Antibacterial and Antioxidant Activities of Crude Extractsfrom Root of *CocciniaGrandis*L.and Isolation of Bioactive Organic Compounds

Khin Myo Myint[1], Su Lay Yee[2], Ni Ni Pe[3], Myint Myint San[4]
drkhinmyo11@gmail.com

Abstract

The roots of *Cocciniagrandis*L. were collected from Shwebo Township, Sagaing region, Myanmar. Phytochemical compounds present in the sample were carried out by common test tube method. It informed the constituents of alkaloid, saponin, glycoside, phenolic, tannin, polyphenol, terpene, steroid and flavonoid respectively. Mineral contents were measured by Energy Dispersive X-ray Fluorescence method. Calcium, Potassium, Silicon, Sulphur, Strontium and Iron are more abundant than others. Antibacterial activity of the sample could be examined by Agar well diffusion method on seven bacteria species using three kinds of solvent. Chloroform extract and ethyl acetate extract of the sample responded only *Enterococcus faecalis* and *Shigella*. Ethanol extract inhibited on *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella* and *Pseudomonus aeruginosa*. According to the result of antioxidant activity by DPPH assay, IC_{50} values of ascorbic acid and crude extract of sample are 0.3316 µg/mL and 3.67 µg/mL. Moreover, the two bioactive organic compounds were isolated from the root by thin layer and column chromatography. Functional groups present in the compounds were determined by FT-IR spectrometry.

Keywords: *Cocciniagrandis*(L.), EDXRF, Agar well, DPPH, FT-IR

Introduction

A vast majority of the population, particularly those living in rural depends largely on medicinal plants for treatment of diseases. Herbal drugs have many advantages over the synthetic formulations in having a longer pharmacological effect and lesser metabolic toxicity. The WHO estimates that about 80% of the populations living in the developing countries rely almost on traditional medicine for their primary health care needs. Plants have played a significant role in maintaining human health and improving the quality of human life (Pekamwar, 2013).During the past several years, there has been increasing interest among the uses of various medicinal plants from the traditional system of medicine for the treatment of different ailments. Medicinal plants are a source of great economic value all over the world (Arshad Husain *et al*., 2010).

However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the plant. Importance of the plant lies in their biologically active principles. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls, etc. The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds (Arora and Kaur, 1999).

*Cocciniagrandis*L. is not only a medicinal plant but also editable vegetable. The leaves and fruits are eaten as vegetable.The plants are widely found in Myanmar.Traditionally, this plant is used in the treatment of diabetes, laxatives, and fevers. The aim of the research work is to investigate thephytochemical and mineral constituents, antibacterial activity, and antioxidant activity of the crude extract and to isolate the bioactive organic compounds from the root of the plant.

Figure 1. Plant of *Cocciniagrandis*L.

Cocciniagrandis L. belongs to family Cucurbitaceae, commonly known as Ivy gourd and locally known as Kinbon; Taw-kinbon. *Cocciniagrandis* is used by humans mostly as a food crop in several countries in India, Australia, Myanmar, Southern United States and Pacific Island. *Cocciniagrandis* is a fast-growing perennial vine that grows several meters long. It can form dense mats on lands that readily cover shrubs and small trees.The roots and stems are succulent, tuberous and most likely facilitate the plant to survive prolonged drought. (Sureshbabu*et.al*., 2001; Maurice *et.al*., 2012; Ajmal*et.al*.,2006). *Cocciniagrandis* has the ability to control blood sugar levels in a natural way. This is because of the liver enzyme in the vegetable that controls the metabolism rate of sugar in the body with the process of improving glucose production (Sridevi, 2014).

Materials and Methods

Sample collection and prepration

The root of *Cocciniagrandis* were collected from Shwebo Township, Sagaing Region, Myanmar. The root sample was washed into natural water and it was cut into small pieces and dried at room temperature. And then, the sample was stored in clean and dried bottle.

Preliminary phytochemical tests of the root of *Cocciniagrandis*

Phytochemical Screening of the root of *Cocciniagrandis* was investigated by using preliminary phytochemical screening (Harbone, 1993).

Elemental analysis

Elemental contents in the root of *Cocciniagrandis*were determined by EDXRF method at Department of Chemistry, Monywa University and EDXRF spectrum was shown in Figure 3.

Determination of free radical scavenging activity by DPPH assay

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g.,a free radical scavenging antioxidant) and is reduced to the DPPHH and as

Figure 2. Sample preparation ofthe root of *Cocciniagrandis*

consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured (Philips, et al., 2010).

Determination of antibacterial activity of the root of *Cocciniagrandis*

Antibacterial activities of the root of *Cocciniagrandis*were tested by ager-well diffusion method at Biotechnology, Mandalay Technology University.

Isolationof bioactive organic compounds from the Root of *Cocciniagrandis*

Ethanol crude extract from raw sample (650 g) was re-extracted with ethyl acetate. Ethyl acetate crude extract (3.2 g) was separated by column chromatography using silica gel (70 -230 Mesh Size) as stationary phase and eluent as n-hexane and ethyl acetate. The functional groups present in the bioactive compound were determined by FT-IR spectrometry.

Results and Discussion

Table 1. Phytochemical Constituents ofthe Root of *Cocciniagrandis*

No.	Tests	Reagents	Observation	Results
1.	Alkaloid	Wagner's	Orange ppt	+
		Dragendroff's solution	Yellow ppt	+
2.	Saponin	distilled water	frothing	+
3.	Glycoside	distilled water, 10% lead acetate	White ppt	+
4.	Phenolic	distilled water, 10% $FeCl_3$	Bluish greensolution	+
5.	Tannin	distilled water,10% $FeCl_3$,1% Gelatin	Brown solution	+
6.	Reducing sugar	distilled water, Benedict's solution	Reddish browns olution	-
7.	Polyphenol	EtOH, 10% $FeCl_3$,1% K_3 $[Fe(CN)_6]$	Greenish bluesolution	+
8.	Terpene	$CHCl_3$, acetic anhydride, conc:H_2SO_4	Reddish brownlayer	+
9.	Steroid	Pet-ether, acetic anhydride, conc:H_2SO_4, $CHCl_3$	Reddish brown ring	+
10.	Flavonoid	EtOH, conc: HCl, Mg turning	Pink solution	+

(+) = present, (-) = absent

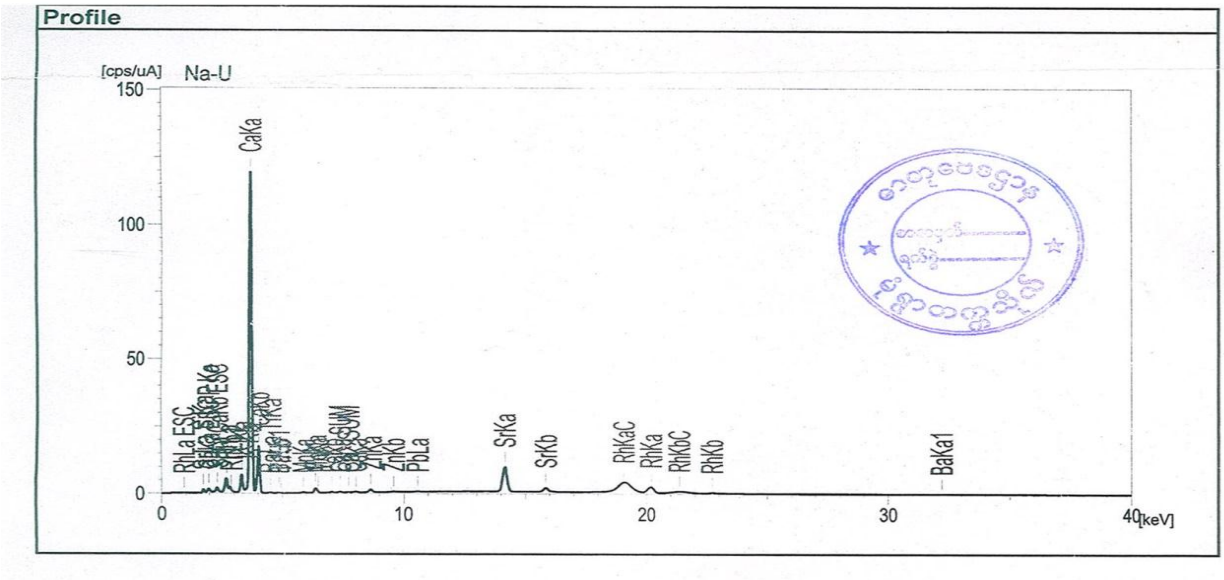

Figure 3. EDXRF Spectrum of Root of *Cocciniagrandis*L.

Table 2. Relative Abundance of Elements Present in the Root of*Cocciniagrandis* L.

No.	Elements	Relative Composition (%)
1.	Ca	5.728
2.	K	0.486
3.	Si	0.417
4.	S	0.160
5.	Sr	0.038
6.	Fe	0.022
7.	Ba	0.017
8.	P	0.008
9.	Zn	0.007
10.	Ti	0.006
11.	Mn	0.005
12.	Cu	0.003

In accordance with the result of elemental analysis, calcium (Ca) was found to be the highest content in the root sample. In the sample, calcium (Ca), Potassium (K), Slicion (Si), Sulphur (S) and Strontium (Sr), Iron (Fe), and Barium (Ba) were observed as 5.728%, 0.486%, 0.417%, 0.160%, 0.038%, 0.022, 0.017%, and the remaining elements are very small amount.

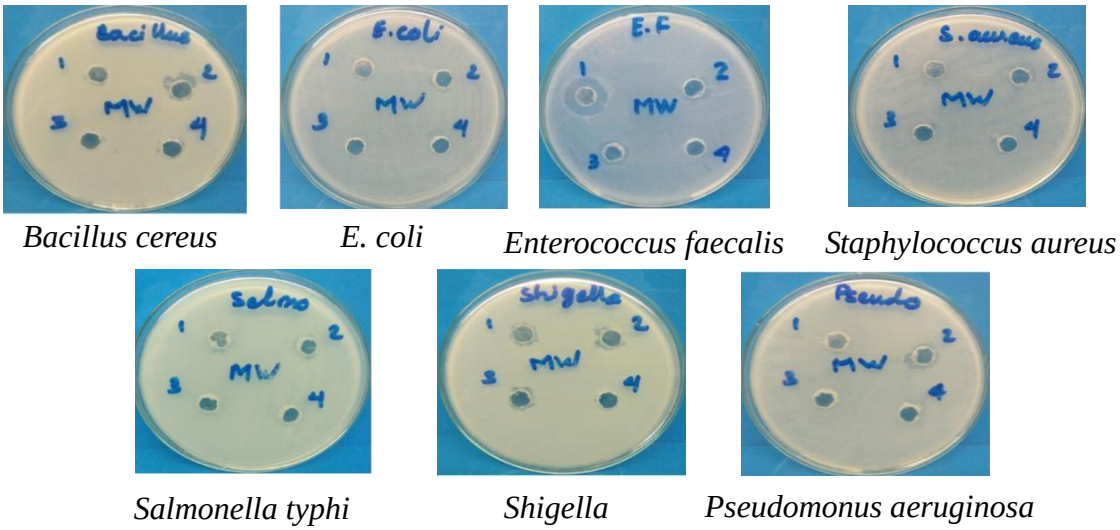

Figure 4.Antibacterial activity of the Root of*Cocciniagrandis*

Antibacterial activities of the Root of*Cocciniagrandis*were tested by Agar-well diffusion method at Biotechnology Department, Mandalay Technology University. The results are shown in Table.According to the results, chloroform extract was significantly responded on *E.faecalis*and low responded on *Shigella* but other bacteria were not inhibited. Ethanol extract was high activity on two bacteria such as *B.cereus*and *P.aeruiginosa*. Ethyl acetate extract gave raise to low activity on *E.faecalis*and *Shigella*.

Table 3. Antibacterial Activity of Aerial Parts ofthe Root of*Cocciniagrandis* L.

No.	Test microorganisms	Inhibition zone of different crude extracts(mm)		
		$CHCl_3$	EtOH	EtOAc
1.	*B.cereus*	-	12	-
2.	*E.coli*	-	-	-
3.	*E.faecalis*	18	-	10
4.	*S.aureus*	-	10	-
5.	*S.typhii*	-	10	-
6.	*Shigella*	10	10	9
7.	*P.aeruginosa*	-	12	-

Agar well diameter= 8 mm

Table 4. DPPH Free Radical Scavenging Activity ofEthanol Extract And Standard Ascorbic acid

Extract	Concentration (μg/ mL)	Mean absorbance	Mean % Inhibition	IC_{50} (μg/ mL)
EthanolExtract (Sample)	2	0.2183	46.84685	3.67
	4	0.2028	50.62089	
	8	0.2003	51.22961	
	16	0.1728	57.92549	
	32	0.1204	70.6842	
Ascorbic Acid	0.25	0.2156	47.50426	0.3316
	0.5	0.1842	55.14974	
	1	0.1588	61.33431	
	2	0.1147	72.07207	
	4	0.0508	87.63087	

According to the result, the ethanol crude extract of the root gave the IC_{50} values as 3.67μg/mL and standard ascorbic acid was 0.3316 μg/mL. It was found that high contents of antioxidants are present in the root of the plant.

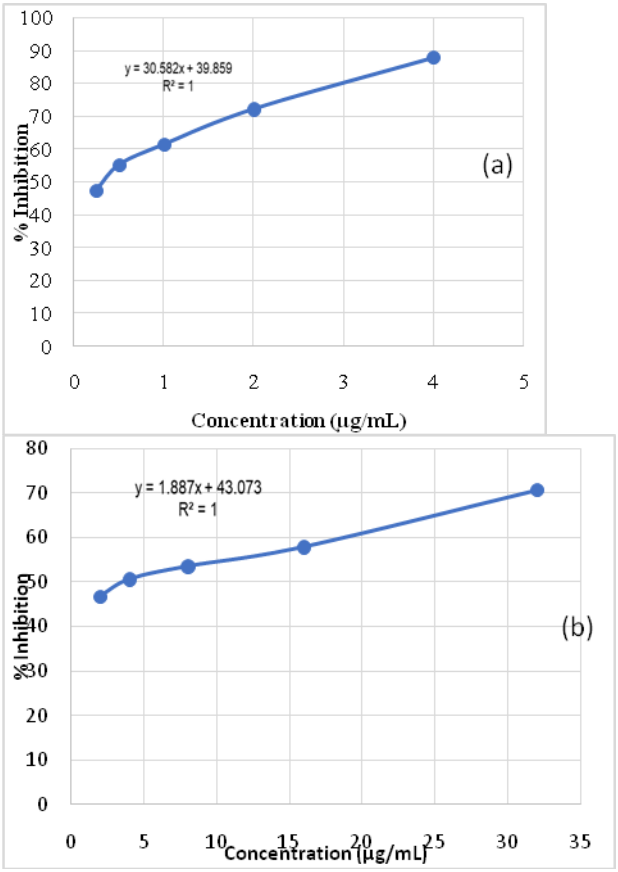

Figure 5. (a) The plots of % inhibition Vs concentration of ascorbic acid
(b) The plot of % inhibition Vs concentration of sample

In the FT-IR spectrum of compound-I, the presence of alcohol group were observed at 3354.50 $cm^{-1}$and 3203.8 cm^{-1}. The band appeared at 2921.57 cm^{-1} and 2851.19cm^{-1} indicated the CH stretching vibration of asymmetrical and symmetrical sp^3 hydrocarbons. The C=C stretching mode of unconjugated methylene group displayed at 1660 cm^{-1}.The C-H bending vibration of asymmetrical methyl group was found at 1455.79 cm^{-1}. The band appeared at 1390.32 $cm^{-1}$and 1269.17cm^{-1}showed the C-H bending vibration of gem-dimethyl group and methylene group. The appearance of O-H bending vibration was also observed at 1168.51 cm^{-1}. In addition, the C-O stretching vibration of alcohol group was shown at 1123.53 $cm^{-1}$and 1038.44 cm^{-1}.

According to FT-IR spectral data of pure compound -II, the N-H stretching vibration of amide was observed at 3331.08 cm^{-1} and 3206.63 cm^{-1}. The peak at 2953.96 cm^{-1} indicated C-H stretching vibration of sp^2hydrocarbons. The C-H stretching vibration of asymmetrical and symmetrical stretching of sp^3 hydrocarbons were occurred at 2915.03 cm^{-1} and 2847.50 cm^{-1}. The band appeared at 1666.90 cm^{-1} indicated C=O stretching vibration of methylene group. Moreover, the N-H bending vibration was observed at 1618.62 $cm^{-1}$and 1542.44cm^{-1}. The presence of C-N stretching vibration of amide was observed at 1465.43cm^{-1}.Furthermore, the N-H in plane bending vibration of amide was observed at 1021.20cm^{-1}, 963.49 cm^{-1} and 873.59 cm^{-1}. In addition, the peak at 799.38cm^{-1}indicated N-H out of plane bending vibration of amide group, respectively.

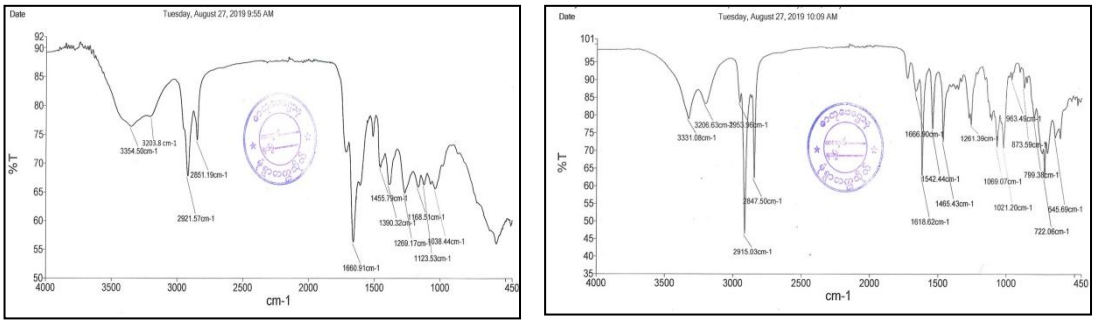

Figure 6.FT-IR spectrums of compound-I and coumpound-II

Conclusion

In accordance with the preliminary phytochemical screening, the root of *Cocciniagrandis*consists of alkaloid, glycoside, saponin, tannin, phenolic, polyphenol, terpene, steroid and flavonoid respectively. But reducing sugar was not observed in this plant sample. Due to the constituent of alkaloid it may be useful for diabetes. It can provide to reduce blood sugar level. Analysis of elemental composition indicated that Calcium is the most abundance mineral. It can help to build up strong bone condition.

Chloroform extract of the sample was high antibacterial activity on *Enterococcus faecalis*. Ethanol extract responded on five bacteria species except *E.coli* and *E.faecalis*. Inhibition of the ethyl acetate extract was found on *E.faecalis* and *Shigella*. Therefore, the sample is useful for prevention of infectious diseases and intestinal diseases such as diarrhea.In accordance with the IC_{50} values, crude extract

sample has free radical scavenging activity. So, some bioactive compounds may be present in the root of *Cocciniagrandis*.

FT-IR spectrum of compound-I informed that hydroxyl group, methyl group, methylene group and gem dimethyl group were observed in the compound. Furthermore,FT-IR spectrum of compound-II showed constituents of amide, carbonyl and C-N groups, respectively.

Therefore, the research work can providesome medicinal capacity due to the constituents of bioactive phytochemical compounds, antibacterial activity and antioxidant property of the root of *Cocciniagrandis*.

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