

Detection of Fungus from Old Palm-leaf Manuscripts Stored in the University of Yangon Library and Biochemical Properties of Citronella Grass



Penicillin



Aspersilla



Microrrhizal

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ABSTRACT

Citronella grass possess many useful biological activities which has been the reason for the selection of this plant for the present paper. The present paper is concerned with detection of fungus from old palm-leaf manuscripts stored in the University of Yangon Library and biochemical properties of citronella grass (*Cymbopogon winterianus* Jowitt). *Penicillin*, *Aspergillus*, *Microrrhizal* fungi were detected from old palm-leaf manuscripts by agar plate diffusion method. Essential oil content of citronella grass was found to be 0.6% by steam distillation. Biological activities such as acute toxicity, antifungal, antibacterial, antioxidant and mosquitoes repellent activities of its extracts and essential oil were determined. No acute toxic effect was found in both water and 70% EtOH extracts from citronella grass leaves at maximum dose of 12 g/kg bw. Citronella oil showed significant antifungal activity by using agar well diffusion method. According to GC-MS spectrum, citronellal was the major component of essential oil of citronella grass.

Keywords: Fungi, citronella oil, palm-leaf manuscripts

INTRODUCTION

Citronella oil is widely used in perfumery, cosmetics, soaps, detergents, confectionary and in the synthesis of vitamin A. The essential oil of citronella grass has an industrial profile; it is used in beverages, foodstuffs, fragrances, household products, personal care products, pharmaceuticals, and in tobacco. In our library, citronella oil was also used for the conservation of palm-leaf manuscripts. In the present review article, research carried out on different activities of citronella oil by different person at the department of chemistry, University of Yangon were presented. “Ei Ei Soe (2006) studied on the mosquito repellent activity of citronella oil”, “HlaThida Aung (2008) on antimicrobial, antidiarrhoeal and antioxidant activities of *Cymbopogon flexuosus* Stapf.” and “Khin San Nwe (2011) on the citronella oils from local and China *Cymbopogon winterianus* Jowitt. leaves and their bioactivities”. The materials and methods employed and the significant results they obtained were reviewed.

MATERIALS AND METHODS

Plant material : The leaves of citronella grass were collected from Paunggyi, Hlegu Township, Yangon Region. Plant sample was washed, followed by cutting, air-drying grinding into powder, and finally stored separately in air-tight containers for further works.

Extraction of essential oil : Extraction of essential oil from citronella grass was carried by steam distillation method. The dried powder (100 g) and distilled water (500 mL) were placed in 1L round bottomed flask. The flask was fitted for steam distillation and heated. The steam was passed into the flask. The condensed oil and water were collected in a flask and the oil was extracted with PE in a separating funnel. The PE extract was dried over anhydrous sodium sulphate. After filtration, the filtrate was evaporated to get the essential oil which was weighed and kept in air tight bottle for further analysis.

Screening of fungus : Detection of fungus from old palm-leaf manuscripts stored in the University of Yangon Library. This study was carried out by agar plate diffusion method.

Acute toxicity test

- (a) Tested Animals : 70 numbers of both sexes of albino mice
(ddy strain) having 25-35g body weight
- (b) Tested Extracts : 70% ethanolic extract and watery extract of citronella grass
- The acute toxicity test was done by the method of Litchfield and Wilcoxon, (1949). In this study, a total of 80 adult mice (ddy strain) of either sex weighing (25-35g) were used for ethanolic and watery extracts of citronella grass (Khin San Nwe, 2011).

Antifungal activity test

Screening of antifungal activity of citronella oil was carried out by agar well diffusion method. *Penicillin*, *Aspergillus* and *Microrrhizal* fungi were used in the investigation of antifungal activity.

Screening of antibacterial activity : The antibacterial activity of citronella grass oil was determined by agar disc diffusion method against *Shigella dysenteriae*, *Salmonella typhi*, *E.coli EPEC*, *Salmonella cholerae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholerae* and *Proteus morganii* (Finegold *et al*, 1978).

Determination of minimum inhibitory concentration (MIC) of citronella grass oil by agar plate dilution method

The MIC of citronella oil was determined by agar plate dilution method testing on *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella dysenteriae*, *Salmonella typhi*, *Proteus morganii*, *Bacillus subtilis*, *E.coli EPEC*, *Salmonella cholerae*. Prior to the testing, the bacterial suspension was prepared by inoculation of the test organisms in trypticase soy broth to obtain the turbidity which is equivalent to $10^5 - 10^7$ organisms per mL. The essential oil to be tested was dissolved in its respective solvent and mixed thoroughly with trypticase soy agar (TSA) to obtain 2 mg mL^{-1} . Serial double dilution was performed up to 10 flasks so as to obtain $0.0039 \text{ mg mL}^{-1}$. Then 20 mL each was transferred in petridish and allowed to settle. The solvent itself in TSA was also carried out in a same procedure to serve as control. After drying the plates, 0.02 mL of the bacterial suspensions was transferred by using a micropipette and allowed to dry the suspension. The plates were incubated at 37°C for 18-24 hours. The least concentration of extracts with no growth of the organism was termed minimum inhibitory concentration (MIC) expressed in $\mu\text{g mL}^{-1}$.

In vivo screening of antidiarrhoeal activity by using castor oil-induced albino mice model

In vivo anti-diarrhoeal effect of the citronella oil was examined by castor oil-induced diarrhoeal test, castor oil-induced entropooling test and castor oil-induced small intestinal transit test (Khin San Nwe, 2011).

Mosquito repellent test of crude extracts of citronella grass

Mosquito repellent tests were carried out by the methods described by Schreck (1985) and Frances (1993). Mosquito repellent tests were performed on two human volunteers (Ei Ei Soe, 2006).

Mosquitoes and bioassays: Laboratory reared 6 to 7 days-old female *Ae.aegypti* mosquitoes were used for mosquito repellent tests. These mosquitoes were obtained from Medical Entomology Research Division, Department of Medical Research, Lower Myanmar. For each test, 50 mosquitoes were placed overnight in a screen wire cage (30 cm x 30 cm x 30 cm) and 10% sugar solution was used for feeding. Mosquitoes were starved for 12 hours before testing. Three replicates were carried out for each run (Ei Ei Soe, 2006).

Determination of antioxidant activity : DPPH (2,2-Diphenyl-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant material. $160 \mu\text{g/mL}$ stock solutions of 70%EtOH and watery extracts of the plant samples were prepared. The

clear stock solutions were made by using 70% ethanol solvent for ethanol extract. Water solvent was used for watery extract and standard BHT. Each stock solution obtained was serially diluted with respective solvents to obtain desired concentration of 10, 5, 2.5, 1.25, 0.625 $\mu\text{g/mL}$ of each test sample solution (Khin San Nwe, 2011).

RESULTS AND DISCUSSION

In this paper *Cymbopogon winterianus* Jowit. leaves originated from Myanmar was chosen. According to preliminary phytochemical investigation, it was found that alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, steroids, tannins and terpenoids were present. However, there is no cyanogenic glycosides and saponins observed in tested sample, indicating that no toxic effect due to these compounds in tested sample. In this work, relative abundance of elements present in citronella grass were determined by EDXRF spectrometer. It can be seen that, potassium and chloride peaks are the most predominant peaks. Other elements such as Rb, Mn, and Br are also present in tested sample (Table 1).

Screening of fungus

Three fungi namely *Penicillin*, *Aspergillus* and *Microrrhizal* fungus were detected from old palm-leaf manuscripts stored in University of Yangon Library (Figure 1).

The acute toxicity of citronella grass leaves

From acute toxicity test of citronella grass leaves extracts on albino mice it was observed that even with the maximal permissible dose (12 g/kg bw) of 70% EtOH and aqueous extracts, the mice were found to be alive and healthy during the observation period of two weeks (Table 2). All of the animals remained alive and did not show any visible symptoms of toxicity like respiratory disorders, convulsions, and death etc. at the dosage tested.

Therefore, it can be inferred that both 70% EtOH and aqueous extracts of citronella grass leaves were free from acute toxic harmful effect at 12 g/kg bw dose. The medium lethal dose (LD_{50}) of plant extracts were more than 12g/kg body weight.

Antifungal activity of citronella oil

In *vitro* antifungal activity of citronella oil has been screened on three fungi: *Penicillin*, *Aspergillus* and *Microrrhizal* by agar well diffusion method (Figure 2 and Table 3). According to this experiment, the citronella oil may be used for the treatment of fungal infections.

Antibacterial screening of citronella grass oil by agar disc diffusion method

Antibacterial activity of citronella grass oil was investigated against 8 bacterial strains; *Shigella dysenteriae*, *Salmonella typhi*, *E-coli* EPEC, *Salmonella cholerae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Vibrio cholerae* and *Proteus morgani* by agar disc diffusion method. The inhibition zones including the diameter of filter paper disc in mm reflects the degree of

antibacterial activity of the samples. Essential oil of citronella grass inhibited all strains of bacteria and significant zone of inhibition range from (18~27 mm diameter). Therefore, it can be deduced that citronella grass oil has higher antibacterial activity (Figure 3 and Table 4).

The minimum inhibitory concentration (MIC) of citronella grass oil against different bacteria are shown in figure 4 and Table 5. The MIC values were found to be ranged from 1.562 $\mu\text{g/mL}$ to 12.50 $\mu\text{g/mL}$ for citronella grass oil concentration. The lowest MIC value for citronella grass oil showed 1.562 $\mu\text{g/mL}$ against *Shigella dysenteriae* and *E.coli*.

Effect on defecation of castor oil induced albino mice model

The antidiarrhoeal index (ADI) was found to be 135.87 % for citronella oil (6 mg/kg bw) and 134.44 % for standard loperamide (6 mg/kg bw). Since the higher the ADI, the larger the antidiarrhoeal efficacy of the sample, the citronella oil showed higher antidiarrhoeal activity than standard loperamide (Table 6).

Mosquito repellency test of citronella grass oil

The protection provided by citronella oil is proportional the dose; higher concentrations of citronella oil provide longer-lasting protection. The complete-protection times of citronella oil correlated positively with its concentration. 100% and 25% citronella oil provided at least 1 ½ h of complete appropriate to use under circumstances in which the biting pressures are intense, the risk of mosquito transmitted disease is great, or environmental conditions promote the rapid loss of repellent from the surface of the skin (Maibach *et al*, 1974). In this study, a formulation containing 25% citronella oil provided an average of 1½ h of complete protection against *Ae. Aegypti* bites after a single application. In addition, the repellency effect was comparable to 5% of DEET solution. It was also found that 5% solution of citronella oil in acetone-water provided quite enough % protection against *Ae. aegypti*, though it did not exhibit complete-protection. Moreover, 2% solution did not provide satisfactory repellent activity. Therefore, 5% concentration was preliminary chosen to compare the mosquito repellency between different tested solutions (Figure 5).

The IC_{50} values were found to be 1.25 $\mu\text{g/mL}$ for citronella oil, 2.12 $\mu\text{g/mL}$ for citronella leaves of (70 % EtOH extract), 1.76 $\mu\text{g/mL}$ for watery extract (Table 7). All of these extracts were found to possess the lower antioxidant activity than standard BHT (IC_{50} = 1.18 (g/mL).

GC-MS spectroscopic study on essential oil of citronella grass

According to GC-MS spectroscopic study, it was found that citronella grass oil contained citronellal, tetrahydro 4-methyl 2H-pyran-2-one. It was identified by matching its MS spectrum with the library data (Figure 6).

CONCLUSION

Penicillin, *Aspergillus*, *Microrrhizal* fungi were detected from old palm-leaf manuscripts stored in the University of Yangon Library. Citronella oil can be inhibited the detected fungi by agar well diffusion method.

Essential oil extracted from citronella grass can be used not only in the medicinal formulation for the treatment of bacterial infection, fungus infection but also for mosquitoes repellent and as natural antioxidants.

Now, citronella oil has already been used for the conservation of palm-leaf manuscripts in University of Yangon Library.

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