Extraction, Isolation and Identification of Active Constituents from *Curcuma aromatica* Salisb (Taw-Sanwin)

Moe Moe Myint Aung[1], Yee Yee Thu[2]

Abstract

Curcuma aromatica Salisb (Taw-Sanwin) belongs to the family Zingiberaceae. In this paper, the rhizomes of *Curcuma aromatica* Salisb were collected from Hpa-An Township during June to December 2022. Crude constituents from the powdered rhizomes of *Curcuma aromatica* Salisb were extracted with 100 percent ethanol three times. The active compounds were isolated from the ethanol extract (10 g) of the powered rhizomes of *C. aromatica* Salisb by the column chromatography method with various solvent systems at Department of Botany, Dagon University. The eleven fractions (F1 to F11) were collected from the crude extract silica gel column. The four compounds were isolated from the three fractions (F2, F7 and F9). Then, the isolated compounds were identified by Thin Layer Chromatography (TLC) behavior, R_f value, Ultra violet (UV) spectrum, and Fourier Transform Infrared (FT-IR) spectroscopic techniques. The yields of the four compounds were 6.7 mg, 7.5 mg, 4.5 mg and 3.1 mg respectively. Their R_f values were 0.97, 0.69, 0.67 and 0.53 respectively. The isolated compound I was the flavonoid phenolic compound and it may be curcuminoid as it gave the yellow colour in normal vision. The isolated compound II gave orange spot on TLC plate with dragendroff reagent, thus it was the alkaloid compound. As the isolated compounds III and IV gave the purple spots on TLC plates with vanallin/H_2SO_4, they were the terpenoid compounds and they may be diterpenes.

Keywords: Alkaloid, *Curcuma aromatica* Salisb., Curcuminoids, Terpenoid

Introduction

Curcuma aromatica Salisb belongs to the 'ginger family' Zingiberaceae. It is growing wild in Myanmar. It is known as 'Vanaharidra' in Ayuveda, wild turmeric in English, 'jangli haldi' in Hindi, and 'Yu Jin' in Chinese. It is commonly used as a coloring and flavoring agent, as well as in many traditional medicines in Southeast Asian countries (Kanase and Khan, 2018).

The rhizomes and roots of *Curcuma aromatica* Salisb are often used in cosmetics and spas for skincare in Thailand, (Choochote *et al*., 2005). The rhizomes of *Curcuma aromatica* Salisb are used as bitter, appetizer, and useful in leucoderma and diseases of blood. *Curcuma aromatica* Salisb is used as a colour indicator in the apparatus used for purification of tap water. Its roots are also used as an ingredient in the preparation of body deodorants (Ahmed *et al*., 2008).

In the northeastern part of India, aqueous extracts and paste (with milk) of *Curcuma aromatica* Salisb rhizomes and leaves are used for the treatment in indigestion, rheumatism, wound healing, dysentery (Sikha *et al*., 2015). *Curcuma aromatica* Salisb has been used for the treatment of gastrointestinal, ailments, arthritic pain, skin infections, insect bites (Umar *et al*., 2020).

Curcuma aromatica Salisb consists of important chemical constituents as curcumin, xanthorrhizol, 1,8-cineole, carvone, camphor, borneol, limonene etc. belonging to class of mainly essential oils (Ahmed *et al*., 2008). The essential oils of *Curcuma aromatica* Salisb revealed the presence of various mono- and sesquiterpenes (Pant *et al*., 2010). The rhizome of *Curcuma aromatica* has to be rich in medicinally

[1] Daw, Lecturer, Department of Botany, Mawlamyine University
[2] Dr., Professor, Department of Botany, Dagon University

essential phytochemicals: alkaloids, flavonoids, curcuminoids, tannins and terpenoids (Kanase and Khan, 2018).

The objectives of present research work are to extract the active constituents from the rhizomes of *Curcuma aromatica* Salisb, to separate and isolate the active constituents from the rhizomes of *C. aromatica* Salisb and to identify the active constituents from the rhizomes of *C. aromatica* Salisb.

Materials and Methods

Preparation of crude extract from rhizomes

The rhizomes of *Curcuma aromatica* Salisb used in this research were collected from Hpa-An Township during June to December 2022. The samples were washed, cut into small pieces and then air-dried at room temperature. When constant weight of the samples was obtained, the dried samples were pulverized by grinding machine and stored in an air tight container. Dried powdered sample of rhizomes (300 g) was extracted with ethanol (3 liters) for four weeks and filtered. The filtrate was concentrated on the water bath and finally they were evaporated to dryness.

Extraction and isolation of organic compounds from the rhizomes of *Curcuma aromatica* Salisb

The crude ethanol extract (10 g) column was eluted with selected solvent system, and one fraction was collected at the rate of 3 ml per minute. Elution was successively performed with petroleum ether: ethyl acetate (100%, 95:5, 9:1, 8:2, 75:25, 60:40, 50:50, 30:70, 20:80, 100% v/v) and ethyl acetate: ethanol (1:1, 100% v/v) respectively. The collected fractions were checked on TLC plate in Figure 1.

According to the TLC behavior, the collected small fractions were combined into the large fractions. After the crude extract column, the second columns for the selected large fractions (F2, F7 and F9) were undertaken in order to isolate the pure compounds in Figures 2, 3 and 4. The reagents used for TLC plate behavior were iodine solution, Dragendroff reagent, 1 % $FeCl_3$ solution, 5% H_2SO_4 solution, vinillin-H_2SO_4 solution and Benedict solution (Grabley *et al*., 1999).

Fig. 1. Silica gel column of crude extract

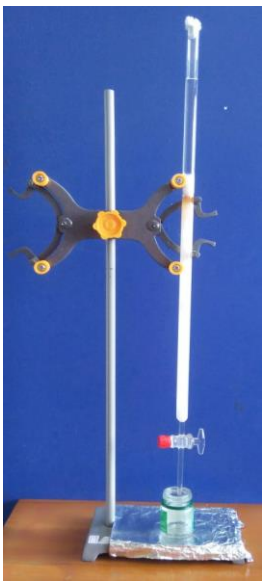


Fig. 2. Second silica gel column of fraction 2

Fig. 3. Second silica gel column of fraction 7

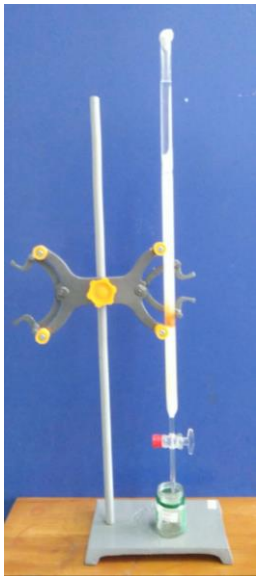


Fig. 4. Second silica gel column of fraction 9

Identification of isolated compounds by spectroscopic analysis

Thin layer chromatography

Thin layer chromatography (TLC) was covered on 0.25 mm per coated silica gel (GF_{254} aluminum plates, Merck). It was cut into small plates (1 x 5 cm size). A small amount of isolated compounds was dissolved in minimum amount of solvent and spotted on silica gel plate by using capillary tube. The spots were allowed to dry and run with solvent systems. The solvent systems were petroleum ether, ethyl acetate and ethanol. The spots were visualized by 1% $FeCl_3$, dragendroff, vanillin/sulphuric acid for the isolated compounds I, II, III and IV respectively.

Ultraviolet spectroscopic study

The UV spectra of isolated compounds were determined by using Perkin Elmer (Lambda 25) UV 240 at the Universities' Research Center, Yangon.

Infra-red spectroscopic study

The Infra-red spectra of the isolated compounds were recorded by using FT-IR 8400 spectrophotometer (Shimadzu, Japan) at Universities' Research Center, Yangon.

Results

Isolation of active constituents from *Curcuma aromatica* Salisb

If the fractions from the crude extract column gave the similar appearance on TLC plate, these small fractions were combined and finally eleven main fractions (F_1 to F_{11}) were obtained.

The compound I was obtained from the fraction F_2 by the second silica gel column with petroleum ether: ethyl acetate (PE: EA, 95:5). The compound II was obtained from the fraction F_7. This compound was more purified by using the second silica gel column with eluting solvent system (PE: EA, 9:1).

The compounds III and IV were isolated from the fraction F_9. They were more purified by using the second silica gel column with eluting solvent system (Hexane: EA, 5:1). The flow diagram of extraction, separation and isolation of the compounds I to IV as shown in Figure 5.

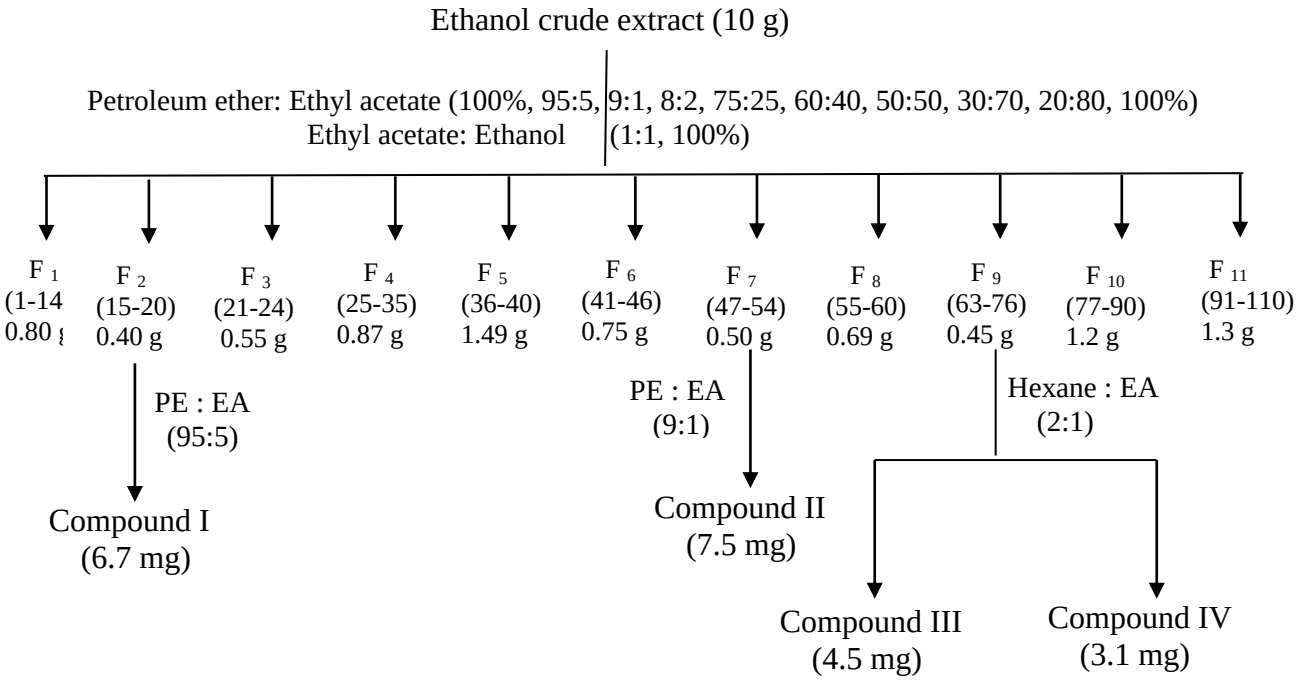
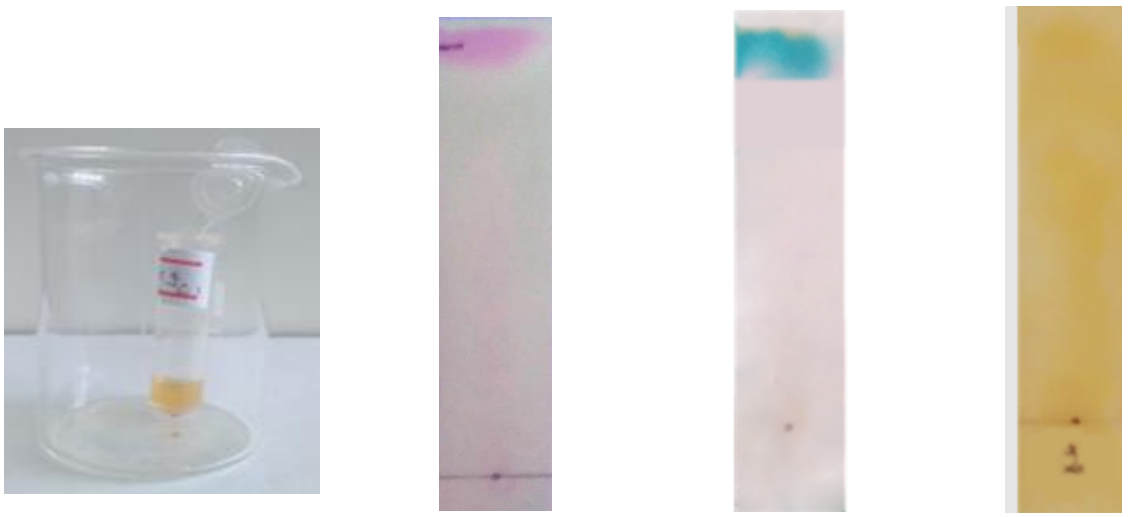


Figure 5. Isolation procedure of active constituents from *Curcuma aromatica* Salisb

Characterization and identification of isolated compounds

Compound I

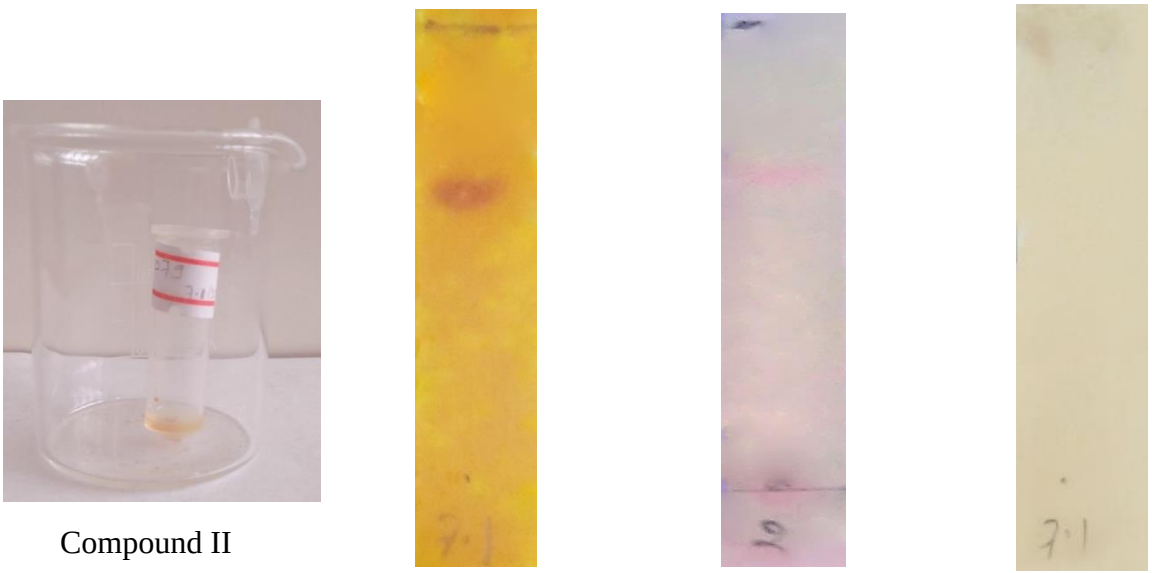


Compound I | Under UV 254 nm | Vanallin/H_2SO_4 | 1% $FeCL_3$

Figure 6. Identification of the isolated compound I by TLC behavior

The isolated compound I (6.7 mg) gave the blue spot on TLC plate with vanallin/H_2SO_4. It was light orange with 1% $FeCL_3$. It was the UV active compound under 254 nm. Its R_f value was 0.97 (petroleum ether: ethyl acetate, 95:5) in Figure 6.

Compound II

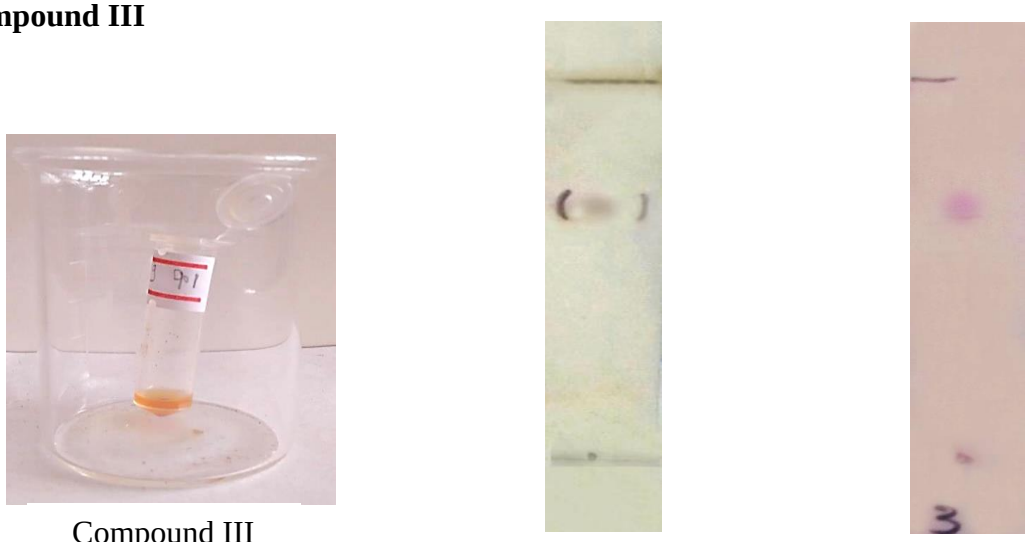


Compound II | with dragendroff | Under UV 254 | with 1% $FeCL_3$

Figure 7. Identification of the isolated compound II by TLC behavior

The isolated compound II (7.5 mg) gave the dark orange color with dragendroff reagent. It was the UV active compound under 254 nm. It was no color with 1% $FeCL_3$. Its R_f value was 0.69 (petroleum ether: ethyl acetate, 9:1) in Figure 7.

Compound III

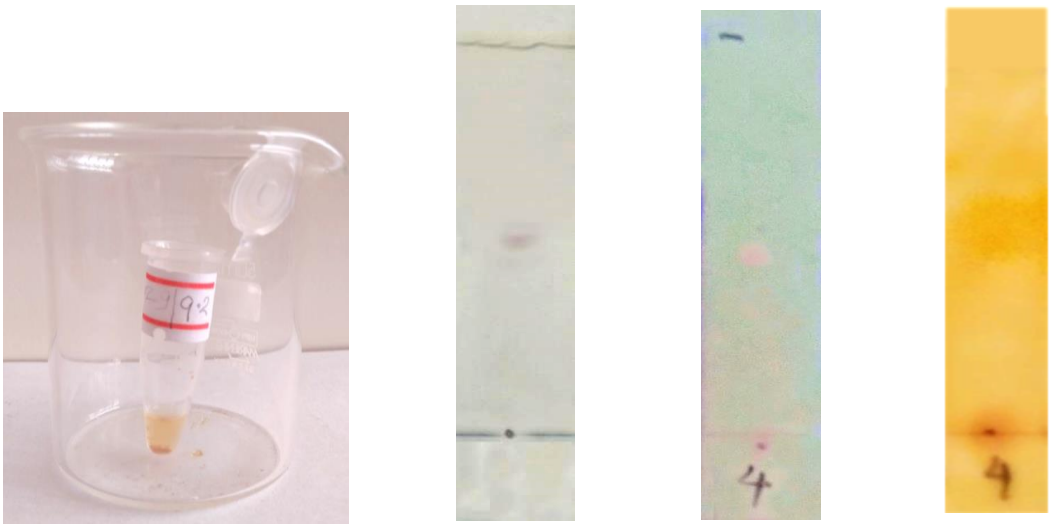


Compound III Spot on TLC plate Under UV 254 nm

Figure 8. Identification of the isolated compound III by TLC behavior

The isolated compound III (4.5 mg) gave the purple spot on TLC plate with vanallin/H_2SO_4. It was UV active compound under 254 nm. Its R_f value was 0.67 (hexane: ethyl acetate, 5 : 1) in Figure 8.

Compound IV



Compound IV Vanillin / H_2SO_4 Under UV 254 nm $FeCL_3$

Figure 9. Identification of the isolated compound IV by TLC behavior

The isolated compound IV (3.1 mg) gave the dark purple spot on TLC plate with vanallin/H_2SO_4. It was UV active compound under 254 nm. It was dark yellow with 1% $FeCL_3$. Its R_f value was 0.53 (hexane: ethyl acetate, 5:1) in Figure 9.

Ultra violet spectroscopic study of isolated compounds

The absorption λ_{max} of the four isolated compounds (I, II, II and IV) were observed at 282, 276, 280 and 262 respectively, and these data showed that n - л* (lone pair system) and 4 or more units of double bond conjugated system were found in the compounds.

Infrared spectroscopic study of isolated compounds

Compound I

In the FT-IR spectrum of the compound I, the wave number 3539 cm^{-1} and 3419 cm^{-1} showed the presence of O-H stretching vibration. The C-H stretching vibration of -CH_3, -CH_2- and CH groups appeared at 2926 cm^{-1} and 2852 cm^{-1}. The wave number 1726 cm^{-1} indicated the presence of C=O stretching vibration. C=C stretching vibration was found at 1641 cm^{-1} and an aromatic ring was observed at 1627 cm^{-1} and 1460 cm^{-1}. C-O-C bending vibration appeared at 1116 cm^{-1}, 1103 cm^{-1} and 1020 cm^{-1}. C-H bending vibration was observed at 731 cm^{-1} in Table 1 and Figure 10.

Table 1. FT-IR spectral data assignment of the isolated compound I

Wave number (cm^{-1}) compound I	Wave number (cm^{-1}) Literature	Assignment
3539, 3419	3528, 3420	O-H stretching vibration
2926, 2852	2910, 2840	C-H stretching vibration of -CH_3,-CH_2- and CH groups
1726	1750	C=O stretching vibration
1641	1650	-C=C- stretching vibration
1627, 1460	1465	Aromatic ring
1116, 1103, 1020	1109, 1000	C-O-C bending vibration
731	750	C-H bending vibration

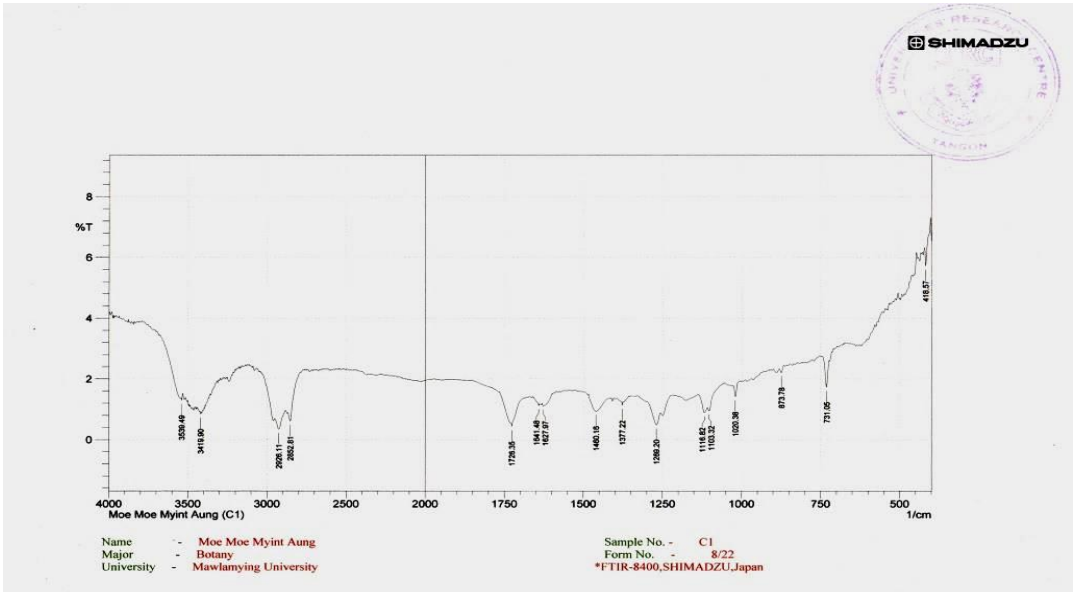


Figure 10. FT-IR spectrum of compound I

According to the results of TLC behavior, R_f value, UV spectral data and IR spectral data, the isolated compound I was the flavonoid polyphenol compound. It is may be a curcuminoid compound.

Compound II

In the FT-IR spectrum of the compound II, the wave number 3450 cm^{-1} showed the presence N-H stretching vibration (alcohol and phenol groups). The C-H stretching vibration for $–CH_3$, $-CH_2-$ and –CH- groups appeared at 2926 cm^{-1} and 2854 cm^{-1}. The wave number 1710 cm^{-1} showed the presence of C=O stretching vibration. The absorption band at 1660 cm^{-1} showed the -C=C- stretching vibration. The bands of aromatic ring were observed at 1537 and 1460 cm^{-1}. C-H bending vibration was 1410 cm^{-1}, CH_3 bending vibration found at 1369 cm^{-1}, C-O-C bending vibration were found at 1242 cm^{-1}, 1114 cm^{-1} and 1074 cm^{-1}. A band at 1030 cm^{-1} was due to the presence of C-N bending vibration. C-C bending vibration was found at 933 cm^{-1}, 895 cm^{-1} and C-H bending vibration were observed at 758 cm^{-1} and 723 cm^{-1} in Table 2 and Figure 11.

Table 2. FT-IR spectral data assignment of the isolated compound II

Wave number (cm^{-1})	Assignment
3450	N-H stretching vibration (alcohol and phenol groups)
2926, 2854	C-H stretching vibration of $–CH_3$, $-CH_2-$ and CH groups
1710	C=O stretching vibration
1660	-C=C- stretching vibration
1537, 1460	Aromatic ring
1410	C-H bending vibration $–CH_3$, $-CH_2-$ and CH groups
1369	CH_3 bending vibration
1242, 1114, 1074	C-O-C bending vibration
1030	C-N bending vibration
933, 895	C-C bending vibration
758, 723	C-H bending vibration

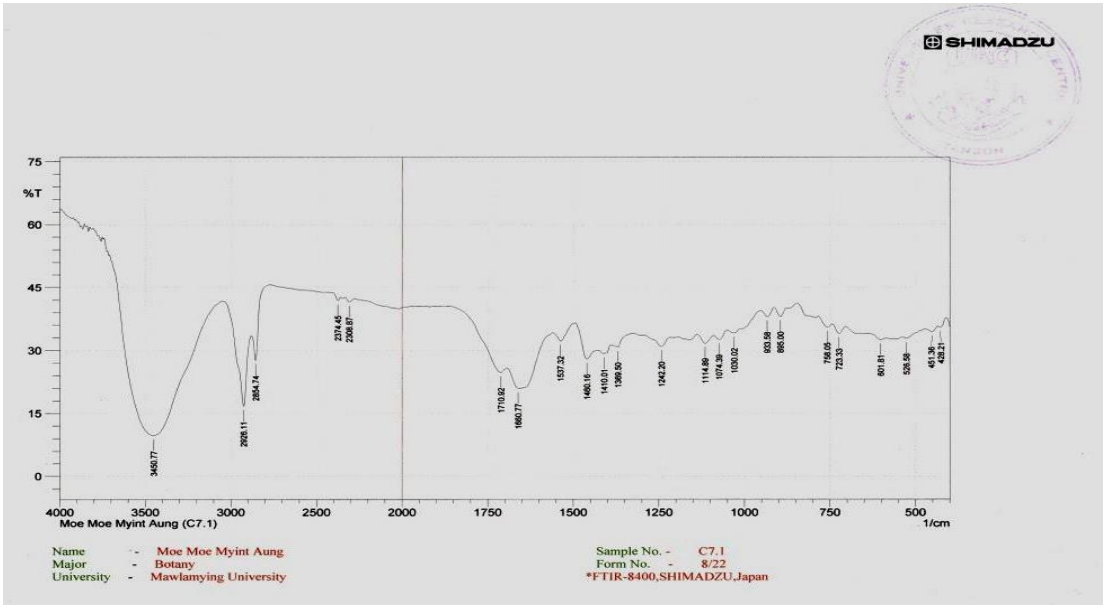


Figure 11. FT-IR spectrum of compound II

According to the results of TLC behavior, UV spectral data and IR spectral data, the isolated compound II was an alkaloid compound.

Compound III

In the FT-IR spectrum of the compound III, O-H stretching vibration at 3549 cm^{-1}, 3421cm^{-1}, 3252 cm^{-1}. The C-H stretching vibration for $–CH_3$, $-CH_2-$ and CH groups appeared at 2926 $cm^{-1}$and 2858 cm^{-1}. The wave number 1755 cm^{-1} showed the presence of C=O stretching vibration. Aromatic ring found at 1624 cm^{-1} and 1519 cm^{-1}. The CH_3 bending vibration showed at 1381 cm^{-1}. C-O-C bending vibration appeared at 1165 cm^{-1}, 1103 cm^{-1} and 1014 cm^{-1}. The C-C bending vibration at 941 cm^{-1} was observed in Table 3 and Figure 12.

Table 3. FT-IR spectral data assignment of the isolated compound III

Wave number (cm^{-1})	Assignment
3549, 3421, 3252	O-H stretching vibration
2926, 2858	C-H stretching vibration of $–CH_3$, $-CH_2-$ and CH groups
1755	C=O stretching vibration
1624, 1519	Aromatic ring
1381	CH_3 bending vibration
1165, 1103, 1014	C-O-C bending vibration
941	C-C bending vibration

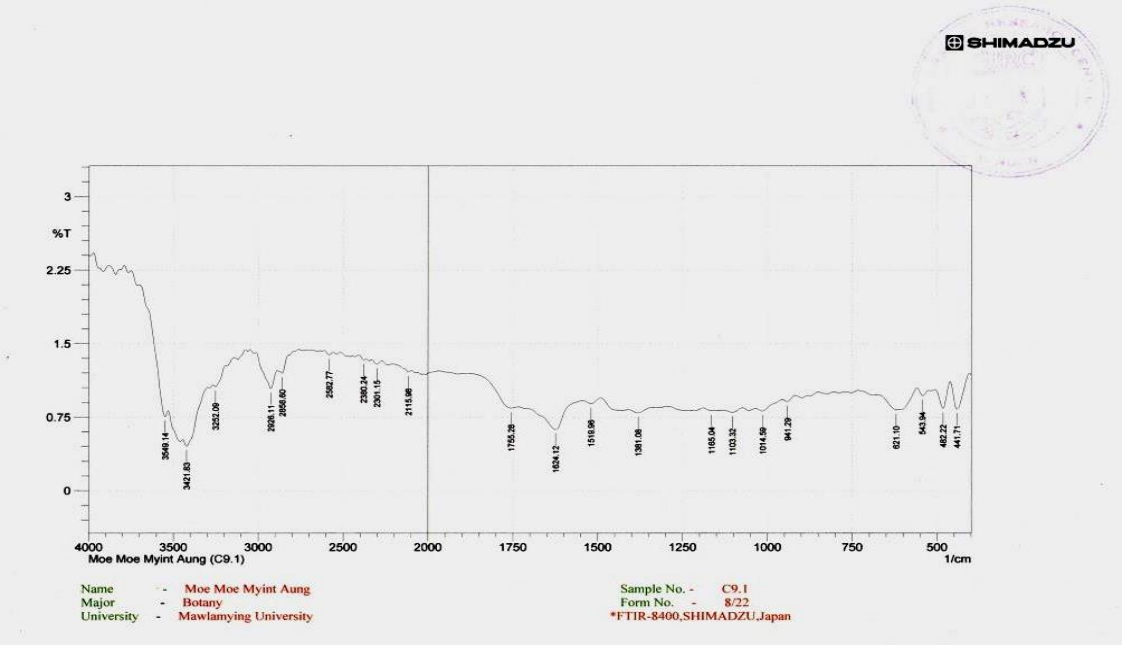


Figure 12. FT-IR spectrum of compound III

According to the results of TLC behavior, UV spectral data and IR spectral data, the isolated compound III was a terpenoid compound. It may be diterpenoid compound due to its spot color (dark purple) on TLC plate.

Compound IV

In the FT-IR spectrum of the compound IV, the wave number 3556 cm^{-1}, 3477 cm^{-1}, 3412 cm^{-1} and 3246 cm^{-1} showed the presence of O-H stretching vibration. The C-H stretching vibration for $–CH_3$, $-CH_2-$ and CH groups appeared at 2922 cm^{-1} and 2852 cm^{-1}. C=O stretching vibration at 1732 cm^{-1}, an aromatic ring at 1622 $cm^{-1}$and 1454 cm^{-1}, CH_3 bending vibration at 1384 cm^{-1}, C-O-C bending vibration at 1118 cm^{-1} and 1030 cm^{-1}, C-C bending vibration at 923 cm^{-1} and C-H bending vibration at 748 cm^{-1}. The FT-IR spectrum of the compound IV was shown in Table 4 and Figure 13.

Table 4. FT-IR spectral data assignment of the isolated compound IV

Wave number (cm^{-1})	Assignment
3556, 3477, 3412, 3246	O-H stretching vibration
2922, 2852	C-H stretching vibration of $–CH_3$, $-CH_2-$ and CH groups
1732	C=O stretching vibration
1622, 1454	Aromatic ring
1384	CH_3 bending vibration
1118, 1030	C-O-C bending vibration
923	C-C bending vibration
748	C-H bending vibration

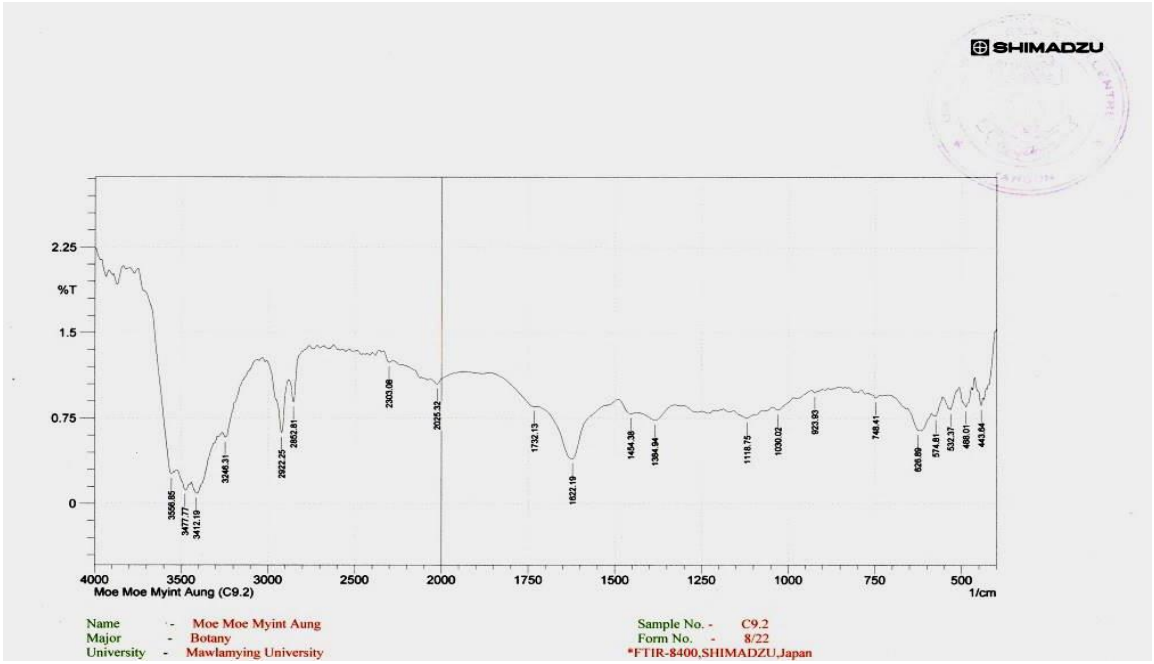


Figure 13. FT-IR spectrum of compound IV

According to the result of TLC behavior, UV spectral data and IR spectral data, the isolated compound IV was a terpenoid compound. It may also be a diterpene.

Discussion and Conclusion

In this study, the four compounds were isolated from the ethanol extract of *Crcuma aromatica* Salisb by column chromatography with various solvent systems. The compounds I was the coloured compound in normal vision, but the other three compounds were not coloured in normal vision. The isolated compound I was the flavonoid phenolic compound and it may be one of the curcuminoids. The compound II was an alkaloid compound. The isolated compounds III and IV were terpenoids, and they may be diterpenoids.

Ahmad *et al*. (2011) reported that three new homosesterpenoids isolated from the methanolic extract of *Curcuma aromatica* Salisb (rhizomes) and the chemical structures of isolated new compounds have been elucidated on the basis of structure data analysis. They have isolated three new phytoconstituent from the chloroform extract of the rhizomes *Curcuma aromatica* Salisb.

Pant *et al*. (2010) reported that the essential oils of *Curcuma aromatica* Salisb revealed the presence of various mono- and sesquiterpenes. The oil contains sequiterpenes (mainly 1- α and 1- β curmenes), two monocyclic tertiary sesquiterpene alcohols, d-camphor, d-camphene, p- methoxycinnamic and other acids, and unidentified residues.

Anoop (2015); Kanase and Khan, (2018) reported that the rhizome of *Curcuma aromatica* has to be rich in medicinally essential phytochemicals (alkaloids, flavonoids, curcuminoids, tannins and terpenoids). Pangesti and Masruri (2020) extracted curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) of spectra and antimicrobial activity of the extract mixture of *Curcuma longa* L.

In conclusion, the ethanol extracts and the isolated compounds of *Crcuma aromatica* Salisb possess some bioactivities according to the literatures. Therefore, as a further research bioactivities of these compounds should be evaluated. Antimicrobial

activity, antitumor activity, and antioxidant activity of the extracts and the compounds of *Curcuma longa* L. would be presented in the next paper.

Knowledgements

We would like to mention our sincere thanks to Rector, Pro-Rectors from Dagon University, and Professor and Head of Botany Department, DU for their kind permission to summit this paper at 4th Myanmar-Korea Conference on Plant Tissue Culture and Genetics (Useful Plants and Life Science) sponsored by Jeonbuk National University, Korea.

References

Ahmed S, Ansari SH, Ali M, Bhatt D, Ansari F. (2008). Phytochemical and biological investigations on *Curcuma aromatic*a: a review. Pharmacogn Rev., 2: 151 – 156

Ahmad S, Mohammed Ali, Shahid H. Ansari and Faheem Ahmed. (2011). Phytoconstituents from the rhizomes of Curcuma aromatica Salisb Journal of Saudi Chemical Society, Volume 15, issue 3, Pages 287-290

Anoop K. (2015). *Curcuma aromatica* Salisb:a multifaceted spice. Int. J. Phytopharm Res, 6: 10 –5.

Chini C., Bilia A.R., Keita A., Morelli I. (1992). Protoalkaloids from Boscia angustifolia. Planta Med. 58: 476

Choochote W, Chaiyasit D, Kanjanapthi D, Rattanachanpichai E, Jitpakdi A, Tuetun B, Pitasawat B. (2005). Chemical composition and anti – mosquito potential of rhizome extract and volatile oil derived from *Curcuma aromatica* against Aedes aegypti (Dipterai Culicidae). J Vector Ecol, 30:302-309

Grabley S., Thiericke R. & Zeeck A. (1999). The Chemistry Screening Approach, In Drug Discovery from Nature; Springer-Verlag, Berlin, Heidelberg, New York, p 125-148

Kanase V, Khan F. 2018. An overview of medicinal value of *Curcuma* species. Ansian J Pharm Clin Res, 11: 40 – 5; doi: 10.22159/Ajpcr. V 11 I 12.20145

Pangesti Dwika Putri and Masruri. (2020). Consisttency of Spectra and Antibacterial Activity of the Extract Mixture of Curcuma longa, Zingiber officinale, and Syzigium aromaticum. *https:www.researchgate.net/figure*

Pant, N., Himanshu, M., D.C. Jain. (2010). Phytochemical investigation of ethyl acetate extract from *Curcuma aromatica* Salisb. rhizomes: Arabian Journal of Chemistry (2013) 6, 279-283

Sikha A, Harini A, Hegde Prakash L. (2015). Pharmacological activities of wild turmeric (*Curcuma aromatica* Salisb)

Umar Nura Muhammad, Thaigarajan Parumasivam, Nafiu Aminu and Seok-Ming Toh. (2020). Phytochemical and pharmacological properties of Curcuma aromatic Salisb. (wild turmeric). Journal of Applied Pharmaceutical Science Vol. 10 (10), pp 180-194