ISOLATION OF A BIOACTIVE SESQUITERPENOID COMPOUND AND EVALUATION OF SOME BIOLOGICAL ACTIVITIES OF STEMS AND ROOTS OF *ALLAMANDA CATHARTICA* L. (SHWEWA-PAN)

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Abstract

This research focused on the screening of phytochemical constituents, nutritional values and biological activities such as antioxidant, antimicrobial and anti-tumor activities of the stems and roots of *Allamanda cathartica* L. (Shwewa-pan). These samples were collected from Bago University Campus and identified at Botany Department, Bago University. According to the phytochemical tests, alkaloids, flavonoids, terpenoids, steroids, glycosides, organic acids, phenolic compounds, saponins, tannins and carbohydrates were found to be present in both samples. The nutritional values were determined by AOAC method resulting 6.91 and 18.64 % of proteins, 2.04 and 3.04 % of moisture, 2.71 and 3.06 % of ash, 62.42 and 38.65 % of fiber, 10.33 and 16.15 % of fat in stems and roots of *A. cathartica*, respectively. By thin layer and silica gel column chromatographic methods, one compound, plumericin (1.51 %, m.pt 210°C) was isolated from ethyl acetate extract of the roots. The antioxidant activity of ethanol and watery extracts of the stems and roots determined by DPPH radical scavenging activity assay was found to be in the order of stem ethanol extract (IC_{50}= 65.89 µg/mL) > root ethanol extract (IC_{50}= 110.46µg/mL) > root watery extract (IC_{50}>200 µg/mL) ≈ stem watery extract (IC_{50}> 200 µg/mL) . The antimicrobial activity of PE, EtOAc, EtOH and H_2O extracts of stems and roots were screened on six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* by agar well diffusion method. All of the extracts from the roots have more pronounced antimicrobial activity with inhibition zone diameters ranged between 14 mm ~ 35 mm than stems extracts (12 mm ~ 18 mm). Among them EtOAc extract of the roots has the highest activity against *B. pumilus.* Antitumor activity of various crude extracts such as PE, EtOAc and EtOH extracts of stems and roots in different concentrations (0.5, 1.0, 1.5g/mL) and plumericin (10, 20, 30 mg/mL) were also determined on tumor producing bacteria by using PCG (Potato Crown Gall) test. It was found that EtOAc extract of roots, PE and EtOH extracts of both samples and plumericin exhibited the inhibition of tumor formation.

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Keywords **:** *Allamanda cathartica* L. Antioxidant activity, Antimicrobial activity, Antitumor activity, plumericin

Introduction

Plants based drugs have been used worldwide in traditional medicines for the treatment of various diseases. Shwewa-pan scientifically known as *A.cathartica* is of the plant family Apocynaceae. Genus is *Allamanda* and species is *cathartica.* Myanmar name is Shwewa-pan and also called Shwe-pan-nwe. Other common names are Golden trumpet, Yellow bell, Buttercup flower and Angle's trumpet (Chandrasekhar *et al*., 2012). Allamanda species are apparently native to northern Brazill Guyana, Surinam and probably French Guiana. It is a genus of climbing shrubs. The distribution of this species is global but is mainly presented in subtropical to tropical (Uduak and Esther, 2015). In Myanmar it is cultivated as ornamental garden plants. *A.cathartica* has long been used in traditional medicine for treating malaria and jaundice. The leaf extract was found to promote wound healing. The flower is also used as a laxative. The chemical constituents are allamandin, allamandicin, allamdin, plumericin, isoplumericin, plumieride, ursolic acid, beta-amyrin, beta-sitosterol, fluvoplumeirin, lupeol, quercetin, kaempferol, glabridin and naringenin (Fah, 2013).

A. cathartica (Shwewa-pan) has been chosen for this research because it has various biological activities and bioactive chemical constituents, and also due to the lack of scientific report on the locally grown *A. cathartica*. In this research work, screening of phytochemical constituents and investigation of antioxidant, antimicrobial and antitumor activities of stems and roots of *A.cathartica* (Shwewa-pan) are carried out on the respective various crude extracts. Furthermore one of the organic compound, plumericin is isolated as a major constituent from the roots and studied on its antitumor property.

Materials and Methods

Plant materials

The stems and roots of *A. cathartica* were collected from Bago University Campus, Bago Township, Bago Region, during October, 2015. Some pharmacological activities such as antimicrobial activity, antioxidant

activity and antitumor activity of various crude extracts of the stems and roots were determined in *in vitro*.

Phytochemical Screening

Preliminary phytochemical tests such as alkaloids, flavonoids, terpenoids, steroids, glycosides, organic acids, starch, phenolic compounds, saponins, tannins, carbohydrates, cyanogenic glycosides, reducing sugars and α - amino acids tests on the two samples were carried out according to the appropriate reported methods (Sofowora, 2000).

Determination of Nutritional Values

The nutritional values such as moisture, ash, fiber, protein and fat contents of the stems and roots of *A. cathartica* were determined by the respective AOAC method.

Isolation and Identification of Phytochemical Constituent from EtOAc Extract of Roots of *A. cathartica* by Column Chromatography

Dried powdered root sample (1000 g) were percolated in EtOH (70 %) (1000 mL) for one week and filtered. This procedure was repeated for three times. Then the filtrate was concentrated by using a vacuum rotatory evaporator to get EtOH extract (25 g). Then the EtOH extract was defatted by using pet-ether and the defatted EtOH extract was successively partitioned with between EtOAc and water. The EtOAc layer was concentrated under reduced pressure using vacuum rotatory evaporator. The EtOAc extract (8 g) was then separated by silica gel column chromatographic method successively eluting with n-Hexane:EtOAc in the ratios of 4:1, 2:1, 1:3 and 1:5 v/v solvent systems. On chromatographic separation, seven combined fractions (F-I to F-VII) were collected after examining on percoated TLC plates. Among the seven fractions, fraction (F-III) gave a white crystal (1.51 %, 121.1 mg, based on EtOAc extract).

The isolated compound was then identified by using joint application of its physicochemical properties and modern spectroscopic techniques such as UV, FT IR, NMR and Mass spectroscopies, and compared with the reported data. The NMR and Mass spectra of the isolated compound were measured at Division of Natural Product Chemistry, Institute of Natural Medicine, University of Toyama, Japan.

Screening of Antioxidant Activity of Ethanol and Watery Extracts of the Stems and Roots of *A. cathartica*

In this experiment, DPPH (2 mg) was thoroughly dissolved in EtOH (100 mL). This solution was freshly prepared in the brown coloured reagent bottle and stored in the fridge for no longer than 24 h. Each tested sample (2 mg) and 10 mL of EtOH were thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. By adding with EtOH, the sample solutions in different concentrations of 200, 100, 50, 25, 12.5 and 6.25μ g/mL were prepared from the stock solution. The effect on DPPH radical was determined using the method by Marinova and Batchvarov (2011). The control solution was prepared by mixing 1.5 mL of 50 μM DPPH solution and 1.5 mL of EtOH using shaker. The test sample solution was also prepared by mixing thoroughly 1.5 mL of 50 μM DPPH solution and 1.5 mL of each sample solution. The mixture solutions were allowed to stand at room temperature for 30 min. Then, the absorbance of each solution was measured at 517 nm by using UV-1650 spectrophotometer. Absorbance measurements were done in triplicate for each concentration and then mean values so obtained were used to calculate percent inhibition of oxidation, the capability to scavenge the DPPH radical was calculated by using the following equation:

$$\%\,\mathrm{RSA} = \frac{A_c - (A - A_b)}{A_c \times 100}$$

Where, %RSA = Radical Scavenging Activity

A_c = absorbance of the control (DPPH only) solution

A_b = absorbance of the blank (EtOH + Test sample solution) solution

A = absorbance of the test sample solution

Screening of Antimicrobial Activity of Various Crude Extracts from the Stems and Roots of *A. cathartica* by Agar Disc Diffusion Method

The screening of antimicrobial activity of various crude extracts such as PE, EtOAc, EtOH, watery extracts from the stems and roots of *A. cathartica* were carried out by agar disc diffusion method at Fermentation Laboratory, Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar. Six microorganisms namely *Bacillus subtilis*,

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* were used for this test.

Screening of Antitumor Activity of the Various Crude Extracts from the Stems and Roots of *A. cathartica*

The antitumor activity of ethanol, ethyl acetate and petroleum ether extracts from the Stems and Roots of *A. cathartica* was examined by Potato Crown Gall (PCG) or Potato Disc Assay (PDA) method (Coker *et al.,* 2003) at the Fermentation Laboratory, Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar.

Fresh disease-free potatoes were purchased from a local market. Tubers of moderate size were surface sterilized by immersion in 0.1 % sodium hypochlorite for 20 min. Ends were removed and the potatoes were soaked an additional 10 min. A core of the tissue was extracted from each and discarded. The remainder of the cylinder was cut into 1.0 cm thick discs with a surface sterilized scalpel. The discs were then transferred to agar plates (1.5 g of agar dissolved in 100 mL deionized distilled water, autoclaved for 20 min at 121 ºC, 20 mL poured into each Petridish). Each plate contained four potato discs and 4 plates, were used for each of the sample solution.

Sample (0.05, 0.10, 0.15 g) was separately dissolved in DMSO (1 mL) and filtered through Millipore filters (0.22 μ m) into sterile tube. This solution (0.5 mL) was added to sterile distilled water (1.5 mL), and broth culture of *A. tumefaciens* in PBS (2 mL) was added. Controls were made in this way; DMSO (0.5 mL) and sterile distilled water (1.5 mL) were added to the tube containing 2 mL of broth culture of *A. tumefaciens.* By using a sterile disposal pipette, 1 drop (0.05 mL) each from these tubes was used to inoculate each potato disc by spreading it over the disc surface. After inoculation, Petri dishes were sealed by film and incubated at 27~30 ºC for 3 days. Observation was made on appearance of tumors on potato discs after 3 days under stero-microscope followed by staining with Lugol's iodine (10 % KI and 5 % I_2) after 30 min and compared with control. The antitumor activity was examined by observation of tumor produced or not on the potato discs. The results are shown in Table 4.

Results and Discussion

Types of Phytochemicals Present in the Stems and Roots of *A. cathartica*

In order to find out the types of phytochemical constituents present in the stems and roots of *A. cathartica*, the phytochemical tests were preliminary carried out according to the reported procedure. From the data findings, it was observed that various secondary metabolites such as alkaloids, flavonoids, terpenoids, steroids, glycosides, organic acids, starch, phenolic compounds, saponins and tannins together with starch and carbohydrates were present, however cyanogenic glycosides, reducing sugars and α - amino acids were not detected in both of the stems and roots samples. According to these results, it can be seen that the stems and roots samples might contain potent bioactive secondary metabolites.

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, antiinflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc. These two samples for screening were found to possess tannins. Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes. Flavonoids are also present in two samples as a potent water-soluble antioxidant and free radical scavengers, which prevent from the oxidative cell damage and also have strong anticancer activity. It also helps in managing diabetes induced oxidative stress. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immune modulatory properties. In addition, terpenoids can be used as protective substances in storing agricultural products as they are known to have insecticidal properties as well (Yadav *et al*., 2014).

Some Nutritional Values of the Stems and Roots of *A. cathartica*

The nutritional values were determined by AOAC method resulting 6.91 and 18.64 % of proteins, 2.04 and 3.04 % of moisture, 2.71 and 3.06 % of ash, 62.42 and 38.65 % of fiber and 10.33 and 16.15 % of fat in the stems and roots of *A. cathartica*, respectively. According to these results, both of the samples were found to be high in fiber content. Fiber is a type of carbohydrate

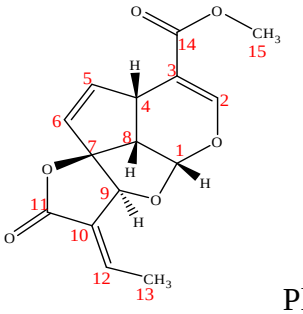
that the body cannot digest. Though most carbohydrates are broken down into sugar molecules, fiber cannot be broken down into sugar molecules, and instead it passes through the body undigested. Fiber helps to regulate the body's use of sugars, helping to keep hunger and blood sugar in check.

Identification of the Compound Isolated from the EtOAc Extract from the Roots of *A. cathartica*

From the silica gel column chromatographic separation of EtOAc extract of the roots, a compound was isolated in 1.51 % of yield based on EtOAc extract as a white crystal. Its melting point was found to be 210 ~ 211°C and its R_f value was observed to be 0.4 (PE: EtOAc, 4:1 v/v). It is soluble in chloroform, methanol and ethanol but insoluble in pet-ether, ethyl acetate, acetone and water. Since the melting point of the isolated compound was observed to be identical with the reported melting point (m.pt. 205-206 ºC) of plumericin (Maged *et al.,* 1997), a major constituent of *A. cathartica*, this isolated compound can be assigned as plumericin. It was confirmed by using modern spectroscopic methods.

It is UV active and the band with maximum absorption at λ_{max} 230 nm was observed in the UV spectrum (Figure 1), indicating the presence of conjugated double bonds in the compound. It could be also confirmed by FT IR spectral data. In the FT IR spectrum (Figure 2) the absorption band appeared at 3084 cm^{-1} was due to the =C-H stretching indicating the presence of alkenic =C-H groups and these alkenic groups were defined to be *trans*- and *cis*- disubstituted according to the bands occurred at 950 and 762 cm^{-1}. The absorption bands observed at 2950 and 2910 cm^{-1} were due to the asymmetric and symmetric C-H stretching vibrations indicating the presence of –CH, CH_2 and CH_3 alkyl groups and their respective C-H bending vibration were observed at 1647, 1616 and 1437 cm^{-1}. The absorption band at 1757 cm^{-1} was appeared due to the C=O stretching of cyclic ester and lactone and the absorption band occurred at 1714 cm^{-1} the C=O stretching of ester. These observations indicate that the compound might possess two ester groups, *ie.*, a lactone ring and an ester. The absorption band at 1083 cm^{-1} was due to the C-O-C stretching of ether. Therefore from UV and FT IR spectral data assignment, it can be inferred that the isolated compound is bearing the conjugated double bonds, alkyl groups, lactone ring, ester group and ether

functional groups and it indicates that the isolated compound must have the plumericin skeleton as follows.



Plumericin

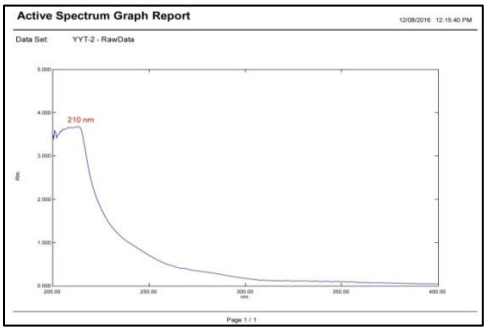


Figure 1: UV spectrum of the isolated compound (Methanol)

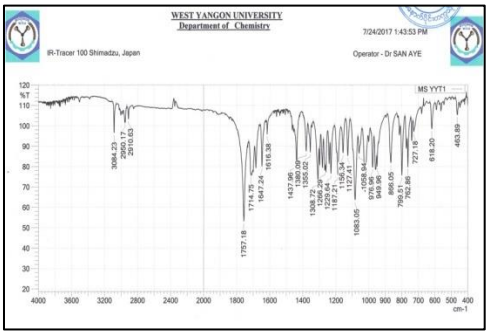


Figure 2: FT IR spectrum of the isolated compound

In addition, the structure of the isolated compound was identified by 1 D and 2 D NMR spectra. The integration of ^{1}H NMR (500 MHz, $CDCl_3$) spectrum (Figure 3) indicated the presence of fourteen protons which further supported the suggested molecular formula. The doublet signal with coupling constant of 7.25 Hz appeared at δ_H 2.1ppm (J =7.25 Hz) and a singlet signal at δ_H3.7 ppm were assigned to be two methyl groups: a aliphatic –CH_3 and a methoxy methyl group (-O-CH_3). The signals appeared at δ 5.55 ppm (1H, d, J = 5.85 Hz), δ 7.4 ppm (1H, s), δ 4.02 ppm (1H, ddd, J = 9.45, 2.25, 2.15 Hz), δ 6.05 ppm (1H, dd, J =2.2, 5.5 Hz), δ 5.65 ppm (1H, dd, J =2.2, 5.5 Hz), δ 3.4 ppm (1H, dd, J = 5.85, 9.45 Hz), δ 5.11 ppm (1H, s) and δ 7.2 ppm (1H, dq, J =7.25, 1.5 Hz) were assigned to be eight methine protons.

The ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of the isolated compound is shown in Figure 4. The spectrum revealed the presence of fifteen carbon signals including two methyl carbons at the chemical shifts of δ_C 16.07 and δ_C 51.64 ppm, eight methine carbons at δ_C152.67, 145.26, 141.05, 126.35, 102, 80.27, 53.66 and 38.38 ppm, three quartenary carbons at δ_C 127.41, 109.33 and 104.57 ppm and two carbonyl groups at δ_C 168.15 and 166.64 ppm.

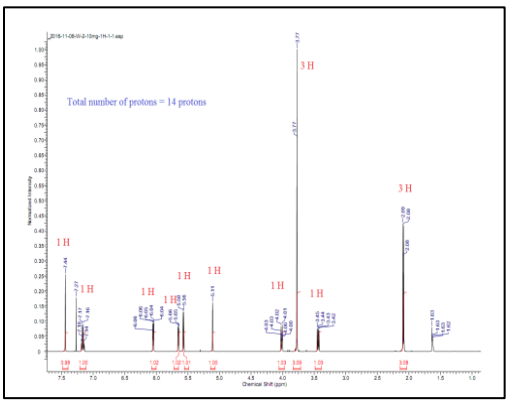


Figure 3: ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of the isolated compound

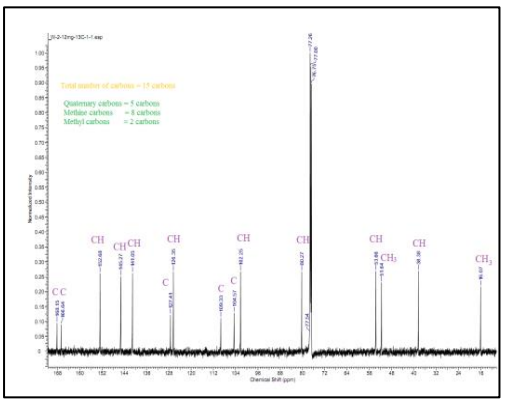


Figure4: ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of the isolated compound

The correlation between the protons directly attached to the carbons, *ie.*, types of carbons (methyl, methine and quarternary carbons) was studied by HMQC spectrum (Figure 5) and proton-proton correlation stduied by ^{1}H-^{1}H COSY spectrum (Figure 6). Furthermore, the long range proton-carbon correlation was also examined by using 2 D HMBC spectrum (Figure 7). The resultant data are shown in Table 1. It was found that the observed data of the isolated compound were similar to the reported data of plumericin (Maged *et al.*, 1997).

Finally, the structure of the isolated compound was confirmed by using EI-LRMS mass spectrum (Figure 8). According to FT IR and NMR spectral data, there were two ester groups, 15 carbons, 14 protons indicating the partial structural formula might be $C_{15}H_{14}O_4$ with molecular weight of m/z 258. The molecular weight of the isolated compound was found to be 290 as observed in MS spectrum. Consequently, the isolated compound must contain

another two oxygen atoms as two ether groups and the complete structural formula of this isolated compound must be assigned as $C_{15}H_{14}O_6$ with the molecular weight 290 and it was identified as plumericin.

Table 1: 1 D and 2 D NMR Spectral Data of the Isolated Compound and the Reported Data of Plumericin

Carbon Position	Isolated Compound				Plumericin *	
	δ_H(ppm)	δ_C(ppm), HMQC	COSY	HMBC	δ_H(ppm)	δ_C(ppm)
1	5.55 (1H, d, 5.85)	102.25,CH	H-8	C-3, C-7, C-8	5.55(1H, d, 5.9)	102.49, CH
2	7.4 (1H, s)	152.67,CH		C-1, C-4	7.42 (1H, s)	152.94,
3		109.33,C				109.58, C
4	4.02, (1H, ddd, 9.45, 2.25, 2.15)	38.38, CH	H-8	C-3, C-14	4.00(1H, ddd, 9.5, 2.2, 2.1)	38.63, CH
5	6.05(1H, dd, 2.2, 5.5)	141.05, CH	H-6	C-6, C-7, C-8	6.03(1H, dd, 5.4, 2.2)	141.31, CH
6	5.65 (1H, dd, 2.2, 5.5)	126.35, CH	H-5	C-5, C-7	5.63(1H, dd, 5.4, 2.1)	126.61, CH
7		104.57, C				104.83, C
8	3.4 (1H, dd, 5.85, 9.45)	53.66, CH	H-4, H-1	C-3, C-7	3.42(1H,dd, 9.5, 5.9)	53.91, CH
9	5.11(1H, s)	80.27, CH		C-11	5.09(1H, s)	80.53, CH
10		127.41, C				127.68, C
11		168.15, C				168.42, C
12	7.2 (1H, qd, 7.25, 1.5)	145.26, CH	H-13	C-9, C-11	7.15 (1H, qd, 7.2, 1.4)	145.54, CH
13	2.1 (3H, d, 7.25)	16.07,CH_3	H-12	C-10, C12	2.07 (3H, d, 7.2)	16.33, CH_3
14		166.64, C				166.90,C
15	3.7 (3H, s)	51.64, CH_3		C-14	3.75 (3H, s)	51.90, CH_3

*Jutamas, 2015

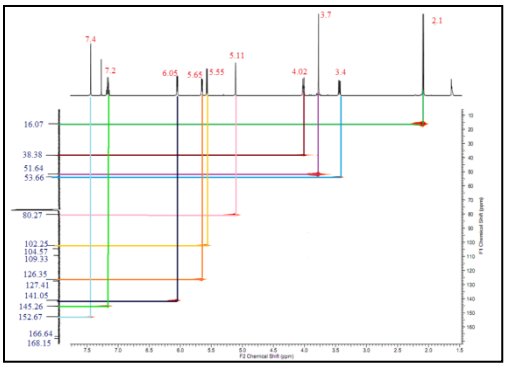


Figure 5: HMQC (500MHz, $CDCl_3$) spectrum of the isolated compound

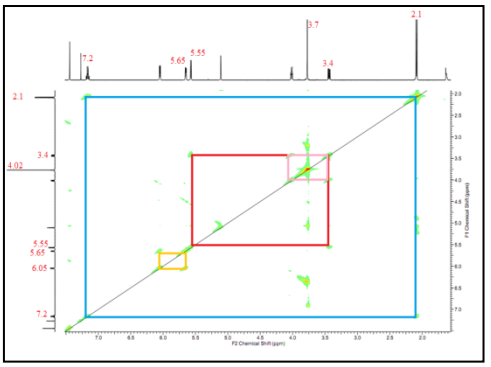


Figure 6: ^1H ^1H COSY (500MHz, $CDCl_3$) spectrum of the isolated compound

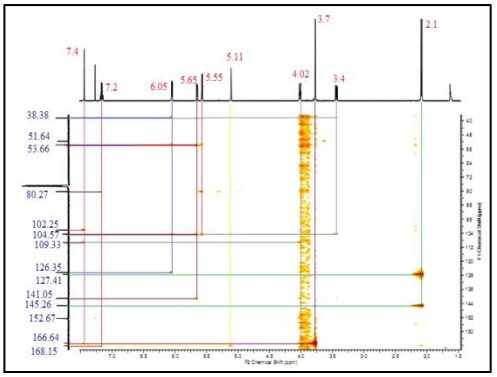


Figure 7: HMBC (500MHz, $CDCl_3$) spectrum of the isolated compound

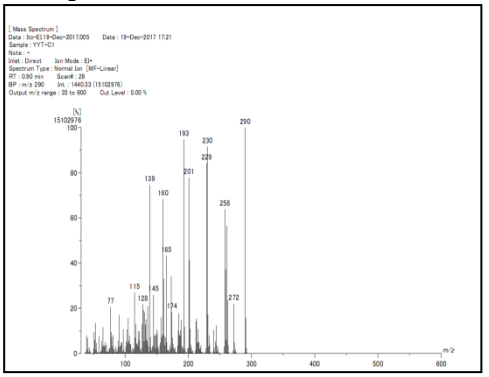


Figure 8: EI-LRMS spectrum of the isolated compound

Antioxidant Activity of the Stems and Roots of *A. cathartica*

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability of the EtOH and watery extracts of the samples by using the stable radical DPPH. The results are shown in Table 2 and Figure 9. From these observations, the radical scavenging activity of EtOH extracts was found to be greater than watery extracts in both samples. EtOH extracts of the roots inhibited 50% of free radical scavenging at the concentrations (IC_{50}) of 110.46 μ g/mL and that of the stems was 65.89 μ g/mL. Watery extracts of the both samples did not show antioxidant activity up to optimum dose of 200 μ g/mL.

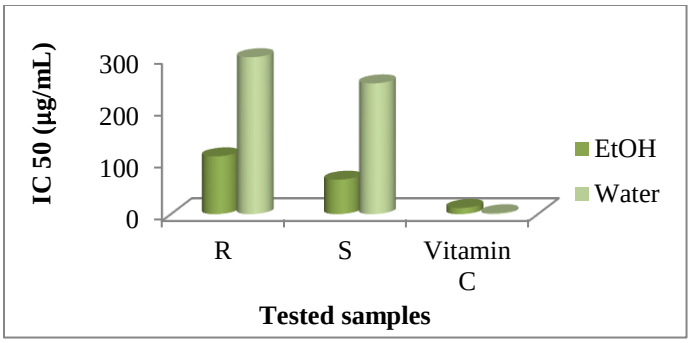


Figure 9: A bar graph of IC_{50} (µg/mL) of EtOH and watery extracts of the roots and stems of *A. cathartica*

Table 2: Percent Radical Scavenging Activity of Crude Extracts of Roots of *A. cathartica*

Sample	% Radical Scavenging Activity at Different Concentrations (µg/mL) ± SD of the sample						IC_{50} (µg/mL)
	6.25	12.5	25	50	100	200	
R-EE	21.70 ± 0.32	26.62 ± 0.95	28.64 ± 0.00	35.46 ± 0.48	48.43 ± 1.11	61.75 ± 0.95	110.46
S-EE	4.25 ± 0.63	12.64 ± 1.42	23.15 ± 3.32	39.26 ± 0.16	68.68 ± 1.58	101.5 ± 0.95	65.89
R-WE	7.54 ± 0.00	8.85 ± 0.02	10.95 ± 0.00	15.68 ± 0.00	26.81 ± 0.00	33.06 ± 0.10	>200
S-WE	28.35 ± 0.00	27.65 ± 0.00	28.39 ± 0.02	31.41 ± 0.00	38.15 ± 0.00	45.62 ± 0.00	>200
Vitamin C	25.20 ± 1.40	53.58 ± 0.88	65.53 ± 1.13	74.82 ± 0.59	83.32 ± 0.78	91.21 ± 0.48	11.7

R-EE = Shwewa Root EtOH Extract
S-EE = Shwewa Stem EtOH Extract
R-WE = Shwewa Root Watery Extract
S-WE = Shwewa Stem Watery Extract

Antimicrobial Activity of Crude Extracts of the Roots of *A. cathartica*

Four crude extracts such as PE, EtOAc, EtOH and water extracts from the stems and roots of *A. cathartica* were subjected to screening of antimicrobial activity against six different pathogenic microbes such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Escherichia coli* using agar well diffusion method. This method is based on zone diameter including the well diameter, in millimeter (mm). The larger the zone diameter, the higher theactivity is. According to the results, watery extract of the stems did not show any antimicrobial activity against *P. aeruginosa* and *E. coli.* Moderately significant antibacterial activities were possessed by PE and watery extracts of both samples. Moreover, EtOAc and EtOH extracts of the roots inhibited significantly all strains of bacteria with the zone of inhibition ranged from 20 to 35 mm (Table 3). Hence in general, the extracts from the roots of *A. cathartica* were found to be high in antimicrobial activity against all tested microorganisms.

The results of present research highlight the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Britto, 2001). The present observation suggested that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigators (Krishna *et al.*, 1997; Singh and Singh 2000; Natarajan *et al*., 2003 & 2005).The present study justifies the claimed uses of *A. cathartica* in the traditional system of medicine to treat various infectious diseases caused by the microbes.

Table 3: Inhibition Zone Diameters of Various Extracts of the Roots of *A. cathartica* against Six Microorganisms by Agar Well Diffusion Method

No.	Microorganisms	Samples	Inhibition Zone Diameters (nm) of Different Crude Extracts			
			PE	EtOAc	EtOH	Watery
1	*Bacillus subtilis*	I	15 (++)	30 (+++)	33 (+++)	24 (+++)
		II	13 (+)	14 (+)	16 (++)	13 (+)
2	*Staphylococcus aureus*	I	14 (+)	30 (+++)	30 (+++)	20 (+++)
		II	13 (+)	15 (++)	14 (+)	14 (+)
3	*Pseudomonasaeruginosa*	I	15 (++)	20 (+++)	20 (+++)	14 (+)
		II	18 (++)	18 (++)	15 (++)	-
4	*Bacillus pumilus*	I	16 (++)	35 (+++)	34 (+++)	24 (+++)
		II	12 (+)	13 (+)	14 (+)	15 (++)
5	*Candida albicans*	I	15 (++)	32 (+++)	30 (+++)	24 (+++)
		II	12 (+)	14 (+)	15 (++)	15 (++)
6	*Escherichia coli*	I	15 (++)	33 (+++)	33 (+++)	21 (+++)
		II	14 (+)	14 (+)	14 (+)	-

I = roots, II = stems
Agar well – 10mm, 10mm ~ 14mm (+), 15mm ~ 19 mm(++), 20mm and above (+++)

Antitumor Activity of the Stems and Roots of *A. cathartica*

Antitumor activity in this study was investigated by potato crown gall (PCG) assay as it is a valuable tool that indicates antitumor activity of the tested samples by their inhibition of the crown gall formation that was induced in wounded potato tissues by *Agrobacterium tumefaciens*.

Table 4:Tumor Inhibition by the Crude Extracts and Ioslated Plumericin from the Stems and Roots of *A. cathartica*

Test Samples	Extracts/Compound	Concentrations of Samples (g/mL/disc)	Tumor Inhibition
Control	-	0.00	+
Roots	PE	0.05	-
	PE	0.10	-
	PE	0.15	-
	EtOAc	0.05	-
	EtOAc	0.10	-
	EtOAc	0.15	+
	EtOH	0.05	-
	EtOH	0.10	-
	EtOH	0.15	-
Stems	PE	0.05	-
	PE	0.10	-
	PE	0.15	+
	EtOAc	0.05	+
	EtOAc	0.10	+
	EtOAc	0.15	+
	EtOH	0.05	-
	EtOH	0.10	-
	EtOH	0.15	-
Plumericin	-	10, 20, 30 mg/mL/disc	-

(+) Tumor appeared, (-) No tumor appeared

It could be clearly seen from the Table 4 that except EtOAc extracts of stems, all of the samples inhibited tumor growth in a concentration dependent manner. Significant tumor inhibition was observed at the concentrations of 0.05, 0.10 and 0.15 g/mL/disc. Plumericin showed antitumor activity at the concentrations of 10, 20 and 30 mg/mL/disc.

Since, tumor inhibition significantly occurred by the extracts of *A. cathartica* on potato discs, it could be concluded that the plant might be used as a potential source of antitumor agent.

Conclusion

From the overall assessment concerning with the investigation of phytochemicals and biological activities on the stems and roots of *A. cathartica*, the following inferences could be deduced. The various types of phytochemical constituents such as alkaloids, flavonoids, terpenoids, steroids, glycosides, organic acids, starch, phenolic compounds, saponins, tannins and carbohydrates were present in the stems and roots of *A. cathartica*, except cyanogenic glycosides, reducing sugars and α - amino acids. The approximate nutritional compositions were observed to be 6.91 and 18.64 % of proteins, 2.04 and 3.04 % of moisture, 2.71 and 3.06 % of ash, 62.42 and 38.65 % of fiber and 10.33 and 16.15 % of fat in stems and roots of *A. cathartica*, respectively. One organic compound, plumericin (1.51 %, mpt. 210 °C) was isolated as a major bioactive chemical constituent from ethyl acetate crude extract of the roots by using silica gel column chromatographic separation technique. The isolated compound was characterized by some physical and chemical properties and structurally identified by the combination of UV, FT IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and EI-LRMS spectroscopic methods and also by comparing with the reported data.

The ethanol extract of stems possesses higher antioxidant activity than that of the roots. Although watery extract of the stems showed low activity and PE and watery extracts of the stems and roots showed medium activity, EtOAc and EtOH extracts of roots were found to possess high antimicrobial activity against all strains of microorganisms tested (inhibition zone ranged between 20 ~ 35 mm). Furthermore EtOAc extract of the roots, PE and EtOH extracts of roots and stems were also found to inhibit the formation of tumor. Plumericin has pronounced antitumor activity.

In conclusion, antimicrobial, antioxidant and antitumor activities of different extracts obtained from the stems and roots of *A. cathartica* grown in Myanmar could be evaluated. Strong antimicrobial activity was found in all tested samples. However the antioxidant activity of tested samples were very weak compared with the potency of standard vitamin C (IC_{50} = 1.17 μg/ mL). In addition, strong antitumor activity was also observed. With these results, the tested plant has some scientific justification as a medicinal plant that might be useful for the treatment of bacteria infected diseases and be used as antitumor agent.

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