INVESTIGATION OF CHEMICAL PROPERTIES AND BIOLOGIICAL ACTIVITIES OF STEM OF *Coccinia cordifolia* COGN. (KIN-PON) AND BARK OF *Dolichandrone serrulata* SEEM. (THA-KHUT)

Kyaw Khaing[1], Aung Phyo Naing[2], Khine Zar Wynn Lae[3], Khin Thida Nyo[4], Daw Hla Ngwe[5]

Abstract

In this research work, stem of *Coccinia cordifolia* Cogn. (Kn-pon) and bark of *Dolichandrone serrulata* Seem. (Tha-khut) were collected from Yekyi Township, Ayarwaddy Region for the investigation of chemical and biological properties such as antimicrobial activity, antioxidant activity, anti-arthritic activity and antitumor activity due to lack of scientific report on these two locally cultivated medicinal plants. Relative abundances of elements analysed by EDXRF showed the presence of calcium, potassium, sulphur, manganese, iron, stronium, zinc and copper in the stems of Kin-pon and calcium, potassium, sulphur, zinc, copper and rubidium in the barks of Tha-khut. Determination of nutritional values has also screen carried out by AOAC method resulting moisture (10.69 %), ash (11.81 %), protein (8.71 %), fiber (36.90 %), fat (0.89 %), carbohydrate (31.00 %) and energy value (169 kcal/100 g) in stems of Kin-pon and moisture (11.25 %), ash (9.55 %), protein (2.29 %), fiber (36.17 %), fat (0.08 %), carbohydrate (40.66 %) and energy value (173 kcal/100 g) in the barks of Tha-khut. In the stems of Kin-pon and the barks of Tha-khut, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starchs, tannins, terpenoids and steroids were found to be present according to preliminary phytochemical tests. However, cyanogenic glycosides were not found in these samples Total phenol contents, total flavonoid contents and reducing ability of the ethanol extracts have been respectively determined by using Folin-Ciocalteu (F-C) method, Kiranmai *et al*. method and Oyaizu method in the ethanol crude extracts from the selcted samples. In addition, antimicrobial activity screening was done on various crude extracts by agar well diffusion method against on *Bacillus subtilis, Staphylococus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans and Eschericha coli.* It was observed that EtOAc extract exhibited higher antimicrobial activity than

1. 4 PhD, Candidate, Department of Chemistry, University of Yangon
2. 4 PhD, Candidate, Department of Chemistry, University of Yangon
3. Lecturers, Department of Chemistry, University of Yangon
4. Lecturers, Department of Chemistry, University of Yangon
5. Professor and Head, Department of Chemistry, University of Yangon

other extracts in the stem of Kin-pon whereas EtOH extract showed higher potency in antimicrobial activity than other extracts in the bark of Tha-khut. The antioxidant activity of ethanol and watery crude extracts of the samples was investigated by using DPPH free radical scavenging assay, resulting in the order of Tha-Khut bark (EtOH extract) (IC_{50} = 42.71 µg/mL) > Kin-pon stem (EtOH extract) > Tha-khut bark (watery extract) > Kin-pon stem (watery extract) in antioxidant activity. Antitumor activity screening by Potato Crown Gall (PCG) test revealed that the EtOH and H_2O extracts of stem of Kin-pon and bark of Tha-khut possess the tumor inhibition. Furthermore, antiarthritic activity of the ethanol crude extracts has been studied according to their proteinase inhibitory action and inhibition of protein denaturation action. In antiarthritic activity, ethanol extract of bark of Tha-khut showed proteinase inhibitory action and inhibiion of protein denaturation, however that of stem of Kin-pon did not show activities.

Keywords*:* *Coccinia cordifolia* Cogn., *Dolichandrone serrulata* Seem., chemical properties, antimicrobial activity, antioxidant activity, antitumor activity, antiarthritic activity

Introduction

Coccinia cordifolia Cogn., (Kin-pon), the family Cucurbiace is distributed in Africa country and tropical Asia country. It is a perennial and herbaceous climber with glabrous stems and tuberous roots. Phytochemical screening of stem of Kin-pon reported the presence of saponin, cardenoloids, flavonoids and poly phenols which may be attributed to anti bacterial activity. Major phytoconstituents present in stem of Kin-pon are cardenolides, saponins, flavonoids and polyphenols. *C. cordifolia* have pharmacological activities like analgesic, antipyretic, anti-inflammatory, antimicrobial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, antimalarial, antidyslipidemic, anticancer, antitussive and mutagenic (Bounmy *et al.,* 2006).

Dolichandrone serrulata Seem., (Tha-khut), the family Bignoniace is distributed in South East Asia. Some chemical constituents of bark of Tha-khut are dolichandroside, decaffeoyl-verbascoside, isoverbascoside, markhamioside, luteocoside B, ixoside, and iridoide glycoside. It is used as anti-fever, anti-inflammatory agent and anti-mutagenicity (Bunbun *et al.,* 2011).

Since Kin-pon and Tha-khut have a lot of useful biological activities, the locally cultivated the stem of Kin-pon and the bark of Tha-khut were chosen to evaluate scientifically some of their bioactivities in this study. In the present work, analyses of some biochemicals such as inorganic elements, nutritional values, total phenol contents, total flavonoid contents and screening of some biological activities such as reducing ability, antimicrobial activities, antitumor activities, antioxidant activities and antiarthritis activities were carried out on the stem of Kin-pon and the bark of Tha-khut.

Materials and Methods

Collection and Preparation of Plant Materials

The stem of Kin-pon and the bark of Tha-khut were collected from Yekyi Township, Ayarwaddy Region, Myanmar in the month of June- July, 2014. The plants were identified and authenticated at the Department of Botany, Yangon University.

After collection, the stem and bark were cleaned thoroughly with distilled water to remove any type of contamination. The washed stem and bark were air dried in shade for about two weeks and ground into the coarse powder with the help of a mechanical grinder. The powders of the samples were separately stored in air tight bottles and kept in a cool, dark and dry place until analyses were commenced.

Qualitative Elemental Analysis by Energy Dispersive X- Ray Fluorescence

Shimadzu EDX-8000 spectrometer can analyze the elements from Na to U under vacuum condition. In this research work relative abundance of elements present in stem of Kin-pon and the bark of Tha-khut was determined by EDXRF spectrometer.

Determination of Nutritional Values

The nutritional values such as moisture, ash, crude protein, crude fiber, crude fat, carbohydrate contents and energy value of the stem of Kin-pon and the bark of Tha-khut were determined according to AOAC method at Food

Industries Development Supporting Laboratory (FIDSL), Myanmar Food Processors and Exporters Association (MFPEA), Yangon, Myanmar.

Preliminary Phytochemical Screening

The stem of Kin-pon and the bark of Tha-khut were subjected to qualitative phytochemical tests for the identification of various bioactive constituents. Phytochemical screenings were carried out by using standard procedures to detect the presence of alkaloids, glycosides, carbohydrates, α-amino acids, phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, starch, reducing sugars and organic acids. After the addition of specific reagents to the test solution, the tests were detected by visual observation of colour change or by precipitate formation.

Preparation of the Extracts from the Samples

The crude extracts of stem of Kin-pon and bark of Tha-khut were prepared by extracting the sample with different solvents like petroleum ether, ethyl acetate, ethanol and water by cold percolation method. All of these extracts were kept for the determination of total phenol contents, total flavonoid contents, reducing ability, antimicrobial activity, antioxidant activity and antiarthritic activity.

Determination of Total Phenol Contents

The total phenol content (TPC) in ethanol extract of each sample was estimated by the Folin-Ciocalteu method according to the procedure described by Saxena *et al.* (2013). The sample solution (50 ppm) was prepared by dissolving 0.005 g of extract in methanol making up to 100 mL solution. First, 0.5 mL of the prepared sample was mixed with 0.5 mL methanol. Then, 0.5 mL of Folin-Ciocalteu reagent (FCR: H_2O, 1: 10) was added to the mixture and incubated for 5 min. 4 mL of 1 M sodium carbonate was added to each tube and the tubes were kept at room temperature for 2 h and the UV absorbance of each reaction mixture was read at λ_{max} 765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as mg gallic acid equivalents per g of EtOH extract.

Determination of Total Flavonoid Contents

Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho – dihydroxyl groups in the A- or B- ring of flavonoids. For building the calibration curve, quercetin is used as construction standard materials. Various concentrations of standard solutions were used to make a standard calibration curve (Kalita *et al.,* 2011).

In this method, quercetin was used to make the calibration curve. 0.01 g of quercetin was dissolved in methanol and then diluted to (6.25, 2.5, 25, 50 and 100μg/mL). A calibration curve was made by measuring the absorbance of the dilutions at 415nm (λ_{max} of quercetin) with a Shimadzu UV-1800 spectrophotometer (Kalita *et al.,* 2011).

Each ethanol extract solution in 50 ppm was prepared by dissolving 0.005 g of extract in 100 mL MeOH solution. 0.5 mL of each extract stock solution, 1.5 mL methanol, 0.1 mL of aluminium chloride, 0.1 mL of potassium acetate solution and 2.8 mL of distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. The absorbance was measured at 415 nm (Kalita *et al.,* 2011).

Determination of Reducing Ability

Like the antioxidant activity, the reducing power increased with increasing amount of the extract, when potassium ferricyanide react with ferric chloride in the presence of anti oxidant, potassium ferrocyanide and ferrous chloride are found as a product. Presence of reducers causes the conversion of the Fe^{3+}/ ferricyanide complex used in this method to the ferrous form.

1 mL of different concentrations (6.25, 12.5, 25, 50, 100 μg/mL) of each of the extract was mixed with 2.5 mL of 1 % potassium ferricyanide, 2.5 mL of phosphate buffer (pH 6.6). The mixture was incubated at 50 °C for 20 min. 2.5 mL of 10 % tri chloroacetic acid was added to it and centrifuged at 3000 rpm for 10 min. 2.5 mL of supernatant was taken and 2.5 mL water and

0.5 mL of 0.1 % $FeCl_3$ were added to it. The absorbance was measured at 700 nm. Higher absorbance of the reaction indicated higher reducing power (Kalita *et al.,* 2011).

Determination of Antimicrobial Activity by Agar Well Diffusion Method

Antimicrobial activities of different crude extracts of the stem of Kin-pon and the bark of Tha-khut were screened in *vitro* by agar well diffusion method on nutrient agar medium (Perez *et al*., 1990). In the present study, petroleum ether, ethyl acetate, ethanol, methanol and aqueous extracts were used to study the antimicrobial property of plant sample. Bacterial cultures used in the research were three strains of gram positive (*Bacillus subtilis, Staphyloccous aureus* and *Bacillus pumilus*), two strains of gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and one strain of fungi (*Candida albicans*). These experiments were carried out at Pharmaceutical Research Department, Insein, Yangon, Myanmar.

About 0.1 mL of crude extracts of petroleum ether, ethyl acetate, ethanol, methanol and water were added to agar wells. The plates were allowed to stand for 1 h for prediffusion of the extracts to occur. Then, the bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruiginosa*, *Bacillus pumilus* and *Escherichia coli*) and fungal (*Candida albicans*) media were incubated at 37 ºC for 24 h. The diameters of inhibition zones including 10 mm well were measured. In the study, the respective pure organic solvents (petroleum ether, ethyl acetate, ethanol, methanol and water) were used as negative control to determine possible inhibitory activity of the solvent. Antimicrobial activity was defined as the diameter (mm) of the clear inhibitory zone formed around the well.

Antioxidant Activity Screening by DPPH Radical Scavenging Assay

The antioxidant activity of 95 % EtOH and H_2O extracts were studied by DPPH Assay Method. DPPH radical scavenging activity was determined by spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95 % ethanol with vortex mixer. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solutions and 1.5 mL of test sample solution. The solutions

were allowed to stand at room temperature for 30 min. After 30 min, measurement of absorbance at 517 nm was made by using spectrophotometer UV1800, Shimadzu corporation. Absorbance measurements were done in triplicate for each solution and the mean value was obtained, and then used to calculate % inhibition of oxidation by the following equation,

$$\% \text{ oxidative inhibition} = \frac{A_c - (A - A_b)}{A_c} \times 100\,\%$$

% oxidative inhibition = % oxidative inhibition of test sample

A_c = absorbance of the control (DPPH alone)

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

A = absorbance of test sample solution

Then IC_{50} (50 % inhibitory concentration) values were also calculated by linear regressive excel program (Brand-Williams *et al.,* 1995).

Screening of Antitumor Activity by Potato Discs Assay Method (Potato Crown Gall Test)

95 % EtOH and H_2O extracts of stem of Kin-pon and bark of Tha-khut were studied the tumor activity by Potato Discs Assay Method. Tumor producing bacteria, *Agrobacterium tumerfacien*, isolated from *Sandoricum koetjape* Merr. (Thitto) leaves were used in this study. All of these strains have been maintained as solid slants under refrigeration. For inoculation of the potato discs, 48 h broth cultures containing 5×10^7-5×10^9 cell / mL were used. Fresh, disease free potato tubers were obtained from local markets and were used within 48 h for transfer to the laboratory.

Tubers of moderate sizes were surface- sterilized by immersion in 50% sodium hypochlorite (Clorox) for 20 min. The ends were removed and soaked for 10 min more in Clorox. A core of the tissue was extracted from each tuber by using surface-sterilized (ethanol and flame) 2.5 cm wide cork borer and 2 cm pieces were removed from each end and discarded and the remainder of the cylinder is cut into 1.0 cm thick discs with a surface-sterilized cutter. The discs were then transferred to 1.5 % agar plates (1.5 g of Difco agar was

dissolved in 100 mL of distilled water, autoclaved and 20 mL poured into each petri dish). Each plate contained three discs. This procedure was done in the clean bench in the sterile room. 0.1, 0.2 and 0.3 g of sample was filtered through Millipolefilters (0.22 μm) into a sterile tube. A 0.5 mL of this solution was added to 1.5 mL of sterile distilled water and 2 mL of broth culture of *A. tumefaciens* strain (48 h culture containing 5×10^7-5×10^9cells/mL) were added aseptically.

Controls were made in this way; 0.5 mL of DMSO and 1.5 mL of sterile distilled water were added to the tube containing 2 mL of broth culture of *A. tumefaciens* (from the same 48 h culture). Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc, surface. The process of cutting the potatoes and incubation must be conducted within 30 min. The plates were sealed with tape to minimize moisture loss and incubated at room temperature counted with microscope and compared with control. The antitumor activity was examined by observation if tumor formation was inhibited or not (Collin, 2001).

Determination of Antiarthritic Activity

The reaction mixture consisted of 0.5 mL of 5 % aqueous solution of bovine serum albumin and 0.05 mL of various concentrations (6.25, 12.5, 25, 50, 100 ug/mL) of the ethanol extract solutions of stems of Kin-pon and that of barks of Tha-khut. The pH of the reaction mixture was adjusted to 6.3 by using 1 M hydrochloric acid and it was then incubated at 37 °C for 20 min and then heated at 57 °C for 3 min. The reaction mixture was allowed to cool and added 2.5 mL of phosphate buffer saline. Turbidity was measured at 340 nm. In control, 0.05 mL distilled water was used instead of test extract while product control lacked bovine serum albumin and diclofenac sodium was used as the standard. The percentage inhibition of protein denaturation was calculated. The control represents 100% protein denaturation. All determinations were done in triplicate.

The reaction mixture consisted of 2.0 mL of 0.06 mg/mL trypsin, 1.0 mL of 0.25 mM tris-HCl buffer pH adjusted to 7.4 and 1.0 mL of ethanol extracts of stems of Kin-pon and barks of Tha-khut. The mixtures were

incubated for 37° C for 5 min. It was then added with 1.0 mL of 0.8 % casein. The mixtures were incubated for additional 20 minutes. Then 2.0 mL of 70 % perchloric acid was added and the cloudy solution was centrifuged at 2500 rpm for 5 min. Diclofenac was used as the standard. Absorbance of the supernatant was measured at 217 nm and buffer was kept as blank. The percentage inhibition of protein denaturation was calculated. All determinations were done in triplicate (Jayaprasam and Ravi, 2012).

Results and Discussion

Relative Abundances of some Elements in the Stem of Kin-pon and the Bark of Tha-khut

As shown in Tables 1 and 2, it can be seen that organic compounds are predominant in the samples, and other elements such as Ca, K and S are also present in reasonable composition but P, Fe and Mn were present in medium amount and Cu, Zn and Rb were present in very little amounts based on the relative abundance of elements.

Table 1: Relative Abundance of Some Elements in Stem of Kin-pon

Element	Relative Abundance (%)
Calcium	3.119
Potassium	1.025
Sulphur	0.172
Manganese	0.015
Iron	0.012
Strontium	0.008
Zinc	0.007
Copper	0.001
Organic Composition	95.641

Table 2: Relative Abundance of Some Elements in Bark of Tha-khut

Element	Relative Abundance (%)
Calcium	3.283
Potassium	0.742
Sulphur	0.128
Iron	0.008
Zinc	0.001
Copper	0.001
Rubidium	0.001
Organic composition	95.836

Nutritional Values of the Stem of Kin-pon and the Bark of Tha-khut

The nutritional values of the stem of Kin-pon and bark of Tha-khut such as moisture, ash, crude protein, crude fiber, crude fat, carbohydrates and energy values were determined by using standard methods for food analysis (AOAC, 2000) and the nutritional composition of the samples are described in Table 3. These analyses revealed some interesting findings. Kin-pon stem was found to contain higher protein content and crude fat content but lower carbohydrate content compared with that in the bark of Tha-khut. The other parameters were observed to be similar in two samples. According to the results, the presence of the important nutrients like fat, fiber, protein, carbohydrate and moisture and ash means that the two samples could be used as a nutritionally valuable and healthy ingredient to improve traditional medicinal formulation and to treat many diseases.

Table 3: Nutritional Compositions of Stem of Kin-pon and Bark of Tha-khut

Parameter	Nutritional Composition (%)	
	Stem of Kin-pon	**Bark of Tha-khut**
Moisture	10.69	11.25
Ash	11.81	9.55
Crude protein	8.71	2.29
Crude fiber	36.90	36.17
Crude fat	0.89	0.08
Carbohydrate	31.00	40.66
Energy value (kcal/100g)	169	173

Preliminary Phytochemical Screening in the **Stem of Kin-pon and the Bark of Tha-khut**

Preliminary Phytochemical screening was performed to investigate the plant materials in terms of its active constituents. In order to detect the various constituents present in the stem of Kin-pon and bark of Tha-khut, the plant extracts were subjected to the qualitative test analysis using standard methods. Test reagents, observations and inferences for the analyses are summarized in Table 4. Various types of organic constituents were found in both samples, except cyanogenic glycosides in both samples.

Table 4: Results of Phytochemical Screening of Stem of Kin-pon and Bark of Tha-khut

Chemical Constituents	Test Reagent	Observation	Inference	
			I	II
Alkaloids	(i) Dragendorff's	Orange ppt.	+	+
	(ii) Wagner's	Brown solution	+	+
	(iii) Mayer's	White ppt.	+	+
	(iv) Hager's	Yellow ppt.	+	+
Glycosides	10% Lead acetate	White ppt.	+	+
Carbohydrates	Molisch's	Red ring	+	+
α-Amino acids	Ninhydrin	Purple colour	+	+
Phenolic	5% Ferric chloride	Brown ppt.	+	+
Compounds	Shinoda's	Green solution	+	+
Flavonoids	LibermanBurchard	Greenish yellow	+	+
Steroids	LibermanBurchard	Pink	+	+
Terpenoids	Foam test	Marked frothing	+	+
Saponins	Ferrous sulphate	Green solution	+	+
Tannins	Sodium picrate	No change	_	_
Cyanogenic	Iodine	Deep violet	+	+
Glycosides	Benedict's	Reddish brown	+	+
Starch		ppt.		
Reducing	Bromocresol green	Deep green	+	+
Sugars		solution		
Organic Acids				

I =Stem of Kin-pon II = Bark of Tha-khut + = present - = absent ppt = precipitate

Total Phenol Contents in the Stem of Kin-pon and the Bark of Tha-khut

Total phenol contents were determined on the ethanol extracts of Kin-pon stem and Tha-khut bark by using Folin-Ciocalteu (F-C) method, where gallic acid was used as the standard. The absorbance values obtained at different concentrations of gallic acid were used for the construction of calibration curve (Figure 3). F-C method is based on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybedic phosphotun-gallic acid complexes to form blue coloured complexes, $(PMoW_{11}O_{40})^{-4}$ that are determined spectrophotometrically at 760 nm. Total phenol content of the extracts was calculated from the regression equation of calibration curve ($Y = 0.003 x + 0.016$; $R^2 = 0.994$) and expressed as mg

gallic acid equivalents (GAE) per gram of sample in dry weight. The results are presented in Table 5.

It can be seen (Table 6) that the total phenol content (30.49 µg GAE/ mg of the extract) of Tha-khut (75.90 µg GAE/ mg of the extract) is higher than that of Kin-pon indicating that Tha-khut bark might possess more phenolic compounds than Kin-pon stem. Generally, extracts with a high amount of phenolic compounds also exhibit high antioxidant activity.

Table 5: Absorbance of Standard Gallic Acid

Concentration (µg/mL)	Absorbance (Mean value) at 760 nm
6.25	0.024
12.5	0.034
25	0.072
50	0.178
100	0.384

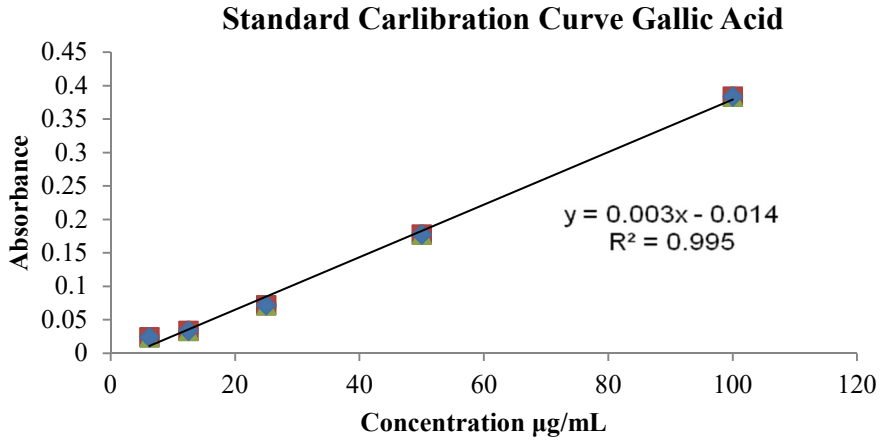


Figure 3: Plot of absorbance vs concentration of standard gallic acid

Table 6. Total Phenol Contents in Ethanol Extracts of Kin-pon Stem and Tha-khut Bark

Ethanol Extract	**Total Phenol Content (mg GAE/g of EtOH extract)**
Kin-pon stem	30.49 ± 2.67
Tha-khut bark	75.90 ± 4.2

Total Flavonoid Contents in the Stem of Kin-pon and the Bark of Tha-khut

To perform the calculation of the total flavonoid content in the samples by using Kiranmai *et al.* (2011) method, a standard curve is needed which is obtained from a series of asorbance of different quercetin concentrations (Table 7 and Figure 4). The total flavonoid content of ethanol extract (51.7 ± 2.9 mg of QE /g of EtOH extract) from the stem of Kin-pon was observed to be similar to that of the bark of Tha-khut (52.4 ± 1.9 ± 2.9 mg of QE /g of EtOH extract) (Table 8). Generally, extracts with a high amount of flavonoid contents also exhibit high antioxidant activity.

Table 7: Absorbance of Standard Quercetin

Concentrations (μg/mL)	Absorbance (Mean value) at 760 nm
6.25	0.005
12.5	0.015
25	0.041
50	0.109
100	0.234

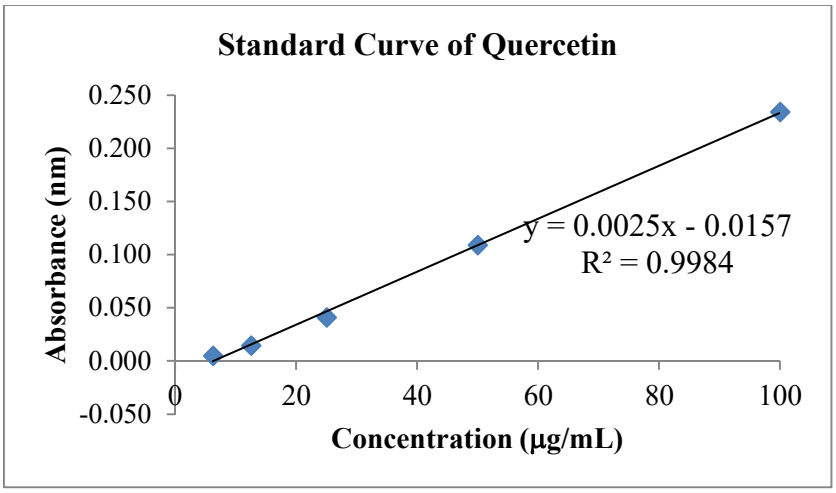


Figure 4: Plot of absorbance vs concentration of standard quercetin

Table 8: Total Flavonoid Contents in Stem of Kin-Pon and Bark of Tha-Khut

Samples	Total Phenolic Content (mg of QE /g of EtOH extract)
Kin-pon EtOH extract	51.7 ± 2.9
Tha-khut EtOH extract	52.4 ± 1.9

Reducing Ability of the Stem of Kin-pon and the Bark of Tha-khut

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The estimation of the reductive ability was investigated the Fe^{3+} to Fe^{2+} transformation by using the method of Oyaizu *et al*.,2013, where the change in the absorbance of the final mixture was measured at 700 nm (Table 9). Increase in the absorbance indicates higher reductive ability. The reducing capabilities of the ethanol extracts of bark of Tha-khut and stem of Kin-pon were found to be in dose dependent manner and were found to be slightly lower than standard quercetin (Figure5).

Table 9: Reducing Ability of Ethanol Extracts of Stem of Kin-pon and Bark of Tha-khut

Samples	**Absorbance of different concentrations (µg/mL) at 700 nm**				
	6.25	12.5	25	50	100
Quercetin	0.30	0.41	0.57	0.57	0.61
Tha-khut barks	0.32	0.36	0.39	0.43	0.45
Kin-pon stems	0.27	0.30	0.31	0.35	0.38

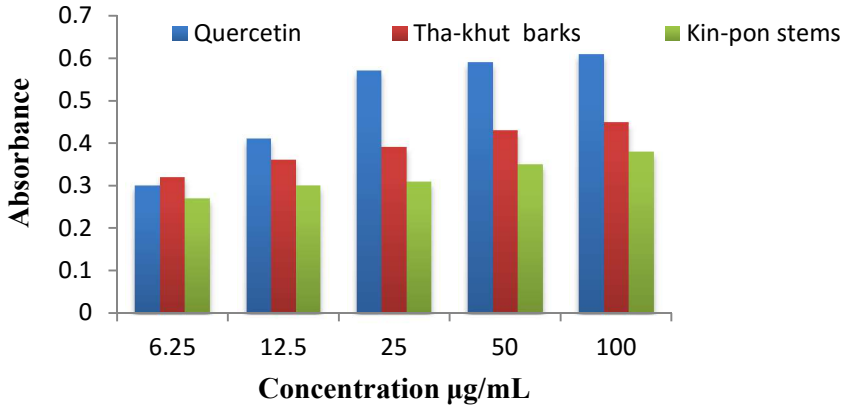


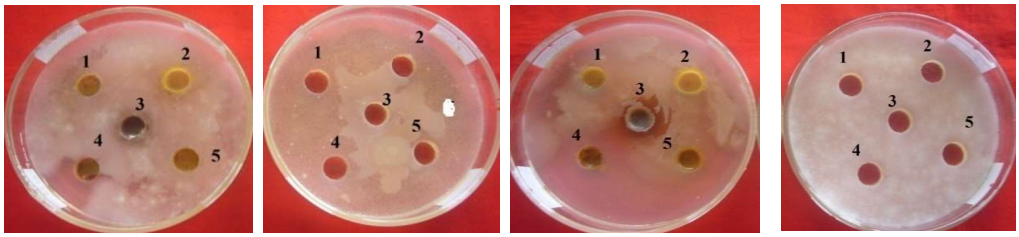
Figure 5: Reducing ability of ethanol extracts from the stem Kin-pon and the bark Tha-khut

Antimicrobial Activity of the Stem of Kin-pon and the Bark of Tha-khut

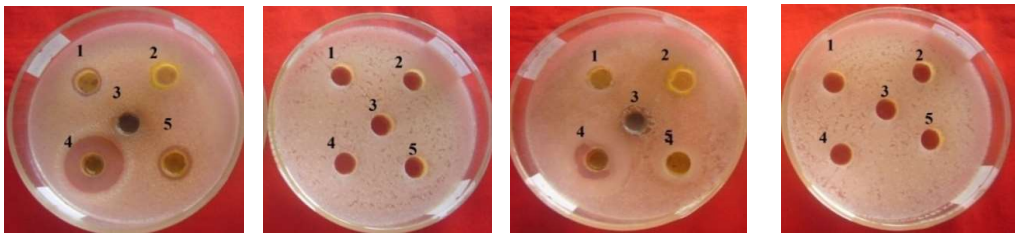
Antimicrobial activity was studied by agar well diffusion method according to Perez *et al*., 1990. In *vitro* antimicrobial screening of both samples extracts was carried out at Pharmaceuticall Research Department, Insein, Yangon, Myanmar. The antimicrobial activity was assessed by agar well diffusion method which is equally suited to the screening of antibiotics or the products of plant evaluation and is highly effective for rapidly growing microorganisms and the activities of the test extracts are expressed by measuring the zones (mm) of inhibition. Generally the more susceptible the organism, the bigger is the zone of inhibition.

Petroleum ether, ethyl acetate, ethanol, methanol and aqueous extracts of the stem of Kin-pon and the bark of Tha-khut were used to determine th antimicrobial activity against five bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Escherichia coli,* and one fungal strain: *Candida albicans*.

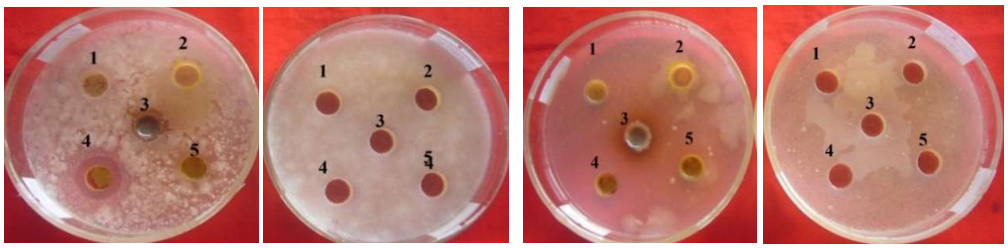
The observed antimicrobial activity of stem of Kin-pon and bark of Tha-khut was expressed as the zone diameters of inhibition shown in Tables 10 and 11. The photographs of agar plates showing the inhibition zones are illustrated in Figures 6 and 7. Among the extracts, EtOAc extract of Kin-pon (14~32 mm) was observed to possess higher antimicrobial activity than EtOAc extract of Tha-khut bark (11 ~ 19 mm). Generally, stem of kin-pon was found to be more potent than bark of Tha-khut in inhibition of microorganisms. Watery extract of Tha-khut bark did not show any activity against all microorganisms.



B.subtilis ***S.aureus***



P.aeruginosa ***B.pumilus***



C.albicans ***E.coli***

Figure 6: Agar wells indicating inhibition zones of various extracts from the stem of Kin-pon

1 = EtOH extract 2 = PE extract 3= H_2O extract

4 = EtOAC extract 5 = EtOH extract

Table 10: Inhibition Zone Diameters of Stems of Kin-pon Extracts

Microorganisms	**Inhibition zone diameters (mm) of different extracts**				
	PE extract	**EtOAc extract**	**EtOH extract**	**H_2O extract**	**MeOH extract**
B.subtilis	15 (+)	15 (+)	–	–	–
S.aureus	–	28 (+++)	–	15(++)	–
P.aeruginosa	-	24 (+++)	13 (+)	–	–
B.pumilus	11(+)	14 (+)	–	13(+)	13 (+)
C.albicans	12 (+)	16 (++)	–	12(+)	–
E.coli	-	32 (+++)	–	–	–

Agar well = 10 mm
(+) = 10-14 mm (low activity)
(++) = 15-19 mm (moderate activity)
(+++) = 20 mm & above (high activity)
(-) = no zone of inhibition

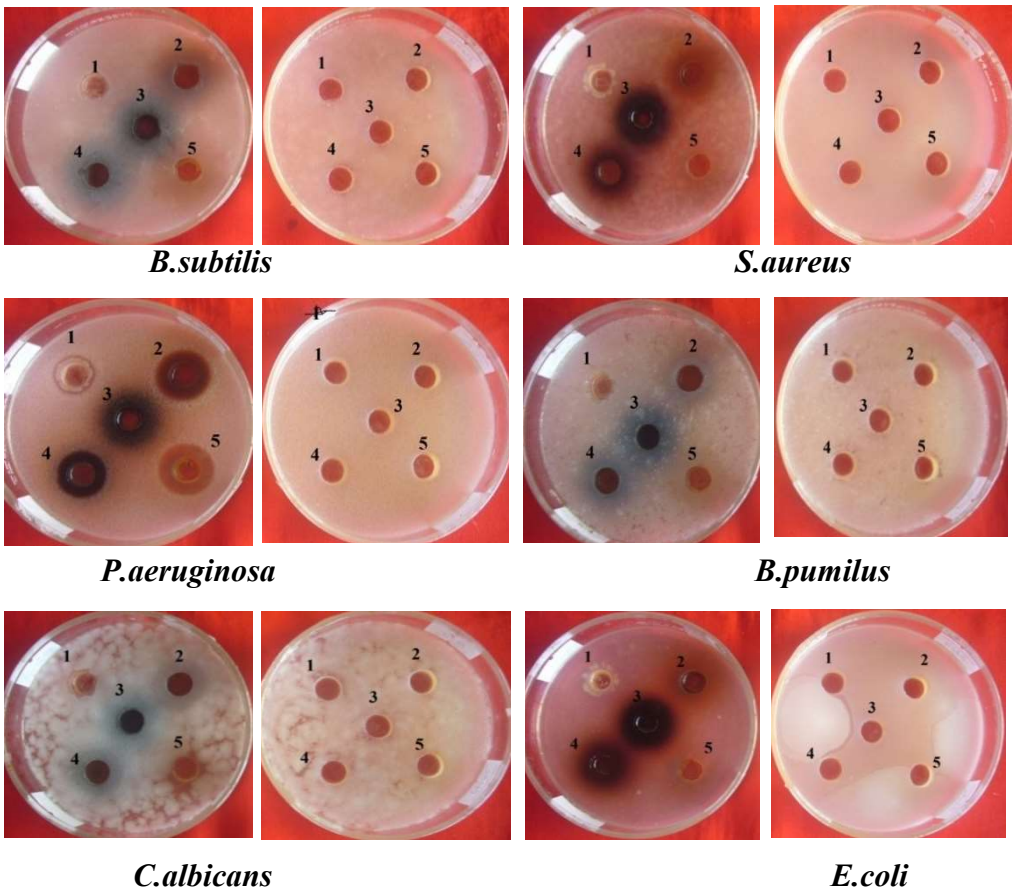


Figure 7: Agar wells indicating inhibition zones of various extracts from the bark of Tha-khut

1 = EtOH extract 2 = PE extract 3= H_2O extract
4 = EtOAC extract 5 = EtOH extract

Table 11: Inhibition Zone Diameters of Barks of Tha-kut Extracts

Microorganisms	Inhibition zone diameters (mm) of different extracts				
	PE extract	**EtOAc extract**	**EtOH extract**	**H_2O extract**	**MeOH extract**
B.subtilis	13 (+)	11(+)	–	–	–
S.aureus	–	13(+)	15 (+)	–	14(+)
P.aeruginosa	14 (+)	19(++)	19 (++)	–	17(+)
B.pumilus	12 (+)	–	–	–	–
C.albicans	13(+)	13(+)	–	–	–
E.coli	–	13(+)	20(+++)	–	14(+)

Agar well = 10 mm
(+) = 10-14 mm (low activity)
(++) = 15-19 mm (moderate activity)
(+++) = 20 mm & above (high activity)
(-) = no zone of inhibition

Antioxidant Activity of the Stem of Kin-pon and the Bark of Tha-khut

The antioxidant activity of ethanol and watery crude extracts of stem of Kin-pon and bark of Tha-khut were investigated with five different concentrations (12.5, 25, 50, 100, 200 µg/mL) by DPPH free radical scavenging assay. According to IC_{50} values, EtOH extract of bark of Tha-khut was more pronounced than other extracts. The antioxidant activity of the tested crude extracts was suggested to be very weak in comparing with the activity of standards ascorbic acid. IC_{50} value of ascorbic acid was found to 21.92µg/mL illustrated in (Table 12).

Table 12: Radical Scavenging Activity (%RSA) and IC_{50} Values of Crude Extracts from the Stem of Kin-pon, the Bark of Tha-khut and Standard Ascorbic Acid on Antioxidant Activity

Test sample	% RSA ± SD at Different Concentration (µg/mL)					IC_{50} (µg/mL)
	12.5	25	50	100	200	
Stem of Kin-pon (EtOH)	21.011±3.358	26.034±1.028	38.371±4.140	64.413±0.211	72.4±1.823	72.33
Stem of Kin-pon (Watery)	26.624±3.241	39.245±3.125	48.136±0.754	83.641±2.336	98.37±0.613	52.62
Bark of Tha-khut (Watery)	20.342±3.607	28.025±1.370	44.436±1.435	68.074±0.754	83.78±0.943	59.14
Bark of Tha-khut (EtOH)	24.012±2.023	41.392±1.963	53.541±0.796	83.193±0.869	92.161±0.827	42.71
Ascorbic acid	42.191±2.443	52.554±0.701	56.382±0.704	83.193±1.001	94.59± 1.365	21.92

Antitumor Activity of the Stem of Kin-pon and the Bark of Tha-khut

The antitumor activity of EtOH and H_2O of stem of Kin-pon and bark of Tha-khut were investigated by using PCG test with the isolated bacterium *A. tumefaciens.* For inoculation of the potato disc, 48 h broth cultures containing $5x10^9$ cells/mL were used. The tested samples were dissolved in DMSO to dilute and the diluted samples were mixed with the bacterial culture for inoculation. After preparing the inoculums, the bacterial suspension was inoculated on the cleaned and sterilized potato discs, and incubated for 3 days, at room temperature. After that, the tumors appeared on potato discs and these were checked by staining the knob with Lugol's (I_2-KI) solution. In the control, the formation of white knob on the blue background indicated the presence of tumor cells because there is no protein in tumor cells. The active test samples did not form any tumors on the potato discs and its surface remained blue.

Antitumor activity screening revealed that two crude extracts of stems of Kin-pon barks of Tha-khut can inhibit tumor growth until the minimum

dose of 0.1 g/mL of EtOH and watery extract of the sample (Tables 12 and 13).

Table 13: Antitumor Activity of Different Crude Extract from the Stem of Kin-pon

No.	Test Sample	Concentration of samples g/mL	Tumor	Remark
1.	Control	0	+	tumor occur
2.	EtOAc extract	0.1	-	No tumor occur
3.	EtOAc extract	0.2	-	No tumor occur
4.	Watery extract	0.1	-	No tumor occur
5.	Watery extract	0.2	-	No tumor occur

(+) Tumor appeared (-) no tumor appeared

Table 14: Antitumor Activity of Different Crude Extract from the Bark of Tha-khut

No.	Test Sample	Concentration of samples (g/mL)	Tumor	Remark
1.	Control	0	+	Tumor occur
2.	Ethanol extract	0.1	-	No tumor occur
3.	Ethanol extract	0.2	-	No tumor occur
4.	Watery extract	0.1	-	No tumor occur
5.	Watery extract	0.2	-	No tumor occur

(+) Tumor appeared (-) No tumor appeared

Anti-arthritic Activity of the Stem of Kin-pon and the Bark of Tha-khut

Inflammatory arthritis is a synovial disease characterized by chronic inflammation of the joints and can result in disability owing to joint destruction. *In vitro* anti-arthritic activity was performed using most popular methods such as inhibition of protein denaturation and proteinase inhibitory (Jayaprakasam and Ravi, 2012). The inhibition of protein denaturation was found in the ethanol extract of bark of Tha-khut but ethanol extract of stem of Kin-pon did not show in the concentrations between 6.25 ~ 100 μg/mL. The

IC_{50} value of the ethanol extract of bark of Tha-khut was 39.42 µg/mL. In this method diclofenac was used as standard for comparing its anti-arthritic potential at much lower concentration with an IC_{50} value of 15.99 µg/mL (Table 14 and Figure 8). In case of arthritis, auto antigens were produced due to protein denaturation. Proteinase inhibitory action was also studied. The ethanol extract of bark of Tha-khut exhibited the proteinase inhibitory action (IC_{50} = 52.33 µg/mL) but ethanol extract of stem of Kin-pon did not show in these concentrations 6.25 ~ 100 µg/mL (Table 15).

Table.15: Inhibition of Protein Denaturation of Stem of Kin-pon and Bark of Tha-khut

Samples	% Inhibition at different concentrations (mg/mL)					
	6.25	12.5	25	50	100	IC_{50} (µg/mL)
Kin-pon (EtOH)	ND	ND	ND	ND	ND	ND
Tha-khut (EtOH)	ND	12.57± 0.86	35.17±0.02	60.88±0.20	71.43±0.38	39.42
DS	10.28±0.11	46.28±0.02	59.58±0.38	72.10±0.11	92.68±0.30	15.99

Data are expressed as mean ± SD for triplicate experiments
ND =Not detected
DS= diclofenac sodium (China)

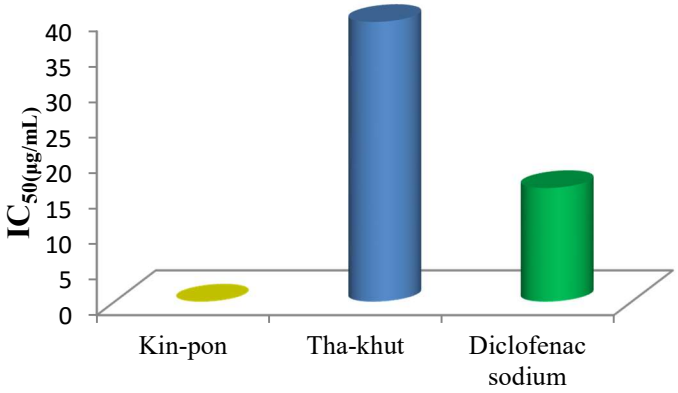


Figure 8: IC_{50} values of protein denaturation of stem of Kin-pon and bark of Tha-khut

Table 16: Proteinase Inhibitory Action Stem of Kin-pon and Bark of Tha-khut

Samples	% inhibition at different concentrations (mg/mL)					
	6.25	12.5	25	50	100	IC_{50} (µg/mL)
Kin-pon (EtOH)	ND	ND	ND	ND	ND	ND
Tha-khut (EtOH)	ND	ND	10.06 ± 0.62	49.66± 0.98	56.37±0.72	52.53
DS	8.96± 0.03	10.48± 0.11	37.71±0.04	68.14±0.96	94.08±0. 52	35.1

Data are expressed as mean ± SD for triplicate experiments
ND =Not detected
DS= diclofenac sodium (China)

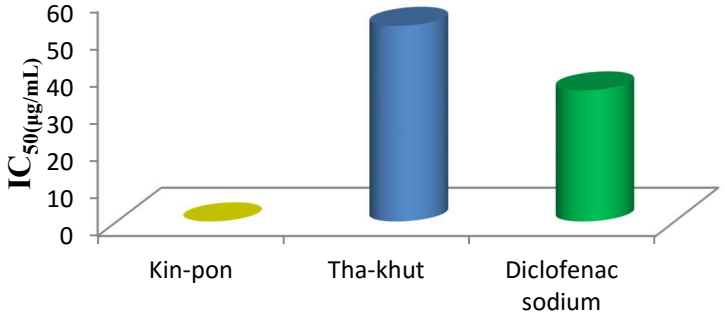


Figure 9: IC_{50} values of proteinase inhibitory action of stem of Kin-pon and bark of Tha-khut

Conclusion

EDXRF analysis showed the presence of calcium, potassium, sulphur, manganese, iron, stronium, zinc, copper in the stem of Kin-pon and calcium, potassium, sulphur, zinc, copper, rubidium in the bark of Tha-khut. According to nutritional composition analyses, the stem of Kin-pon had higher contents in protein and fat than the bark of Tha-khut. Qualitative phytochemical screening of the stem of Kin-pon and the bark of Tha-khut indicated the presence of bioactive constituents like alkaloids, glycosides, carbohydrates, α-amino acids, phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, starch, reducing sugars and organic acids which are medicinally valuable. Total phenol contents, total flavonoid

contents and reducing ability of the ethanol extract of bark of Tha-khut was higher than that of stem of Kin-pon. Therefore, the ethanol extract of bark of Tha-khut has highest antioxidant activity than other extracts. From the results of antimicrobial activity screening, it may be concluded that the stem of Kin-pon was more potent than the bark of Tha-khut extracts in antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Escherichia coli*, and antifungal activity against skin pathogen, *Candida albicans*. Antitumor activity screening revealed that EtOH and H_2O extracts of stem of Kin-pon and bark of Tha-khut possessed tumor inhibition up to the minimum dose of 0.1 g/mL extract. In addition, antiarthritic activity of the ethanol crude extracts of stem of Kin-pon and bark of Tha-khut has been evaluated according to their proteinase inhibitory action and inhibition of protein denaturation. In antiarthritic activity, both of the ethanol extracts from bark of Tha-khut showed proteinase inhibitory effect (IC_{50}= 39.42 μg/mL) and inhibition of protein denaturation (IC_{50}= 52.53 μg/mL) however the ethanol extracts from stem of Kin-pon did not exhibit under same conditions. Consequently, it could be deduced that the bark of Tha-khut was more effective than the stem of Kin-pon for the treatment of antiarthritics.

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