Study on Antioxidant Activity of *Commelinanudiflora* L.

(Myat Kyut)

Khine Khine Lin [1] , Cherry Ohn[2]

khinekhinelin@gmail.com,dr.cherryohn98@gmail.com

ABSRACT

The present work was carried out to study the antioxidant activity of the plant of *Commelinanudiflora*L. (Myatkyut). It is native to Asia and traditionally used as a medicinal plant due to the presence of some effective phytoconstituents. In this work, the sample of whole plant was collected from Hinthada University campus. Preliminary phytoconstituents investigation of the plant was done by standard procedures. Alkaloids, flavonoids, phenolic compounds, terpenoids, steroids,glycosides, amino acids, starch, reducing sugars, tannins and saponins were present in the plant, but cyanogenic glycosides were not detected. The antioxidant activity of the *Commelinanudiflora*wasdetermined by DPPH assay method. Free radical scavenging activity (antioxidant activity) of different crude extracts (pet-ether, acetone, ethanol and water) of the plant against DPPH free radicals was expressed in terms of IC_{50} values. Among the extracts, ethanol extract was found to be higher antioxidant activity (10.13 µg/mL), followed by water extract (11.25 µg/mL), acetone extract (14.78 µg/mL) and pet ether extract (17.71 µg/mL).

Keywords :*Commelinanudiflora*L. , phytoconstituents, antioxidant activity, DPPH, IC_{50}

1, Lecturer,Dr, Chemistry department, West Yangon University

2, Lecturer,Dr, Chemistry department, West Yangon University

INTRODUCTION

Commelinanudiflora, belongs to the family of commelinaceae is a weed. It is ascending branched perennial herb,usually pubescent.*Commelina* species are used to cure various chronic and acute diseases.The stem of the plant is 15–30 cm long, with green leaves and purple flowers. It is mostly found in wet places. It is native to Asia and which has been distributed in various places in India, China, Malaysia, Africa Egypt and Myanmar. The plant is used in the treatment of intestinal obstruction, diarrhea, hemorrhoids, abnormal uterine bleeding, and vaginal discharge. In addition to that, it is also used to cure wart and erysipelas (deep red inflammation of skin). In East Africa, *C. nudiflora* is consumed for sore throats, while in India, the plant is believed to be beneficial in the treatment of leprosy.It control various bacterial and fungal diseases, used traditionally to heal various chronic diseases such as diabetes, skin diseases and atherosclerosis(Anto&Jeya 2014).The plant of *C.nudiflora* is shown in figure 1.

Scientific Classification of the Plant

Botanical name :*Commelinanudiflora* L.

Family : Commelinaceae

Genus : *Commelina*

Species : *nudiflora*

Myanmar name :Myatkyut

Part used : Whole plant

Fig.1 Photograph of plant of *Commelinanudiflora* L.

Free Radicals

Oxygen is the molecules of life for us, aerobic creatures when oxygen gets into our blood stream and cells. Oxygen undergoes chemical reactions with the carbohydrate molecules and generates energy molecules such as ATP (adenosine triphosphate). However oxygen not only generates energy but it also generates undesirable side products such as free radicals. Moreover, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also cause free radicals.These generated radicals damaged DNA and essential molecules in our body. Normally, the body can handle free radicals but if antioxidants are unavailable or if the free radical production becomes excessive, damage can occur.As the molecular damages in proteins and DNA accumulate are the chance for diseases such as cancer and cardiovascular diseases. (Cheeseman& Slater, 1993).

Antioxidant

Antioxidants are a diverse group of chemicals that can be naturally found in vegetables, fruits and plants in general. Examples of dietary antioxidants are vitamin E,C, phenolic acids, selenium, chlorophyll and chlorophyll derivatives, carotenoids, flavonoids, glutathione, coenzyme Q 10, melatonin, and lycopene. Antioxidant have anti-aging effect because they are scavenging of free radicals (and other reactive oxygen species) which are linked with human diseases including cancer, cardiovascular disease, and with aging.

Antioxidants protect the body from oxidative damage induced by free radicals and reactive oxygen species by suppressing their formation; and acting as scavengers; and acting as their substrate. Antioxidants are essential for proper function of the immune system. This is partly because immune cells produce free-radicals for normal defense functions. If the level of free radicals in the immune cells surpasses beyond the normal level, they negatively affect the immunesystem. On the other hand, antioxidants act as scavengersof the free radicals in cells and therefore promote our immunity. Imbalance between free radical and antioxidants in cells, due to deficiency in single or multiple antioxidants has been reported to result in weakness of immunity function (Salvayre, *et.al.,* 2006).

Principle of DPPH assay

DPPH has a free radical that tends to capture hydrogen from the antioxidants. Due to its free radical, the methanolic DPPH solution is violet and absorbs at 517 nm. The colour changes upon neutralization of the free radical from violet to pale yellow. Measuring the absorbance at 517 nm allows to determine the DPPH proportion being neutralized by the test substance. The decolourization of the initial colour is proportional to the test substances anti-radicalizing (oxidant) power.(Yamaguchi *et.al.,* 1998).

EXPERIMENTAL

Sample Preparation

The present research was carried out in the Chemistry laboratory, Department of Chemistry, Hinthada University. The whole plant of *C.nudiflora*L. (Myat-Kyut) was collected from HinthadaUniversity Campus.The collected plants were identified by Botany Department ,Hinthada University. After washing with water, all the plants were sliced into small pieces and dried in shadow for two weeks. Then the dried pieces were ground into powder by the aid of grinding machine and stored in airtight container.

Investigation of Phytoconstituents on *C.nudiflora* L.

Phytochemical screening on the dried sample of *C.nudiflora*L. was done according to standard procedures. It involves testing of different extracts of the sample for their contents of different classes of compound such as alkaloids, terpenoids, flavoids, phenolic compound, steroids, glycosides and amino acid.

Screening of Antioxidant Activity

Accurately weighed 2 mg of each test sample and 10 mL of 95 % EtOH was thoroughly mixed by shaker. The mixture solution was filtered and the stock solution was obtained. Desired concentrations 1.25 μgmL^{-1}, 2.5 μgmL^{-1}, 5 μgmL^{-1}, 10 $\mu gmL^{-1}$and 20 μgmL^{-1} of each solution was prepared from this stock solution by dilution with appropriate amount of 95 % ethanol.

The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95% ethanol using shaker.

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 solution wasallowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the equation. Then IC_{50} (50% inhibitory concentration) value was also calculated by linear regressive excel program.

Blank solution was also prepared by mixing the test sample solution (1.5 mL) with 95 % ethanol (1.5 mL).

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{blank}) - A_{test\ sample}}{A_{control}} \times 100$$

% Inhibition = percent inhibition of sample

$A_{control}$ = absorbance of DPPH in EtOH solution

$A_{test\ sample}$ = absorbance of (sample + DPPH) solution

A_{Blank} = absorbance of (sample + EtOH) solution

RESULTS AND DISCUSSION

Preliminary Phytochemicals Investigation

Before conducting other investigation, it is essential to carry out preliminary phytochemical investigation on *C.nudiflora* L.Test results revealed the presence of phenolic compounds, alkaloids,flavonoids, tannins, steroids, terpenoids, starch, saponins, α-amino acids and glycosides in the sample, whereas cyanogenic glycoside were not found in the plant.All the results obtained were represented in Table 1.

Antioxidant Activity of the *C.nudiflora* L. (Myatkyut)

Free Radical Scavenging Activity**(**antioxidant activity)was studied on petroleum ether, acetone, ethanol and watery extracts of the*C.nudiflora* by DPPH free radical scavenging assay method. DPPH (1,1-diphenyl -2-picryl-hydrazyl) assay method is the most widely reported method for screening of antioxidant activity on many plant drugs. This method is based on the reduction of coloured free radical DPPH in ethanolic solution by different concentration of the samples. In this experiment, five different concentrations (1.25 μ g/mL, 2.5 μ g/mL, 5 μ g/mL, 10 μg/mL and 20 μg/mL)of each crude extract were prepared in ethanol solvent. Ascorbic acid was used as standard and ethanol without crude extract was employed as control. Determination of absorbance was carried out at wave length 517 nm using UV visible spectrophotometer. Each experiment was done triplicate.The antioxidant activity was expressed as 50% oxidative inhibitory concentration (IC_{50}). IC_{50} is the concentration of sample or antioxidant required to inhibit the initial absorbance of DPPH free radical by 50% and a lower IC_{50} value would reflect greater antioxidant activity of the sample.

The percent oxidative inhibition values of crude extracts measured at different concentrations and IC_{50} value are summarized in Table 2. From these experimental results, ethanol extractshowedgreater antioxidant activity as it possesses lower IC_{50} value of 10.13 μ g/mL. According to the results, it was found that as the concentrations increased, the absorbance values decreased i.e, increase in radical scavenging activity of crude extracts.

Table 1.Result of Phytochemical Examination on *C.nudiflora* L.(MyatKyut)

No	Constituents	Extracts	Reagents	Observation	Remark
1.	Alkaloids	1% HCl	Mayer's reagent	White ppt	+
			Dragendroff's reagent	Orange ppt	+
			Wagner's reagent	Reddish-brown ppt	+
2.	Phenolic compounds	H_2O	1% $FeCl_3$, $K_4Fe(CN)_6$	Green colour	+
3.	Flavonoids	EtOH	Mg turning and HCL (con.)	Reddish-pink	+
4.	Steroids	$CHCl_3$	Acetic anhydride, con H_2SO_4	Greenish- blue	+
5.	Terpenoids	$CHCl_3$	I_2 solution	Reddish-brown	+
6.	Tannins	H_2O	3% $FeCl_3$	Green color	+
7.	Saponins	H_2O		Frothing	+
8.	α-amino acids	H_2O	Ninhydrin	Violet spot	+

9.	Glycosides	H_2O	Lead II acetate	White ppt	+
10.	Starch	H_2O	I_2 solution	Blue ppt	+
11	Cyanogenic Glycosides	H_2O	Sodium picrate solution	No Brick-red	_
12.	Reducing sugar	Dil H_2SO_4	NaOH, Benedict's Solution	Brick- red ppt	+

(+) = presence (-) = absence

Table 2.Oxidative Inhibition Percent and IC_{50} Values of Different Extracts of *C.nudiflora*L. and Standard Ascorbic Acid

Extracts	Inhibition percent in different concentrations (μg/mL)					IC_{50} (μg/mL)
	1.25	2.5	5	10	20	
Ethanol	0.54	6.47	44.21	57.47	68.04	0.13
Watery	3.5	0.21	37.73	49.77	61.07	11.25
Acetone	1.21	5.95	33.06	45.23	57.23	14.78
Pet-ether	3.21	6.28	38.05	46.68	58.63	7.71
Ascorbic acid	5.53	57.25	68.38	75.07	82.84	.14

CONCLUSION

In this work, the free radicals scavenging activity (Antioxidant activity) of the plant of *C.nudiflora*L.(MyatKyut) was studied according to DPPH assay method. Preliminary phytochemical examination of the plant indicated the presence of effective constituents such as alkaloids, flavonoids, tannins, steroids, terpenoids and phenolic compounds. Therefore *C.nudiflora* possesses medicinal properties. The antioxidant activity of this plant with different solvents was represented in terms of IC_{50} value (50% inhibition percent). The lower the IC_{50} value, the greater is the antioxidant activity. Ethanol extract showed greater antioxidant activity as it possesses lower IC_{50} value of 10.13μg/mLthan the others. On the basis of the present study, edible weed plant *C.nudiflora* is a potential source of antioxidant activity and eating the plant can prevent oxidative damage and aging in our body. Therefore, *C.nudiflora* L. (MyatKyut) is one of the naturally occurring valuable plantsin Myanmar.

ACKNOWLEDGEMENT

The authors would like to express their profound gratitude to Professor and Head, Dr SandaKharand DrMyintNgwe, Professor, Department of Chemistry West Yangon University for their kind supervision and encouragement to present this research work.

REFERENCES

AntoSuganya R , JeyaJothi G, (2014), " *Preliminary Phytochemical Screening , Antibacterial and Antioxidant activities of Commelinanudiflora L.*

(Commelinaceae) " International Research Journal of Pharmacy , vol.5 (11): pp.2230 - 8407.

Cheeseman, K.H. and Slater,T.F(1993)."Introduction to free Radical Biochemistry".*British*

*Medical Bulletin,*vol.49(3), pp. 481-493

Mohammad AA Khan. *et al (*2011)*, "Antibacterial activity of different fractions of Commelina benghalensis L.*" Der Pharmacia Sinica ;vol.2 (2): pp.320-326

Slavayre, A.N., DoussetN., and Ferretti, G (2006). "Properties of High density Lipoproteins in

Vascular Cells ". *Free Radical Biology and Medicine*, vol.41(7),Pg. 1031-1040

Yamaguchi, T. H., Matoba, T. and Terao, J (1998). "HPLC Method for Evaluation of the Free

Radical Scavenging Activity of Foods by Using 1,1-Diphenyl 2-picryhydrazyl".*Biochemistry,* vol.62(6), 1201-1204