SOME BIOACTIVITIESOF*CURCUMA AERUGINOSA* ROXB. (NA - NWIN–TAIN- PYAR) RHIZOMES

Zin Thu Khaing[1], Y-Mung San Aung[2], Daw Hla Ngwe[3]

Abstract

This research work has focused on the elucidation of chemical constituents and some biological activities of the fresh rhizome of *C. aeruginosa* Roxb. The phytochemical screening of *C. aeruginosa* Roxb. powder reveals the presence of several compounds. The main phytochemical constituents include alkaloids, carbohydrates, amino acid, flavonoids, phenolic compounds, glycosides, reducing sugars, starch, steroids, terpenoids, and tannins. However, cyanogenic glycoside and saponin were not detected at the assay conditions. In the screening of the antioxidant activity, ethanol extract (IC_{50}= 1.56 µg/mL) was found to be more potent than watery extract (IC_{50} =3.95 µg/mL). The total phenol contents (TPC) of ethanol extract (52.31 ±8.70 µg GAE/mg) was found to be higher than watery extract (16.92 ± 0.20 µg GAE/mg).The antimicrobial activities of (PE, EtOAc, EtOH and water) extracts from *C. aeruginosa* Roxb. sample were determined against seven strains of microorganisms (*B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans, E.coli* and *A. tumefaciens*) by agar well diffusion method. The highest antimicrobial activity was observed in EtOH extract whereas H_2O extract showed minimum activity. Antitumor activity was carried out with EtOAc and EtOH extracts by PCG test. From this experiment, both extracts were found to prevent the tumor formation with the dose of 0.1 and 0.15 mg/disc. The rhizome of *C.aeruginosa* Roxb.could be applied not only as the local health remedy to the local indigenous but also for the treatment of some bacterial plant pathogen and diseases in agriculture of Myanmar.

Keywords: *Curcuma aeruginosa* Roxb., phytochemicals, antioxidant activity, antimicrobial activity, antitumor activity

[1] Lecturer, Department of Chemistry, University of Yangon
[2] Candidate, MSc. (2016-2017), Department of Chemistry, University of Yangon
[3]Professor and Head, Department of Chemistry, University of Yangon

Introduction

Many Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today in Myanmar. Modern medicine recognizes herbalism as a form of alternative medicine. Herbal medicine has a great tradition of maintaining human health for centuries. A majority of the world's population living in the developing countries still relies on herbal medicine to meet its health care needs. Although modern medicine may be available in such countries herbal medicine have often maintained popularity for historical and cultural reasons. Currently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs. The use of herbal remedies is more prevalent in patients with chronic diseases such as cancer, diabetes, asthma and end-stage renal disease (WHO, 1991).

Traditional systems of medicine continue to be widely practiced on many accounts. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care (Thomas *et al.,*1998).

Curcuma is a herb with leaves and inflorescences rising to 18 inches from the ground. The rhizome is rather large, branched horizontal externally grey and polished, with white or pink tips, internally blue, greenish-blue or violet-blue and paler in the young parts. The rhizomes are carefully dug up with hand picks.*C.aeruginosa*Roxb.is found from Myanmar and Cambodia to Java (Newman *et al.,* 2004).In Myanmar it is cultivated in Kachin and Shan state.

Rhizome of *C. aeruginosa*Roxb.is used as medicine for rheumatic, cough, asthma and anthelmintic. The traditional medical practitioners in India have identified its usage in amebicdysentery, stomach ache, ulcer and indigestion.The rhizome of *C. aeruginosa*Roxb.isa promising source of potential anti-oxidants. It is employed for making various cosmetic items and for sprains and bruises. The other medicinal uses of rhizome include postcoital contraception, hepatoprotection, and reduced platelet-activation effects. Due to its high medicinal value and indiscriminate harvest from the wild, the natural population has come down and according to International Union for

Conservation of Nature (IUCN) report, the plant is in the critically endangered category (Srivastava *et al*.,2006).

Materialsandmethods

Sample Collection and Preparation of *Curcumaaeruginosa*Roxb.

The rhizomes of*C.aeruginosa*Roxb.(Figure 1)were collected from Mogaung Township, Kachin State. These samples were identified at Department of Botany, University of Yangon. The collected samples were cleaned and air-dried at room temperature. The dried samples were cut into small pieces and ground into powdered by a grinding machine(Figure 2). The dried powdered samples were used for further experiment.

Figure 1 Photographs of *C. aeruginosa*Roxb. Rhizomes

Figure 2 Preparation of dried powder sample (Na-Nwin-Tain-Pyar) Rhizomes

Preliminary Phytochemical Investigation of *Curcuma aeruginosa* Roxb.

In order to find out the types of phytoorganic constituents such as alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, and terpenoids in the sample, preliminary phytochemical tests were carried out according to the appropriate reported methods.

Various crude extracts (PE, EtOH, EtOAc) of *C.aeruginosa* Roxb. were prepared for TLC investigation. Extracts were loaded on the percolated TLC silica gel plate and the chromatography was carried out using an appropriate standard solvent system for *C. aeruginosa* Roxb. The developed chromatograms were first inspected under UV-254nm and 365 nm light and then sprayed with detecting reagents to classify the compounds present and their functional group.

Determination of Phenolic Contents in Crude Extracts of (Na-Nwin-Tain-Pyar) Rhizomes

The total phenolic content (TPC) assay was performed in accordance with modifications. A 0.2 mL of each sample solution was mixed with 1.5 mL of Folin-Ciocalteu Reagent in a test tube covered with aluminum foil. After 5 min, 1.5 mL of 10% Na_2CO_3 was added to each test tube. The sample was then incubated for 90 min at room temperature. The absorbance was measured at 765 nm spectophotometrically (KWF UV-7504). A standard curve of Gallic acid solutions (range from 0 - 250 μg mL^{-1}) was used for calibration. The experiment was done in triplicate. Concentrations of Gallic acid equivalent (GAE) in the plant extracts were calculated from the linear regression equation explored from standard curve construction for Gallic acid. TPC in the plant samples were expressed as (μg GAE/mg) (Reynertson, 2007).

Measurement of DPPH Radical Scavenging Activity by Spectrophotometric Method

Control solution was prepared by mixing (0.002 %) DPPH solutions (1.5 mL) and 95% ethanol (1.5 mL) using vortex mixer. Similarly, a blank solution was also prepared by mixing 95% ethanol (1.5 mL) and the sample solution (1.5 mL). The sample solution was also prepared by mixing the

sample solution (1.5 mL) with 0.002 % DPPH solution (1.5 mL). All these solution were allowed to stand at room temperature for30 min. Then, the absorbance was measured at λ_{max} 517 nm and recorded on spectrophotometer (KWF UV-7504), Shimadzu Corporation. Absorbance of individual solution was measured in triplicate and % inhibition of each sample solution was calculated by using the following formula:

$$\% \text{ RSA} = ([A_{DPPH} - (A_{Sample} - A_{Blank})] / A_{DPPH}) \times 100$$

where, % RSA = % radical scavenging activity

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (µg/mL) that result in a 50% reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50% inhibitory concentration) values were calculated by linear regressive excel program.

$$\text{Standard Deviation (SD)} = \sqrt{\frac{(\bar{x}-x_1)^2+(\bar{x}-x_2)^2+\ldots(\bar{x}-x_n)^2}{(n-1)}}$$

where, $A_{control}$ = Absorbance of control solution

A_{sample} = Absorbance of sample solution

A_{blank} = Absorbance of blank solution

$\overline{X}$ = Average % inhibition

$X_1, X_2 \ldots, Xn$ = % inhibition of test sample solution

n = number of times

Screening of Antimicrobial Activity of Crude Extracts of (Na-Nwin-Tain-Pyar) Rhizomes By Agar Well Diffusion Method

Antimicrobial activities of various crude extracts such as (PE, EtOAc, EtOH and water)extracts of Na-Nwin-Tain-Pyar were studied by agar well diffusion method at the Pharmaceutical Research Department, Ministry of Industry, Yangon. The test procedure is as follow, at first, the extracts (1 g each for 6 species of bacteria) were introduced into sterilized Petri-dishes and dissolved in 1mL of their respective solvents: PE, EtOAc, EtOH and H_2O. Four small holes of 10 mm diameter each were cut out in the inoculated agar to place samples to be tested. The volume of each sample placed in each hole was0.1 mL. The Petri-dishes were incubated of 37ºC for 24 hr and the inhibition zone diameter was measured the extent of antimicrobial activity.

Antitumor Activity Screening of Crude Extracts of(Na-Nwin-Tain-Pyar) Rhizomes by Potato Crown Gall Test or Potato Disc Assay Method

Tumor producing bacteria, *Agrobcterium tumefacien*, was used in this study. All of these strains have been maintained as solid slants under refrigeration. For inoculation of the potato discs, 48 hours broth cultures containing $5 \times 10^7 - 5 \times 10^9$ cell/mL were used.

Results and Discussion

Phytoconstituents of Various Crude Extracts of *C.aeruginosa*Roxb.(Na-Nwin-Tain-Pyar) Rhizomesby TLC Method

Preliminary phytochemical screening of *C.aeruginosa* Roxb. powder revealed the presence of several compounds. The main phytochemical constituents include alkaloids, carbohydrates, amino acid, flavonoids, phenolic compounds, glycosides, reducing sugars, starch, steroids, terpenoids, and tannins. However, cyanogenic glycoside and saponin were not detected at the assay conditions. Qualitative determination of phytoconstituents by thin layer chromatography indicated that essential oils, alkaloids, steroids, terpenoids, phenolic compounds and flavonoids were present in *C.aeruginosa* Roxb (Table 1).

Table 1 Results of Preliminary TLC Screening of *Curcuma aeruginosa* Roxb.

Type of compound	Spray reagent	Observation
Steroids &Terpenoids	10% H_2SO_4	Various color intensity
Steroids, Terpenoids& Essential oils	Vanilin	Bright Fluorescence color intensity
Phenolic compound	5% Ferric chloride	Black color
Flavonoids	1% Aluminium chloride	Formation of color zone
Alkaloids	Dragendorff	Black color intensity

Total Phenol Contents of Crude Extracts of *C.aeruginosa* Roxb. (Na-Nwin-Tain Pyar)Rhizomes By Folin-Ciocalteu Method

In this study, the total phenolic content of *C.aeruginosa* Roxb.was estimated by Folin-Ciocalteu method. Phenols react with an oxidizing agent phosphomolybdate in F-C reagent under alkaline conditions and result in the formation of blue coloured complex, the molybdenum blue which is measured at 765 nm colorimetrically. Total phenolic content (TPC) was expressed as micro gram of Gallic acid equivalent (GAE) per milligram of crude extract (µg GAE/mg).The total phenol content of ethanol extract (52.31 ±8.70 µg GAE/mg) was found to be higher than watery extract (16.92 ± 0.20 µg GAE/mg).

Table2 Total Phenol Content (TPC) of Ethanol and Watery Extracts of (Na-Nwin-Tain-Pyar) Rhizomes

No	Extracts	TPC (µg GAE/mg±SD)
1	Ethanol	52.31 ±8.70
2	Watery	16.92 ± 0.20

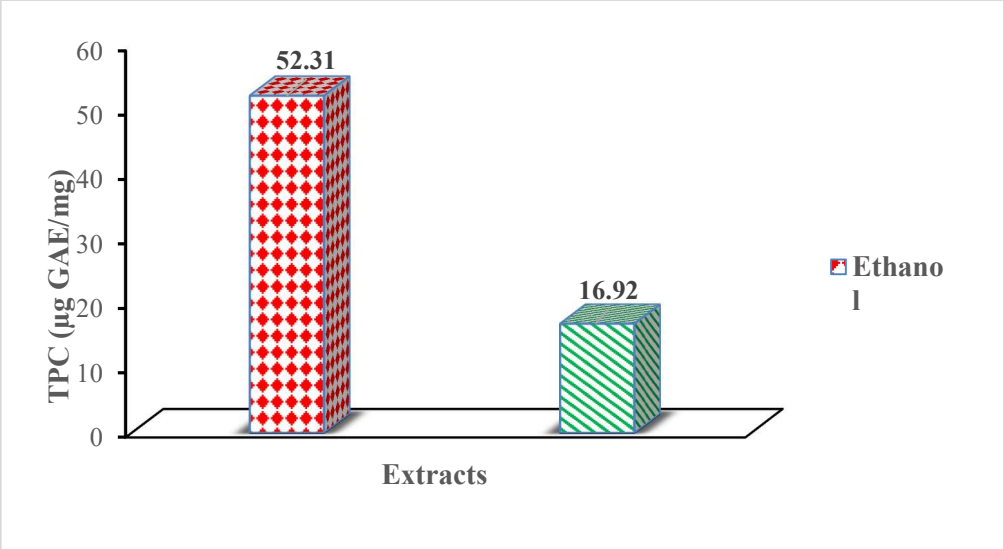

Figure 3 A bar graph of total phenolic contents of ethanol and watery extracts from (Na-Nwin-Tain-Pyar) rhizomes

Antioxidant Activity of Crude Extracts of (Na-Nwin-Tain-Pyar) Rhizomesby DPPH Radical Scavenging Assay

The antioxidant activity of EtOH and water extracts of *C.aeruginosa* Roxb.were studied by DPPH free radical scavenging assay method. DPPH free radical scavenging method is widely used to evaluate the free radical scavenging ability of various samples. DPPH radical is scavenged by antioxidant through the donation of electron forming the reduced DPPH. The color of the sample changes from purple to pale yellow which can be quantified by its decrease of absorbance at wavelength 517 nm (Maw *et al*., 2011).The radical scavenging activity and crude extracts were expressed in term of % RSA and IC_{50} (50 % inhibitory concentration).In this study, six different concentrations (20, 10, 5, 2.5, 1.25 and 0.625 µg /mL) of crude extracts were prepared by serial dilution. Ascorbic acid was used as standard and DPPH and ethanol without crude was employed as control. Absorbance was measured at λ_{max} 517 nm using UV-visible spectrophotometer (UV-1800, Shimadzu).From their average values of percent inhibition, IC_{50}(50 % inhibition concentration) values in µg/mL were calculated by linear regressive

excel program. The IC_{50}values of ethanol extract of Na-Nwin-Tain-Pyar rhizomes was1.56µg/mL and watery extract was 3.95µg/mL (Table 3 and Figure 4).The lower the IC_{50}values, the higher the free radical scavenging activity, the higher the antioxidative property. According to the results, the IC_{50}values of ethanol extract of *C. aeruginosa* Roxb. (IC_{50} = 1.56 µg/mL) was comparable to that of standard ascorbic acid (IC_{50} = 1.21 µg/mL), indicating that ethanol extract has potent antioxidant property than water extract.

Table 3 % Radical Scavenging Activity (% RSA) and IC_{50} Values of Ethanol and Watery Extracts of (Na-Nwin-Tain-Pyar) Rhizomes and Standard Ascorbic Acid

Samples	% Inhibition (Mean ± SD) in Different Concentration (µg/mL)						IC_{50}(µg/mL)
	0.625	1.250	2.500	5.000	10.000	20.000	
Ethanol Extract	42.01 ± 2.58	46.80 ± 0.32	51.14 ± 1.29	57.53 ± 1.93	62.78 ± 2.26	71.68 ± 1.29	1.56
Water Extract	36.11 ± 0.30	40.17 ± 1.21	44.66 ± 1.51	53.85 ± 0.60	57.91 ± 0.91	63.68 ± 1.21	3.95
Ascorbic acid	47.81 ± 0.88	57.18 ± 1.32	61.09 ± 0.22	67.50 ± 0.44	71.71 ± 1.54	79.21 ± 2.43	1.21

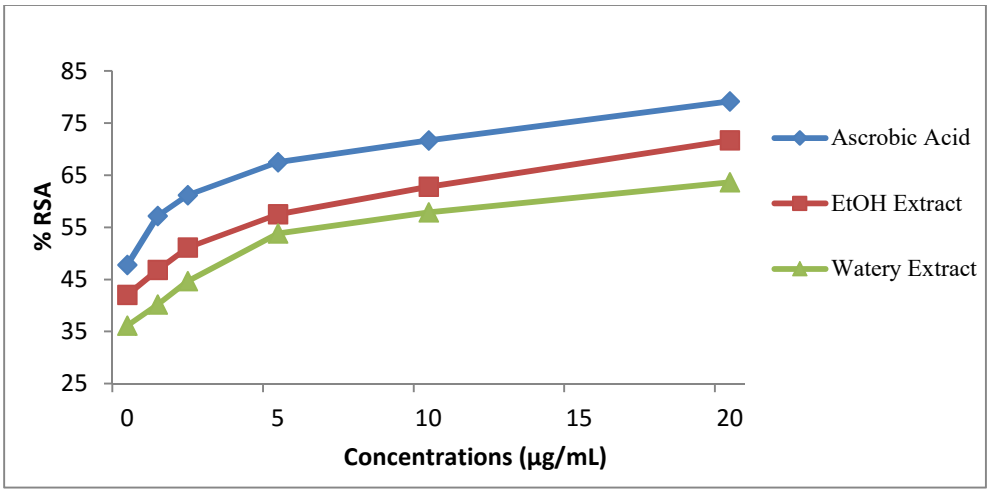

Figure 4 **% RSA vs. concentration of ethanol and watery extracts of (Na-Nwin-Tain-Pyar) rhizomes and standard ascorbic acid**

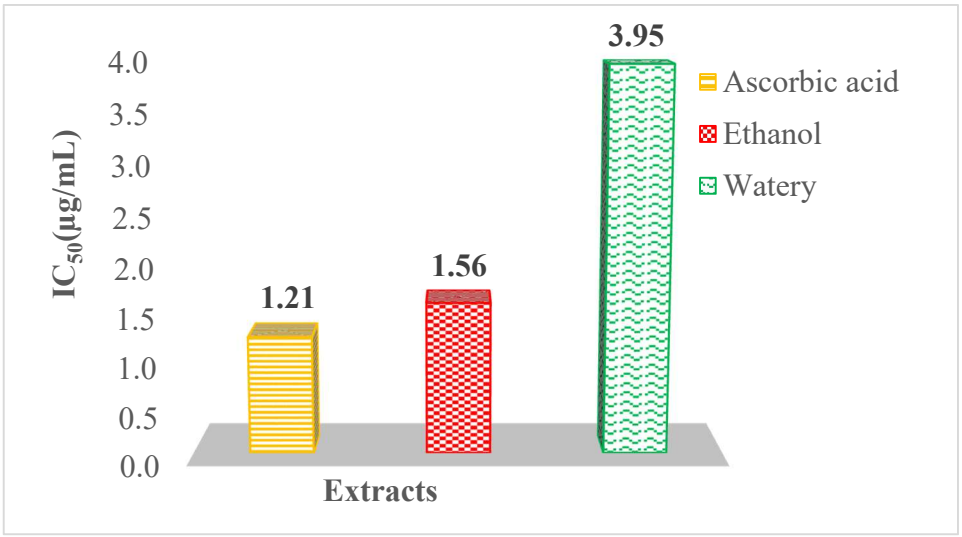

Figure 5 **IC_{50} values ofethanol and watery extracts of (Na-Nwin-Tain-Pyar) rhizomes and standard ascorbic acid**

Antimicrobial Activity of Crude Extracts of (Na-Nwin-Tain-Pyar) Rhizomes by Agar Well Diffusion Method

Screening of antimicrobial activity of crude extracts such as PE, EtOAc, 95 % EtOH and H_2O extracts from (Na-Nwin-Tain-Pyar) rhizomeswere done by agar well diffusion method. In this investigation, the various crude extracts was determined against seven species of microorganisms such as *B. subtilis, S. aureus, P. aeruginosa, B. pumilus, C. albicans* , *E. coli and A. tumefaciens* by using agar well diffusion method. The larger the inhibition zone diameters, the higher the antimicrobial activity.EtOH extract showed high potent antimicrobial activity with the inhibition zone diameter range between (16 ~ 25 mm) against seven microorganisms, whereas it could inhibit for *Agrobacterium* activity with the largest diameter 25 mm (Table 4 and Figure 7). In addition EtoAc extract inhibits all organisms with the same diameter 15 mm. Almost all of PE extract inhibited against all microorganisms except *P.aeruginosa*. However H_2O extract showed low activity on *B. subtilis, P.aeruginosa* and *B. pumilus* with the same diameter 11 mm and inactive against remaining microorganisms. From these findings, it could be concluded that (Na-Nwin-Tain-Pyar) rhizomes could be employed to cure ailment caused by such bacteria.

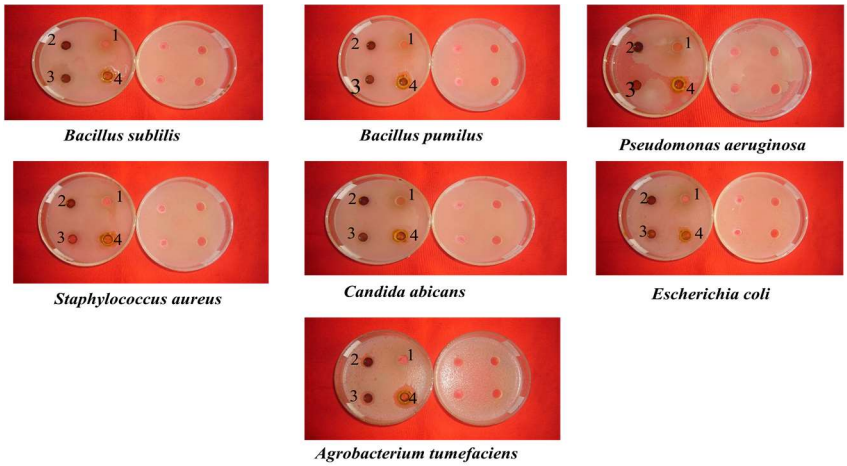

Figure 6 Effect of antimicrobial activity of (1) H_2O, (2) PE, (3) EtOAc, and (4) EtOH extracts from (Na-Nwin-Tain-Pyar) rhizomes on seven microorganisms

Table4 Antimicrobial Activity of Four Crude Extracts from(Na-Nwin-Tain-Pyar) Rhizomes by Agar Well Diffusion Method

No.	Microorganisms	Diameter of inhibition zone (mm)			
		PE extract	**EtOAc extract**	**EtOH extract**	**H_2O extract**
1.	*Bacillus subtilis*	13 (+)	15 (++)	20 (+++)	11 (+)
2.	*Staphylococcus aureus*	14 (+)	15 (++)	20 (+++)	_
3.	*Pseudomonas aeruginosa*	_	15 (++)	16 (++)	11 (+)
4.	*Bacillus pumilus*	14(++)	15 (++)	20 (+++)	11 (+)
5.	*Candida albicans*	14 (+)	15 (++)	18 (++)	_
6.	*Escherichia coli*	14 (+)	15 (++)	17 (++)	_
7.	*Agrobacterium tumefaciens*	14 (+)	15 (++)	25 (+++)	_

Agar Well – 10 mm

10 mm ~ 14 mm	(+)	low activity
15 mm ~ 19 mm	(++)	medium activity
20 mm ~ above	(+++)	highest activity

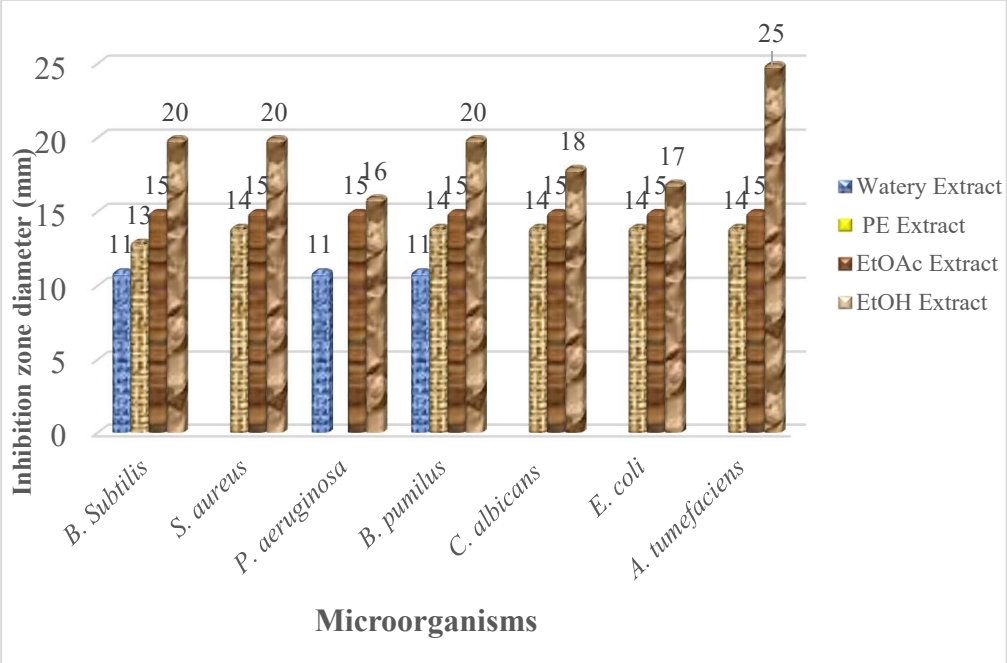

Figure 7 A bar graph of inhibition zone diameters of four crude extracts from (Na-Nwin-Tain-Pyar) rhizomes against seven microorganisms

Antitumor Activity of (Na-Nwin-Tain-Pyar) Rhizomes by Potato Crown Gall Test

The antitumor activity of EtOH and EtOAc extracts of (Na-Nwin-Tain-Pyar) rhizomes were investigated by using PCG test with bacterium *A. tumefaciens*. For inoculation of the potato disc, 48 hr broth cultures containing 5×10^9cells/mL were used. The tested samples were dissolved in DMSO, diluted and mixed with the bacterial culture for inoculated on the cleaned and sterilized potato discs, and incubated for 7 days, at room temperature. After that, the tumors were appeared on potato disc and checked by staining the knob with Lugol's(K_2-KI)solution. In the control, the formation of white knob on the blue background indicated the presence of tumors cells because there is no protein in tumor cells. The activities of test samples did not form any tumors on the potato discs and its surface remained blue. Tumors were counted with the aid of a dissecting scope after staining with Lugol's solution.

From this experiment, it was found that both EtOH and EtOAc extracts of *C. aeruginosa* Roxb. rhizomes were good for preventing the antitumor formation with the dose of 0.1 and 0.15 mg/discin *vitro* potato disc assays. In addition, both EtOH and EtOAc extracts were not significantly inhibited the formation of tumor with the dose of 0.05 mg/disc. The quantitative criteria and results are given as (-) for high inhibition, (+) for less activity and (++) for non inhibition of tumor growth after visual comparison with the control (Table 5 and Figure 8).

Table5 Antitumor Activity of EtOH and EtOAc Extracts of (Na-Nwin-Tain-Pyar) Rhizomes by PCG Test

No.	Test samples	Day	Concentration/disc (mg)		
			0.05	**0.1**	**0.15**
1.	Ethanol Extract	5 days	+	-	-
		7 days	+	-	-
2.	Ethylacetate Extract	5 days	+	-	-
		7 days	+	-	-
3.	Control		++		

Tumor Inhibition: (++) = non activity, (+) = less activity,(-) = high activity

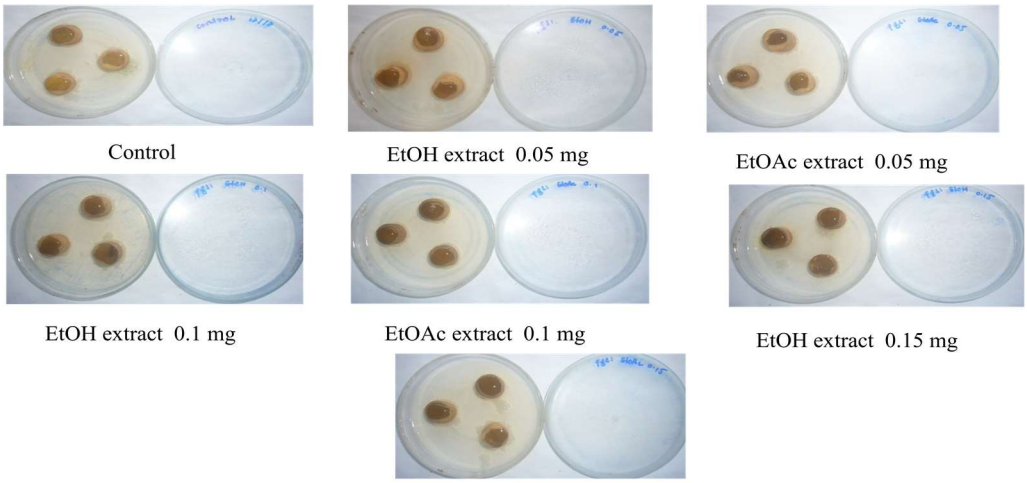

Figure 8 Antitumor screening of EtOH and EtOAc extracts of (Na-Nwin-Tain-Pyar) rhizomes incubated for7 days

CONCLUSION

By the biological activities investigations of *C.aeruginosa* Roxb. rhizome, the following inferences could be concluded.

Preliminary phytochemical screening of (Na-Nwin-Tain-Pyar) rhizomes powder indicated the presence of alkaloids, α-amino acid, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, starch, steroids, terpenoids, and tannins. However, cyanogenic glycoside and saponin were not detected at the assay conditions. From the screening of the antioxidant activity, ethanol extract (IC_{50}= 1.56 µg/mL) was found to be more potent than watery extract (IC_{50} =3.95 µg/mL).For the determination of the total phenol contents (TPC) of watery and ethanol crude extracts, it was observed that ethanol extract (52.31 ±8.70 µg GAE/mg) was found to be higher than watery extract (16.92 ± 0.20 µg GAE/mg) of (Na-Nwin-Tain-Pyar) rhizomes. In addition, there was a positive correlation between the total phenolic content and antioxidant activity in the selected plant sample. The results indicated that high phenolic provided more potent antioxidant activity.

For the screening of antimicrobial activity of the various crude extracts(PE, EtOAc, EtOH and watery extracts) from (Na-Nwin-Tain-Pyar) rhizomes sample the highest antimicrobial activity was observed in EtOH extract whereas H_2O extract showed minimum activity. Antitumor activity was carried out with EtOAc and EtOH extracts by PCG test. From this experiment, both extracts were found to prevent the tumor formation with the dose of 0.1 and 0.15 mg/disc.

The rhizome of (Na-Nwin-Tain-Pyar) could be applied not only as the local health remedy to the local indigenous but also for the treatment of some bacterial plant pathogen and diseases in agriculture of our country.

ACKNOWLEDGMENT

The authors would like to thank the Department of Higher Education, Ministry of Education, Yangon, Myanmar, for the permission of doing this research and Myanmar Academy of Arts and Science for allowing the writing of this paper. I would like to express my sincere thanks to Dr Daw HlaNgwe (Professor and Head), Department of Chemistry, University of Yangon, for her kind advice, guidance and permission to allow this research work. The authors would like to thank for all from the Department of Chemistry.

REFERENCES

Maw, S. S., Mton M. M. and OoZ. K. (2011). "Study on Antioxidant Activities of Some Herbal Extracts". *World Academy of Science, Engineering and Technology*, vol**51**, pp 450 – 452

Newman, M.F., Lhuillier A., Poulsen A.D. (2004). "A Checklist of the Zingiberaceae of Malasia". *Blumea.,*vol **11**,pp 16-25

Reynertson, K. A. (2007). "*Phytochemical Analysis of Bioactive Constituents from Edible Myrtaceal Fruits*". PhD (Dissertation), New York, the City University

Srivastava S., Dan M.., Rawat A. (2006). "Pharmacognostic Evaluation of *Curcuma aeruginosa* Roxb.". *Nat Prod Sci.,vol* **12**,pp 162-165

Thomas, J., Joy P., sSkaria P. (1998). *Medical Plants*. India: 1st Ed., Kerala, 3-4

WHO. (1991). *Guidelines for the Assessment of Herbal Medicines, Programme on Traditional Medicine*.Geneva:1st Ed, World Health Organization,91-94