A STUDY ON SOME BIOLOGICAL ACTIVITIES AND IDENFTIFICATION OF AN ISOLATED COMPOUND FROMTHE LEAVES OF*Eupatorium odoratum* L.

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Abstract

In this study, leaves of a Myanmar indigenous medicinal plant, *Eupatorium odoratum* L. (Taw-bizat) were chosen for the investigation of phytochemical constituents andsome of the biological activities such as antitumor, antimicrobial, antioxidant and acute toxic properties. The phytochemical constituents, nutritional values and elemental contents were analyzed on the leaves sample by using appropriate reported methods. The methanol extract (25 μg/disc) of the leaves sample was found to possess the antitumor activity against the tumor producing bacteria: *Agrobacterium tumefaciens* isolated from gall tissues of leaves of *Sandoricum koetjape* Merr. (Thitto). The crude extracts such as pet-ether, ethanol, methanol and ethyl acetate extracts were observed to be more potent than watery extract in antimicrobial activity against *Bacillus subtilis*, *Staphylococus aureus*, *Pesudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*. However, the watery showed the antioxidant activity (IC_{50}= 13.07 μg/mL) higher than ethanol extract (IC_{50}= 56.14 μg/mL), determined by DPPH radical scavenging assay method. Both of watery and ethanol extracts did not show acute toxic effect up to 5000 mg/kg body weight dose on albino mice. From the separation of silica gel column chromatographic method, a bioflavonoid compound, 2´-hydroxy-4,4´,5´,6´-tetramethoxy chalcone(143-144 °C, 0.12 %) was isolated from the active pet-ether extract of *E. odoratum* leaves. It was identified by UV-Visible, FT IR, ^{1}HNMR, ^{13}C NMR, and EI-MS spectroscopic methods and also by comparing with the reported data. This isolated chalcone compound was observed to exhibit higher antioxidant activity (IC_{50}= 9.69 μg/mL) than the watery and ethanol extracts.

Keywords*: Eupatorium odoratum* L., bioflavonoid, 2´- hydroxy 4,4´,5´,6´ tetramethoxy chalcone, antitumor activity, antioxidant activity, acute toxicity

Introduction

Eupatorium odoratum L. belongs to the family Asteraceae and it is (up to 9 feet) shrubby with rather large, lanceolate, leaf blades coarsely

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toothed, especially near the base on long leaf less branches which spreading from the axils. These flowers are white, flowering time from August to October. It is distributed throughout Indian, Indochina and common in open country. In Myanmar, it can be widely distributed throughout the country (Ahmad and Nabi, 1969). The part of leaves are used externally in traditional medicine as a wound healing, skin abscess, diuretic, cathartic, intermittent fever, ulcers, bilious, catarrh and influenza (Biswal *et al.*,1997). According to the reported data (Baruah *et al.,* 1978), steroids, terpenoids and 13 flavonoids were observed in this plant. This plant was so found that rich in flavonoids. There is no report concerning with investigation of flavonoid and biological activities in this plant so far in Myanmar. Therefore, in this study the phytochemical constituents, antitumor activity, antimicrobial activity, antioxidant activity, acute toxicity and identification of one isolated bioactive flavonoid compound from the leaves of *E.odoratum* were conducted.

Materials and Methods

Collection and Phytochemical Investigation of the Leaves of*E.odoratum*

The leaves of *E. odoratum*were collected from Nwe-kway-ywa, Htauk-kyant Township, Yangon Region, Myanmar. After cleaning, the leaves were air-dried at room temperature for three weeks and the dry sample was ground into powder by grinder. The dried powdered sample was stored separately in air-tight containers to prevent moisture changes and other contamination. These plants were identified at the Department of Botany, University of Yangon. The preliminary investigation of phytochemical constituents was carried out according to reported methods (M-Tin Wa, 1972; Harbone, 1984).

Determination of Nutritional Values and Elemental Analysis of the Leaves of *E. odoratum*

The nutritional values such as moisture content, ash content, fat content, protein content(micro Kjeldahl method), fiber content, total carbohydrate contents and the energy value were determined by AOAC method (AOAC,1990). In addition, some mineral elements such as Ca, Mg, Fe, Cd, Cu and Mn were analyzed on the sample by using AAS (Atomic

Absorption Spectrometry) technique at Universities' Research Center (URC), Yangon.

Isolation, Characterization and Identification of Phytoconstituent from the Leaves of *E. odoratum*

Thedried powdered sample (ca 300 g) was percolated in 1000 mL of pet-ether (60-80 $^{\circ}$C) with occasional shaking for one week and filtered. This procedure was repeated for three times. The combined filtrate was concentrated under vacuum rotatory evaporator to obtain pet-ether crude extract. The pet-ether crude extract was decolourized by charcoal to give decolourized pet-ether crude extract (Robison, 1983).

The decolourized pet-ether extract (3 g) was separated chromatographically on a silica gel column (4 cm diameter x 47 cm length) by gradient elution with different ratios of PE:EtOAc (18:1, 8:1, 3:1 v/v) solvent mixture and a total of 87 fractions (7 mL each) were collected. From the inspection of TLC chromatogram under UV lamp, the fractions which had similar appearance were combined. The combined fraction from fractions 35-79 provided a yellow colour solid after evaporation. It was recrystallized from PE:AcOEt to give a yellowish crystal in 0.12 %(0.36 g) of yield.

The above isolated compound was characterized by visualizing under UV-light, by determination of melting points and R_f values and by some colourtests. The melting point was examined on a Gallenkamp melting point apparatus. Thecolour tests were carried out by spraying with 5% $FeCl_3$ solution, $K_4Fe(CN)_6$ solution, aqueous NaOH solution, conc: H_2SO_4 and by exposure to NH_3vapour on precoated aluminium TLC chromatograms after developing in PE:EtOAc (8:1 v/v) and R_f value was determined. In addition, the chromatograms sprayed with HOAc followed by exposure to NH_3 vapor were examined under a long wavelength (365 nm) UV lamp. Furthermore, it was also treated with Mg/ conc. HCl.

The isolated compound was then identified by modern spectroscopic techniques such as UV-Visible, FT IR, ^{1}HNMR, ^{13}CNMR and MS spectroscopic techniques and also by comparing with the reported spectral data.The UV spectra of the isolated compound were recorded in methanol and also in the presence of some flavonoid shift reagents such as NaOH, $AlCl_3$ and $AlCl_3$/HCl, NaOAc and NaOAc/H_3BO_3 by using Shimadzu UV-240 UV-

Visible spectrophotometer at URC, Yangon. The infrared spectrum of isolated compound was recorded on a Perkin Elmer Spectrum GX FT IR spectrophotometer at URC, Yangon. The isolated compound was sampled as a 1% KBr pellet. ^{1}HNMR spectrum of the isolated compound was recorded in $CDCl_3$ (400 MHz) and ^{13}CNMR spectrum was recorded in $CDCl_3$(100 MHz) with TMS as internal standard at the Department of Chemistry, Kanazawa University, Japan. Electron impact mass spectrum (EI-MS) of the isolated compound was recorded on a JEOL SX-102, a mass spectrometer at the Department of Chemistry, Kanazawa University, Japan.

Screening of some Biological Activities of the Leaves of *E.odoratum*

Screening of antitumor activity of the leaves of *E.odoratum*

In the screening of antitumor activity, the tumor producing bacteria *Agrobacterium tumefaciens* was firstly isolated from gall tissues of leaves of *Sandoricum koetjape* Merr.(Thitto). The isolated bacteria was identified by its morphology, gram staining, spore staining, some biochemical tests and compared with the references. The morphology of isolated bacteria was examined under Microscope (Cruickshank, 1960). In gram staining method, ammonium oxalate crystal violet solution (Hucker's solution), gram's modification of Lugol's solution and counter stain solution were used. In biochemical assay: motility test, catalase test, starch hydrolysis test, gelatin test, nitrates reduction test, indole test and carbohydrate test were carried out. After that, 2 mL(5×10^9 cells/mL) of broth culture of *Agrobacterium tumefaciens* and 0.5 mL of methanol extract of the leaves sample were inoculated on each potato disc, spreading it over the disc surface. The plates were sealed with the tape to minimize moisture loss and incubated at room temperature for one week. After incubation, Lugol's solution (I_2&KI) was added and tumors were observed under the microscope and compared with control disc. The antitumor activity was detected with the result of tumor occurred or not (Ferrigni *et al*., 1982). This experiment was carried out at the Pharmaceutical Research Department, Ministry of Industry, Yangon.

Screening of antimicrobial activity of theleaves of *E. odoratum*

Agar well diffusion method (Balouiri, 2016) was employed for determining antimicrobial activity of the extracts such as PE, EtOH, MeOH,

EtOAc and watery extracts from the sample against six pathogenic microorganisms namely *Bacillus subtilis*, *Staphylococus aureus*, *Pesudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*, conducted at the Pharmaceutical Research Department, Ministry of Industry, Yangon. The antimicrobial activity was determined by measuring the inhibition zone diameters appeared around the agar well indicated that the presence of antimicrobial activity (Cruickshank,1960).

Screening of antioxidant activity of the leaves of *E. odoratum*

Antioxidant activity of 95 % ethanol, watery extracts and the isolated compound was determined UV-visible spectroscopically by using DPPH (1,1-diphenyl, 2-picryl, hydrazyl) radical scavenging assay (Halliwell, 2012).

Firstly, a blank solution was prepared by mixing 1.5 mL of the test sample solution with 1.5 mL of 95 % ethanol and a control solution was prepared by mixing of 60 μM DPPH solution and 1.5 mL of 95 % ethanol using vortex mixer. The sample solution was prepared by mixing thoroughly 1.5 mL of 60 μM DPPH solution and 1.5 mL each of test sample in ethanol with different concentrations (3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 μg/ mL). The solutions were allowed to stand at room temperature for 30 min. After 30 min, the absorbance of these solutions was measured at 517 nm by UV-visible spectrophotometer. Absorbance measurements were done in triplicate for each solution and the mean values so obtained were used to calculate the percent inhibition of oxidation by the following equation.

$$\% \text{ Oxidative Inhibition} = \frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}} \times 100$$

$A_{control}$ = Absorbance of the DPPH solution

A_{sample} = Absorbance of the sample and DPPH solution

A_{blank} = Absorbance of the sample solution

Then, the antioxidant activity of the samples was expressed by theirIC_{50} (50 % oxidative inhibitory concentration) which were calculated by linear regressive excel program (Kahlonene, 1999).

Screening of acute toxicity of the leaves of *E. odoratum*

The acute toxicity of watery and 95 % ethanol extracts of the leaves sample was determined in *in vivo* by using albino mice model according to OECD guideline 425(OECD, 2000). This method is reproducible, used in very few animals and able to rank substances in a similar manner to the other acute toxicity testing methods (OECD, 2000).

In the present study, 24 albino rats were taken and divided into 8 groups (3 in each group) (Group I, II, III, IV, V, VI, VII, VIII). Then, Groups I, II, III and IV were orally treated with watery extract and Groups V, VI, VII and VIII treated with ethanol extract in different concentrations by using a stomach tube or a suitable intubation cannula. The dose levels administrated to each group were 175, 550, 1750 and 5000 mg/kg body weight/day and the sign of animals were observed daily up to 7 days and 14 days.

Results and Discussion

Phytochemical Constituents Present in the Leaves of *E.odoratum*

The leaves of locally grown *E. odoratum* were collected from New-kway-ywa, Htauk-kyant Township, Yangon, Myanmar in the middle of September. According to the preliminary phytochemical tests, it was found that glycosides, flavonoids, alkaloids, α-amino acid, carbohydrates, phenolic compounds, saponins, steroids, tannins and terpenoids are present in the *E. odoratum* leaves sample, whereas, cyanogenic glycosides, the harmful phytoconstituent, were not detected in the collected sample (Table 1).

In addition, from the analysis of nutritional value, the *E.odoratum* leaves were observed to contain 35.37 % of carbohydrate, 19.75 % of protein, 24.31 % of fiber, 3.52 % of fat, 7.51 % of ash and 9.54 % of moisture with 185 kcal/100 g of energy value, based on dry weight.

Furthermore, elemental analysis by using AAS method showed that the leaves of *E.odoratum* contained 122.18 ppm of Ca, 100.27 ppm of Mg and 34.34 ppm of Fe, and Cd, Cu and Mn were not found in the sample under experimental condition.

Table 1: Phytochemical Constituents Present in the Leaves of *E. odoratum*

Types of compounds	Extract	Test reagents	Observation	Remark
Alkaloids	1% HCl	Mayer's Dragendroff'sDragendroff's reagent Wagner's reagent	White ppt Orange ppt Brown ppt	+ + +
α-Amino acids	H_2O	Ninhydrin	Pink colour spot	+
Carbohydrates	H_2O	10 % α naphthol,conc. H_2SO_4	Red ring	+
Flavonoids	EtOH	Conc. HCland Mg ribbon	Pink colour	+
Glycosides	H_2O	10 % lead, acetate solution	White ppt	+
Phenolic compounds	EtOH	1% $K_3Fe(CN)_6$, 1 % $FeCl_3$	Blue/green colour	+
Steroids	PE	Acetic anhydride,conc:H_2SO_4	Greenish blue colour	+
Saponins	H_2O	Shaking	Frothing	+
Terpenoids	$CHCl_3$	Acetic anhydride and conc: H_2SO_4	Pink colour	+
Tannins	H_2O	1 % gelatin	White ppt	+
Cyanogenic glycosides	H_2O	Sodium picrate	No brick red colour	-

\+ = Presence - = Absence

Isolation and Identification of the Isolated Compound from the Leaves of *E. odoratum*

On silica gel coloum chromatographic separation by using PE:AcOEt (8:1 v/v) solvent system as eluent, a yellowish crystal was isolated from the decolourized pet-ether extract (3 g) of the leaves of *E. odoratum*in 0.36 g, 0.12 % of yield. Its R_f value was 0.45 in PE:AcOEt (8:1 v/v) solvent system and its melting point was observed to be 143-144º**C** (PE/AcOEt). This compound could be observed on TLC chromatogram under a long wavelength (365 nm) UV lamp, indicating the presence of conjugated double bonds.

The isolated compound was observed in brown colour on the precoated aluminium TLC chromatograms while spraying with 5 % $FeCl_3$ solution, in greenish blue colour while spraying with $K_4Fe(CN)_6$ solution, in

pale yellow colour while spraying with aqueous NaOH solution, in yellow colour while spraying with conc. $H_2SO_4$and in yellow colour by exposure to NH_3 vapour, indicating that it is a flavonoid compound containing phenolic group.

In addition, when the chromatogram was sprayed with HOAc, deep purple spot was observed to appear under a long wavelength (365 nm) UV lamp. The deep purple spot was found to change red under UV after exposure to NH_3 vapor. Furthermore, it did not give any colouration while treating with Mg/ conc. HCl. From these observation it may therefore, be assigned as a chalcone with a free 2- and 4-OH (Geissman, 1955).

According to UV-Visible spectra in MeOHas shown in Figure 1 (a), of the isolated chalcone, the major absorption band (Band I) occurred at 360 nm with a shoulder at 305 nm and a minor absorption band (Band II) at 230 nm. This information also pointed out that the isolated compound as a chalcone (Markhal, 1982).In the presence of NaOH, a 60 nm bathochromic shift of Band I with a decrease in the peak intensity occurred at 420 nm as a shoulder indicating that the chalcone cannot possess 4-OH group (Figure 1 (a)). According to 70 nm bathochromic shift of Band I (360 nm to 430 nm) in the presence of $AlCl_3$ as well as in $AlCl_3$/HCl (Figure 1 (b)), there may be a 2′ -OH group in the isolated chalcone. Band I of the compound was observed by 10 nm bathochromic shift in the presence of NaOAc as well as in NaOAc/H_3BO_3 (Figure 1 (c)). All of the observed UV-visible spectral data are listed in Table 2. On the basis of UV-Vis spectral data, the isolated compound might be assigned as a chalcone bearing a OH group at 2' position.

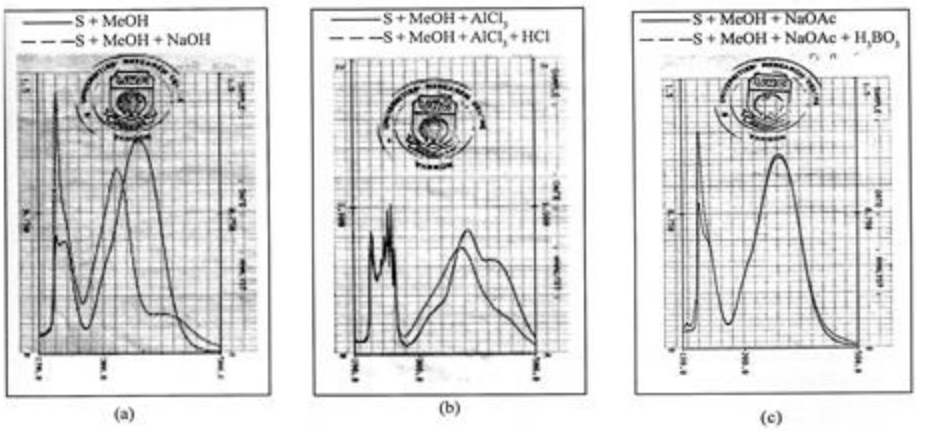


Figure 1: UV-Visible Spectra of the isolated compound from *E.odoratum* leaves

Table 2: UV-Visible Spectral Data of the Isolated Compound from the Leaves of *E. odoratum*

No.	Solvents and Shift reagents	λ_{max}(nm)
1	MeOH	230(II), 305(sh), 360(I)
2	MeOH + NaOH	225(II), 320(sh), 420(I)
3	MeOH + $AlCl_3$	230(II), 390(II), 430(I)
4	MeOH + $AlCl_3$ +HCl	228(II), 390(II), 430(I)
5	MeOH + NaOAc	230 (I), 270 (I), 370 (I)
6	MeOH + NaOAc + H_3BO_3	230(I), 270, 380(I)

The presence of OH group and α, β-unsaturated C=O group could also be confirmed with the peaks respectively appeared at 3450 cm^{-1} and 1625 cm^{-1} in FT IR spectrum (Figure 2 and Table 3) of the isolated compound. The characteristic bands at 2947, 2844, 1345, 1244 and 1020 cm^{-1} also showed the presence of aromatic O-CH_3 group. The bands appeared in the regions: 3010, 1602, 1174, 988, 874 and 836 cm^{-1} showed the presence of aromatic C=C and =C-H groups.

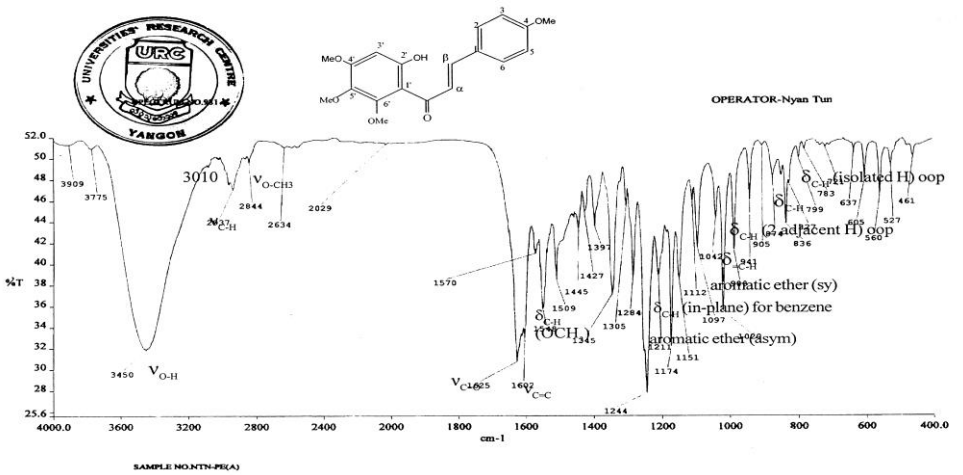
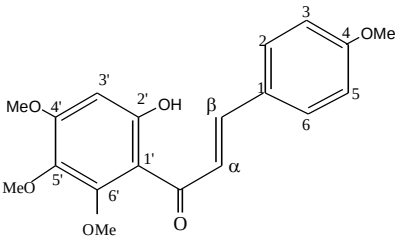


Figure 2: FT IR spectra of the isolated compound from the leaves of *E.odoratum*

Table 3: FT IR Spectral Data of the Isolated Compound from the Leaves of *E. odoratum*

Wave Number (cm^{-1})	Assignment
3450	ν_{O-H} of phenol
3010	$\nu_{=C-H}$ of aromatic ring
2947, 2844	Asymmetric and symmetric ν_{C-H} of CH_3 group
1625	$\nu_{C=O}$ of α, β -unsaturated carbonyl group
1602	$\nu_{C=C}$ of aromatic ring
1345	δ_{C-H} of CH_3 group
1244	Asymmetric ν_{C-O-C} of aromatic ether
1174	δ_{C-H} in plane bending of benzene
1020	Symmetric ν_{C-O-C} of aromatic ether
988	$\delta_{=CH}$ of aromatic ring
874	$\delta_{C-H(oop)}$ of two adjacent H in aromatic ring
836	$\delta_{C-H(oop)}$ of isolated H in aromatic ring

According to ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum and spectral data (Figure 3 and Table 4), there were two *trans* olefinic protons (H-α and H-β) due to a singlet peak at 7.8 ppm. One phenolic protons appeared as a singlet at 13.75 ppm which indicated that the OH group is ortho to the CO group. The signals at 3.76 (s, 3H), 3.87 (s, 3H), 3.92 (s, 3H), 3.97 (s,3H) ppm were due to the protons of four methoxy groups. The H-2 and H-6 protons occurred at 7.73 ppm (2H, d, J= 8.78 Hz) as a doublet with coupling constant 8.78 Hz and H-3 and H-5 protons appeared at 7.03 ppm (2H, d, J=8.78 Hz) also as a doublet with same J value of 8.78 Hz. The H-3′ proton was occurred as a singlet at 6.33 ppm. The ^{1}HNMR spectral data of the isolated chalcone were found to be identical with the reported data (Table 4) for 2′-hydroxy-4,4′,5′,6′-tetramethoxychalcone (Mabry *et al.*, 1970). Therefore the structure of the isolated chalcone was assigned as follows.



2′ -hydroxy-4,4′ ,5′ ,6′ -tetramethoxychalcone

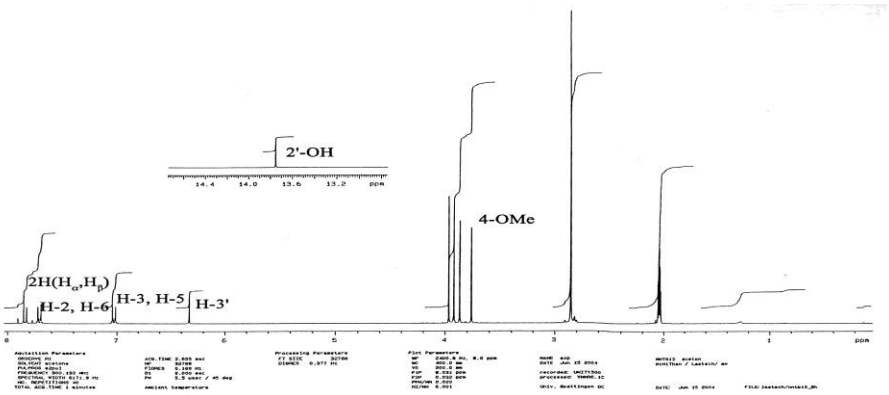


Figure 3: ^{1}HNMR spectrum ($CDCl_3$, 400 MHz) of the isolated compound from *E. odoratum* leaves

Table 4: ^{1}H NMR ($CDCl_3$, 400 MHz) Spectral Data of the Isolated Compound from *E. odoratum* Leaves and the Reported Data of 2□-hydroxy-4,4□,5□,6□-tetramethoxy chalcone

Chemical Shifts (□ /ppm)		Multiplicity	Remark
Observed	**Reported***		
3.76	3.82	s	3H (OMe)
3.87	3.85	s	3H (OMe)
3.92	3.90	s	3H (OMe)
3.97	3.92	s	3H (OMe)
6.33	6.27	s	1H (H–3′)
7.03	6.68	d (J=8.78 Hz)	2 H (H-3, H–5)
7.73	7.59	d (J=8.78 Hz)	2 H (H-2, H–6)
7.83	7.84	s	2 H (Hα , Hβ)
13.75	13.50	s	1 H (2′ –OH)

* Mabry*et al.*, 1970

^{13}CNMR spectrum and the spectral data interpreted are illustrated in Figure 4 and Table 5, respectively. From this spectrum, it was observed that four methoxy groups are appeared at 55.76, 56.48, 61.14 and 62.08 ppm. According to this spectrum, it was found that there were a total of 19 carbon atoms: 4 methyl carbons of 4 methoxy groups (55.76, 56.48, 61.14 and 62.08 ppm), one carbonyl carbon at 193.46 ppm and 14 double bond carbons (97.25, 109.11, 115.34, 124.68, 128.74, 131.11, 136.13, 144.01, 155.07, 161.28, 162.70 and 163.4 ppm) as summarized in Table 5. The numbers of carbon and types of carbons were observed to be that of 2′-hydroxy-4, 4′, 5′, 6′-tetramethoxychalcone.

In EI-MS spectrum (Figure 5), the molecular ion [M^+] peak appeared at m/z = 344 indicating that the molecular formula of the isolated chalcone is $C_{19}H_{20}O_6$. The fragmentation pattern of the compound could be expressed as in Figure 6 and that finally confirmed the isolated compound to be 2′-hydroxy-4, 4′, 5′, 6′-tetramethoxy chalcone

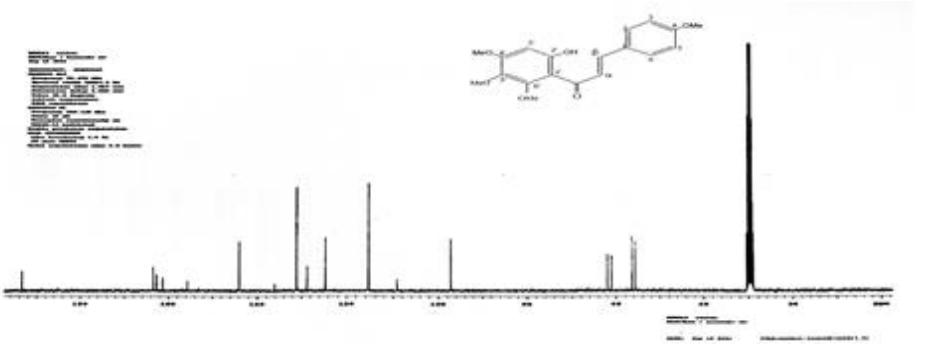


Figure 4: ^{13}CNMR spectrum ($CDCl_3$, 100 MHz) of isolated compound from *E.odoratum* leaves

Table 5: ^{13}CNMR ($CDCl_3$, 100 MHz) Spectral Data of the Isolated Compound from *E. odoratum* Leaves

δ_c (ppm)	Assignment
55.76 56.48 61.14 62.08	Methyl carbons of four methoxy group
97.25 109.11 115.34 124.68 128.74 131.11 136.13 144.01 155.70 161.28 162.70 163.47	Double bond carbons
193.46	Carbon of carbonyl group

Figure 5: EI -MS spectrum of the isolated compound from *E.odoratum* leaves

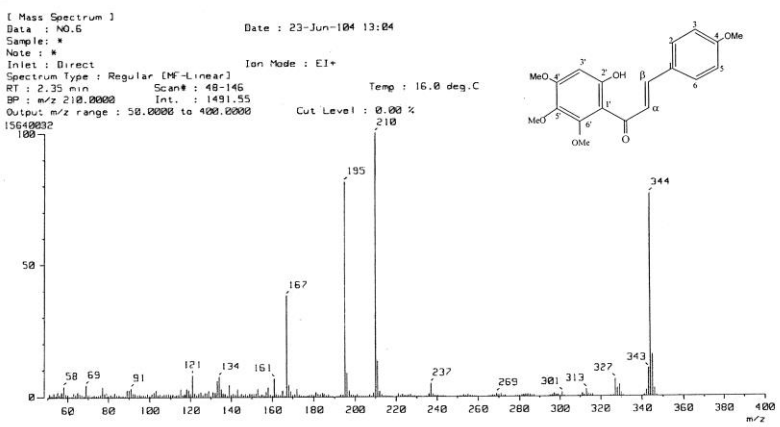


Figure 5:EI -MS spectrum of the isolated compound from *E.odoratum* leaves

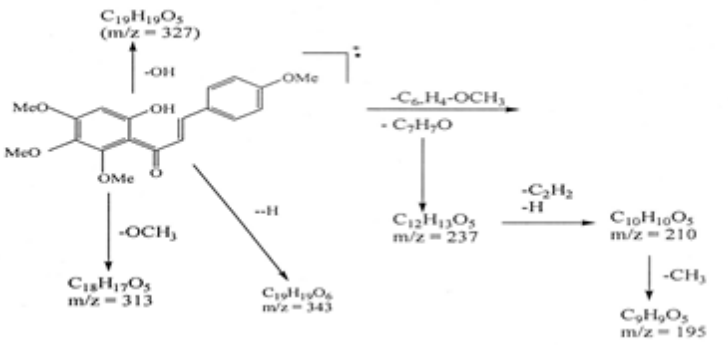


Figure 6: Fragmentation pattern of the isolated compound from *E. odoratum* leaves

Antitumor Activity of *E. odoratum*Leaves

The antitumor activity was determined on the methanol extract of *E. odoratum* leaves by using Potato Crown Gall test. *Agrobacterium tumefaciens* isolated from the leaves of *Sandoricum koetjape* Merr. (Thitto) was used for tumor formation on the potato disc. From this experiment, methanol extract was observed to inhibit the tumor formation with the dose of 25 μ g/disc.

Antimicrobial, Antioxidant and Acute Toxicity Activities of *Eupatorium odoratum* L.

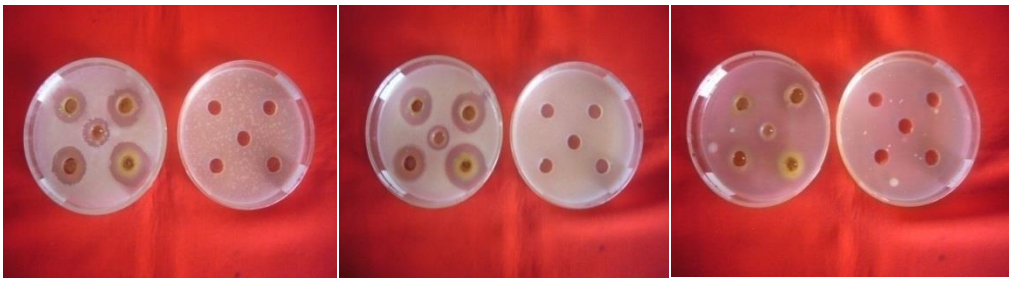
The antimicrobial activity of MeOH extract (inhibition zone diameter35 mm) was exhibited the most potent antimicrobial activity against *Staphylococcus aureus*. The remaining extracts were showed antimicrobial activity against on six strains of microorganisms. The results of inhibition zone diameters are described in Figure 7 and Table 6.

The antioxidant activity was expressed as 50% oxidative inhibitory concentration (IC_{50}). The lower the IC_{50} values, the higher the antioxidant activity of the sample. By using DPPH free radical scavenging assay, the compound A was more potent antioxidant activity than 95 % ethanol and watery extracts. The results of antioxidant activity are shown in Table 7 and Figures 8 and 9.

In acute toxicity test, there is no lethality at the dose of 5000 mg/kg b.w of the extracts and hence LD_{50} was supposed to be higher than 5000 mg/ kg b.w. It can be concluded that the ethanol and watery extracts of *E.odoratum* were practically non-toxic.

Table 6: Inhibition Zone Diameters of Various Extract of *Eupatorium odoratum* L. against Six Microorganisms by Agar Well Diffusion Method

No.	Microorganisms	Inhibition Zone Diameter (mm)				
		PE	EtOH	MeOH	EtOAc	H_2O
1	*Bacillus subtilis*	20	23	18	22	14
2	*Staphylococcus aureus*	18	30	35	24	15
3	*Pseudomonas aeruginosa*	21	23	18	20	15
4	*Bacillus pumilus*	23	24	21	20	14
5	*Candida albicans*	23	20	24	20	16
6	*Escherichia coli*	21	24	18	18	15



Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa

Bacillus pumilus, Candida albicans, Escherichia coli

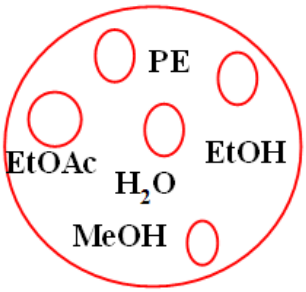


Figure 7: Inhibition zone diameter of crude extracts against six microorganisms

Table 7: Oxidative Percent Inhibitions and IC_{50} Values of Crude Extracts and the Isolated Compound A of *Eupatorium odoratum* L. and Standard BHT

Sample	% Inhibitions (Mean ± SD) in various Concentrations (μg/ml)								IC_{50}
	3.125	**6.25**	**12.5**	**25**	**50**	**100**	**200**	**400**	**(μg/ml)**
95 % EtOH	38.095 ± 3.863	36.395 ± 2.567	30.272 ± 7.167	27.347 ± 18.395	37.551 ± 4.081	54.762 ± 4.248	52.381 ± 5.802	59.372 ± 15.587	56.14
Watery	20.748 ± 0.601	35.034 ± 0.601	41.497 ± 1.202	64.966 ± 0.601	80.952 ± 0.601	94.217 ± 1.202	93.197 ± 0.601	93.06 ± 1.323	13.07
Compound A	20.748 ± 0.601	39.532 ± 0.601	52.262 ± 1.202	21.428 ± 0.601	25.17 ± 0.601	25.489 ± 0.601	24.499 ± 0.601	25.987 ± 0.531	9.69
Standard BHT	43.301 ± 1.40	53.582 ± 2.49	65.53 ± 1.132	74.82 ± 0.621	83.321 ± 0.782	87.412 ± 2.372	91.516 ± 1.113	94.702 ± 0.692	3.16

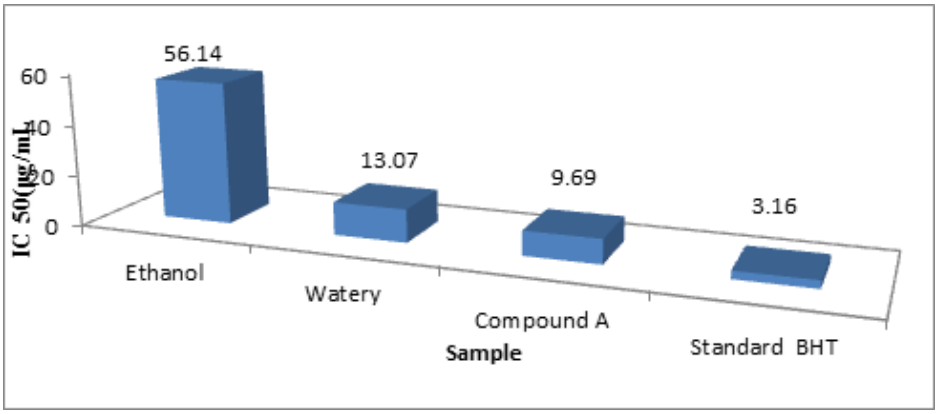


Figure 8: A bar graph of IC_{50} (µg/mL) values of different concentrations of watery, EtOH extracts and the isolated compound A from *E.odoratum*

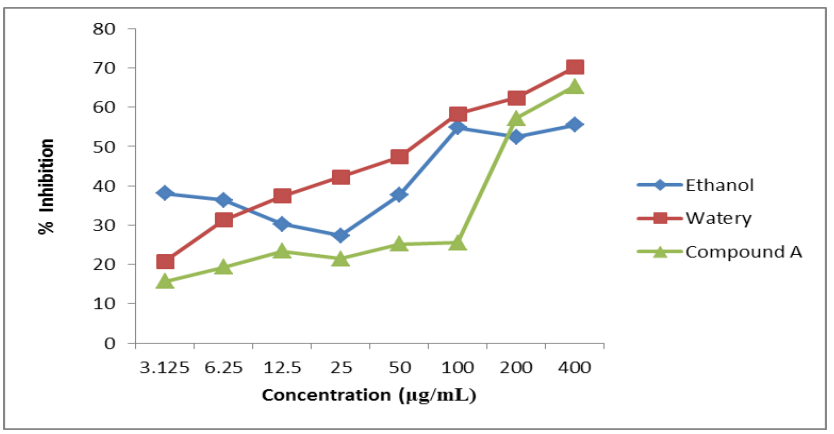


Figure 9: DPPH radical scavenging activity of different concentrations of watery, EtOH extracts and the isolated compound A from *E. odoratum*

Conclusion

The overall assessments of the research work, the preliminary phytochemical investigation indicated that alkaloids, α-amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids were present and *the* cyanogenic glycosides were not detected in *E. odoratum.*The nutritional values as 35.37 % of carbohydrates, 19.75% of protein, 24.31 % of fiber, moisture 9.54 % and ash 7.51 % were observed in it. The energy value was observed to be 185 kcal/ 100g in collected sample.According to qualitative elemental analysis carried out by AAS spectrometry, Ca and Mg were occurred higher than Fe contents in*E.odoratum*.In order to find out the bioactive organic constituents from active decolourized pet-ether extract, silica gel column chromatographic separation using PE/ EtOAc solvent system with variousratio were carried out. One of the bioflavonoid compound: 2´- hydroxy - 4, 4´, 5´, 6´ - tetramethoxychalcone (0.12%, R_f = 0.45, mp = 143-144 °C) was successfully isolated from *E.odoratum* by column chromatographic separation. This compound was identified structurally by modern spectroscopy: UV, FTIR, ^{1}HNMR,^{13}CNMR and EI-MS.*E. odoratum* was observed to possess the prevention of tumor formation with the doses of 25 μg/disc of methanol extract by Potato Crown Gall Test. The MeOH extract of *E.odoratum* was found to show the highest antimicrobial activity (35 mm) against on *Staphylococcus aureus*. The remaining extracts also showed the antimicrobial activity against six microorganisms. The isolated chalcone compound (IC_{50} = 9.69μg/mL) was more potent than the watery extract (IC_{50} = 13.07 μg/mL) and 95% ethanol extract (IC_{50} = 56.14 μg/mL) in the antioxidant activity. There is no lethality up to the dose of 5000 mg/kg of the both watery and ethanol extracts. Consequently, it can be deduced that *E.odoratum* leaves may be useful for the treatment of the diseases related to bacterial infection and antitumor drug in the formulation of Myanmar Traditional medicine.

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