COMPARATIVE STUDY OF SCOPOLETIN, EURYLENE, AND CANTHIN-6-ONE ISOLATED FROM *EURYCOMA LONGIFOLIA* JACK ROOTS AND CRUDE EXTRACTS COLLECTED FROM MYANMAR AND MALAYSIA SAMPLES

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Abstract

The research compared some compounds and some bioactivities of yellow roots of *Eurycoma longifolia* Jack from Myanmar and Malaysia. Various phytochemicals were identified, including alkaloids, flavonoids, terpenoids, steroids, glycosides, organic acids, phenolic compounds, carbohydrates, starch, saponins, and reducing sugars. Cyanogenic glycosides and tannins were not found in the yellow roots from both samples. TLC profiles revealed the presence of coumarins, alkaloids, and terpenoids in yellow roots from both regions. HPLC profiles showed identical retention times for methanol extracts of both samples with the isolated compounds El-a (scopoletin) and El-b (eurylene). Four compounds (*β*-sitosterol, canthin-6-one, scopoletin and eurylene) were isolated from both selected samples. The isolated compounds were characterized by various physicochemical properties (co-TLC, melting point) and structurally identified using ^{1}H NMR, ^{13}C NMR, ESI-MS, and LCMSD-Trap SL spectroscopic methods with comparisons to reported data. In antimicrobial tests, ethyl acetate extract from Myanmar exhibited significant activity against *Candida albicans* (32 mm), while watery extracts from Malaysia showed no antimicrobial effects. Cytotoxicity evaluation using a brine shrimp assay indicated no strong cytotoxic effects for the crude extracts. Antiproliferative activity was observed in ethanol extracts from yellow roots against lung (A549), cervix (HeLa), and breast (MCF-7) cancer cells, with promising activity in Malaysian samples against cervix cancer.

Keywords*:* *Eurycoma longifolia* Jack, antimicrobial activity, cytotoxicity, antiproliferative activity

Introduction

Eurycoma longifolia Jack belongs to the family Simaroubaceae. *E. longifolia* (Tongkat Ali) is one of the most well-known herbal folk medicines in Southeast Asia. It is also called Lambel in Tanintharyi Region and Drongsaw in Mon State in Myanmar. These plants have been used for the treatments of malaria, sexual dysfunction, cancer, diabetes, anxiety, constipation, and to increase strength (Shaheed *et al*., 2016). It is a remarkable medicinal plant that grows wild in Malaysia and throughout Southeast Asia in Myanmar, the roots of the plant have been traditionally used as a natural remedy to treat various health problems in rural areas. The root is often boiled in water to make a decoction, to treat diseases such as fever, and malaria, and to increase strength in rural areas of Mon state and Tanintharyi Region. However, this plant has not been scientifically investigated in Myanmar. Therefore, *E. longifolia* was selected in this study for the investigation and comparison of some bioactivities such as antioxidant, antimicrobial, cytotoxicity, and antiproliferative activities, and their constituents were examined.

Figure 1. Photographs of the plant, flowers, roots and fruits of ***Eurycoma longifolia*** **Jack**

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Materials and Methods

Sample Collection

The yellow roots of *Eurycoma longifolia* Jack were collected from Kuala Lumpur, Malaysia, in June 2019 and Dawei Township, Tanintharyi Region, Myanmar in October 2021. The samples were washed, air-dried (as shown in Figure 1), and ground into powder using a grinding machine. The powdered samples were then stored in air-tight containers to prevent moisture changes and contamination.

Preparation of Extracts

The dried, powdered *E. longifolia* root samples (50 g) were extracted using appropriate solvents (EtOAc, EtOH, and MeOH), with each sample sonicated three times in 1000 mL of each solvent for 1 hour. The filtrates were concentrated using a rotary evaporator to obtain extracts for biological activity studies. Additionally, 1000 g of each dried powder samples was percolated with 14 L of methanol for one week and filtered. This process was repeated three times, and the combined methanol extracts were concentrated under reduced pressure using a rotary evaporator to obtain a methanol extract, which was used for the isolation of bioactive compounds.

Preparation of Watery Extract

A watery extract of the dried powdered sample was obtained by boiling 20 g of sample with 300 mL of distilled water for about 30 min on a hot plate, and it was filtered. Then, the filtrate was concentrated by evaporation on a hot plate.

Preliminary Phytochemical Screening of the Selected Medicinal Plant Samples

A preliminary phytochemical investigation was used to classify phytoconstituents embedded in the samples, through a reported method (M-Tin Wa, 1972; Trease and Evans, 1980; Shriner *et al*., 1980).

Thin Layer Chromatography Profiling of Extracts

Following Biradar *et al*. (2013), TLC profiling was conducted by applying samples to a TLC plate, allowing the mobile phase to rise, and detecting alkaloids, coumarins, phenols, steroids, and terpenoids.

Phytochemical Analysis Using HPLC Techniques

HPLC, an analytical technique for separating, identifying, and quantifying components in a mixture, was used to compare chemical constituents in *E. longifolia* roots from Myanmar and Malaysia. Methanol extracts were analyzed using a mobile phase of H_2O: MeOH (49:51) and a Poroshell 120 EC-C18 column (Tsuda, 2004). The study was carried out at the University Research Center, University of Yangon.

Extraction and Isolation of Chemical Constituents from the Roots of *E. longifolia* Jack Collected from Myanmar and Malaysia

The percolated extracted MeOH was used to achieve five fractions by using column chromatographic separation of the two selected samples. The combined fractions (PE:EtOAc 1:1 and EtOAc only) chromatographed from the roots of yellow *E. longifolia* Jack collected from Myanmar and Malaysia were subjected to column chromatography with gradient elution with PE and EtOAc. Three compounds were isolated from the yellow colour roots of *E. longifolia* collected from Malaysia namely El-a, El-b, and El-c. Only ethyl acetate fraction which chromatographed from the yellow colour roots of *E. longifolia* collected from Myanmar was used to chromatograph. The column was initially eluted with PE:EtOAc (80:1 v/v). The polarity of the eluting solvent was gradient increased to PE:EtOAc (1:5 v/v). The collected fractions were

checked by TLC using a 5 % H_2SO_4 spraying reagent. Three fractions F_1-F_3 were obtained. Then, the compound ElM-1 was obtained from the F_1 fraction by washing with the PE-only solvent and was checked by TLC and co-TLC with authentic *β*-sitosterol. The fraction F_3 was re-chromatographed by isocratic elution with the solvent system $CHCl_3$:EtOAc (9:1). The compound ElM-2 was obtained and TLC checked by the Dragendorff's reagent and gave a positive test with the reagent.

Structural Identification

The structures of isolated compounds were identified by physicochemical properties and modern spectroscopic techniques such as UV, FT IR, ^{1}H NMR, ^{13}C NMR, LC-MSD-Trap-SL, and ESI-MS.

Screening of Antimicrobial Activity of the Sample by Agar Well Diffusion Method

By using the agar well diffusion method (Perez, Paul, and Bazerque, 1990), the antimicrobial activity of crude extracts the two yellow colour roots of *E. longifolia* Jack (Myanmar and Malaysia) was assessed against eight strains of microorganisms, including *Agrobacterium tumefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Micrococcus luteus*, C*andida albicans*, and *Escherichia coli* at Pathein University's Chemistry Department. The results of measuring the zone diameter are shown in Table 1, and photographs of inhibition zones on agar plates are shown in Figures 2 and 3.

Determination of Cytotoxicity Activity

Cytotoxicity of H_2O and EtOH extracts of two selected samples were investigated by brine shrimp bioassay described by Dockery and Tomkins (2000).

Determination of Antiproliferative activity

Antiproliferative activity of crude extracts were studied by using the MTT assay on three human cancer cell lines: A549 (lung cancer), HeLa (cervical cancer), and MCF-7 (breast) (Win *et al*., 2015).

Results and Discussion

Phytochemical Analysis of *Eurycoma longifolia* Jack Roots

From the assessment of the preliminary phytochemical analysis, the phytochemical screening of both *E. longifolia* Jack roots collected from Myanmar and Malaysia was preliminarily done by the test tube methods. Alkaloids, *α*-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, flavonoids, and terpenoids were found, whereas tannins, and cyanogenic glycosides were absent in both samples. In a comparative study of the thin layer chromatography (TLC) profiles of methanol crude extracts from the roots of *E. longifolia* collected in Myanmar and Malaysia, the yellow-coloured roots from both locations displayed similar chemical constituents, including coumarins, alkaloids, and terpenoids. The solvent system used was $CHCl_3$: MeOH at a 20:1 ratio. In HPLC profiles, compounds El-a (scopoletin) and El-b (eurylene), isolated from the yellow roots of *E. longifolia* collected in Malaysia, were used as standards to compare the chemical constituents of samples by retention time. The methanol extracts from yellow roots of *E. longifolia* in both regions showed identical retention times with El-a (scopoletin) and El-b (eurylene). Thes result indicateds that these compounds are common to *E. longifolia* roots from Myanmar and Malaysia.

Isolation and Identification of Compounds from the Yellow Colour Roots of *E. longifolia* Jack (Myanmar and Malaysia)

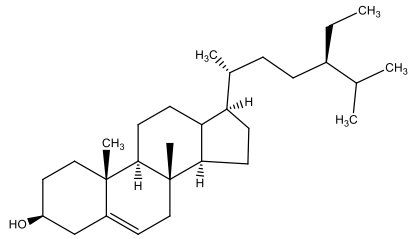
Compound **ElM-1** isolated from the roots of *E. longifolia* in Myanmar and Compound **El-c** isolated from the roots of *E. longifolia* in Malaysia, potentially *β*-sitosterol, displayed Co-TLC profiles similar to standard *β*-sitosterol, along with corresponding physicochemical properties. The compound **ElM-2** was isolated as a pale yellow crystalline solid with a 0.013 % yield and a melting point of 161°C from the methanol extract of yellow *E. longifolia* roots (Myanmar) using silica gel column chromatography. It appears as an orange spot on TLC with an R_f value of 0.48 in a $CHCl_3$: MeOH (20:1, v/v) solvent system. The compound is UV active under UV_{254} and UV_{365} nm, and gives a positive Dragendorffs' test which indicates it is an alkaloid. UV spectral data show absorption maxima at 252, 259, 299, 350, 361, and 379 nm, suggesting $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, indicative of conjugated π-bond systems and carbonyl groups. FT IR analysis reveals peaks at 1672 cm^{-1} (carbonyl C=O), 1633 cm^{-1} (imine C=N), and 1436 cm^{-1} (C=C bonds), confirming the presence of these functional groups. ^{1}H NMR spectra show peaks at δ 8.23 (H-1, d, J = 5.2 Hz), 8.83 (H-2, d, J = 5.2 Hz), 8.08 (H-4, d, J = 10 Hz), 6.98 (H-5, d, J = 10 Hz), 8.60 (H-8, d, J = 7.8 Hz), 7.78 (H-9, d, J = 7.2 Hz), 7.60 (H-10, d, J = 7.2 Hz), and 8.34 (H-11, d, J = 8 Hz). ^{13}C NMR spectra show fourteen carbon signals, including six sp^2 quaternary carbons at δ 160.0 (C-6), 140.3 (C-13), 137.3 (C-16), 133.0 (C-15), 130.7 (C-14), and 125.6 (C-12). The upfield shift of the carbonyl carbon at δ 160.0 (C-6) suggests it is directly linked to the nitrogen of the indole moiety and is *α*, *β*-conjugated. This indicates the presence of a *β*-carboline alkaloid canthin-6-one skeleton. Thus, based on the above spectral data and comparison with literature (Alemu *et al*., 2020), compound ElM-2 was suggested to be identical to canthin-6-one. The mass spectrum of isolated compound ElM-2 shows *m/z* of [M+H] + peak at 221.0 which indicates that the molecular mass is 220. So, it confirms that the isolated compound ElM-2 was canthin-6-one. The compound **El-a** was isolated as a pale yellow solid crystal with a 0.002 % yield from a 1000 g dry sample of *E. longifolia* roots from Malaysia using silica gel column chromatography. It appears as a bright fluorescence spot on a TLC plate under UV light (365 nm) with an R_f value of 0.38 in a PE:EtOAc (1:1 v/v) solvent system, suggesting it is a coumarin-type compound. El-a is soluble in EtOAc, EtOH, and MeOH, but insoluble in pet-ether. The UV spectra of El-a and reported scopoletin show similar characteristic bands at 228, 297, 340 nm and 227, 297, 338 nm, respectively. A bathochromic shift observed after adding NaOH confirms El-a as scopoletin. IR spectral analysis of El-a shows peaks at 3332 cm^{-1} (O-H stretching), 2947 cm^{-1} (C-H stretching), 1698 cm^{-1} (C=O), 1606 cm^{-1} (–CH=CH), 1564 cm^{-1} and 1509 cm^{-1} (benzene ring), and 860 cm^{-1} (disubstitution of benzene ring), identical to finger print region of the spectrum of the reported scopoletin. ^{1}H NMR spectra of reported scopoletin and isolated compound El-a showed reveal two doublets at δ 7.88 and δ 7.86 (H-4), and δ 6.23 and δ 6.21 (H-3), characteristic of coumarins. A methoxyl group singlet at δ 3.91 and aromatic singlets at δ 7.11 and δ 6.77 are also observed, consistent with scopoletin. ^{13}C NMR spectra show ten carbon atoms, including a methoxy carbon at δ 56.8, four aromatic methine carbons (δ 112.6, 146.1, 109.9, 103.9), and five aromatic quaternary carbons (δ 164.1, 147.1, 151.4, 152.5, 112.5). The mass spectrum shows an *m/z* peak at 190.7 $[M-H]^-$ and 214.7 $[M+Na]^+$, indicating a molecular mass of 191.7, confirming that El-a is scopoletin. The compound **El-b** was also isolated as a white crystal with a 0.0048 % yield from the methanol extract of yellow *E. longifolia* roots collected in Malaysia using silica gel column chromatography. It is soluble in EtOAc, EtOH, and MeOH, but not in pet-ether. The R_f value was 0.26 (PE:EtOAc, 3:1, v/v), and it was UV inactive. FT IR analysis showed a peak at 3543 cm^{-1} for O-H stretching. Peaks at 2969 cm^{-1}, 2923 cm^{-1}, and 2849 cm^{-1} indicated sp^3 C-H stretching for CH_3 and CH_2 groups. The absence of peaks for aromatic C-H stretching, along with peaks at 1740 cm^{-1} (C=O stretching) and 1069 cm^{-1} (C-O stretching), suggests that El-b is an aliphatic saturated compound with ester and hydroxyl groups, likely a terpenoid. ^{1}H NMR analysis

revealed signals for six methyl groups [(δ 1.64 (6H), 1.17, 1.12, 1.14, 1.18, and 1.69 (6H)] and two acetyl methyl signals [δ 2.08 and δ 2.07 (each 3H)], suggesting that El-b is the diacetate of a squalene derivative. ^{13}C NMR spectra showed ten methyl signals [δ 17.7, 17.73, 25.88, 23.14, 22.40, 22.08, 22.45, 25.88, 21.22, and 21.15], four carbons with ether groups [δ 87.36, 85.09, 85.11, and 85.78], two methine carbons with acetoxyl groups [δ 79.13 and δ 79.42], and two quaternary carbons with hydroxyl groups [δ 73.68 and δ 74.06]. The mass spectrum of El-b showed an *m/z* peak at 617 $[M+Na]^+$, indicating a molecular weight of 594, confirming that the isolated compound El-b is eurylene.

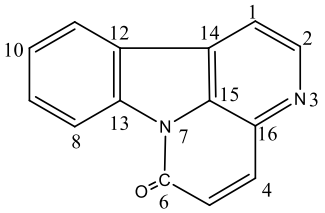
Compound ElM-2: (canthin-6-one), pale yellow crystal, 0.013 %, m.pt. 161 °C); UV spectral data (λ_{max}) in MeOH 252, 259, 299, 350, 361, and 379 nm; FT IR of 3059 (=C-H), 1672 (C=O), 1633(C=N); ^{1}H NMR of (400 MHz, Acetone-d_6) δ 8.23 (1H, d, H-1), 8.83 (1H, d, H-2), 8.08 (1H, d, H-4), 6.98 (1H, d, H-5), 8.60 (1H, d, H-8), 7.78 (1H, t, H-9), 7.6 (1H, t, H-10), 8.34 (1H, d, H-11); ^{13}C NMR (100 MHz, Acetone-d_6) δ160.0 (C-6), 146.8 (C-2), 140.8 (C-4), 140.3 (C-13), 137.3 (C-16), 133.0 (C-15), 131.6 (C-9), 130.7 (C-14), 129.7 (C-5), 126.5 (C-10), 125.6 (C-12), 124.1 (C-11), 117.7 (C-8), 117.5 (C-1); ESI-MS *m/z* 221 $[M + H]^+$ (Lopez *et al.*, 2018).

Compound El-a: (Scopoletin), pale yellow crystal, (0.002 %, m.pt. 205 °C); UV spectral data (λ_{max}) in MeOH 228, 297, and 340 nm; FT IR of 3332 (O-H), 3028 (=C-H), 2947 (C-H), 1698 (C=O); ^{1}H NMR of (600 MHz, CD_3OD) δ 6.21 (1H, d, H-3), 7.86 (1H, d, H-4), 7.11 (1H, s, H-5), 6.77 (1H, s, H-8), 3.91 (3H, s, O-CH_3), ^{13}C NMR (150 MHz, CD_3OD) δ 164.1 (C-2), 152.5 (C-9), 151.4 (C-7), 147.1 (C-6), 146.1 (C-4), 112.6 (C-3), 112.5 (C-10), 109.9 (C-5), 103.9 (C-8), 56.8 (-OCH_3); LC-MSD-Trap-SL *m/z* 190.7 $[M - H]^-$ (Bhatt *et al*., 2011).

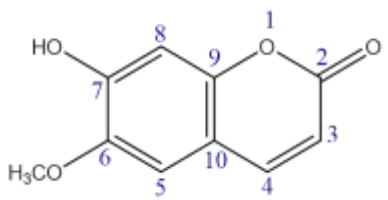
Compound El-b: (Eurylene), white crystals, (0.0048 %, m.pt. 147 °C); FT IR of 3543 (O-H), 2969-2849 (C-H), 1740 (C=O)1069-1021(C-O); ^{1}H NMR of (600 MHz, CD_3OD) δ 5.13 (2H, m, H-3, H-22), 4.85 (4H, m, H-11, 14), 3.85(1H, dd, J = 8.4, 6.6, H-18), 3.77 (1H, t, J = 7.8, H-7), 2.08 (3H, s, H-32), 2.07 (3H, s, H-34), 2.25-2.01 (4H, m, H-21, H-4), 2.01-1.17 (8H, m, H-8, H-9, H-16, H-17), 1.47-1.13 (2H, m, H-5), 1.68-1.48 (4H, m, H-12, H-20), 1.75-1.57 (2H, m, H-13) 1.64 (6H, s, H-1, H-24), 1.69 (6H, s, H-25, H-30), 1.17 (3H, s, H-26), 1.12 (H, s, H-27), 1.14 (3H, s, H-28), 1.18 (3H, s, H-29); ^{13}C NMR (150 MHz, CD_3OD) δ17.7 (C-1), 132.09 (C-2), 125.91 (C-3), 22.98 (C-4), 39.96 (C-5), 73.68 (C-6), 87.36 (C-7), 26.86 (C-8), 35.45 (C-9), 85.09 (C-10), 79.42 (C-11), 28.05 (C-12), 28.20 (C-13), 79.13 (C-14), 85.11 (C-15), 35.45 (C-16), 26.98 (C-17), 85.78 (C-18), 74.06 (C-19), 40.16 (C-20), 23.04 (C-21), 125.93 (C-22), 132.11 (C-23), 17.73 (C-24), 25.88 (C-25), 23.14 (C-26), 22.40 (C-27), 22.08 (C-28), 22.45 (C-29), 25.88 (C-30), 172.81 (C-31), 21.22 (C-32), 172.74 (C-33), 21.15 (C-34); LC-MSD-Trap-SL *m/z* 617.3$[M+Na]^+$ (Ha *et al*., 2020).



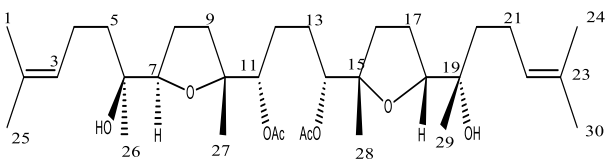
Compound, **ElM-1, El-c ($C_{29}H_{50}O$)**



Compound **ElM-2 ($C_{14}H_8N_2O$)**



Compound **El-a ($C_{10}H_8O_4$)**



Compound **El-b ($C_{34}H_{58}O_8$)**

Antimicrobial Activity of Crude Extracts of Yellow Colour Roots of *E. longifolia* Jack (Myanmar and Malaysia)

The investigation of the antimicrobial activity of the different crude extracts of ethyl acetate, ethanol, methanol and watery extracts of the roots of *E. longifolia* collected from Myanmar and Malaysia was carried out by the agar well diffusion method on eight different pathogenic microbes. It was found that ethyl acetate extract of *E. longifolia* collected from Myanmar exhibited high activities on all tested microorganisms, with an inhibition zone diameter range of 24 to 32 mm, with particularly remarkable activity on *Candida albicans* (32 mm) compared with standard nystatin (24 mm). Based on this assessment, the *E. longifolia* extract from Myanmar exhibited higher antimicrobial activity compared to the extract from Malaysia. So, the compounds embedded in the ethyl acetate extract would be effective in antimicrobial activity.

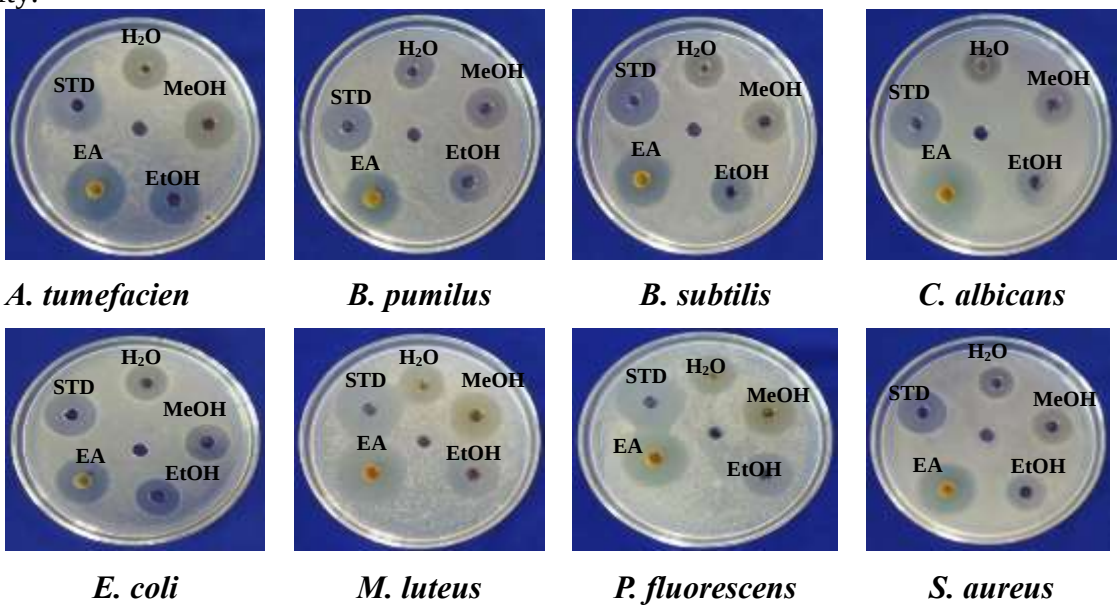


Figure 2. Antimicrobial activity of H_2O, MeOH, EtOH, and EtOAc extracts of the yellow colour roots of *E. longifolia* (Myanmar)

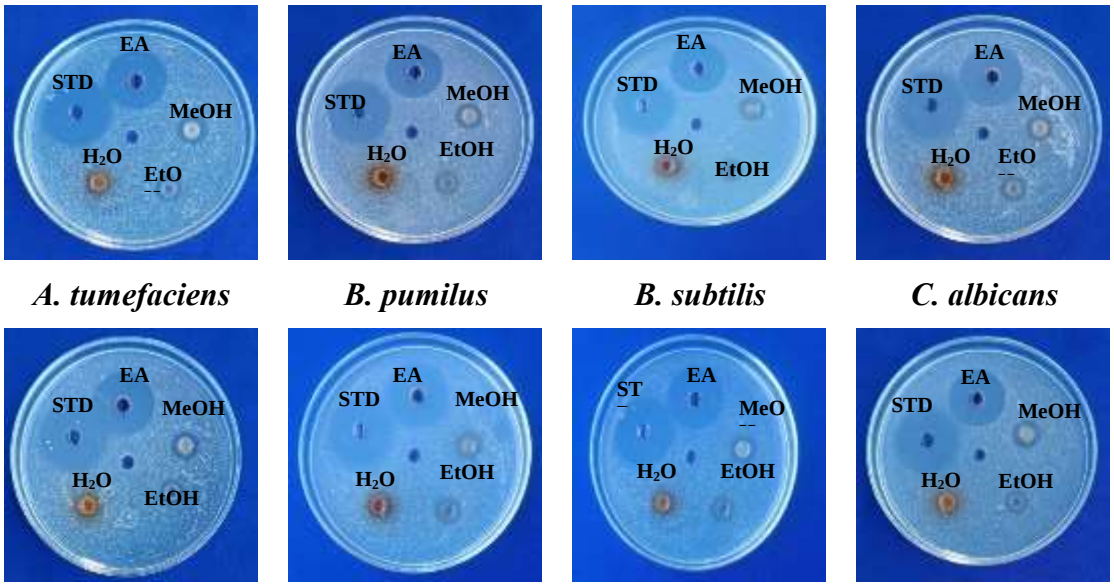


Figure 3. Antimicrobial activity of H_2O, MeOH, EtOH, and EtOAc extracts of the yellow colour roots of *E. longifolia* (Malaysia)

Table 1. Antimicrobial Activity Screening of the Various Crude Extracts of Roots of *E. longifolia* Collected from Myanmar and Malaysia

Extracts	Inhibition zone diameter (mm)															
	A. tumefacies		***B. pumilus***		***B. subtilis***		***C. albicans***		***E. coli***		***M. luteus***		***P. fluorescens***		***S. aureus***	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
H_2O	21	-	18	-	19	-	19	-	21	-	21	-	20	-	19	-
MeOH	23	14	21	14	21	19	19	13	21	14	24	19	24	20	20	14
EtOH	24	13	20	12	21	21	19	13	21	12	25	21	22	22	20	12
EtOAc	27	28	26	26	26	25	32	28	24	27	30	24	29	25	26	27
***STD**	25	29	25	28	25	26	24	30	24	31	30	27	31	27	24	30

Agar well diameter - 8 mm
(-) no activity
9 mm ~ 14 mm, Low activity
15 mm ~ 19 mm, medium activity
20 mm above, high activity

***STD = Chloramphenicol for bacteria**
= Nystatin for fungus

Y_1= *E. longifolia* (Myanmar,Yellow)
Y_2= *E. longifolia* (Malaysia, Yellow)

Cytotoxic Activity of the Roots of Yellow Colour Roots of *E. longifolia* Jack (Myanmar and Malaysia)

Based on the results, watery and ethanol extracts of the selected samples generally tend to exhibit cytotoxic effects. Moreover, in the roots of *E. longifolia* which was collected from Myanmar, the ethanol extract has a lower LD_{50} (233.35 µg/mL) compared to its water extract (254.42 µg/mL). Both the watery and ethanol extracts of Malaysia sample have the lower LD_{50} values than Myanmar sample. Therefore, Malaysia sample extracts show the higher cytotoxicity. When they were compared with the negative control, caffeine (LD_{50} > 1000 µg/mL), both the two selected samples showed cytotoxic activity but were not strong.

Table 2. Cytotoxicity on Ethanol and Watery Extracts of Roots of *E. longifolia* Jack Collected from Myanmar and Malaysia

Samples (Extracts)	% of Dead Brine Shrimp in Different Concentrations (µg/mL)					LD_{50} (µg/mL)
	50	100	200	400	800	
EtOH (My)	6.67 ± 0.57	23.33 ± 0.57	43.33 ± 0.57	83.33 ± 0.57	100 ± 0.00	233.35
H_2O (My)	13.33 ± 1.15	23.33 ± 0.57	40.49 ± 0.57	75.40 ± 1.00	96.67 ± 0.57	254.42
EtOH (Ma)	3.33 ± 0.57	23.33 ± 0.57	53.33 ± 0.57	66.67 ± 0.57	76.66 ± 0.57	188.9

Samples (Extracts)	% of Dead Brine Shrimp in Different Concentrations (µg/mL)					LD_{50} (µg/mL)
	50	100	200	400	800	
H_2O (Ma)	3.33 ± 0.57	26.67 ± 5.77	53.33 ± 0.57	56.66 ± 0.57	73.33 ± 1.15	187.51
***$K_2Cr_2O_7$**	36.67 ± 0.47	56.67 ± 0.47	76.67 ± 0.47	93.33 ± 0.47	96.67 ± 0.57	83.33
****Caffeine**	0.00	0.00	6.67 ±0.82	16.67 ± 0.94	30.00 ± 0.47	>1000

My = *E. longifolia* (Myanmar, Yellow)
Ma = *E. longifolia* (Malaysia, Yellow)

Antiproliferative Activity of the Roots of Yellow Colour Roots of *E. longifolia* Jack (Myanmar and Malaysia)

Antiproliferative activity was observed in ethanol extracts from yellow roots in Myanmar against lung (A549), cervix (HeLa), and breast (MCF-7) cancer cells, with promising activity in Malaysian samples against cervix cancer. The antiproliferative activity of the tested samples is summarized in Table 3. In this comparison of the antiproliferative activity of the yellow roots of *E. longifolia* which was collected from Myanmar and Malaysia on A549 (lung cancer), HeLa (cervix cancer), and MCF-7 (breast) cell lines are evaluated based on their respective IC_{50} values. The IC_{50} values of ethanol extract of the yellow roots of *E. longifolia* collected from Myanmar were 78.59 µg/mL against A549 (Lung cancer), 63.52 µg/mL against HeLa (Cervix cancer) and 39.64 µg/mL against MCF-7 (breast cancer) cell lines. In comparing the antiproliferative activities of the ethanol extract and the watery extracts of the selected samples, the ethanol extract of the yellow roots of *E. longifolia* collected from Myanmar consistently displayed commendable effectiveness across all three cancer types of A549 (lung cancer), HeLa (cervix cancer) and MCF-7 (breast), with particularly remarkable activity against breast cancer cells (MCF-7). The ethanol extract of the yellow colour roots of *E. longifolia* collected from Malaysia demonstrated significant effectiveness against cervix cancer (IC_{50} = 56.56 µg/mL) and lung cancers (IC_{50} = 91.63 µg/mL).

Table 3. Antiproliferative Activity Screening of Ethanol and Watery Extracts of *E. longifolia* Colledcted from Myanmar and Malaysia

Sample (Extracts)	IC_{50} (µg/mL) of Extracts against Tested Cell Lines		
	A 549 (Lung cancer)	HeLa (Cervix cancer)	MCF-7 (Breast cancer)
EtOH (Myanmar)	**78.59**	**63.52**	**39.64**
H_2O (Myanmar)	96.36	75.66	75.66
EtOH (Malaysia, Yellow)	91.63	**56.56**	> 100
H_2O (Malaysia, Yellow)	87.55	65.84	75.65
5-Fluorouracil	**4.29**	**7.42**	**79.11**

Conclusion

Four compounds were isolated from the roots of *E. longifolia* collected from Myanmar and Malaysia. In Myanmar's yellow roots, compounds ElM-1 (*β*-sitosterol) and ELM-2 (canthin-6-one) were found, while in Malaysia's yellow roots, compounds EL-a (scopoletin), EL-b (eurylene), and EL-c (*β*-sitosterol) were isolated. Various methods including co-TLC, melting point, and spectroscopic techniques were used for identification. In antimicrobial tests, ethyl acetate extract from Myanmar exhibited significant activity against *Candida albicans*. In the cytotoxicity screening, both selected samples exhibited some cytotoxic activity, but it was not strong when compared to the negative control, caffeine (LD_{50} > 1000 µg/mL). The ethanol extracts of *E. longifolia* collected from Myanmar showed higher antiproliferative activity than watery extracts. Overall, both watery and ethanol extracts of the yellow roots of *E. longifolia* collected from Myanmar exhibit a more potent antiproliferative effect on the cancer cell types: lung, cervix, and breast cancer cell lines, compared to the yellow roots of *E. longifolia* collected from Malaysia. These findings suggest that the ethanol extract of *E. longifolia* collected from Myanmar would be a potential extract for further investigation.

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