Antifungal Activity of Conyza canadensis ((L.) Cronquist) Collected in Northern Viet Nam

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Abstract— ***Conyza canadensis* (*C. canadensis*) ((L.) Cronquist) has been used as medicinal herb in many countries. The antifungal activity of *C. canadensis* which was extracted by different solvents including ethanol, ethyl acetate and n-hexane was examined by using agar well diffusion method and minimum inhibitory concentration method. Two fungal pathogens used to determine the bioactive activity of these extracts were *Candida albicans* (*C. albicans*) and *Trichosporon insectorum* (*T. insectorum*). Among these three extracts using different polarity solvents, the antifungal activity of *C. canadensis* extracted with ethyl acetate showed the highest antifungal activity against both tested fungal pathogens. Conversely, the extracts with ethanol and n-hexane didn't show any activity towards the tested fungi in the agar well diffusion experiment. *C. canadensis* extracted with ethyl acetate showed its high effect against *T. insectorum* with 45.33 mm of inhibition zone, antifungal activity was lower to *C. albicans* which was about 25.33 mm of inhibition zone. Minimum inhibitory concentration (MIC) of ethanol, ethyl acetate and n-hexane extract against *C. albicans* were 250, 15, and 500 mg/ml, respectively. MIC values with extracts in ethanol and ethyl acetate solvents in case of *T. insectorum* were lower than which of *C. albicans*. The particular MIC values of extracts in ethanol, ethyl acetate and n-hexane against *C. albicans* were 63, 8 and 1000 mg/ml, respectively.***

Keywords— ***C. canadensis*, *C. albicans, T. insectorum*, agar well diffusion method, minimum inhibitory concentration.**

I. INTRODUCTION

Conyza canadensis (*C. canadensis*) is a common medicinal herb found throughout most of North America. It is also named horseweed and belongs to Asteraceae family. In the traditional medicine of some Asian countries, *C. canadensis* functions as an effective treatment for many diseases causing by bacteria, fungi, or viruses including bronchitis, smallpox, cystitis, diarrhea [1, 2]. In China, *C. canadensis* is used as folk medicine to treat wound and pain caused by arthritis [3]. Its essential oil containing bioactive compounds can inactivate the growth of many types of microorganisms. In Viet Nam, data on the antifungal activities of this herbal plant is still limited. Furthermore, the differences in geographical origin of Vietnamese plants to that of other countries may affect their antifungal activity. Besides, many local traditional practitioners have been using *C. canadensis* to treat fungal infections indicating its high effectiveness. Therefore, it is necessary to study thoroughly their antifungal effects in order to improve their application and usage. This study aimed to determine the antifungal activity *C. canadensis* extracting in different solvents by using agar well diffusion and minimum inhibitory concentration (MIC) method.

II. MATERIALS AND METHOD

A. Plant Extract Preparation

The *C. canadensis* was collected randomly in northern Viet Nam particular in Hai Duong province, in May 2013. This plant was cleaned and subjected in drying process at constant temperature of about 50^{o}C until completely dry. Then, it was grinded to fine powder by using blender (Phillips, Japan) at the Laboratory of Chemistry. Ten grams of *C. canadensis* dried powder were macerated in 100 mL of three different solvents including ethanol, ethyl acetate and n-hexane for extraction. They were kept in bottles for 3 days to be extracted according to established protocol [4]. The samples were filtrated through Whatmann filter paper by using Buchner funnel. After that, the gained solutions in the three bottles were subjected to vacuum evaporation to get crude extracts.

B. Antifungal Assay

Antifungal assay was performed with 2 fungal pathogens, *Candida albicans* (*C. albicans*) and *Trichosporon insectorum* (*T. insectorium*) by using agar well diffusion method described in previous studies [5]. The surface of SDA (Sabouroud-dextrose agar) plate was seeded by 1ml of overnight fungal culture inoculum. Three tested wells were made on the agar plate by using Pasteur pipette. Different amount of crude extract were loaded into the wells (40µL/ well). Amphotericin B, a common antifungal drug, was used as positive control (40 µg/ well for *T. insectorum* and 10 µg/ well for *C. albicans*) while solvents were used as negative control. The agar plates were incubated at 37^{o}C for 48h. After 48h, diameters of inhibition zones were recorded.

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C. Minimum Inhibitory Concentration (MIC)

The MIC values were determined by using broth dilution method [6]. The extract was diluted in 10 consecutive tubes with 1:2 ratios to 2^{11} times. Then, the fungal suspension was inoculated in each tube. Medium plus inoculum and extracted solvent plus medium and inoculums were used as positive controls while only medium or only extracted solvent plus medium were used as negative controls. All tubes were incubated at 37^0C for 48 hours. Minimum inhibitory concentrations were determined based on the turbidity of medium in compared with negative control. The concentration of the last turbid test tube was considered as MIC value of that fungi strain with *C. canadensis* extract.

III. RESULTS AND DISCUSSION

We have found a high antifungal activity of *C. canadensis* against tested fungal pathogens. Table 1 showed inhibition zones corresponding to the loading weigh of the crude extract. We observed a proportional relationship between the loading weigh and the inhibition zone, i.e. the more crude extract amount was applied, the larger inhibition zone was achieved. In details, extract in ethyl acetate solvent had highest inhibition value (25.33 mm in *C. albicans* and 45.33 mm in *T. insectorum*). However, crude extract of this plant in polar solvent (ethanol) and non-polar solvent (n-hexane) didn't show the bioactivity. The maximum inhibition zone of *C. canadensis* against *T. insectorum* was 45.33 mm in diameter (with 2 mg/ mL crude extract in ethyl acetate), against *C. albicans*, 25.33 mm in diameter (with 2 mg/ mL crude extract in ethyl acetate). This suggested that ethyl acetate – a medium polar solvent gave better bioactivity than polar solvents such as ethanol, methanol or water. Ethyl acetate solvent is often used for extraction of medium-polar substances including tertpene, coumarin, quinone,…due to the fact that it can penetrate through the cell wall of this plant and forming bonds with aldehyde or cetone group [7]. In *C. canadensis*, there are two main compound groups, Conyzolide and Conyzoflavone of *C. canadensis* with antifungal and antimicrobial activity [8]. It was known that the Conyzaflavone gave superior antifungal activity as compared to Conyzolide. The functional groups of these flavonoids contain methoxyl (-OCH_3) which was well dissolved in ethyl acetate. This result is in accordance with our data that *C. canadensis* extraction in ethyl acetate showed best antifungal effect.

Table 1 The inhibition zone of three types of extract were against *C. albicans* and *T. insectorum*

Fungi	Solvents	Concentration of Conyza canadesis (g/ml)	Zone of inhibition (mm)[a]
T. insectorum	Ethanol	0.5	NA
		1	NA
		1.5	NA
		2	NA
	n- hexane	0.5	NA
		1	NA
		1.5	NA
		2	NA
	Ethyl acetate	0.5	29.33 ± 1.53
		1	36 ± 2.65
		1.5	40.33 ± 1.53
		2	45.33 ± 1.16
C. albicans	Ethanol	0.5	NA
		1	NA
		1.5	NA
		2	NA
	n- hexane	0.5	NA
		1	NA
		1.5	NA
		2	NA
	Ethyl acetate	0.5	NA
		1	16.67 ± 1.53
		1.5	20 ± 1
		2	25.33 ± 1.77

[a] *Diameter of inhibition zone (mm) including diameter of well (6 mm)*
NA: not active
Three types of solvent had no effect on fungal growth.

Table 2 The inhibition zone of Amphotericin B were against *C. albicans* and *T. insectorum*

Fungi	Amount of Amphotericin B (µg)	Zone of inhibition (mm)[a]
T. insectorum	40	9.33 ± 0.58
C. albicans	10	19.67 ± 0.58

[a] *Diameter of inhibition zone (mm) including diameter of well (6 mm)*

The MIC results were similar to the agar well diffusion results described above with some slight difference (Table 3). MIC values of the crude extract ranged from 1000 mg/ml to 7.813 mg/ml. With this method, the bioactivity of the crude extracts in ethanol and n-hexane solvent was revealed (250 mg/ mL of *C. albicans* and 62.5 mg/ mL of *T. insectorum* in ethanol fraction, 500 mg/ mL of *C. albicans* and 1000 mg/ mL of *T. insectorum* in n-hexane fraction) while they were not able to be estimated by agar well diffusion method above.

Table 3 MIC results of extracted plant to *C. albicans* and *T. insectorum*

Types of extracted solvent	MIC (mg/ml)	
	C. albicans	*T. insectorum*
Ethanol	250 mg/mL	62.5 mg/mL
*n-h*exane	500 mg/mL	1000 mg/mL
Ethyl acetate	15.625 mg/mL	7.813 mg/mL

The lowest MIC result was with extract in ethyl acetate (15.625 mg/ mL of *C. albicans* and 7.813 mg/mL of *T. insectorum* in ethanol fraction) indicating the highest antifungal activity which was in accordance with the result done by diffusion method. However, we found that while using MIC test, extract in n- hexane showed exceptionally better activity towards *C. albicans* than to *T. insectorum*.

In general, *C. canadensis* extracts showed much higher activity towards *T. insectorum* than to *C. albicans* for both disc- test and MIC test.

IV. CONCLUSIONS

Our result on antifungal activity of *C. canadensis* revealed that this plant possessed bioactive compounds with potential to serve as antifungal agents against pathogenic fungi in this case, *C. albicans* and *T. insectorum*. In the three types of solvents which were used for extraction, the medium polar solvent such as ethyl acetate was the most appropriate solvent for the extraction process. Data also indicated the clear advantage of *C. canadensis* in inhibiting the new fungus strain *T. insectorum* compared to the commercial common antifungal drug, amphotericin B. The extraction of *C. canadensis* should be further investigated and tested in the in-vivo experiments.

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