Investigation On Antioxidant, Glucose Lowering Activities And Functional Group Determination Of Organic Compounds Isolated From The Plant Of *Phyllanthus virgatus* G. Forster

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Abstract

In this research work, the whole plant of *Phyllanthus virgatus* G. Forster was collected from Shwebo Township, Sagaing Region, for chemical analysis. The phytochemical constituents of sample were determined by phytochemical screening methods. The antimicrobial activities of three extracts such as n-hexane, ethyl acetate and ethanol of sample were tested by Agar-well diffusion method on six tested organisms. Moreover, the glucose lowering activity of sample was examined by iodometric titration method. The antioxidant activity of ethanol extract of sample was determined by using DPPH radical scavenging assay. The elemental contents in sample were measured by EDXRF method. In addition, pure compound I and II were isolated from the sample by using Thin Layer and Column Chromatographic methods. These isolated pure compounds were identified by FT-IR spectral data. From melting point determination, color test and identification of compound II by FT-IR spectral data, the compound II could be assigned as ethyl chlorogenate.

Keywords: *Phyllanthus virgatus* G. Forster, phytochemical, antioxidant, iodometric, chromatographic

Introduction

Phyllanthus virgatus G. Forster belongs to the family Euphorbiaceae. These families comprise about 300 genera and 7500 species. In Myanmar, *Phyllanthus virgatus* G. Forster is locally an abundant weed plants occurring in the whole country. It's commonly known as Taw-zee-phyu in Myanmar. In Myanmar, Taw-zee-phyu is natural abundant weed plant in rainy season. (Han Shu, 2012)

Medicinal plants from the backbone of traditional medicine in the last few decades with intense pharmacological studies. Infusion of the young shoots is given in dysentery. Fresh root is used as a remedy for jaundice. A decoction of the leaves is used a refrigerant for scalp; leaves and roots are made into a populace with rice water for application on edematous, swellings and ulcers.(Website 2)

In this research work, one of Myanmar indigenous medicinal plants, *Phyllanthus virgatus* G. Forster was selected for chemical investigation. Organic compounds were isolated from the plant of *Phyllanthus virgatus* G. Forster by using Thin Layer and Column chromatographic methods. These isolated compounds were identified by FT-IR spectroscopy.

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Botanical Description

Family name : Euphorbiaceae
Scientific name: *Phyllanthus virgatus* G. Forster
Myanmar name: Taw-zee-phyu
English name : Quail Grass

Figure (1) The Plant of *Phyllanthus virgatus* G. Forster

EXPERIMENTAL

Instrumentation and Materials

The occurrence of UV absorption on TLC plate was checked by UV detector and iodine vapor. Analytical and preparative thin-layer chromatography was performed by using precoated silica gel plates. Silica gel (Merck-Co, Inc., Kiesel gel 60, 70-230 Mesh ACTM) was used for column chromatography.

Sampling

The sample of *Phyllanthus virgatus* G. Forster for chemical analysis was collected from Shwebo Township, Sagaing Region.

The sample was cut into small pieces and dried in air for about three weeks. It was stored in a well- stopper bottle and used throughout the experiment.

Preliminary Phytochemical Test

Preliminary phytochemical test of sample was carried out by usual method. The results are shown in Table (1)

Antimicrobial Activities

Antimicrobial activities of the crude extract of the whole plant of *Phyllanthus virgatus* G. Forster were tested in various solvent systems by using Agar well diffusion method on six selected organisms in CRDT (Central Research Development and Technology), Insein, Yangon as shown in Figure (2). The results are shown in Table (2).

Glucose Lowering Activity

Glucose lowering activity of ethanol extract of the whole plant of *Phyllanthus virgatus* G. Forster was done by using iodometric titration method. The results are shown in Table (3).

Elemental Composition

Elemental contents of sample were measured at Department of Physics, University of Mandalay by Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF). The results are shown in Table (4).

Extraction and isolation

The sample 500 g was percolated with 95 % ethanol 1200 ml for about two months and then filtered and the filtrate was concentrated. The residue was re-extracted with 300 ml of ethyl acetate.The EtOAc extract (2.1g) was separated by column chromatography using silica gel and eluent as n-hexane and ethyl acetate. The pure compound I (yellow amorphous) and compound II (colourless amorphous) were obtained.

Antioxidant Activity

The antioxidant activity of ethanol extract of the whole plant of *Phyllanthus virgatus* G. Forster was done by using DPPH Radical Scavenging Assay.

Functional Groups Determination

The FT-IR spectrum informs the prominent functional groups containing in the compound. The FT-IR spectra of the isolated compound I and II were measured at the Department of Chemistry, University of Mandalay. The infrared spectrum of compound I and II were described in Figure (5) and Figure (6).

Determination of Melting Point

The melting point of isolated compound II was measured by melting point Apparatus (SMP-30).

Results and Discussion

The results of phytochemical constituent of the whole plant of *Phyllanthus virgatus* G. Forster are shown in Table (1).

Table (1) Results of Preliminary Phytochemical Test of Sample

No.	Constituents	Reagent used	Observation	Result
1.	Alkaloid	(1)Wagner's reagent (2)Dragendorff's reagent	Reddish brown ppt Orange ppt	+ +
2.	Tannin	10%$FeCl_3$,dilH_2SO_4	Red color solution	+
3.	Saponin	Distilled water	Froth like comb	+
4.	Flavonoid	conc: HCl, Mg turning, Δ	Pink color solution	+
5.	Terpene	Pet-ether,acetic anhydride,$CHCl_3$, conc: H_2SO_4	No Red color solution	–
6.	Glycosides	10 % lead acetate	White ppt	+
7.	Steroids	Acetic anhydride,$CHCl_3$, conc: H_2SO_4	Green color solution	+
8.	Phenolic	10 % $FeCl_3$	Greenish blue color solution	+
9.	Polyphenol	1 % $FeCl_3$, 1 % $K_3[Fe(CN)_6]$	Greenish blue color solution	+
10.	Reducing sugar	Benedict solution	Brick-red ppt	+
11.	Lipophilic	0.5 M KOH,NaOH	Deep color solution	+

(+) = presence of constituent (–) = absence of constituent According to this table, *Phyllanthus virgatus* G. Forster extract consist of alkaloid, tannin, saponin, flavonoid, glycosides, steroid phenolic, polyphenol, reducing sugar and lipophilic respectively.

The results of antimicrobial activities of the whole plant of *Phyllanthus virgatus* G. Forster are shown in Table (2).

Table (2) Antimicrobial Activities of *Phyllanthus virgatus* G. Forster

Sample	Solvents	Inhibition Zone					
		I	II	III	IV	V	VI
Taw-zee-phyu	n-hexane	11mm (+)	11mm (+)	–	–	11mm (+)	–
	EtOAc	14mm (+)	14mm (+)	–	13mm (+)	13mm (+)	14 mm (+)
	EtOH	12mm (+)	13mm (+)	–	13mm (+)	13mm (+)	13 mm (+)

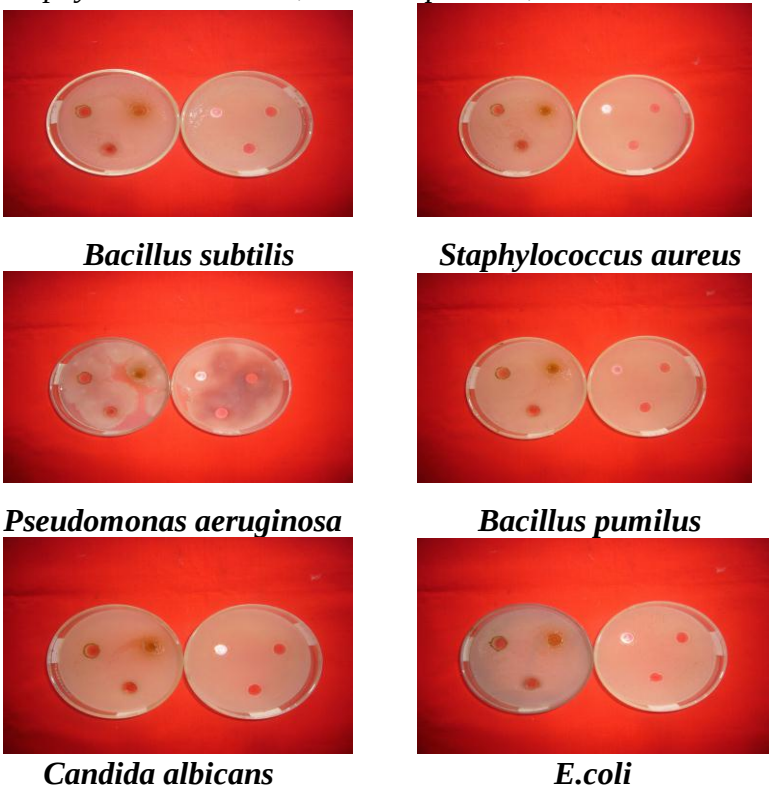
Agar well – 10 mm

10 mm ~ 14 mm (+)
15 mm ~ 19 mm (++)
20 mm above (+++)

Organisms

I= *Bacillus subtilis*
II = *Staphylococcus aureus*
III= *Pseudomonas aeruginosa*
IV= *Bacillus pumilus*
V= *Candida albicans*
VI= *E. coli*

According to this table, n-hexane extract of the whole plant of *Phyllanthus virgatus* G. Forster responds low activities on *Bacillus subtilis, Staphylococcus aureus* and *Candida albicans.* Ethyl acetate and ethanol extracts of the sample respond low activities on *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus, Candida albicans* and *E. coli.*



Bacillus subtilis ***Staphylococcus aureus***

Pseudomonas aeruginosa ***Bacillus pumilus***

Candida albicans ***E.coli***

Figure (2) Antimicrobial Activities of *Phyllanthus virgatus* G. Forster

The results of glucose lowering activity of the whole plant of *Phyllanthus virgatus* G. Forster are shown in Table (3).

Table (3) Percent of Decrease in Amount of Glucose

No.	Contact time (min)	Initial amount of glucose (mmol)	Left amount of glucose (mmol)	Decrease amount of glucose (mmol)	**%** of decrease inamountof glucose
1.	30	7.5	7.36	0.14	1.867

According to this table, the percent of decrease in amount of glucose for *Phyllanthus virgatus* G. Forster was found to be 1.867.So; this plant may be beneficial for diabetes patient.

The results of elemental contents of the whole plant of *Phyllanthus virgatus* G. Forster are shown in Table(4).

Table(4) Results of Elemental Contents of Sample

Elements	**Relative Abundance (%)**
Chlorine	1.666
Potassium	1.556
Calcium	1.201
Silicon	0.564
Phosphorus	0.239
Aluminum	0.199
Iron	0.151

According to this table, sample consists of Chlorine, Potassium, Calcium, Silicon, Phosphorus, Aluminum and Iron respectively.

The results of antioxidant activity of sample by using DPPH assay in standard ascorbic acid are shown in Table (5).

Table (5) % Inhibition of Various Concentration of Standard Ascorbic Acid

Concentration (μg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (μg/mL)
50	0.168	82.80	8.23
25	0.279	71.44	
12.5	0.4	59.06	
6.25	0.506	48.21	
3.125	0.608	37.77	

IC_{50} value was calculated by using linear regressive equation

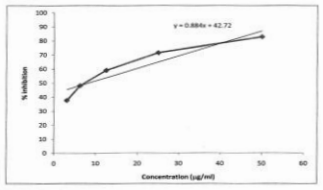


Figure (3) Plot of % Inhibition Vs Concentration of Standard Ascorbic Acid

The results of antioxidant activity using DPPH assay in ethanol extract of sample is shown in Table (6).

Table (6) %inhibition of Various Concentration of Sample

Concentration (μg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (μg/mL)
200	0.123	71.26	110.9
100	0.227	46.96	
50	0.243	38.31	
25	0.278	30.37	
12.5	0.331	22.98	
6.25	0.352	18.22	

IC_{50} value was calculated by using linear regressive equation.

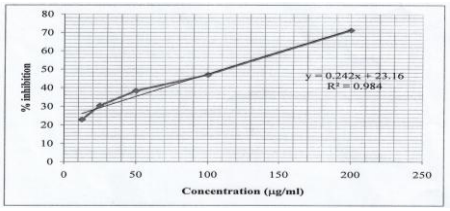


Figure (4) Plot of % Inhibition Vs Concentration of the *Phyllanthus virgatus* G. Forster

According to this table, the IC_{50} value of sample was found to be 110.9 μg/mL. It was higher than that of standard ascorbic acid (IC_{50} = 8.23 μg/mL). So, the sample extract has lower antioxidant activity than standard ascorbic acid. From FT-IR spectrum Figure(5), the functional groups of compound I consists of N-H stretching,sp^2 and sp^3 hydrocarbons, carbonyl group,N-H bending,C-N stretching, ether group,cis or Z alkenic group and N-H wagging. From FT-IR spectrum Figure(6), the functional groups of compound II consists of OH group,sp^2 andsp^3hydrocarbons, carbonyl group , aromatic benzene ring, O-H in plane bending vibration ,C-H stretching vibration, C-O stretching vibration, ester group, trans or E alkenic group and out of plane O-H bending vibration respectively.

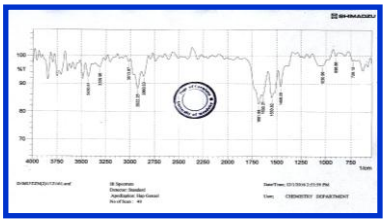


Figure (5): FT-IR Spectrum of Compound I

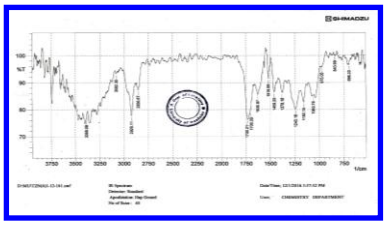


Figure (6): FT-IR Spectrum of Compound II

The result of melting point of pure compound II was found to be 207°C-208°C which is closely satisfied with the reference value of ethyl chlorogenate (207°C-209°C).

Conclusion

In this research work, one of Myanmar indigenous medicinal plants, *P. virgatus* G. Forster was collected from Shwebo Township, Sagaing Region. According to phytochemical screening, sample gave positive test for alkaloid, tannins, saponin, flavonoid, glycoside, steroid, phenolic, polyphenol, reducing sugar and lipophilic respectively. From antimicrobial activity result n-hexane extract of sample gave low activities *on Bacillus subtilis, Staphylococcus aureus* and *Candida albicans.* EtOH and EtOAc extracts of sample also show low activities on *Bacillus subtilis, Staphylococcus, Bacillus pumilus, Candida albicans, E.coli.* The percent of decrease amount of glucose for sample was found to be 1.867%.According to experimental data, the ethanol extract of sample(IC_{50} 110.9µg/mL)was found to be lower antioxidant activity than standard ascorbic acid(IC_{50} 8.23µg/mL),According to EDXRF data, the sample consists of Cl (1.666%), K(1.556%), Ca(1.201%), Si(0.564%), P(0.239%), Al(0.199%) and Fe(0.151%).

The yield percents of compound I and II were found to be 1.90% and 1.43% based upon ethyl acetate crude extract. According to FT-IR assignments, the compound I consists of N-H stretching, sp^2 hydrocarbon, sp^3 hydrocarbon, carbonyl group, alkenic group, N-H bending, C-N stretching and N-H wagging respectively. So, the compound I may be amide compound. The compound II contains O-H group, sp^2 hydrocarbon, sp^3 hydrocarbon, carbonyl group, aromatic benzene ring, C-O stretching, ester group, trans or E and cis or Z alkenic groups. According to the prominent functional groups, color test and melting point value (207°C-208°C) of compound II could be assigned as ethyl chlorogenate.

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