoids, terpenes, phenolic compounds, saponins, tannins and glycosides can have various pharmacological effects such as antibacterial, antifungal, anti-malarial, antimicrobial, anti-diarrhoeal, antioxidant, anti-diabetic, anti-inflammatory, anti-allergenic, antitumor and anthelmintic activities. The primary metabolites, carbohydrates are known to be a major source of energy for human's body and hence are valuable as dietary supplements. These phytochemicals are unique compounds for health improvement and disease prevention.

Elemental Analysis of Karaway Leaves

The results of mineral contents in Karaway leaves are shown in Figure 7 and Table 2.



West Yangon University
Chemistry Department

EDX Report

Report No.

Sample Information

Sample Name	Karaway
Meas. Date	2017/12/14 13:07:27
Comment	Quick&easy Air-Metal
Group	easy
Operator	Dr Cherry Ohn

Measurement Condition

Channel	kV	uA	Filter	Acq.	Collimator	10mm	Atmos.	Air
					Analysis	Time	DT%	
Al-U	50	49-Auto	----	0 - 40	0.00-40.00	Live- 30		30
C-Sc	15	388-Auto	----	0 - 20	0.00- 4.40	Live- 30		30

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
Ca	49.231	%	[0.320]	Quan-FP	CaKa	16.6391
K	28.801	%	[0.219]	Quan-FP	K Ka	12.2168
Fe	9.467	%	[0.098]	Quan-FP	FeKa	48.6437
Mn	3.264	%	[0.060]	Quan-FP	MnKa	12.2500
S	2.786	%	[0.072]	Quan-FP	S Ka	0.8121
Al	2.339	%	[0.994]	Quan-FP	AlKa	0.0280
Si	2.247	%	[0.307]	Quan-FP	SiKa	0.0991
Ti	0.667	%	[0.112]	Quan-FP	TiKa	0.8902
Zn	0.351	%	[0.042]	Quan-FP	ZnKa	3.4238
Sr	0.285	%	[0,021]	Quan-FP	SrKa	6.3425
P	0.198	%	[0.165]	Quan-FP	P Ka	0.0219
Rb	0.186	%	[0.023]	Quan-FP	RbKa	3.8063
Cu	0.178	%	[0.040]	Quan-FP	CuKa	1.4685

Profile

Figure 7: EDXRF Spectrum of Karaway Leaves

Table 2: Results of Qualitative Analysis of Mineral Contents in Karaway Leaves

No.	Elements		Relative abundance (%) in Karaway leaves
1	Calcium	(Ca)	49.231
2	Potassium	(K)	28.301
3	Iron	(Fe)	9.467
4	Manganese	(Mn)	3.264
5	Sulfur	(S)	2.786
6	Aluminum	(Al)	2.339
7	Silicon	(Si)	2.247
8	Titanium	(Ti)	0.667
9	Zinc	(Zn)	0.351
10	Strontium	(Sr)	0.285
11	Phosporus	(P)	0.198
12	Rubidium	(Rb)	0.186
13	Copper	(Cu)	0.178

The results of mineral analysis indicate the presence of calcium (49.231 %), potassium (28.301 %), iron (9.467 %), manganese (3.264 %), sulfur (2.786 %), aluminium (2.339 %), silicon (2.247 %), titanium (0.667 %), zinc (0.351 %), strontium (0.285 %), phosphorus (0.198 %), rubidium (0.186 %) and copper (0.178 %). The macro minerals, calcium, potassium, sulfur and phosphorus are needed for proper fluid balance, nerve transmission, muscle contraction; they prevent blood clotting, regulate blood pressure and interfere in the protein metabolism. They ensure the health of teeth, bones and connective tissue.

Trace minerals, iron, manganese, aluminum, silicon, titanium, zinc, strontium, rubidium and copper are essential for growth and development because they are involved in oxygen transport and various metabolic functions. They participate in all enzyme reactions in the body and help in the assimilation. These macro and trace minerals are an essential part of a healthy diet. Despite the minute amounts of minerals needed by the body, their lack can cause rious health conditions.

Antimicrobial Activities of Karaway Leaves

The crude extracts of Karaway Leaves were prepared by using various solvents such as n-hexane, pet-ether, chloroform, methanol, Ethyl acetate, ethanol and Water. The results of antimicrobial activities of Karaway leaves are shown in Figures 8(a) and (b), and Table 3.

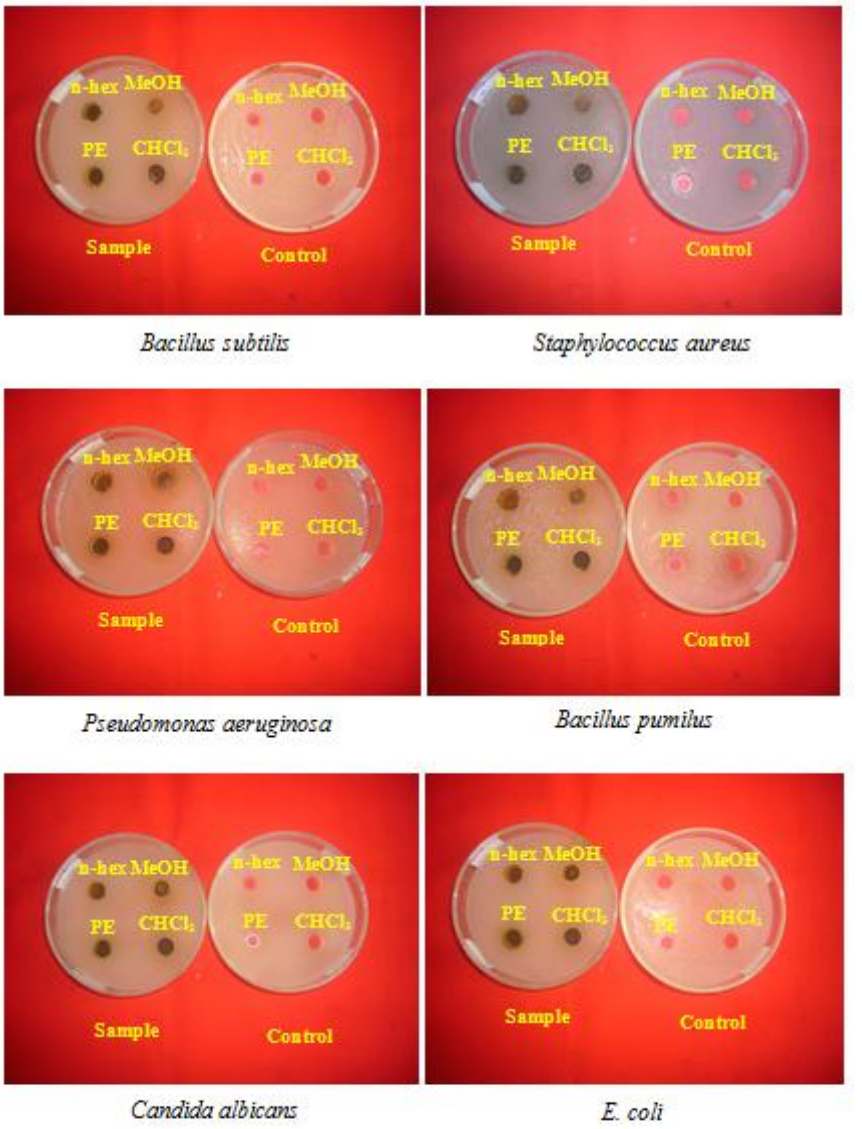


Figure 8: (a) Antimicrobial Activities of crude extracts from Karaway Leaves

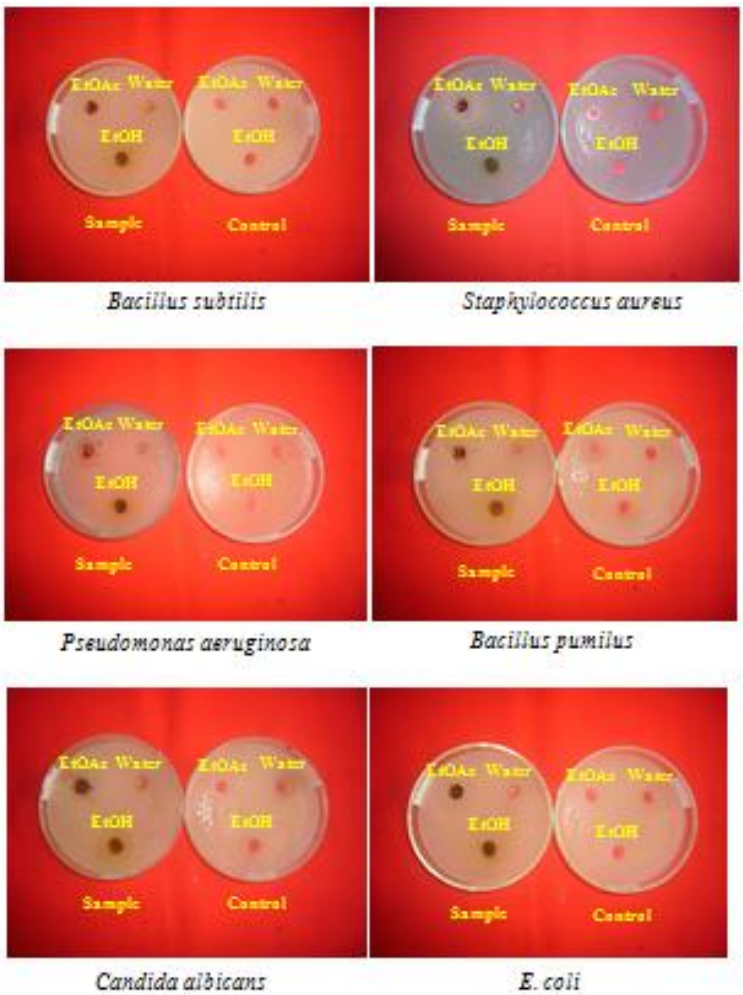


Figure 8: (b) Antimicrobial Activities of crude extracts from Karaway Leaves

Table 3: Results of Antimicrobial Activities of Karaway Leaves

Sample	Extracts	Inhibition zone diameters of different extracts against six microorganisms (mm)					
		I	**II**	**III**	**IV**	**V**	**VI**
Karaway (leaves)	n-hexane	13 (+)	14 (+)	13 (+)	12 (+)	12 (+)	-
	Pet-ether	14 (+)	14 (+)	13 (+)	12 (+)	12 (+)	11 (+)
	$CHCl_3$	12 (+)	13 (+)	13 (+)	12 (+)	11 (+)	11 (+)
	MeOH	12 (+)	13 (+)	15 (++)	12 (+)	11 (+)	11 (+)
	EtOAc	11 (+)	13 (+)	13 (+)	11 (+)	-	11 (+)
	EtOH	12 (+)	13 (+)	13 (+)	12 (+)	11 (+)	11 (+)
	Water	12 (+)	-	14 (+)	12 (+)	12 (+)	12 (+)

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) *E. coli*

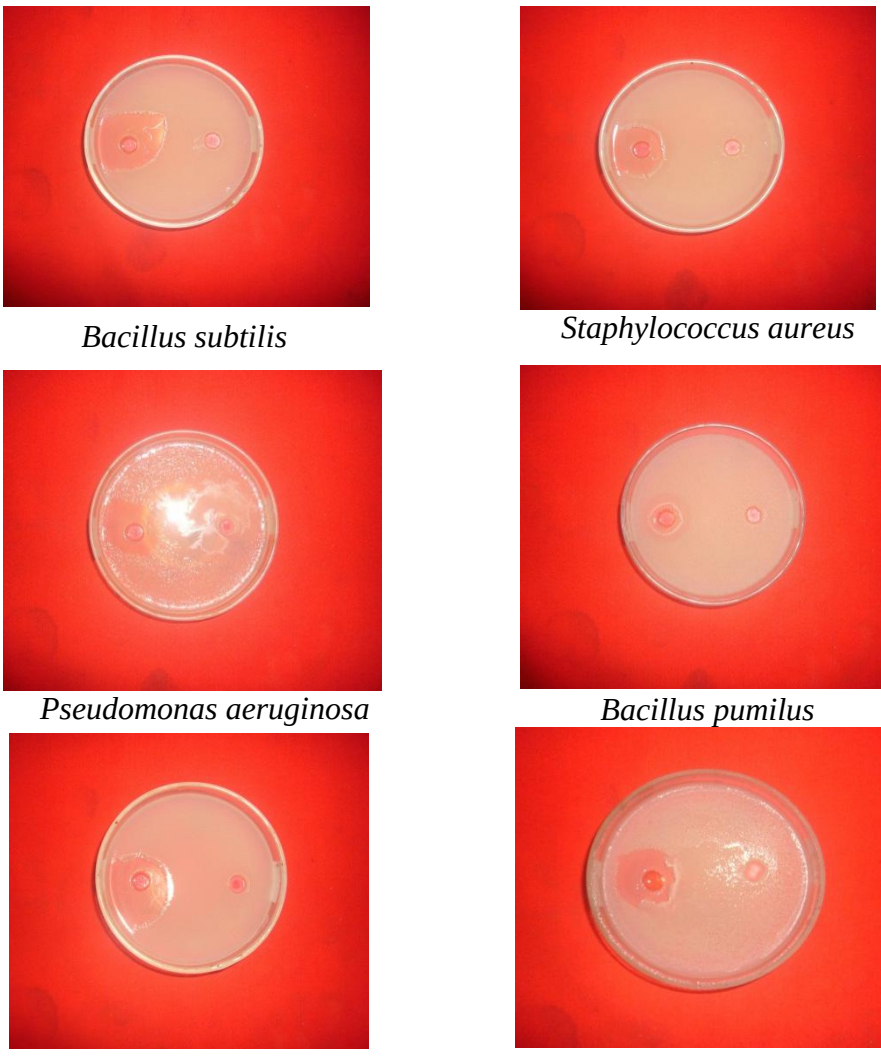
According to Table 3, pet-ether, chloroform and ethanol crude extracts of Karaway leaves showed low activities on all tested organisms. The n-hexane crude extract of Karaway leaves showed low activities on five tested organisms but did not respond *E. coli*. Methanol crude extract of Karaway leaves showed low activities on five tested organisms and medium activity on *Pseudomonas aeruginosa* which responded medium activity. Ethyl acetate crude extract of Karaway leaves showed low activities on all tested organisms except *Candida albicans*. Water extract of Karaway leaves showed low activities on all tested organisms except *Staphylococcus aureus*.

Percent Yield of Essential Oil Extracted from Karaway Leaves

3.21g (1.07 %) of essential oil was extracted from steam distillation of 300g of Karaway leaves. It indicates that Karaway Leaves can be a source of essential oil. It indicates that Karaway leaves can be a source of essential oil.

Antimicrobial Activities of Essential Oil Extracted from Karaway Leaves

Antimicrobial activities of essential oils from Karaway leaves are given in Figure 9 and Table 4.



Bacillus subtilis *Staphylococcus aureus*

Pseudomonas aeruginosa *Bacillus pumilus*

Candida albicans *E. coli*

Figure 9: Antimicrobial Activities of Essential Oil from Karaway Leaves

Table 4: Results of Antimicrobial Activities of Essential Oil Extracted from Karaway leaves

Sample	Inhibition zone diameters of essential oil against six microorganisms (mm)					
	I	**II**	**III**	**IV**	**V**	**VI**
Essential Oil	30 (+++)	25 (+++)	30 (+++)	18 (++)	30 (+++)	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) *E. coli*

According to these results, essential oil of Karaway leaves showed high activities on all tested organisms except *Bacillus pumilus* which responded the medium activity. The antimicrobial activities of essential oil demonstrated that folk medicine could be as effective as standard drug to combat pathogenic microorganisms.

GC-MS Analysis of Essential Oil Extracted from Karaway Leaves

The gas chromatogram of the essential oil is displayed in Figure 10. The GC-MS analysis of Karaway leaves led to the identification and quantification of twenty compounds as shown in Table 5. It was found that It was found that linalool (35.04 %) as the main component and, trans-cinnamaldehyde (28.88 %), benzaldehyde (10.76 %), cinnamyl acetate (7.81 %) D-Limonene (3.70 %), eucalyptol (2.81 %), hydrocinnamaldehyde (1.36 %), 2-Methyl cumarone and geraniol (1.11 %) and estragole (1.08 %) as the significant components. The other ten components are found as trace volatile compounds (<1 %). The contents of volatile organic compounds were within the value reported in the literature and the percentage contents of these constituents were similar to those given in the literature. But eucalyptol and hydrocinnamaldehyde has no literature data (Nath, *et al*.,1994). This possibly may be due to the fact that the trees are grown in different regions which exhibit differences in their chemical constituents. Linalool is a high-valued aromatic chemical extensively used for flavour applications. Trans-cinnamaldehyde has antimicrobial activity and anti-diabetic property. It is used as a flouring agent in liquid refreshments, ice-creams, chewing gums and

candy. Benzaldehyde is commonly employed to confer almond flavor to foods and also used in cosmetic products. Cinnamyl acetate is used as a flavour ester in for example bread and animal feed and has a sweet floral-fruity fragrance. It is used in several cosmetics, some toiletries but also in non-cosmetic products, for example detergents. D-limonene and eucalyptol is used especially as flavouring agent in foods, beverages, and chewing gum. Hydrocinnamaldehyde has a floral odor and is used in perfumes. Geraniol possesses anti-fungal and antiseptic properties. Due to the aromas and flavour, geraniol is an important ingredient in a variety of consumer products made by the flavour and fragrance industries. 2-Methyl cumarone and estragole are used as flavouring agents. The remaining ten compounds in essential oil have antimicrobial, anti-inflammatory, antioxidant, anti-diarrhea and antiulcer properties. The present source being chemotye of a well-known commercial crop can be useful to the industry in this regard.

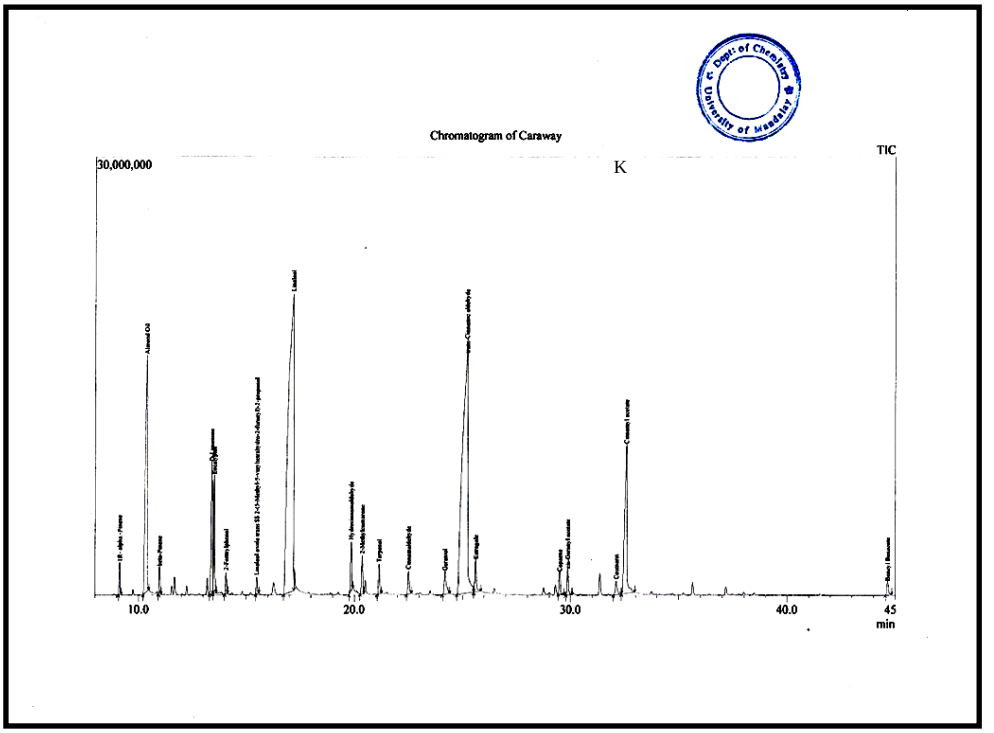
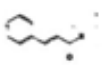
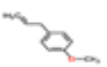
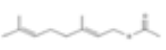
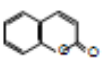
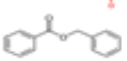


Figure 10: Gas Chromatogram of Essential Oil from Karaway Leaves

Table 5: Chemical Compositions of Essential Oil Extracted from Karaway Leaves

No.	Retention time	Compound Name	Structure	Molecular Formula	M W	Area %
1	9.136	alpha-Pinene		$C_{10}H_{16}$	136	0.69
2	10.393	benzaldehyde		C_7H_6O	106	10.76
3	10.981	beta-Pinene		$C_{10}H_{16}$	136	0.61
4	13.401	D-Limonene		$C_{10}H_{16}$	136	3.70
5	13.510	Eucalyptol		$C_{10}H_{18}O$	154	2.81
6	14.060	2-Formylphenol		$C_7H_6O_2$	122	0.53
7	15.490	Linalool oxide		$C_{10}H_{18}O_2$	170	0.46
8	17.175	Linalool		$C_{10}H_{18}O$	154	35.04
9	19.850	Hydrocinnamaldehyde		$C_9H_{10}O$	134	1.36
10	20.365	2-Methylcumarone		C_9H_8O	132	1.11
11	21.155	alpha-Terpineol		$C_{10}H_{18}O$	154	0.82
12	22.505	Cinnamaldehyde		C_9H_8O	132	0.72
13	24.190	Geraniol		$C_{10}H_{18}O$	154	1.11
14	25.210	trans-Cinnamaldehyde		C_9H_8O	132	28.88
15	25.620	Estragole		$C_{10}H_{12}O$	148	1.08
16	29.500	Copaene		$C_{15}H_{24}$	204	0.64
17	29.870	Geranyl acetate		$C_{12}H_{20}O_2$	196	0.77
18	32.115	Coumarin		$C_9H_6O_2$	146	0.59
19	32.585	Cinnamyl acetate		$C_{11}H_{12}O_2$	176	7.81
20	44.650	Benzyl Benzoate		$C_{14}H_{12}O_2$	212	0.50

Conclusion

Medicines derived from plants act as a source of inspiration for novel drug compounds. Essential oil has been called nature's medicine cabinet, and with good reasons. Phytochemicals and minerals of Karaway Leaves are the major source of pharmaceuticals and help human's body growth and stay healthy. The antimicrobial activities of crude extracts and essential oil of Karaway leaves possess vast curative properties since they have fewer side effects as compared to synthetic antimicrobial drugs. And twenty compounds of leaves essential oil prove that this essential oil is a source of medicinal activities. Therefore, due to different chemical compounds present in the essential oil of Karaway leaves, further investigation concerned with properties, activities and application of the essential oil to replace to medicines or supplement in diseases, kinds of cancer and chemotherapy is recommended.

Acknowledgements

I would like to gratefully acknowledge the Myanmar Academy of Arts and Science, for their valuable advices and discussion on my research work. I am also thankful to Dr Ni Ni Aung, Professor and Head and Dr Htay Htay Win, Professor, Department of Chemistry, Myitkyina University, for their constant encouragement.

References

Adams, R.P. (1995). "Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy." Carol Stream: Allured Publishing Corp. pp. 78–330.

Cragg, G.M. and Newman, D.J. (2005). "Biodiversity. A Continuing Source of Novel Drug Leads". *Pure. Appl. Chem.*, vol. 77 (1), pp.7-24.

Gulati, B.C. (1982)."*Essential Oils of Cinnamomum species.* In *Cultivation and Utilization of Aromatic Plants*". At al and Kapur (Eds.). Regional Research Laboratory (CSIR), Jammu-Tawi, pp. 607-619.

Natch S.C., Hazarika, A.K. and Singh, R.S. (1994). "Essential oil of leaves of Cinnamomum tamala Nees & Eberm. from North East India." *Journal of Spices and Aromatic Crops*, vol.3 (1), pp. 33-35.

Showkar, R.M., Ali, M. and Kapoor, R. (2004). "Chemical Composition of Essential Oil of *Cinnamomum tamala* Nees et Eberm. Leaves". *Frag. J*., vol.19, pp.112-114.

Sood, R.P., Padha, C.D., Talwar, Y.P., Jamwal, R.K., Chopra, M.M. and Rao, P.K. (1979). "Essential oil from the leaves of *Cinnamomum tamala* Nees & Eberm. Growing in Himachal Pradesh." *Indian Perfumer*, vol.23(2), pp.75-78.