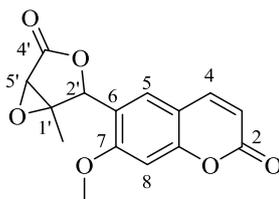


ISOLATION OF MICROZELANICUM FROM THE PLANT OF *CLAUSENA HEPTAPHYLLA* (ROXB.) WIGHT & ARN

Zar Chi Myint¹, Ko Ko Myo², Hnin Yu Win³, Myint Myint Sein⁴

Abstract

The present research paper describes the first isolation of 7-methoxy-6-(1'-methyl-4'-oxo-3',6'-dioxabicyclo[3.1.0]hexan-2'-yl)-2H-chromen-2-one (Microzelanicum, C₁₅H₁₂O₆) from the plant of *Clausena heptaphylla* (Roxb.) Wight & Arn which belongs to the family Rutaceae. The structure of isolated pure compound was confirmed by spectroscopic data measurements (¹H- and ¹³C-NMR, 2D NMR, and MS). The structure of isolated pure compound, Microzelanicum (C₁₅H₁₂O₆), is shown below.



1

7-methoxy-6-(1'-methyl-4'-oxo-3',6'-dioxabicyclo[3.1.0]hexan-2'-yl)-
-2H-chromen-2-one

Microzelanicum

Keywords: Isolation, *Clausena heptaphylla*, Rutaceae, Microzelanicum

Introduction

Clausena heptaphylla (Roxb.) Wight and Arn. (Bengali name: Panbilash, Karanphul, Pomkaphur and Myanmar name is Taw-Pyin-Taw-Thein) is a small bushy shrub and widely distributed throughout Bangladesh, India and other parts of south East Asia. *Clausena* species are known to be useful in the treatment of paralysis, ulcerated nose, headache, muscular pain and malarial fever (Begum *et al.*, 2011) and are also reputed as diuretic, astringent, insecticide, tonic and vermifuge. The leaves of the plants possess antimicrobial properties (Fakruddin *et al.*, 2011). Previous phytochemical investigations of *Clausena* species have been reported that led to the isolation of 7-Demethylmurralonginol isovalerate and Murralonginol (Lekphrom *et al.*, 2011), Micromelin (Cassady *et al.*, 1979), Clausenolide-1-methyl ether (Begum *et al.*, 2011), Lunamarins A and B (Sohrab *et al.*, 1999), Lunamarin C (Sohrab *et al.*, 2000), Carbazole Alkaloid (Chakraborty *et al.*, 1995), Clausenalene (Bhattacharyya *et al.*, 1993), Clausmarin-A (Sohrab *et al.*, 2000), and **Microzelanicum** (1) which is the first report of its occurrence from this plant.

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Materials and Methods

General Experimental Procedures

NMR spectra were measured on a Bruker Ascend TM (400 MHz) spectrometer. Mass spectra were measured on an Agilent liquid chromatography/mass spectrometry (LC/MS, Triple Quadrupol mass spectrometer). Column chromatography was carried out on silica gel (70-230) mesh. Analytical preparative thin layer chromatography was conducted on Kiesel gel 60 (F254, Merck). In thin layer chromatography, visualization was taken via UV lamp (Lambada-40, Perkin-Elmer Co, Japan) and iodine developing vapour. Crude and purified extracts were measured in Electric Balance. Common laboratory apparatus and column and thin layer chromatographic method were used for the isolation and purification of pure compounds. Commercial grade reagents and solvents were purchased from Chemico Co. Ltd., Yangon.

Sample Collection

The specimen of *Clausena heptaphylla* (Roxb.) Wight & Arn. was collected from Kalay Township, Sagaing Region, Myanmar and identified by Dr Thet Naing Oo, Pro-rector of Monywa University. The fresh stem barks of *C. heptaphylla* were chopped into small pieces and allowed to air dried at room temperature for about two weeks.

Extraction and Isolation

The air-dried sample of *C. heptaphylla* (1 kg) was percolated with methanol (2.5 L) for about one month. Then, the methanol crude extract was filtered and evaporated to concentrate at room temperature. The residue was extracted with ethyl acetate to get (13.2 g) of ethyl acetate crude extracts. Among them, the crude extract (4 g) was dissolved in ethyl acetate and 12 g of silica gel were added. The mixture was allowed to dryness under reduced pressure. The resulting crude powder extracts were subjected to silica gel column chromatographic separation by using stepwise gradient of n-hexane and ethyl acetate to give a pure compound ZCM-3 (Microzelanicum, 20.7 mg), which was isolated as pale-yellow crystals.

Results and Discussion

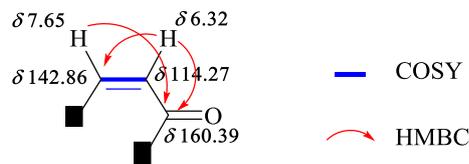
Structure Elucidation of Microzelanicum (ZCM-3)

Microzelanicum was isolated as pale-yellow crystals. The IR absorption band of the compound at 1727, 1562 and 1500 cm^{-1} showed common features of a coumarin framework. Another IR absorption at 3061 cm^{-1} was due to the presence of sp^2 hydrocarbons and 2972 and 2931 cm^{-1} were due to the presence of sp^3 hydrocarbons (methoxy and C-H stretching of methyl groups respectively).

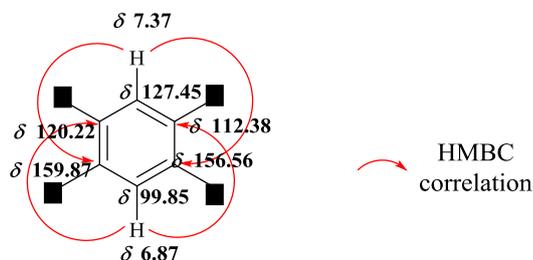
Furthermore, the structural elucidation of pure compound ZCM-3 was done by applying ^1H NMR (400MHz), splitting patterns and coupling constant (J values) of some prominent protons, ^{13}C NMR (100 MHz), DEPT, DQF-COSY, HMQC, HMBC and NOESY spectral data.

In DQF-COSY spectrum (Figure 1) shows two doublets at δ_{H} 6.32 ppm and δ_{H} 7.65 ppm, integrating 1 H of each and could be confirmed by splitting pattern and coupling constant of these alkenic protons (δ_{H} 6.32 ppm, doublet, $J = 9.5$ Hz, H-3 and δ_{H} 7.65 ppm, doublet, $J = 9.5$ Hz, H-4) which indicates that the two protons are oriented as *cis* position. Moreover, HMQC spectrum (Figure 2) shows ^1H - ^{13}C direct correlation of the two protons (δ_{H} 6.32 ppm, H-3) and (δ_{H} 7.65 ppm, H-4) with their respective carbons (δ_{C} 114.27 ppm, C-3

and δ_C 142.86 ppm, C-4). The observation of α ^1H - ^{13}C long range coupling of alkenic proton (δ_H 6.32 ppm, H-3) in the HMQC spectrum, with the sp^2 methine carbon (δ 142.86 ppm, C-4) and carbonyl carbon (δ 160.39 ppm, C-2), also shows β ^1H - ^{13}C correlation of alkenic proton (δ 7.65 ppm, H-4) with carbonyl carbon (δ 160.39 ppm, C-2) which leads to the following fragment~A.

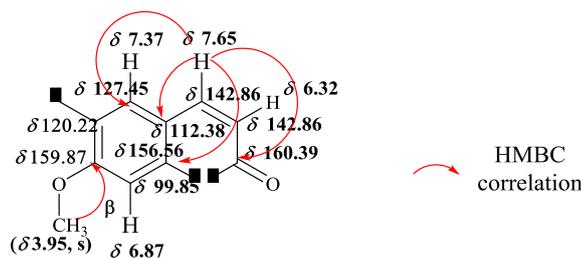
Fragment \approx A

In ^1H NMR spectrum (Figure 3), the occurrence of two singlet aromatic protons at (δ_H 7.37 ppm, H-5 and δ_H 6.87 ppm, H-8) are positioned as para position, which produces the following tetra-substituted benzene ring. In HMQC spectrum (Figure 2) displays ^1H - ^{13}C direct correlation of the two aromatic protons (δ_H 7.37 ppm, H-5 and δ_H 6.87 ppm, H-8) with their respective carbons as shown below. In the HMBC spectrum, there was observed β ^1H - ^{13}C long range coupling of aromatic proton (δ_H 6.87 ppm, H-8) with the aromatic quaternary carbons (δ_C 112.38 ppm, C-10 and δ_C 120.22 ppm, C-6). Furthermore, in HMBC spectrum (Figure 4), the observation of β ^1H - ^{13}C long range coupling of aromatic sp^2 methine proton (δ_H 7.37 ppm, H-5) with the two aromatic quaternary carbons (δ 156.56 ppm, C-9 and δ 159.87 ppm, C-7) produces the following fragment ~ B.



Fragment ~ B

The connection between fragment ~ A and B could be done by α ^1H - ^{13}C long range coupling of sp^2 methine proton (δ_H 7.65 ppm, C-4) with sp^2 quaternary carbon (δ_C 112.38 ppm, C-10). Moreover, the observation of β ^1H - ^{13}C long range coupling of sp^2 methine proton (δ_H 7.65 ppm, C-4) with carbonyl carbon, sp^2 methine carbon and sp^2 quaternary carbon (δ_C 160.39 ppm (C-2), δ_C 127.45 ppm, C-5 and δ_C 156.56 ppm, C-9) in HMBC spectrum. The presence of aromatic methoxy at C-7 (δ_C 159.87 ppm) was confirmed by ^1H NMR singlet single peak occurs at δ_C 3.95 ppm (OCH_3), ^{13}C NMR signal at δ_C 56.46 ppm and supported by $3J$ correlation to C-7 (δ_C 159.87 ppm) which produced the following extended fragment ~ C.



Fragment ~ C

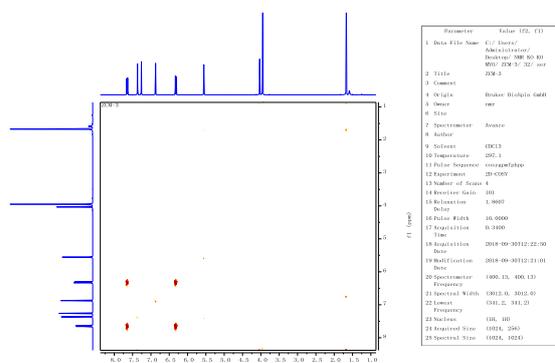


Figure 1 DQF-COSY Spectrum (CDCl₃, 300 MHz)

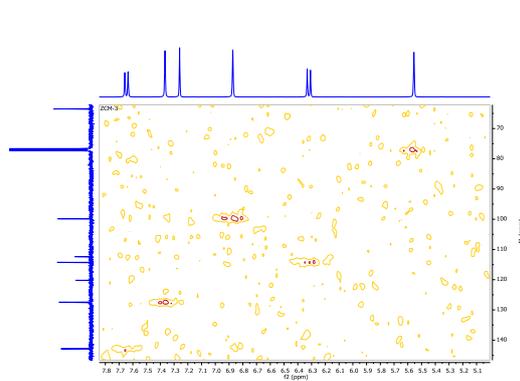


Figure 2 HMQC Spectrum (CDCl₃, 300 MHz)

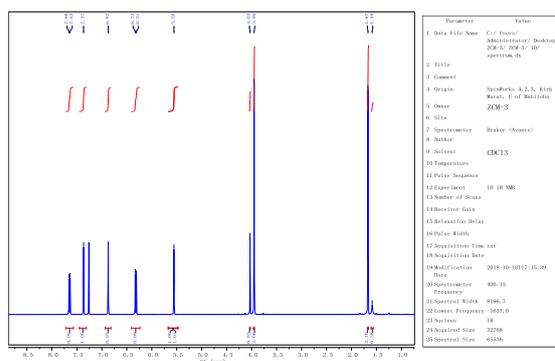


Figure 3 ¹H NMR Spectrum (400 MHz) (CDCl₃)

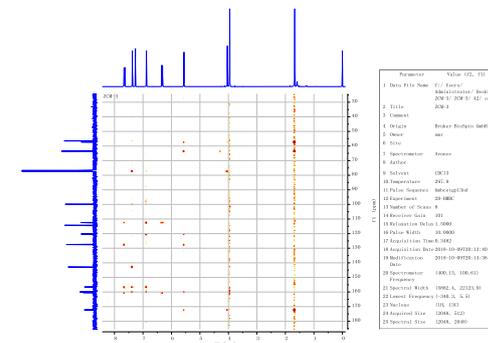
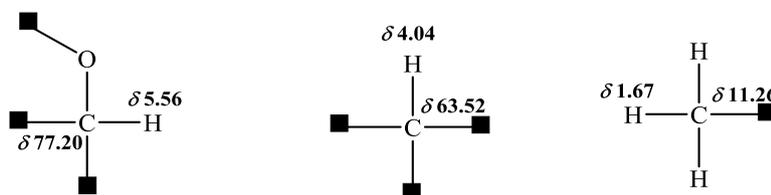
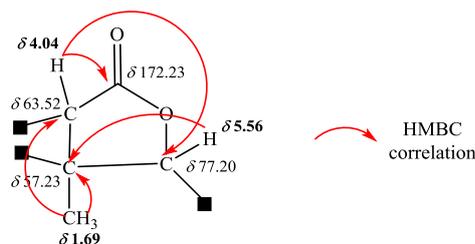


Figure 4 HMBC Spectrum (CDCl₃, 300 MHz)

In the ¹H NMR spectrum (Figure 3), the down field chemical shift of sp³ methine proton (δ_H 5.56 ppm, singlet, 1 H, H-2') must be connected to oxygen and also shows ¹H-¹³C direct correlation with sp³ methine carbon (δ_C 77.20 ppm, C-2') in HMQC spectrum (Figure 2). Moreover, in HMQC spectrum, ¹H-¹³C direct correlation of down field chemical shift of sp³ methine proton (δ_H 4.03 ppm, singlet, 1 H, C-5') and sp³ methyl proton (δ_H 1.67 ppm, singlet, 3 H, H-1') are connected with their respective carbons (δ_C 63.52 ppm, C-5' and δ_C 11.26 ppm, H-7') generates the following fragments.

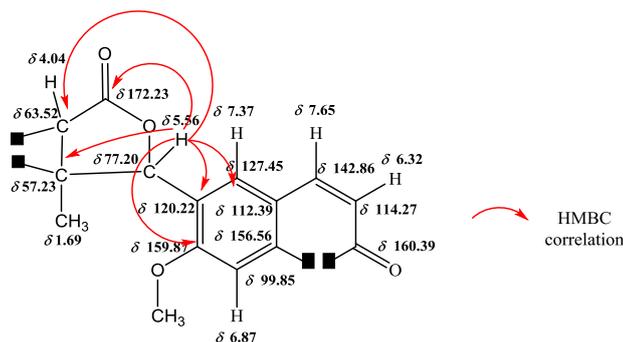


In the HMBC spectrum, the occurrence of α and β ¹H-¹³C long range coupling of sp³ methyl proton (δ_H 1.67 ppm, H-1') with sp³ quaternary carbon (δ_C 57.23 ppm, H-1') and sp³ methine carbon (δ_C 63.52 ppm, C-5'). Furthermore, in HMBC spectrum, sp³ methine proton (δ_H 4.03 ppm, H-5') has α and β ¹H-¹³C long range coupling of carbonyl carbon (δ_C 172.23 ppm, C-4') and sp³ methine carbon (δ_C 77.20 ppm, C-2'). The other substituent on aromatic ring at C-6 (δ_C 120.22 ppm) is made up of prenyl unit which cyclized to form γ -lactone moiety. Moreover, the attachment of these protons in the lactone ring is further supported by $2J$ and $3J$ cross peak correlation which reveals the following fragment ~ **D**.



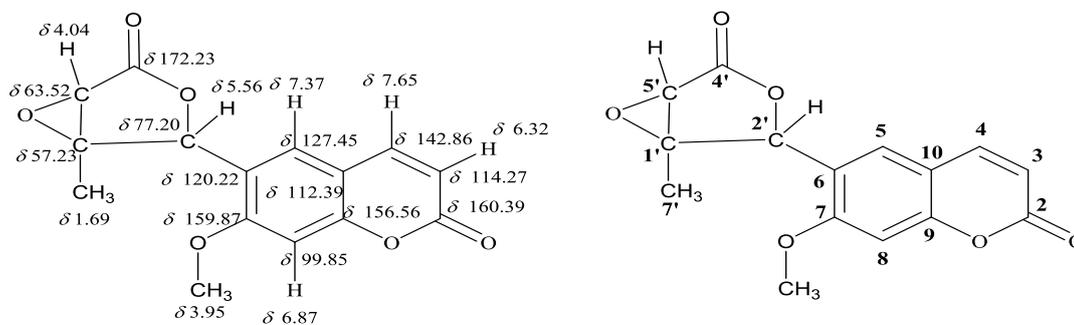
Fragment ~ D

The connection between fragment ~ C and D could be done by α ^1H - ^{13}C long range coupling of sp^3 methine proton (δ_{H} 5.56 ppm, H-2') with sp^2 quaternary carbon (δ_{C} 120.22 ppm, C-6) and sp^3 quaternary carbon (δ_{C} 57.23 ppm, C-1'). In addition, β ^1H - ^{13}C long range coupling of sp^3 methine proton (δ_{H} 5.56 ppm, H-2') with sp^3 methine carbon (δ_{C} 63.53 ppm, C-5'), sp^2 methine carbon (δ_{C} 127.45 ppm, C-5), sp^2 quaternary carbon (δ_{C} 159.87 ppm, C-7) and carbonyl carbon (δ_{C} 172.23 ppm, C-4') in HMBC spectrum produces the following partial fragment ~ E.



Fragment ~ E

In this stage, the partial molecular formula of the fragment ~ E could be calculated as $\text{C}_{15}\text{H}_{12}\text{O}_4$. In EI-MS spectrum showed the molecular ion peak and m/z is $289.0[\text{M} + \text{H}]^+$, $311.1[\text{M} + \text{Na}]^+$, and $599.1[2\text{M} + \text{Na}]^+$ (which represents its molecular mass of this compound). Thus, the remaining molecular mass of this compound is $288 - 256 = 32$. It must be two 'O' atoms. The remaining two 'O' atoms must be connected to downfield chemical shift of aromatic carbon (δ_{C} 156.56 ppm and δ_{C} 160.39 ppm) and sp^3 carbons (δ_{C} 63.52 ppm and δ_{C} 57.23 ppm). Therefore, the molecular formula of this compound was determined as $\text{C}_{15}\text{H}_{12}\text{O}_6$ on the basis of EI-MS spectrum.



7-methoxy-6-(1'-methyl-4'-oxo-3',6'-dioxabicyclo[3.1.0]hexan-2'-yl)-2H-chromen-2-one

Chemical Formula: $\text{C}_{15}\text{H}_{12}\text{O}_6$

Table 1 ^1H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT Spectral Data of Microzelanicum (ZCM-3) in CDCl_3

No.	^{13}C NMR δ (ppm)	^1H NMR (J in Hz)	DEPT
<i>Carbon</i>			
4'	172.23	-	C
2	160.39	-	C
7	159.87	-	C
9	156.56	-	C
4	142.86	7.65 (d, $J = 9.5$ Hz, 1H)	CH
5	127.45	7.37 (s, 1H)	CH
6	120.22	-	C
9	114.27	6.32 (d, $J = 9.5$ Hz, 1H)	CH
10	112.38	-	C
8	99.85	6.87 (s, 1H)	CH
2'	77.20	5.56 (s, 1H)	CH
5'	63.52	4.04 (s, 1H)	CH
1'	57.23	-	C
OCH_3	56.46	3.95 (s, 3H)	CH_3
7'	11.26	1.67(s, 3H)	CH_3

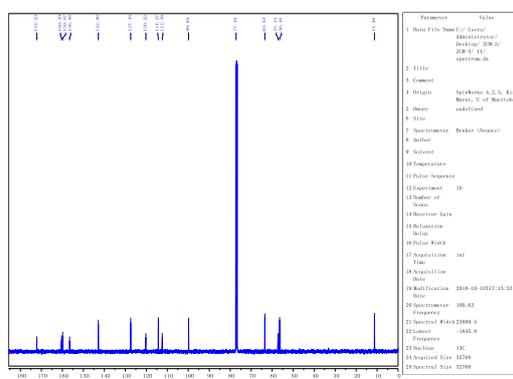


Figure 5 ^{13}C NMR Spectrum (100 MHz, CDCl_3)

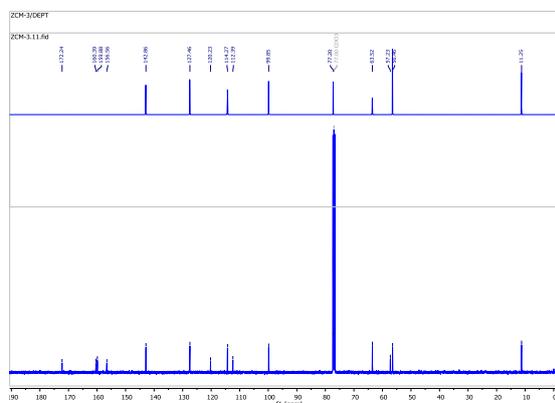
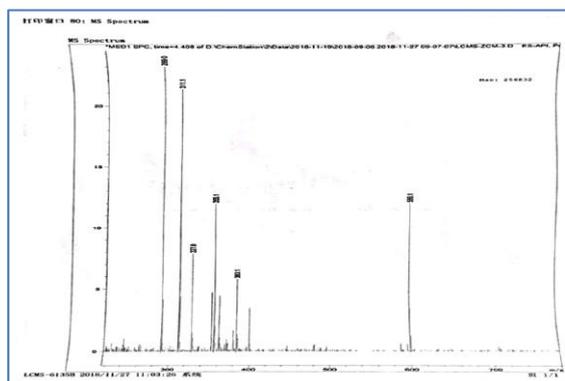


Figure 6 DEPT Spectrum



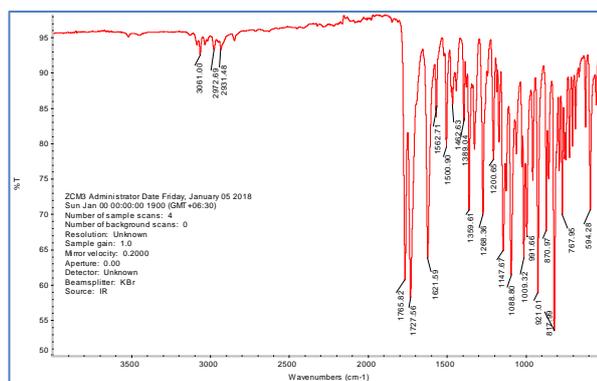


Figure 9 FT-IR Spectrum

Conclusion

In this study, the first isolation of 7-methoxy-6-(1'-methyl-4'-oxo-3', 6'-dioxabicyclo [3.1.0] hexan-2'-yl)-2*H*-chromen-2-one (Microzelanicum, $C_{15}H_{12}O_6$) from the plant of *Clausena heptaphylla* (Roxb.) Wight & Arn was presented. Although the compound was identified as a known compound, no literature data had been reported for this compound. Therefore, further investigations such as antimicrobial, anti-inflammatory and cytotoxic properties on this compound will also be studied.

Acknowledgements

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References

- Begum, R.M.A., Kaisar, M.S., Rahman, A.M., Chowdhury, S., Rahman. M.M., Hasan, C.M. and Rashid, M.A. (2011). "Clausenolide-1-methyl ether from *Clausena heptaphylla* W&A". *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, vol.10 (2), pp.136 – 138
- Bhattacharyya, P., Biswas, G.K., Barua, A.K., Saha, C., Royt, I.B. and Chowdhury, B.K. (1993). "Clausenalene, A Carbazole Alkaloid from *Clausena heptaphylla*". *Phytochemistry*, vol.33 (1), pp.248-250
- Cassady, J.M., Ojima, N., Chang, C. and Mclaughlin, J.L. (1979). "An Investigation of the Antitumor Activity of *Micromelum integerrimum* (Rutaceae)". *Journal of Natural Products*, vol.42 (3), pp.274-278
- Chakraborty, A., Saha, C., Podder, G., Chowdhury, B.K. and Bhattacharyya, P. (1995). "Carbazole Alkaloid with Antimicrobial Activity from *Clausena heptaphylla*". *Phytochemistry*, vol.38 (3), pp.787-789
- Fakruddin, M., Mannan, K.S.B., Mazumdar, R.M. and Afroz, H. (2012). "Antibacterial, Antifungal and Antioxidant Activities of the Ethanol Extract of the Stem Bark of *Clausena heptaphylla*". *BMC Complementary and Alternative Medicine*, vol. 1, pp.232
- Lekphrom, R., Kanokmedhakul, S., Kukongviriyapan, V. and Kanokmedhakul, K. (2011). "C-7 Oxygenated Coumarins from the Fruits of *Micromelum minutum*". *Arch Pharm Res*, vol.34 (4), pp.527-531
- Sohrab, M.H., Hasan, C.M. and Rashid, M.A. (1999). "Lunamarins A And B: Two Novel Terpenoid Coumarins from *Clausena heptaphylla*". *Natural Product Letters*, vol.14 (1), pp.47-53
- Sohrab, M. H., Hasan, C.M. and Rashid, M.A. (2000). "Clausmarin-A from the Leaves of *Clausena heptaphylla*". *Biochemical Systematics and Ecology*, vol.28, pp.91-93