Isolation and Functional Groups Identification of Pure Organic Compound fromthe Whole Plant of *Tradescantiazebrina* Bosse (YaeWarr) Khin Win*, Swe Swe Lwin**, San San Lwin***

Abstract

In this research, a pure organic compound was isolated from the whole part of YaeWarr. The sample was collected from Chan Aye Thar San Township in Mandalay Region. Before analyzing, phytochemical screening of the plant was carried out. Moreover, mineral contents of YaeWarr were determined byusing Energy Dispersive X-Ray Fluoresence Spectroscopy(EDXRF). Antimicrobial activities of this plant in various solvents were examined by means of Agar well diffusion method. In addition, the active compound (KW) was isolated from YaeWarr applying column chromatography. Then, thin layer chromatographic separation method was used for its purity. The pure compound could be identified by Fourier-Transform Infrared (FT-IR) spectrum.

Keywords—*Tradescantiazebrina*, phytochemical screening, elemental analysis, antimicrobial activity

Introduction

*Tradescantiazebrina*Bosse syn. *Zebrinapendula*Schnizl., commonly known as *Wandering Jew* is a herbaceous perennial plant with creeping shoots and fleshy leaves. The leaves are purple green in colour with broad, silvery stripes and purple undersides, simple, alternate, ovate with entire margin. Flowers are purple-pink and periodically throughout the year, fruits are oval and about less than 0.5 inch. The plant is native to the Gulf Coast region of eastern Mexico but naturalized in various parts of the world. It thrives well in a variety of soils and commonly distributed as a weed of waste areas, roadsides and forests in subtropical and warmer temperate regions (Gouri Kumar Dash *et al.,* 2017).

The chemical constituents of plants are complicated and monomeric compounds must be obtained via extraction and isolation before structure identification, bioactivity screening and so on.

Plants contain many kinds of natural organic compounds and their compoundspossesses many useful properties. Natural organic compounds are important for the development of medicines, pesticides, fragrances, cosmetics and synthetic chemicals.

The phytochemicals rich in plants have shown to be beneficial for prevention of diseases as well as long-term health. Plants are generally consumed as sources of essential

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compounds such as saccharides, coumarins, flavonoids, terpernoids and steroids. The aim of the research is to study and isolate the pure organic compounds from *Tradescantiazebrina* which was collected from Chan Aye Thar San Township in Mandalay region.

Botanical Description

Scientific Name	:	<i>Tradescantiazebrina</i> Bosse
Family	:	Commelinaceae
Myanmar Name	:	YaeWarr
Parts Used	:	whole plant

Medicinal Uses : Treatment of high blood pressure, coughs ,tuberculosis, to purify the blood, to reduce swellings hemorrhoids, to treat kidney infections, to treat gastrointestinal disorders, treatment of poisonous snake bite, leucorrhea, urinary infection, nephritis and inflammation. (Gouri Kumar Dash *et al.,* 2017)



Figure 1. The plant of *Tradescantiazebrina* Materials and Methods

Materials

Commercial grade reagents and solvents were used for analytical preparative thin layer chromatography and it was performed by using precoated silica gel (Merk, Co. Inc., Kieselgel 60 F_{254}) silica gel (70- 230 mesh ASTM) was used for column chromatography. Iodine vapour and UV detector were used for location of the spot on the TLC plates.

Instruments

- 1. FT-IR Spectrometer (Shimadzu, Japan)
- 2. UV lamp (Lambda 40, Perkin- Elmer Co., England)
- 3. The apparatus for extraction and chromatography were used with common laboratory tools.

Sample Collection

The whole plant of YaeWarr was collected from Chan Aye Thar San Township in Mandalay Region. These plants were washed and chopped into pieces and allowed to air dry for two months. The air dried sample was ground to powder, stored in well stoppered bottle and used throughout the experiments.

Phytochemical Screening of Tradescantiazebrina

The phytochemical screening of crude extract was carried out by using Harbone methods (Harbone, 1998).

Determination of Elemental Constituents of Tradescantiazebrina

The Energy Dispersive X-ray Fluorescence(EDXRF) spectrum of the sample was recorded on a Perkin Elmer 700, EDXRF Spectrometer of Department of Chemistry, Monywa University.

Antimicrobial Activities of Tradescantiazebrina

Different kinds of solvent extracts were checked for antimicrobial activities by using agar well diffusion method.

Extraction and Isolation of Pure Compound from Tradescantiazebrina

The air dried sample (YaeWarr) 200g was percolated with 2 liters of 95% ethanol for about two months. Ethanol percolated solution was filtered and concentrated by evaporation. The ethanol extract was dissolved in 200 mL of ethyl acetate solution. Ethyl acetate crude extract (2.33g) was separated by column chromatographic method by using silica gel with EtOAc and n-hexane with various ratios as eluents. Totally 110 fractions were obtained. Each fraction was checked by TLC and the same R_f values of the fractions were combined. Ten combined fractions were obtained. The colour of the compound was brown(oily-crystal form). The total yield of pure compound was 2.25% based on the ethyl acetate crude extract. The functional groups of the pure compound were investigated by FT-IR spectrum.

Results and Discussion

Phytochemical Screening of YaeWarr

Table 1.Phytochemical Screening of YaeWarr

No.	Constituents	Reagent Used	Observation	Inference
1.	Alkaloid	(I) Dragendorff'sreagent(2)Mayer's reagent	Brown solution Reddish brown color solution	+
2.	Flavonoid	ConcHCl+ Mg	green color solution	+
3.	Phenolic	10% FeCl ₃	Brown color solution	+

4.	Polyphenol	1%FeCl ₃ +1%K ₃ [Fe(CN) ₆]	Dark brown color solution	+	
5.	Glycoside	10% lead acetate	White ppt	+	
6.	Sugar	Benedict's solution	Red ppt	+	
7	Lipophilic		No deep color	_	
1.	проринис		solution	-	
8.	Saponin	NaHCO ₃	No frothing	-	
a	0 Tannin	Conc. H.SO. 1% FeCh	Pale yellow color	+	
9. Tannin		CONC. 112304 ,170 FEC13	solution		
10 Terpene		CHCl ₃ ,	Pale green color	+	
to. Terpene	Conc: H ₂ SO ₄	solution	•		
11	11. Steroid	CHCL Conc. H.SO. CH.COOH	Pale green color	+	
11.			solution		
	12. Carbohydrat	Molish's test			
12.		(α -naphthol and Conc:	white ppt	+	
	C	$H_2SO_4)$			

(–) = absence of constituent

ppt = precipitate

According to table (1), alkaloid, flavonoid, pheonlic compound, polyphenol, glycoside, sugar, tannin, terpene, steroid and carbohydrate were found respectively in the sample YaeWarr.

Quantitative Elemental Analysis of YaeWarr

Table 2. Elemental Analysis of YaeWarr

No.	Symbol	Elements	Concentration (%)
1	Са	Calcium	1.752 %
2	Si	Silicon	1.571 %
3	К	Potassium	1.421 %
4	Cl	Chlorine	0.789 %
5	Al	Aluminium	0.685 %
6	S	Sulphur	0.173 %
7	Fe	Iron	0.125 %
8	Р	Phosphorus	0.093 %
9	Ti	Titanium	0.015%

10	Mn	Manganese	0.005%
11	Zn	Zinc	0.004%
12	Cu	Copper	0.002%

According to the results of elemental analysis, calcium, silicon, potassium and chlorine were found to be high amount in the sample. Decreasing order of elemental composition of sample was Ca>Si >K >Cl>Al>S >Fe >P >Ti >Mn>Zn >Cu. The toxic metals such as copper were observed in this sample but the amount of this metal was very traceable. So, it was not toxic for human health.



Figure 2. The Elemental Contents of the Sample by EDXRF Method

Antimicrobial Activities of YaeWarr

The antimicrobial activities of the crude extracts with various solvent systems are recorded in Table 3.

Table3.Antimicrobial Activities of YaeWarr

Sample	Organisms	Solvents			
		n-hexane	H ₂ O	EtOAc	EtOH
YaeWarr	I	-	+	++	++
	П	-	+	++	++
	Ш	-	+	+++	++
	IV	-	+	+++	++
	V	-	+	+++	++
	VI	-	+	++	++
Agar well - 8mm	(-) = no activ	vity	•	

9 mm ~ 14 mm (+)

(+) = Low activity

- 15 mm ~ 20 mm (++) (++) = Moderate activity
- 21 mm above (+++) (+++) = High activity

Organism; I = *Bacillus subtilis*

II = Candida albicans

III = Escherichia coli

IV = Pseudomonousaeruginosa

- V = *Staphylococusaureus*
- VI = Salmonella typhi

According to the results of antimicrobial activities, EtOAc extract of YaeWarr respond the highest activities on three microorganisms and medium activity on three microorganisms. The EtOH extract of this sample gave the medium activity on all organisms. The results of antimicrobial activities agree to treat diuretic, dysenteric, diarrheal, cold and cough, tuberculosis, swellings hemorrhoids, cancer, kidney infections, gastrointestinal disorders and inflammation.



Figure 3. FT-IR Spectrum of Pure Organic Compound (YaeWarr)

Determination of functional Groups in Isolated Pure Compound

FT-IR spectrum was taken at the Department of Chemistry, Monywa University to confirm the pure compound as isolated. This spectrum represents some prominent functional groups containing in the pure compound. In FT-IR spectrum, the broad band which appears at 3423.99 cm⁻¹ should be –OH stretching vibration of alcohol group. The peaks at 2932.36 cm⁻¹ and 2863.95cm⁻¹

¹ indicate the asymmetric and symmetric stretching vibration of sp³ hydrocarbon. On the other hand, C=C stretching vibration of alkenic group is detected at 1620.3 cm⁻¹.

Moreover, C-H out of plane bending vibration of gem-dimethyl group is also observed at 1462.85 cm⁻¹. The band at 1381.61 cm⁻¹ implies the C–O stretching vibration of secondary alcohol. The peak which appear at 1268.26 cm⁻¹ indicates the C-O stretching vibration of alcohol group. In addition, C-O-C stretching vibration of ether group could be detected at 1102.35 cm⁻¹. On the other hand, the two bands at (970.17 and 800.65) cm⁻¹ are also due to C-H out of plane bending vibration of cis or Z and trans or E alkenic groups respectively. The functional groups containing in this pure compound are tabulated in Table 4.

Table 4. Functional Groups Presented in FT-IR Spectrum of Pure Organic Compound from
YaeWarr

No	Wave Number (cm ⁻¹)	Functional Groups
1	3423.99	O-H Stretching vibration (broad band)
2	2932.36, 2863.95	Symmetric and asymmetric sp ³ C-H Stretching vibration of sp ³
		hydrocarbon
3	1620.3	C = C Stretching vibration of alkenic group
4	1462.85	sp ³ –CH out of plane bending vibration of gem-dimethyl group
5	1381.61	C–O stretching vibration of secondary alcohol
6	1268.26	C-O stretching vibration of alcohol group
7	1102.05	C-O-C ether group
8	970.17	C-H out of plane bending vibration of cis or Z alkene
9	800.65	C-H out of plane bending vibration of trans or E alkenic group

Conclusion

The sample(YaeWarr) was collected from Chan Aye Thar San Township in Mandalay Region. In this research, the phytochemical tests, elemental analysis and antimicrobial activities of the sample (YaeWarr) were performed. The sample revealed the presence of alkaloid, flavonoid, phenolic compound, polyphenol, glycoside, sugar, tannin, terpene, steroid and carbohydrate respectively.

Elemental analysis of this sample was performed by using EDXRF method and the results indicated that calcium content is the highest in the sample. (Calcium that helps regulate the passage of nutrients in and out of the cell wall and lowers blood pressure, is important to normal kidney function and reduces blood cholesterol levels.) (http://www.ijrpbsonline.com, 2013)

Moreover, the antimicrobial activities of the crude extract in various solvent system were tested by agar well diffusion method on six selected organisms. According to the results of antimicrobial activities, the ethyl acetate extract of YaeWarr gave rise to high activities on all tested

organisms and the ethanol extract of the sample gave rise to medium activities on all tested organisms.

A pure compound was isolated by using column and thin layer chromatography. The yield percent of this compound was found to be 2.25 % based upon the crude extract. The FT-IR spectrum of this compound revealed the presence of alcohol, alkenic hydrocarbon, sp³ hydrocarbon, allylic hydrocarbon, gem dimethyl, ether group, and (Cis or Z and Trans or E) alkenic functional groups, respectively.

Isolation and characterization resulted in the identification of the compound from the extract of YaeWarr. This compound may be β -sitosterol which possesses potent hypotensive activity.

The molecular formula of β -sitosterolis C₂₉H₅₀O. It is a sterol (phytosterol) compound which has strong antioxidant and antitumor activity. In this regard, further studies need to explore the plant of YaeWarr for its potential in preventing and treating diseases.

Acknowledgements

We would like to thank Rector Dr Tin Moe Thu Zar and Pro-rectors U KhinMyot, Dr. MyintMyintOo and Dr. Khin Maw MawSoefor their permission to do this research. Moreover, we are also grateful to DrSuuSuu Win, Professor and Head and Dr Chaw TheingiKhin, Professor, Department of Chemistry, Yadanabon University, for their permission, suggestion and supporting all the requirements to do this research. We are also thankful to our friends for their kind support.

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