ISOLATION OF ASARONE FROM THE RHIZOME OF *Acorus calamus* L. (LIN-NE) AND ANTIBACTERIAL SCREENING OF THE CRUDE EXTRACTS AND ASARONE

Ei Ei Khaing[1]

ABSTRACT

The rhizome of *Acorus calamus* L. (Lin-ne) used in the treatment of diarrhoea and dysentery in traditional Myanmar medicinal system was chosen for present study. The aim of the study is to isolate asarone from the rhizome of *Acorus calamus* L. (Lin-ne) and antibacterial screening of its crude extracts and asarone. At first, four crude extracts of this plant were prepared by using various solvents; petroleum ether, ethyl acetate, 96 % ethanol and 50 % ethanol. *In vitro* antibacterial activity of four crude extracts was investigated against 19 bacterial strains by using agar disc diffusion method. Among the four crude extracts, the most active ethyl acetate extract was selected for isolation of active compound by using column chromatographic method. The isolated compound, asarone (0.712 %) was identified by TLC and spectroscopic methods; UV, FT IR, GC-MS and then tested on 11 bacteria; *Klebsiella* species, *Salmonella paratyphi* A, *Citrobacter* species, *Escherichia coli* ATCC, *Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* YCH 149, *Shigella flexneri, Proteus* species, *Staphylococcus aureus* and *Vibrio cholerae* O1 by agar disc diffusion method. In addition minimum inhibitory concentration (MIC) of asarone was determined by microtitre plate dilution method and the MIC value 0.06 mg/mL of asarone on tested bacteria, *E. coli* LT and *S. aureus* was found.

Keywords: *Acorus calamus* L., asarone, antibacterial activity, MIC

Introduction

Diarrhoea and dysentery are important health problems in worldwide especially developing countries. So the Government of Myanmar has initiated a national programme for the development of Traditional Medicine System in

[1] Associate Professor, Dr, Department of Chemistry, Sagaing University of Education

combating six major types of diseases: namely; malaria, tuberculosis, diarrhoea, dysentery, diabetes and hypertension.

Diarrhoea is the host response to infection of the gastrointestinal tract by a variety of viruses, bacteria and parasites. There are three types of diarrhoea, namely acute diarrhoea, persistent diarrhoea and chronic diarrhoea. Acute diarrhoea is usually defined as the passage of 3 (or) more liquid motions within 7 days. Persistent diarrhoea have a usually long duration, more than 2 weeks, but usually less than 2 weeks duration. Chronic diarrhoea lasts for more than three weeks (Khan, 2001).

Dysentery is an inflammatory disorder of the lower intestinal tract, usually caused by bacteria, parasitic, or protozoa infection and resulting in pain, fever, and severe diarrhoea, often accompanied by the passage of blood and mucous. Dysentery is caused by an *Amoeba* or *Bacillus* that infects the colon (Boyd and Marr, 1980).

In this study, Myanmar medicinal plant, *Acorus calamus* L. (Lin - ne or Lin - lay) (Figure 1) was selected to find out of active principle for the treatment of dysentery and diarrhoea. *A. calamus* plants are found in the north temperate regions and South East Asia. These plants are found wild or cultivated throughout India and Srilanka, ascending to 6000 ft. *A. calamus* L. is indigenous and grows wild and extensively in Myanmar, especially in "Myitkyina". It can be also found in Pan Ta Naw Township in Ayeyarwady Region and Shan State. The rhizome in this study was collected from Ywa Thar Gyi, Yangon Region. The yellow oil, asarone is major constituent responsible for peculiar warm and spicy odour of *A. calamus* L. (Bose *et al*., 1960; Fluck, 1976). In Myanmar, *A. calamus* L. is used in treating dyspepsia, chronic diarrhoea, dysentery of children, fever, bronchitis and insecticide (Mar Mar Nyein *et al*., 2006).

In *A. calamus* L., essential oil mainly contains cis or trans asarone and it shows antioxidant, antibacterial activity as well as antimicrobial activity. The rhizome of *A. calamus* L. has been also used in the treatment of gastrointestinal (GI) problems, anorexia, flatulence and then used as spice, perfume in food and industry (Bruno *et al*.,2005; Devi and Ganjewala, 2011; Raina *et al*., 2003). Therefore, antibacterial activity investigation on four crude extracts (PE, EtOAc, 96% EtOH, 50% EtOH) and some isolated phytoconstituents

from the rhizome of *A. calamus* L. were carried out by using agar disc diffusion method. Minimum inhibitory concentration (MIC) of major constituent was also determined by microtitre plate dilution method in this study.

Botanical Aspects of *Acorus calamus* L.

Name : Lin-ne (or) Lin-lay (in Myanmar),
The Sweet-Flag (in English)

Botanical Name : *Acorus calamus* L.

Family : Acoraceae
(Kress *et al*., 2003)

Genus : *Acorus*

Rhizome : Root-stock creeping wit
5 feet long horizontal and
much branched aromatic rhizome

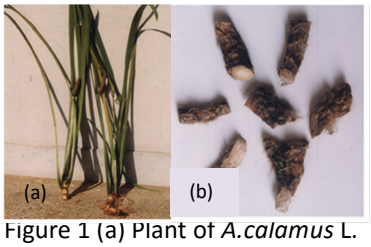


Figure 1 (a) Plant of *A.calamus* L.
(b) Rhizome of *A.calamus* L.

Distribution

A. calamus L. (Lin-ne) is widely distributed in Myanmar and often planted for its medicinal properties.

Chemical Constituents

The rhizome of *A. calamus* L. (Lin-ne) contained volatile oil, α-pinene, camphene, calamene, calamenol, calamenone, asarone, aromatic glucoside (acorin), a strong base calamine, a soft resin, tannin, mucilage, starch and calcium oxalate.

Materials and Methods

Plant Materials

The rhizome of *A. calamus* L. was collected from Ywa Thar Gyi, Yangon Region. The plant was identified at Department of Botany, Yangon University. The rhizome of *A. calamus* L. was washed, cleaned and dried at room temperature for two weeks. Then the dried sample powdered and stored in air- tight container.

Instruments: Shimadzu UV-240, (MeOH), Shimadzu FT IR- 8400, GC-MS; Turbo Mass Perkin Elmer, URC and University of Goettingen, Germany,

Chemicals : CC; Merck Silica gel 60 (70-230) mesh, eluents; Petroleum - ether (PE)-ethyl acetate(EtOAc), only ethyl acetate, TLC; precoated silica gel 60 (F_{254}, Merck)

Extraction and Isolation of Asarone from Rhizome of *Acorus calamus* L.

Preparation of extracts from rhizome of *Acorus calamus* L.

The air-dried powder (1kg) was cold extracted with (2500 mL) of various solvent; petroleum-ether (60-80°C), ethyl acetate, 96% ethanol and 50% ethanol, respectively for 7 days and then filtered. The filtrate was evaporated to dryness at normal pressure on a water bath and dessicated. The yield % of petroleum-ether extract, ethyl acetate extract, 96% ethanol extract and 50% ethanol extract were determined.

Isolation of phytoconstituents from EtOAc extract of rhizome of *Acorus calamus* L.

The EtOAc extract was subjected to isolate the active phytoconstituents from Lin-ne rhizome by column chromatography. The column was packed with silica gel (400g) by the wet method using petroleum-ether the column was eluted consecutively with the solvent system (v/v) (PE : EtOAc) (30:1, 19:1, 9:1, 2:1, 1:1) and only ethyl acetate according to their increasing polarity. The column was completely filled with the solvent system and fractionation was started. Flow rate was adjusted to about 1 drop per second. Fractions were monitored by thin layer chromatography (TLC). The fractions

that gave similar spots on thin layer chromatography (TLC) plates were combined together and the solvent was removed. Finally, isolated compound (yellow oil, 0.712 %) was characterized as asarone by spectroscopic methods.

In vitro Studies on the Antibacterial Activity of Rhizome of *A. calamus* L. by Agar Disc Diffusion Method

Screening of antibacterial activity of crude extracts against 19 tested bacterial strains

Agar disc diffusion method was used for the detection of antibacterial activity for four crude extracts from *A. calamus* L. rhizome. The test procedure was as follows: the extracts (1g each for testing 19 cultural bacterial strains) were introduced into sterile petridishes and dissolved in 1 mL of their respective solvents, petroleum ether, ethyl acetate, 96 % ethanol and 50% ethanol. Discs obtained by filter paper (Toyo No.26, Japan) punched to 6 mm diameter, were used to impregnate the extracts. To obtain approximately 20 μg/disc and prior to adherence on the culture plates the discs were allowed to dry at 42° C in incubator.

The bacterial suspension from trypticase soy broth was streaked evenly into three places on the surface of the trypticase soy agar plates with sterile cotton swab (Puritan, USA). After the inoculums had dried for 5 min, the dried disc impregnated with extracts were placed on the agar with flamed forceps and gently pressed down to ensure proper contact. A disc impregnated with solvent only was placed alongside the test discs for control and comparing purposes known antibiotics tetracycline was also used as positive control.

The plates were incubated immediately (or) within 30 min after inoculation. After overnight incubation at 37°C, the zones of inhibition diameter including 6 mm discs were measured, by means of a thin transparent ruler or by a divider. The most active extract (EtOAc) was selected for isolation of active compound and MIC determination.

Screening of Antibacterial Activity of EtOAc Extract and Iolated Asarone from Active EtOAc extract of rhizome (*A. calamus* L.) against 11 tested bacterial strains

The selected most active EtOAc extract and isolated compound asarone (0.712 %) were subjected to study antibacterial activity against 11 tested bacteria from clinical sources, National Health Laboratory (NHL), Yangon; related to acute diarrhoea (cholera), dysentery, abscess, pneumonia and typhoid.

Determination of Minimum Inhibitory Concentration (MIC) by Microtitre Plate Dilution Method

The isolated compound, asarone, from *A. calamus* L. was tested with 5 strains; *Escherichia coli* ATCC, *Escherichia coli* LT, *Escherichia coli* STLT, *Staphylococcus aureus* ATCC, *Staphylococcus aureus* by microtitre plate dilution method.

Microtitre plate dilution method was done by using trypticase soy broth by dissolving with appropriate soluble compound, asarone in 2-fold dilutions. First, an inoculum of pure culture of respective bacteria was seeded in 5 mL of trypticase soy broth (TSB) and incubated at 37˚C for 3-4 h to obtain a turbidity of 0.05 by MacFarland nephelometer which corresponded to a bacterial suspension of 10^6 organisms per mL. Prior to the experiment, 50 L of TSB was introduced into all wells of 96-well microtitre plate. The compound (asarone) was dissolved in ethyl acetate and diluted with trypticase soy broth to obtain the following concentration: 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.32 mg/mL, 0.16 mg/mL, 0.08 mg/mL, 0.04 mg/mL, 0.02 mg/mL, 0.01 mg/mL, 0.005 mg/mL, 0.0025 mg/mL in 96 - well microtitre plates.

Then 0.02 µL of the prepared inoculum was introduced to its respective wells and the microtitre plates were incubated at 37°C for 18 h. Prior to taking spectrophotometer readings, contents of all wells were thoroughly mixed with a multi-channel pipetter to resuspend clamped cells at the bottom of the wells in a solution. Growth of the bacteria was determined by automated microplate reader (Bio Rad) at a wavelength of 450 nm as well as confirmed by the growth of culturing onto trypticase soy agar to incubate at

37°C for overnight. The last well with no growth of bacteria was taken to represent the minimum inhibitory concentration (MIC) of the compound.

Results and Discussion

Isolation of Asarone from Ethyl Acetate Extract of *A.calamus* L. Rhizome

The dried rhizome powder collected from Ywa Thar Gyi, Yangon Region was extracted using various solvents and the yield % of petroleum-ether extract (1.1%), ethyl acetate extract (1.5%), 96% ethanol extract (2.0%) and 50% ethanol extract (3.2%) were obtained respectively. Asarone (0.712%) has been isolated from ethyl acetate extract of *A. calamus* L. by column chromatographic separation method. The structure of isolated compound as asarone have been identified by TLC determination, UV(Figure 2), FT IR (Figure 3), and GC - MS (Figure 4 a, b) spectrometry. Isolated compound gave R_f value 0.43 with petroleum ether - ethyl acetate (4:1) system and red purple colour was observed with vanillin-conc. H_2SO_4 in TLC chromatogram.

Asarone : Yellow oil (1.78 g, 0.712 % yield); λ_{max}^{MeOH} 250, 300 (nm) (Obs. value), λ_{max}^{EtOH} 252, 302 (nm) (Lit. value) ; FT IR $(CHCl_3)/cm^{-1}$ 2995.2, 2933.5, 2829.4 (ν_{CH} for asym & sym of – CH_3), 1604.7, 1506.3 ($\nu_{C=C}$ of aro-), 1458.1(δ_{asym} – CH_3) , 1392.5, 1321.1 (δ_{sym} – CH_3), 1201.6, 1126.3 ($\nu_{asym\ C\text{-}O\text{-}C}$ of aro-), 1039.6 ($\nu_{sym\ C\text{-}O\text{-}C}$) , 981.7 ($\delta_{CH}$ of trans double bond), 850.5, 763.8 (δ_{CH} of aro - system); GC-MS (m/z) 208 (M^{+o}), 209 $(M + H)^+$, 193 $(M – CH_3)^+$ [C_{12} H_{16} O_3] (Figures 2,3 and 4) (Silverstein *et al*., 1991)

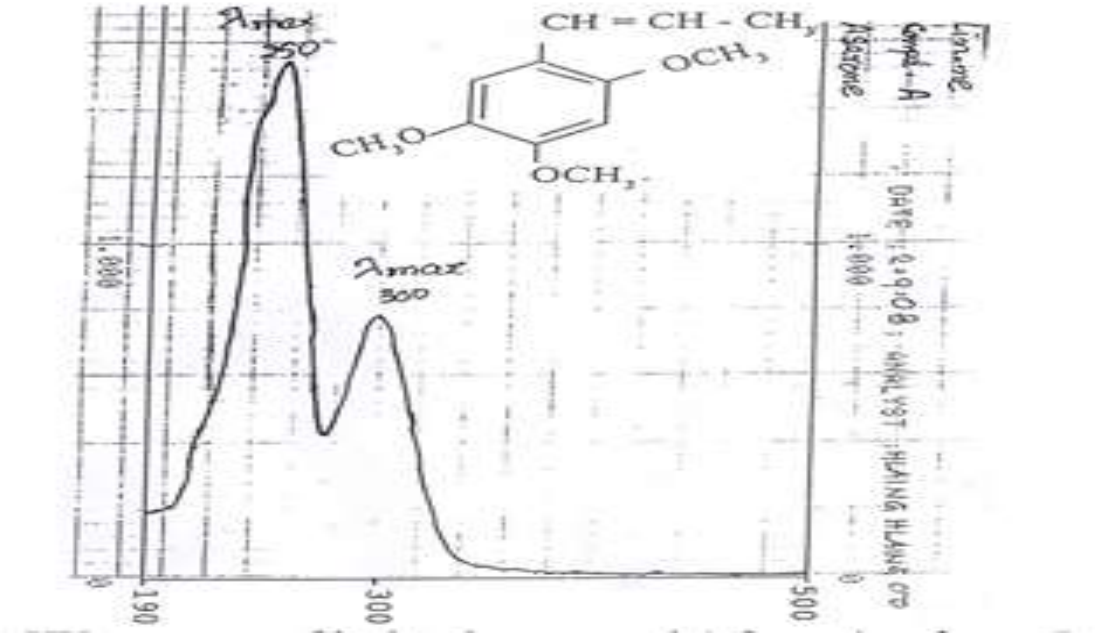


Figure 2 Ultraviolet spectrum of isolated compound from *Acorus calamus* L.

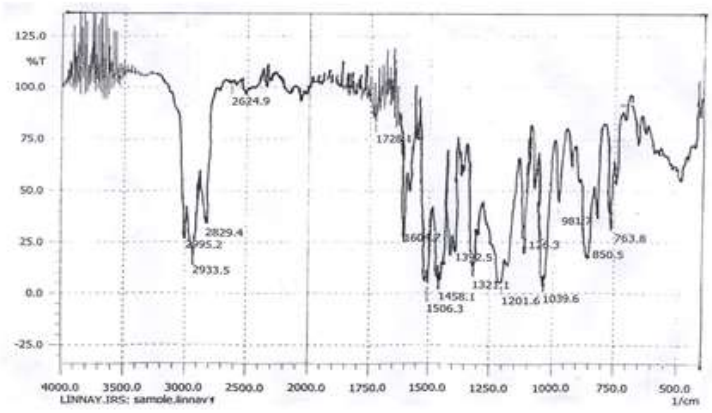
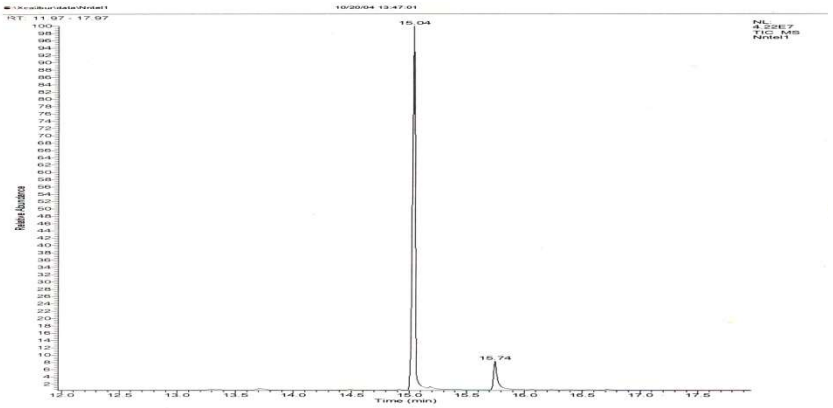
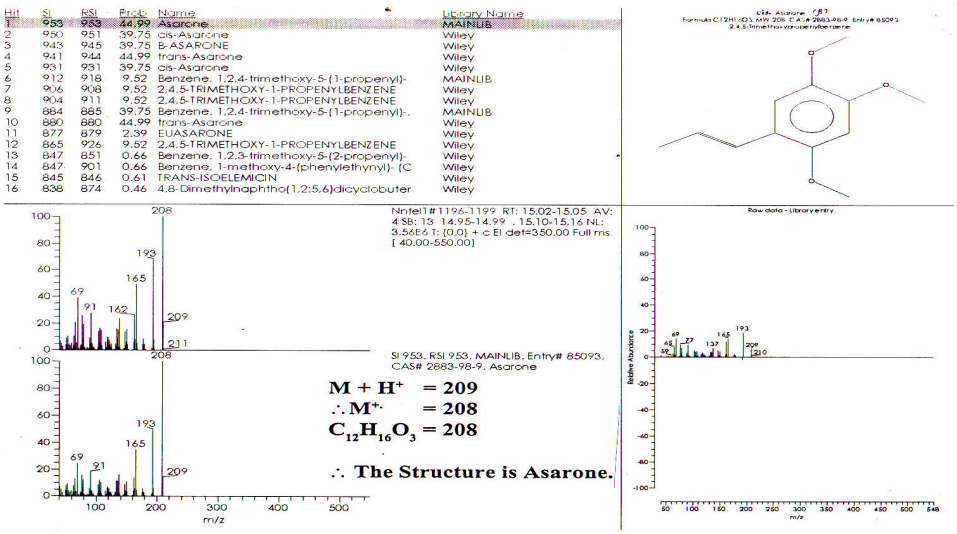


Figure 3 FT IR spectrum of isolated compound from *Acorus calamus* L.



(a)



(b)

Figure 4 GC-MS spectrum of isolated compound from *Acorus calamus* L.
(a) GC spectrum of isolated compound from *Acorus calamus* L.
(b) MS spectrum of isolated compound from *Acorus calamus* L.

Screening of Antibacterial Activity of Crude Extracts and Isolated Asarone

Screening of antibacterial activity of 4 crude extracts has been done by agar disc diffusion method. The inhibition zone diameters of extracts tested with 19 strains of bacteria from clinical sources are shown in Table 1. The most active ethyl acetate extract with the range of inhibition zone diameter (12-22) mm against 7 strains, namely *Escherichia coli* LT, *Escherichia coli* EHEC, *Salmonella paratyphi, Vibrio chlolerae* Inaba, *Pseudomonas pyocyanea, Salmonella typhi* and *Staphylococcus aureus* was selected for isolation of active compound. Antibacterial activity of ethyl acetate extract and isolated asarone were being compared on 11 tested bacteria shown in Figure 5. In Table 2 it was found that the isolated asarone showed more potent activity with inhibition zone diameters (12-33) mm of all strains except *Proteus* species but ethyl acetate extract exhibited less potent to 8 out of 11 strains with inhibition zone diameter (7-11) mm. According to these zone diameters, the antibacterial activity of asarone against *Klebsiella* species, *Salmonella paratyphi* A, *Citrobacter* species, *Escherichia coli* ATCC, *Salmonella typhi, Escherichia coli* YCH 149 and *Shigella flexneri* are more potent than tetracycline, the control except *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Vibrio cholerae* O1. From the screening results, it can be generally deduced as follows. The EtOAc extract and isolated asarone from *A. calamus* L. were found to inhibit the tested bacteria with regard to acute diarrhoea (cholera), dysentery, pneumonia, typhoid, urinary tract infection, sepsis and abscess. In addition, asarone, major constituent yielded (0.712%) from *A. calamus* L. was employed by microtitre plate dilution method for minimum inhibitory concentration (MIC) determination with 3 strains of *Escherichia coli* and 2 strains of *Staphylococcus aureus* obtained from clinical sources at Bacteriology Research Division, DMR (LM) are shown in Table 3. The microtitre plate dilution method also elaborates the specificity, sensitivity and the least amount required for media, reagents and glassware. It also saves time and working space in conducting the experiments. The lowest minimum inhibitory concentration (MIC), 0.06 mg/mL of asarone with *Escherichia coli* LT and *Staphylococcus aureus* showed the plant possess bactericidal activity on them. From the MIC elucidation, asarone isolated from the rhizome of Linne would be more effective for the treatment in diarrhoea and abscess. Since it

has bacterial action against *E. coli* responsible for diarrhoea and *Staphylococcus aureus* responsible for abscess occurred in skin, mouth and nose.

Table 1. Results of Antibacterial Activity of Four Extracts of *A. calamus* L. on 19 Species of Bacteria

No.	Type of bacteria	Inhibition zone diameter (mm)			
		EtOAc extract	96%EtOH extract	50%EtOH extract	PE extract
1	*Salmonella derby*	-	-	-	-
2	*Escherichia coli* LT	14	12	-	-
3	*Escherichia coli* O128	-	-	-	-
4	*Escherichia coli* EHEC	13	-	-	11
5	*Staphylococcus aureus* ATCC	-	18	12	12
6	*Salmonella paratyphi*	20	15	12	20
7	*Salmonella stanley*	-	-	-	-
8	*Shigella boydii*	-	-	-	-
9	*Salmonella pollorum*	-	-	-	-
10	*Shigella dysenteriae*	-	-	-	-
11	*Vibrio cholerae* Inaba	12	-	10	-
12	*Escherichia coli* ATCC	-	-	-	-
13	*Pseudomonas pyocyanea*	12	-	10	-
14	*Vibrio cholerae* O1	-	-	-	-
15	*Salmonella typhi*	12	-	-	-
16	*Vibrio cholerae* O139	-	-	-	-
17	*Shigella flexneri*	-	-	-	-
18	*Bacillus subtilis*	-	-	-	-
19	*Staphylococcus aureus*	22	20	15	20

(-) = no activity

Disc diameter = 6 mm

Table 2. Antibacterial Activity of EtOAc Crude Extract and Asarone, Isolated from Lin- ne or Lin - lay Rhizome

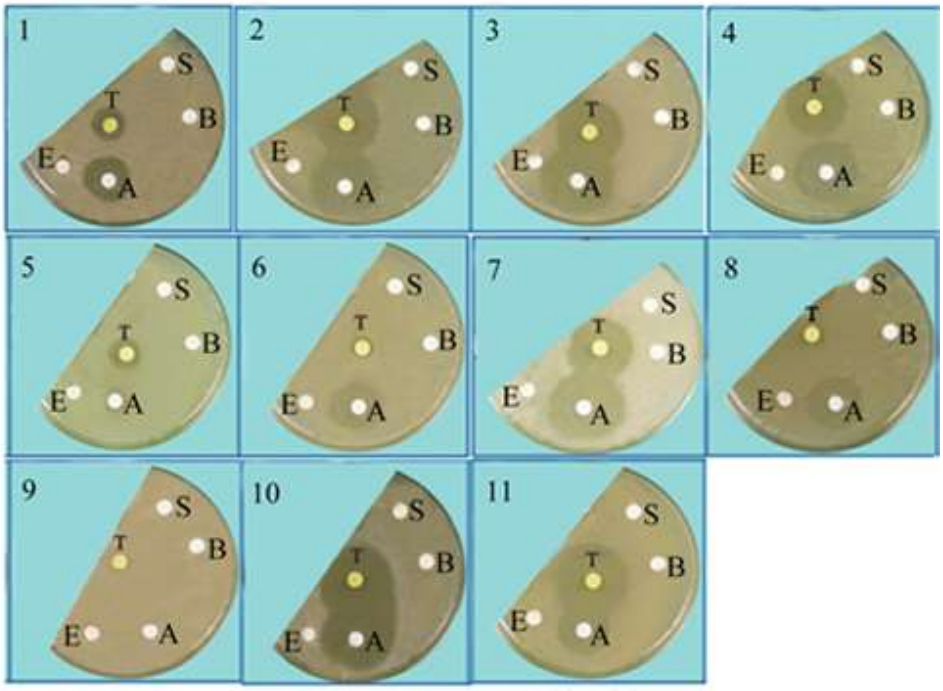
Sample	Inhibition zone diameter (mm) against test bacteria										
	1	2	3	4	5	6	7	8	9	10	11
Crude (Lin-ne)	8	7	-	7	-	7	11	10	7	7	-
Asarone	20	33	31	27	12	22	33	24	-	28	22
Blank disc	-	-	-	-	-	-	-	-	-	-	-
EtOAc solvent (-) control	-	-	-	-	-	-	-	-	-	-	-
Tetracycline (+) control	13	25	25	25	15	10	25	12	-	29	29

Tested Bacteria (From Clinical Sources*)

1 = *Klebsiella* species
2 = *Salmonella paratyphi* A
3 = *Citrobacter* species
4 = *Escherichia coli* ATCC
5 = *Pseudomonas aeruginosa*
6 = *Salmonella typhi*
7 = *Escherichia coli* YCH 149
8 = *Shigella flexneri*
9 = *Proteus* species
10 = *Staphylococcus aureus*
11 = *Vibrio cholerae* O1

Disc diameter = 6 mm
- = no activity

* National Health Laboratory (NHL), Yangon



1 = *Klebsiella* species

2 = *Salmonella paratyphi* A

3 = *Citrobacter* species

4 = *Escherichia coli* ATCC

5 = *Pseudomonas aeruginosa*

6 = *Salmonella typhi*

7 = *Escherichia coli* YCH 149

8 = *Shigella flexneri*

9 = *Proteus* species

10 = *Staphylococcus aureus*

11 = *Vibrio cholerae* O1

E = EtOAc crude extract (Lin – ne or Lin - lay)

A= Asarone

S = EtOAc solvent (-) control

B = Blank

S = EtOAc solvent (-) control

T = Tetracycline (+) control

Figure 5 Antibacterial activity of EtOAc crude extract and asarone, isolated from Lin - ne or Lin – lay

Table 3. Minimum Inhibitory Concentration (MIC) of Active Isolated Compound Asarone (mg/mL) of *Acorus calamus* L.

No.	Bacteria	MIC(mg/mL)
1	*Escherichia coli* ATCC	0.12
2	*Escherichia coli* LT	0.06
3	*Escherichia coli* STLT	0.63
4	*Staphylococcus aureus* ATCC	0.12
5	*Staphylococcus aureus*	0.06

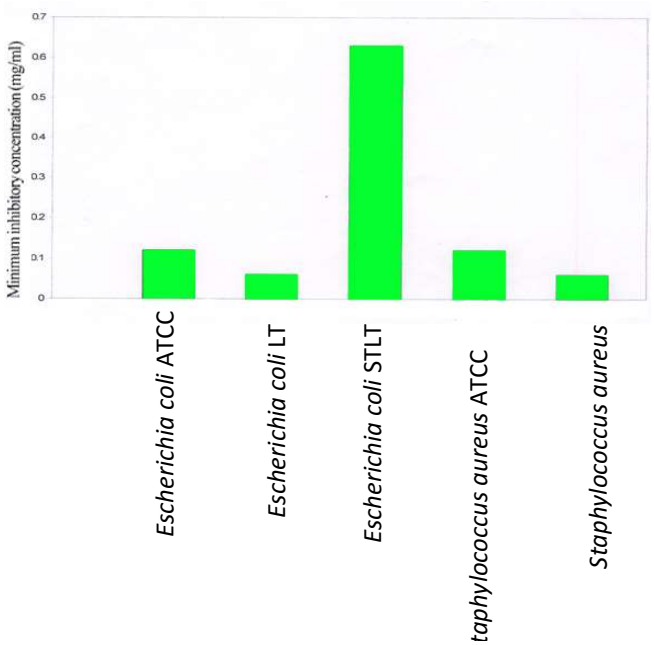


Figure 6 Minimum inhibitory concentration (MIC) of asarone from EtOAc extract of *Acorus calamus* L. rhizome against different bacteria by microtitre plate dilution method

CONCLUSION

From the rhizome of *A. calamus* L., four crude extracts: PE extract (1.1 %), EtOAc extract (1.5 %), 96 % EtOH extract (2.0 %) and 50 % EtOH extract (3.2 %) were obtained and screened antibacterial activity against 19 tested bacteria by agar disc diffusion method. Among the four crude extracts of Lin-ne, only EtOAc extract showed the most potent antibacterial activity with the related larger zone diameter (12-22) mm on *Escherichia coli* LT, *Escherichia coli* EHEC, *Salmonella paratyphi, Vibrio cholerae* Inaba, *Pseudomonas pyocyanea, Salmonella typhi* and *Staphylococcus aureus* out of 19 bacterial strains. Using column chromatographic separation, yellow oil (1.78 g, 0.712 %) was isolated from the most active EtOAc extract of *A. calamus* L. (Lin-ne) and identified as asarone by UV, FTIR and GC-MS spectrometry.

In vitro antibacterial activity of ethyl acetate crude extract and asarone were also investigated and it was found that asarone showed the range of inhibition zone diameter (12-33) mm whereas inhibition zone diameter of EtOAc extract ranged between (7-11) mm. It was concluded that asarone, pure compound was more effective on 10 out of 11 tested bacteria than that of the crude EtOAc extract and tetracycline for the treatment of dysentery, abscess and diarrhoea.

Acknowledgements

I would like to express my thanks to Dr Saw Pyone Naing, Rector and Dr. Myo Thant, Pro-Rector, Sagaing University of Education, for giving the permission to report this research work. I would also like to extend my gratitude to Dr. Kathy Myint Thu, Professor (Head), Department of Chemistry, Sagaing University of Education, for her enthusiastic guidance in this research work. I am also grateful to Dr Than Soe, Rector, University of Myitkyina and Dr Mar Mar Nyein, Director (Retired), Bacteriology Research Division, Department of Medical Research (Lower Myanmar) for their valuable suggestions.

References

Bose, B.C., Vijayvargiya, R., Saifi, A.Q. and Sharma, S.K. (1960). "Some Aspects of Chemical and Pharmacological Studies of *A. calamus* Linn.". *J. of the American Pharmaceutical Asso*, vol. 49 (1), pp. 32-34

Boyd, R.F. and Marr, J. J. (1980). *Medical Microbiology.* Boston: 1st Ed., Little Brown and Company, p. 597

Bruno, M., Alessandra, P., Silvia, P. and Andrea, S. (2005). "Chemical Composition of the Essential Oil and Supercritical CO_2 Extract of *C. myrrha* N. and *A. calamus* L.". *Journal of Agricultural and Food Chemistry,* vol. 53 (20), pp. 7939-7943

Devi, A.S. and Ganjewala, D. (2011). "Antioxidant Activities of Methanolic Extracts of Sweet- Flag (*A. calamus* L.) Leaves and Rhizomes". *Journal of Herbs Species & Medicinal Plants,* vol. 17, pp. 1-11

Fluck, H. (1976). *Medicinal Plants and their Uses*. London: 2nd Ed., W. Foulsham & Co. Ltd., pp. 101-110

Khan, M.R. (2001). "Antibacterial Activity of Some Tanzanian Medical Plants". *Pharmaceutical Biology*, vol. 39 (3), pp. 206-212

Kress, W.J., Defilipps, R.A., Farr, E. and Daw Yin Yin Kyi. (2003). *A Checklist of the Trees, Shrubs, Herbs and Climbers of Myanmar.* Washington, DC: 1st Ed., Department of Systematic Biology-Botany, National Museum of Natural History, p. 37

Mar Mar Nyein, Mi Mi Htwe, Ba Han and Ei Ei Khine. (2006). "Antibacterial Activity of Some Plants and Formulations and Determination of Minimum Inhibitory Concentration (MIC) by Microtitre Plate Dilution Method". *The Myanmar Health Sciences Research Journal,* vol. 18 (1), pp. 26-30

Raina, V.K., Srivastava, S.K. and Syamasunder, K.V. (2003). "Essential Oil Composition of *A. calamus* L. from the Lower Region of the Himalayas". *Flavour and Fragrance Journal,* vol. 18 (1), pp. 18-20

Silverstein, R.M., Bassler, G.C. and Terence, C.M. (1991). *Spectrometric Identification of Organic compounds*, Singapore: 5th Ed., John Wiley and Sons, Inc., pp. 289-315

ISOLATION OF ASARONE FROM THE RHIZOME OF *ACORUS CALAMUS* L. (LIN-NE) AND ANTIBACTERIAL SCREENING OF THE CRUDE EXTRACTS AND ASARONE

A Research Paper Submitted to the Myanmar Academy of Arts and Science

By

Ei Ei Khaing
BSc (Hons.), MSc, PhD
Associate Professor
Department of Chemistry
Sagaing University of Education

June, 2017