Phytochemical Screening, Antimicrobial Activities and Isolation of Bioactive Constituents from the Stem of *Schisandra sphenanthera* Rehd.E.H.Wils (Sayn-myit)

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Abstract

World Health Organization estimated that 80% of population in developing countries depends on traditional medicines, mostly natural plant products, for their primary health care needs. In this research work, one of Myanmar medicinal plants, *Schisandra sphenanthera* Rehd.E.H.Wils (Say-ni-myit) was selected for chemical analysis and antimicrobial activities. Phytochemical compounds present in test plants were carried out according to Harbone J.B (1984). The antimicrobial activities of six solvent extract ofsay-ni-myit were determined on six selected organisms (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia Coli*), by applying agar-well diffusion technique, according to modified Kirby and Bauer method. In addition, pure compounds were separated by Thin Layer and Column Chromatographic separation techniques.Moreover, FT-IR spectrum of isolated compound was measured.The phytochemical screening of stem of Say-ni-myit consists of alkaloid, flavonoid, glycoside, phenol, polyphenol, protein, reducing sugar, saponin, steroid, tannin and terpene respectively. According to antimicrobial activities,pet- ether and n-hexane extracts showed higher activity than other extracts. The pure organic compound, LLW -1 (pale pink colour crystal, 90.6 mg, R_f = 0.688, 4.53 %) was isolated from ethyl acetate crude extract. The prominent functional groups present in the isolated compounds, LLW - 1 was assigned.

KeyWords: S*chisandra sphenanthera*, Chemical Compositions,Antimicrobial Activities

Introduction

World Health Organization estimated that 80% of population in developing countries depends on traditional medicines, mostly natural plant products, for their primary health care needs (WHO, 2013).The use of herbal medicine has been significant increased due to their minimal side effects, availability and acceptability to the majority of the population, vital role in traditional medicine and are widely consumed as home remedies since past decade (Gjorgieva D, 2011).Plants have played an important role in Myanmar for traditional medicine, since ancient time. It is widely practiced by the majority of population, partly as a supplement

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and partly alternate to modern medicine (TheinSwe, 2005). Medicinal plants research has continued the area of major interest and priority for researcher since last decade (DMR Bulletin, 1996). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants and also used as food and medicine (Nyamai DW, 2016). These compounds have many pharmacological activities against various chronic diseases. The development of plant based antimicrobial compounds and new antibiotics are effective against the resistant organism (Sebiomo A, 2011). The effectiveness of plant extracts on microorganism has been studied worldwide (Mujeeb F, 2014).Many local people fromMoegyoke Township, commonly used stem of Say-ni-myit in folk medicine for chronic cough, liver diseases, cardiovascular diseases, lymphoma and scald. In this research work, one of Myanmar medicinal plants, *Schisandra sphenanthera* Rehd.E.H.Wils (Say-ni-myit) was selected for phytochemical screening and antimicrobial activities. And then, pure organic compound (LLW-1) was isolated from stem of Say-ni-myit based on ethyl acetate crude extract by using chromatographic separation techniques.The prominent functional groups present in isolated compound, (LLW- 1) was assigned.

Aim and Objectives

Research work aim to investigate phytochemical screening, antimicrobial activities and to isolate bioactive constituents from the stem of *Schisandra sphenanthera* Rehd.E.H.Wils (Say-ni-myit)

Specific Objectives

1. To do botanical identification of selected medicinal plant
2. To investigate the phytochemical constituents of the stem of Say-ni-myit
3. To determine the antimicrobial activities of the stem of Say-ni-myit
4. To isolate bioactive organic compounds from the stem of Say-ni-myit
5. To assign the functional groups of isolated compound

Materials and Methods

Materials

Analytical grade reagents from Merck, Iodine vapor, Silica gel (Merck Co. Inc, Kiesel gel 60 F_{254}, 70-230 mesh), thin layer chromatography TLC silica gel plate (Merck, F245), and ceftriaxone were used. UV-Lamp (Lambda-40, Perkin-Elmer Co, England), FT-IR spectrometer (Shimadzu, Japan), Rotary evaporator, KRUSS melting point meter (Germany) and common laboratory apparatus were used. The test organisms, (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus pumilus, Candida albicans and Escherichia Coli)* were used for this study.

Botanical identification

The collected fresh specimens with their inflorescences had been used for identification.The collected fresh samples were studied and identified at the Department of Botany, University of Mandalay and University of Taungoo. (Medicinal Plants of China,Flora of Ceylon and Flora of Hong Kong)

Sample Collection

The stems ofSay-ni-myit were collected within June, 2017, from Kyat Pyin village, Moegyoke Township, Mandalay Region. These samples were washed with tap water to remove dust and other particles and rinsed with distilled water. After that, samples were cut into small pieces and allowed to air dry at room temperature.

Preliminary Phytochemical Screening of Say Ni Myit

The investigation of phytochemical constituents in Say-ni-myit was carried out according to the standard procedures (Harborne, J.B, 1984).

Determination of Antimicrobial Activities onVarious Solvent Extracts

The antimicrobial activities of stem of Say-ni-myitextracts were determined by applying agar -well diffusion technique according to modified Kirby and Bauer method (WHO, 2003). The tests were carried outwith six solventsextracts (n-hexane, pet – ether, ethyl acetate, chloroform, ethanol and water) at Pharmaceutical Research Department, Yangon.Ceftriaxone was used as positive control, reference standard. The negative control was prepared using same solvent employed to dissolve plant extract.

Extraction and Isolation of Pure Organic Compound

Three hundred grams of air dried sample were macerated in 95 % methanol (1 L) for six weeks. These extracts were filtered and concentratedby using rotatory evaporator. The resulting extract was dried in the air, at room temperature. Methanol crude extract 23.28 g was obtained. Thesemethanol crude extract was re-extracted with pet-ether. And then, the remaining methanol layer was repartitioned with ethyl acetate by using separating funnel. 9.5 g of pet-ether extract and 7.5 g of ethyl acetate extract were obtained respectively. Ethyl acetate crude extract (2 g) was chromatographed by silica gel column, using gradient ratio of n-hexane and ethyl acetate solvent system. Totally (256) fractions were collected. Each and every fraction was checked by TLC for purity of separated compound. The fractions with same R_f values were combined to give (8) combined fractions. Among them, fraction (V) was further chromatographed by micro column using same procedure as described above. All fractions were checked again by TLC plate, UV lamp and combined. Among these combined fractions of micro column, fraction IIgave pure pale pink colour crystal, 90.6 mg, R_f = 0.68, with solvent system of n-hexane: ethyl acetate, 1:1 (v/v). The yield percent of pure compound (LLW-1) was found to be (4.53 %) based upon ethyl acetate crude extract.

Melting Point Determination of Pure Organic Compound

A few amounts of pure organic compounds were inserted into the capillary tube and the melting point was determined by the help of KRUSS melting point meter.

Determination of Functional Groups of Isolated Compound by FT-IR Spectrometry

The infrared spectra of isolated compound (LLW-1), was determined by using Fourier Transform Infrared Spectrophotometer at Department of Chemistry, University of Mandalay. The resulting peaks were applying the FT-IR spectrum (Silverstein R.M. *et al.*, 2005).

Results and Discussions

Botanical identification

The collected fresh samples were studied and identified at the Department of Botany, University of Mandalay and University of Taungoo.

Figure 1.S*chisandra sphenthera* Rehd.E.H.Wils. (Say-ni-myit)

Scientific Name - S*chisandra sphenanthera* Rehd.E.H.Wils
Family - Magnoliaceae
English Name - Chinese Magnolia vine.
ChineseName -Nan wuwei -zi
Korean Name - Omiza, Omija
Local Name - Say-ni-myit
Part Used - Stem
Flowering Period - May – June
Medicinal Activity - liver disorder, anti-viral, antioxidant, anticancer & anti- inflammatory

Plant Description

A deciduous woody climbing vine, about 8 mm long. Leaves are alternate, petiolate, ovate or oblong- ovoid, 5-11 cm long and 3-7 cm wide, apex acute or acuminate; base cuneate or broadly cuneate, membranous. Inflorescences are a few flower axillary cluster, uni-sexual, dioecious. Flowers have many pistils on the round receptacle, which grows long and hangs like anear when the fruit ripens.Fruits areglobose, red, of different size. The skin and pulp of the fruit are sweet and sour, the kernels pungent and bitter, the whole has a salty taste. This has given rise to the Chinese name "Five Flavours Fruit".

Phytochemical Screening of S*chisandra sphenanthera* Rehd.E.H.Wils

The preliminary phytochemical screening of Say-ni-myit were shown in Table (1)

Table (1) Phytochemical Constituents of stem of S*chisandra sphenanthera* Rehd.E.H.Wils

No	Constituents	Reagents	Observation	Result
1.	Alkaloid	Dragendorff's solution	Orange ppt	+
2.	Carbohydrate	α – naphthol, Con: H_2SO_4 sol^n	No Pink color sol^n	–
3.	Flavonoid	Con: HCl, Mg turning	Reddish brown sol^n	+
4.	Glycoside	10 % lead acetate sol^n	Yellow ppt	+
5.	Phenol	10% $FeCl_3$sol^n	Bluish green sol^n	+
6.	Polyphenol	1 % $FeCl_3$, 1% K_3 [$Fe(CN)_6$] sol^n	Dark green sol^n	+
7.	Protein	10 % Na OH, 10 % $CuSO_4$ sol^n	Red sol^n	+
8.	Reducing sugar	Benedict'ssol^n	Brick red ppt	+
9.	Saponin	H_2O, Con: H_2SO_4 , Shaken	Frothing	+
10.	Steroid	acetic anhydride, Con: H_2SO_4sol^n	Greenish blue sol^n	+
11.	Tannin	1% Fe Cl_3, Dil: H_2SO_4 sol^n	Yellowish brown sol^n	+
12.	Terpene	$CHCl_3$, acetic anhydride, Con: H_2SO_4 sol^n	Reddish brown sol^n	+

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

According to the Table (1) Say-ni-myit contains many chemical constituents, such as alkaloid, flavonoid, glycoside, phenol, polyphenol, protein, reducing sugar, saponin, steroid, tannin and terpene respectively.

Antimicrobial Activities of stem of S*chisandra sphenanthera* Rehd.E.H.Wils

The study of antimicrobial activities was performed by agar well diffusion method on six microorganisms. The resulting data are shown in Figure (2) and Table (2).

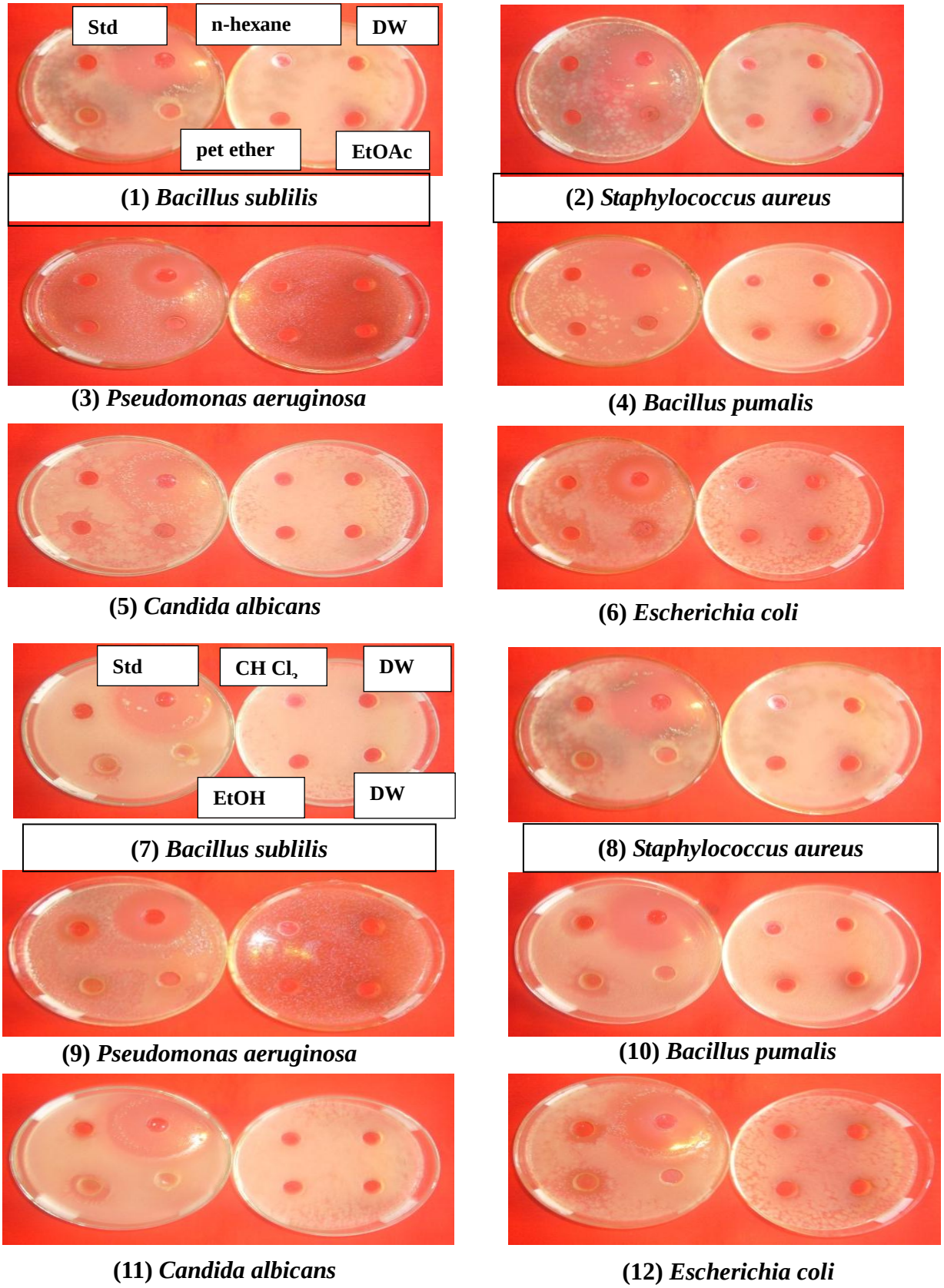

Figure 2. Antimicrobial activities of various solvent extracts of *Schisandra sphenanthera* Rehd.E.H.Wils

Table (2) Antimicrobial Activities of Various Solvent Extracts of *Schisandra sphenanthera* Rehd.E.H.Wils

Solvent		Zone inhibition (mm)					
	Organism	I	II	III	IV	V	VI
Test Sample	n-hexane	**19**	**18**	13	17	13	**17**
	Pet - ether	**20**	**20**	12	18	15	**19**
	EtoAc	13	15	**17**	**17**	13	16
	$CHCl_3$	14	17	16	15	15	14
	EtOH	16	17	13	15	18	15
	H_2O	11	11	12	11	11	11
Negative Control	n-hexane	-	-	-	-	-	-
	Pet –eher	-	-	-	-	-	-
	EtoAc	-	-	-	-	-	-
	$CHCl_3$	-	-	-	-	-	-
	MeOH	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-
	H_2O	-	-	-	-	-	-
Standard Ceftriaxone	DW	35	37	33	4 0	35	36

Organisms Agar well – 10 mm
I = *Bacillus subtilis* 10mm~14mm - low activity
II = *Staphylococcus aureus* 15 mm ~ 19 mm - medium activity
III = *Pseudomonas aeruginosa* 20 mm above - high activity
IV = *Bacillus pumilus*
V = *Candida albicans*
VI = *Escherichia coli*

According to Figure (2) and Table 2, pet - ether and n-hexane extracts showed higher activity than other extracts of Say-ni-myit. The aqueous extract responds the lowest activity on all organisms.

Isolation of Pure Organic Compound (LLW-1)

The column chromatographic separation of the stem of Say-ni-myit obtained pale pink colour crystal, 90.6 mg, R_f = 0.68, with solvent system of n-hexane: ethyl acetate,1:1 (v/v). The yield percent of pure compound (LLW-1)was found to be (4.53 %) based upon ethyl acetate crude extract.

Melting Point of Pure Organic Compound(LLW-1)

The melting point of pure organic compound LLW-1was found 136°C -138 °C.

FT-IR Assignments of Pure Organic Compound (LLW-1)

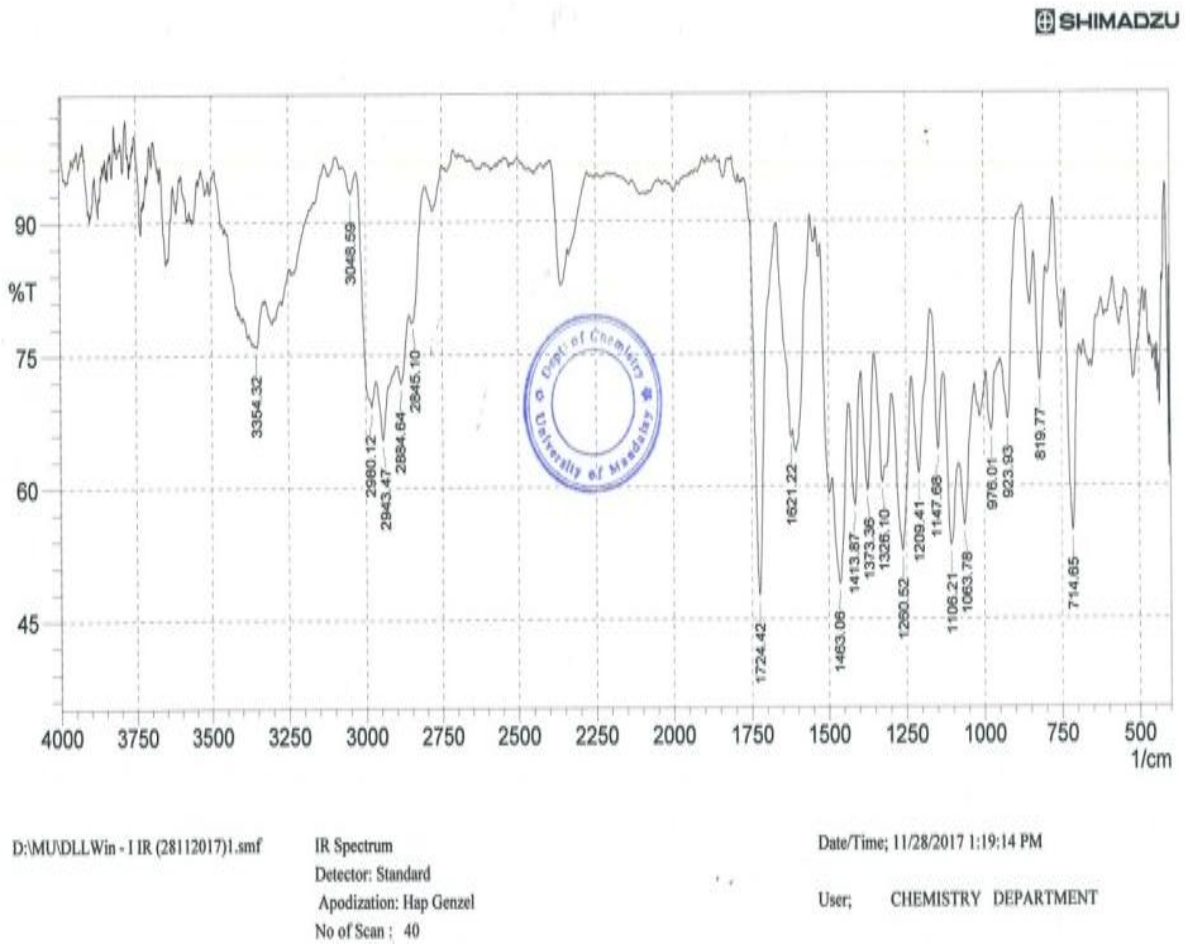

Figure (3) FT-IR Spectrum of Compound (LLW-1)

According to FT-IR spectrum, (LLW-1) was observed hydroxyl group, sp^2 hydrocarbon, sp^3 hydrocarbons, carbonyl group, aromatic ring skeletal, allylic hydrocarbon, ether group, trans or *E* and cis or *Z* alkenic groups respectively.

Table (3) FT-IR Assignments of Pure Organic Compound (LLW-1)

No.	Wave number (cm^{-1})	Functional groups
1.	3354.32	O-H stretching vibration of hydroxyl group
2.	3048.59	C-H stretching vibration of sp^2 hydrocarbon
3.	2980.12, 2943.47, 2884.64, 2845.10	Asymmetrical and symmetrical C-H stretching vibration of sp^3 hydrocarbons
4.	1724.42	C=O stretching vibration of carbonyl group
5.	1621.22, 1463.06	C=C stretching vibration of aromatic ring
6.	1413.36	in plane C-H bending vibration of allylic hydrocarbon
7.	1373.37	in plane C-H bending vibration of sp^3 hydrocarbon
8.	1260.52, 1209.41	C-C-O stretching vibration of alcohol group
9.	1147.86,1106.78, 1063.78	C-O-C stretching vibration of ether group
10	976.01	C-H out of plane bending vibration of E or trans alkenicgoup
11.	819.77	C-H out of plane bending vibration of Z or cisalkenic group
12.	714.65	O-H out of plane bending vibration

Conclusion

From this research work, selected plant, Say-ni-myit was identified as*Schisandrasphenthera*Rehd.E.H.Wils. Phytochemical analysis of Say-ni-myitrevealed that alkaloid, flavonoid, glycoside, phenol, polyphenol, protein, reducing sugar, saponin, steroid, tannin and terpene were present.According to antimicrobial activity, pet- ether and n-hexane extracts showed higher activity than other extracts of Say-ni-myit. The aqueous extract responds to lowest activities on all organisms. The pure organic compound (LLW -1), pale pink colourcrystal (90.6 mg), R_f = 0.68, n- hexane: EtOAc 1 : 1 (v/v)was isolated from ethyl acetate soluble portion of stem of Say-ni-myit. The yield percent of the isolated compounds (LLW-1) was found to be 4.53%based upon ethyl acetate crude extract. FT-IR spectral data of isolated pure organic compound (LLW-1) observed hydroxyls group, sp^2 hydrocarbon, sp^3 hydrocarbons, carbonyl group, aromatic ring skeletal, allylic hydrocarbon, ether group, trans or *E* and cis or *Z* alkenic groups respectively.

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