Chemical Characterization and Pharmacological Action of *Cynodondactylon*(L.) Pers. (myin-sa-myet)

Aye Myint Sein[1] and Kyaw Khine Oo[22]

[1]ayemyintsein2018@gmail.com

Abstract

The selected medicinal plant,*Cynodondactylon*(L.)Pers. (myin-sa-myet) was chosen for the investigation of chemical analysis and biological action. Preliminary phytochemical investigations on dried powder of myin-sa-myet sample indicated the presence of alkaloids, α -amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, reducing sugars, saponins, steroids, terpenoids and tannins. The elemental analysis of myin-sa-myet sample was carried out by EDXRF method. By EDXRF method, it was found that potassium was the most abundant element and no heavy toxic elements were detected. In antimicrobial screening, H_2O, EtOH, EtOAc and PE extracts for this plant was examined by using agar well diffusion method. Among these extracts EtOAc (ID 15-21 mm) and EtOH (ID 15-20 mm) show more significant antimicrobial activity than that of other crude extracts.MeOH crude extract from myin-sa-myet may was investigated by using rapid screening of antioxidant activity by dot-blot and DPPH staining method. Therefore, myin-sa-myet may contribute significantly to potent anti-microbial activity and antioxidant activity.

Keywords:*Cynodondactylon*(L.)Pers.(myin - sa - myet), EDXRF, Antimicrobial activity, Antioxidant activity

Introduction

The Grass *Cynodondactylon* is also known as the Bermuda grass (creeping grass), light green in color, very tough and has a rough texture. The English name of *Cynodon* is Bermuda grass and belongs to family of Poaceae. The roots are whitish, tough and creeping, almost woody with smooth fibers. Leaves tapering to a sharp point, ribbed with smooth sheath and hairy stipules. It is native to East Africa, Asia, Australia and southern Europe (Mohamed Shabil. M., *et al*., 2013). Cynodonis used as control diabetes agent in India. The leaf extract is used to be anti-diabetic, antioxidant and hypolipidemic efficacy. The rhizomepart isalso used for anti-emetic, purifying agent and dysentery. The plant extract isalso used to significant application in secondary syphilis, wounds and cardio protective. Decoction of whole plant is orally taken in a dose of 300 - 600 ml, for oliguria, neurasthenia and eye diseases. Fresh plant is cooked with fish or prawn and it is taken for renal diseases, diarrhoea diseases, edema and skin infections. Myin-sa-myetis found in wild everywhere in Myanmar.

Materials and Methods

Collection and Preparation of Plant Sample

*C. dactylon*was collected from Maubin Township from June to September, 2017.The collected sample was identified by authorized botanists, in Department of Botany, Maubin University.Sample was air-dried for two to three weeks.Thedried plantwas made into powder by using blender and then stored in air-tight container.

[1]Associate Professor, Dr, Department of Chemistry, Bago University
[2]Lecturer, Department of Chemistry, Maubin University

Figure 1. Photograph of *Cynodondactylon* (L.) Pers. (myin-sa-myet)

Chemical and Reagents

Chemicals used were petroleum-ether, ethyl acetate, methanol and ethanol which are procured, from internationally established companies such as BDH, Kento, Merck, Hopkin and Williams, and also locally from the commercial chemical stores.The chromatographic separation of chemical constituents includedSilicagel GF_{254}precoated plates (250 μm layers thickness; Sigma-Aldrich, Germany).The reagents used for colour reaction tests were Dragendoff's, Mayer's,Sodium picrate reagents, Ninhdrin reagent,Liebermann-Burchard, 10 % $FeCl_3$, 10 % α-napthnol, 10 % lead acetate, and aceticanhydride.

Preliminary Phytochemical Test on *Cynodondactylon*(L.) Pers.

Preliminary phytochemical examination was carried out on dried powder of*C. dactylon*with a view to determine the presence or absence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids and tannins(Finar, I. L.1969).

Elemental Analysis of Plant Sample by EDXRF

The element contents of *C. dactylon*powder was determined by EDXRF(Shimadzu EDX-8000); C-H balance method. In order to determine the heavy toxic metals and macronutrient elements in*C. dactylon*plant was determined by EDXRF method (Griken et al., 1986)at the West YangonUniversity.

Screening of Antimicrobial Activity Effects of Crude Extracts

For the examination of *in vitro* antimicrobial effects of PE, EtOAc, EtOH and H_2O extracts of the sample from *C. dactylon*plant were investigated by *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* species using agar well diffusion method in Pharmaceutical Research Department, Yangon.

Rapid Screening of Antioxidant by Dot-Blot and DPPH Staining

DPPH (1, 1-diphenyl-2-picryl–hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food systems. In this experiment, the antioxidant activity was studied on MeOH extracts from the sample.Each diluted sample of the methanol extractswas carefully loaded onto a 6 cm × 6 cm TLC layer (silica gel GF_{254}precoated plates; Merck) and allowed to dry (3 min). Drops of each sample were loaded, in order of decreasing concentration (400, 200,100, 50, 25 and 12.5 μg/mL), along the row. The sheet bearing the dry spots was placed upside down for 10 s in a 60 μM DPPH

solution. Then the excess of solution was removed with a tissue paper and the layer was dried with a hair-dryer blowing cold air. Stained silica layer revealed a purple background with white spots at the location where radical-scavenger capacity presented. The intensity of the white color depends upon the amount and nature of radical scavenger present in the sample (Huang et al., 2004).

Results and Discussion

Preliminary Phytochemical Investigation of *Cynodondactylon*(L.) Pers.

From the preliminary phytochemical tests, it was observed that alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins and steroids were present in *C. dactylon*plant.

Elemental Analysis of Plant Sample by Energy Dispersive X-Ray Fluorescence (EDXRF) Method

With a view to determine the heavy toxic metals and macronutrient elements in the *C. dactylon*, the elemental analysis was performed by EDXRF in Section 2.As a results K (1.68%), S (0.54%), P (0.17 %), Ca (0.09%), Fe (0.03 %) and CH (97.37 %) detected in*C. dactylon*. They are essential elements for various metabolism and activity of enzyme of body.Among them, potassium peak was also the most predominant and so it showed potassium was the highest contentand no heavy toxic elements were detected.

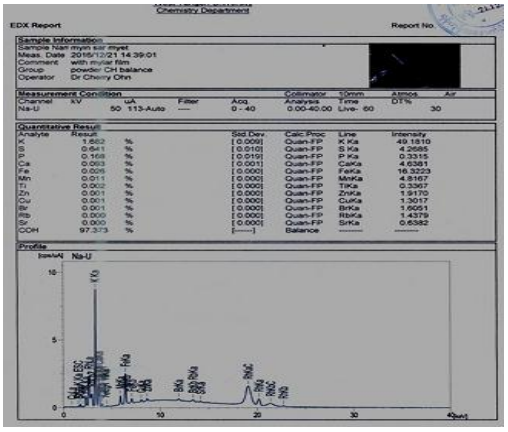

Figure 2. EDXRF spectrum of*Cynodondactylon*(L.) Pers.

Screening of Antimicrobial Activity of Crude Extracts by using Agar Well Diffusion Method

Antimicrobial activities of PE, EtOH, EtOAc and H_2O extracts were screened by agar diffusion method. In this investigation, the samples were tested on

six species of microorganisms; *Bacillus subtils*, *Staphlococcusaureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans*and *Escherichia coli* species.

EtOAc and EtOH extracts of *C. dactylon*plant showed more significant zone of inhibition when compared with other extracts of this plant. The activities of EtOAc extract on the organisms are considerably high. (zone of inhibition ranged from 15 to 21mm). The EtOH extract also showed antimicrobial activity against six species of microorganisms (zone of inhibition ranged from 15 to 20mm).

The results obtained from tested samples are shown in Figure 3and Table1.

Table 1. Antimicrobial Activity of Crude Extracts of *Cynodondactylon*(L.) Pers.by Agar Well Diffusion Method

Organisms	Diameter of inhibition zone (mm)			
	PE extract	EtOH extract	EtOAc extract	H_2O extract
B. subtilis	12	15	17	15
	(+)	(++)	(++)	(++)
S. aureus	15	17	17	14
	(++)	(++)	(++)	(+)
P. aeruginosa	15	16	20	15
	(++)	(++)	(+++)	(++)
B. pumilus	15	20	21	12
	(++)	(+++)	(+++)	(+)
C. albicans	14	17	21	15
	(+)	(++)	(+++)	(++)
E. coli	15	17	15	16
	(++)	(++)	(++)	(++)

Agar Well – 10 mm

10 mm ~ 14 mm (+) (lower activity),15 mm ~ 19 mm (++)(higher activity)

20 mm above (+++) (highest activity)

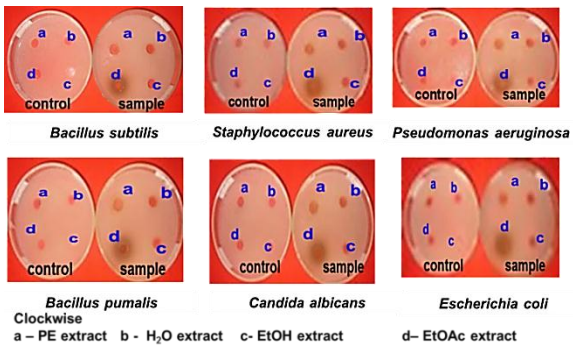

Figure 3. Antimicrobial screening of crude extracts of *Cynodondactylon*(L.) Pers.

Rapid Screening of Antioxidant by Dot-Blot and DPPH Staining

The antioxidant activity was studied on the methanol extracts from the selected plant samples by rapid screening of antioxidant by dot-blot and DPPH staining method . The principle of this method is that, in the presence of a stable free radical (DPPH), an antioxidant donates a hydrogen atom to quench the stable free radical. DPPH method is a simple method and can be applied either when the antioxidant is in its pure form or in a mixture.

Antioxidant capacity of the *C. dactylon*was eye-detected semiquantitatively by a rapid DPPH staining TLC method. Each diluted crude MeOH extract fractions were applied as a dot on a TLC layer that was then stained with DPPH solution (Figure 4). Rapid screening of free radical scavenging activity of MeOH extract of myin-sa-myet plant by dot-blot and DPPH staining using test amounts 400 µg,200 µg,100 µg,50 µg,25 µg and 12.5 µg (conc: 2mg/mL to 0.062 mg/mL) respectively. The appearance of yellow colored spots have a potential value of antioxidant activity.

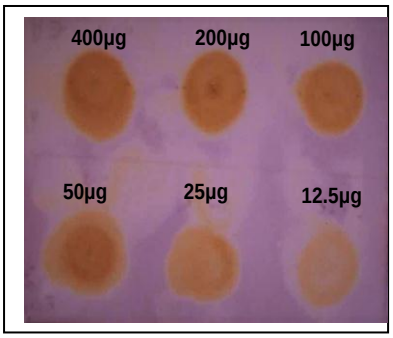

Figure 4. Screening of antioxidant activity of MeOH extracts (12.5µg to 400µg) of *Cynodondactylon*(L.) Pers.by dot-blot and DPPH staining

Conclusion

As the biological screenings, antimicrobial activity and antioxidant activity were demonstrated from activity guided plant extracts.

From previous results of phytochemical investigations by test tube method showed that the presence of α-amino acid, alkaloid, carbohydrate, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, steroids and tannins.

The elemental analysis of *Cynodondactylon*(L.) Pers. (myin-sa-myet) sample was carried out by EDXRF method. By EDXRF method, it was found that potassium was the most abundant element and no heavy toxic elements were detected. In the myin-sa-myet, K (1.68%), S (0.54%), P (0.17 %), Ca (0.09%), Fe (0.03 %) and CH (97.37 %) were found to be present.

In antimicrobial activity of the different crude extracts (H_2O, EtOH, EtOAc and PE) were screened by using agar well diffusion method against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*and *Escherichia coli* species. Among these extracts EtOAc(ID 15-21 mm) and EtOH(ID 15-20mm) show more significant antimicrobial activity than the other crude extracts.

MeOH crude extract from *Cynodondactylon*(L.) Pers. was investigated by using rapid screening of antioxidant activity by dot-blot and DPPH staining method. In this method, MeOH extracts of *C. dactylon*showed potent activity at dry matter amount

(12.5µg to 400µg dry matter/ml). The appearance of yellow colored spots have a potential value of antioxidant activity. Methanol extracts from *Cynodondactylon*(L.) Pers. was analyzed, yellow colored spots with strong intensity appeared fast up to the dilution of 12.5µg dry matter/mL.

Finally, the overall results of this study revealed a scientific finding;*Cynodondactylon*(L.) Pers. can be used for antimicrobial and antioxidant agents in traditional medicine. Phytochemical constituents such as phenolic compounds and flavonoids present in this plant may be responsible for these activities.

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