Advances in Microbiology, 2016, 6, 471-478
Published Online June 2016 in SciRes. http://www.scirp.org/journal/aim
http://dx.doi.org/10.4236/aim.2016.67046

Antifungal Activities of Commercial Rice Wine Extracts of Taiwanese *Allium fistulosum*

Tsan-Chang Chang[1*], Hung-Der Jang[2], Wang-De Lin[3], Peng-Fu Duan[4]

[1]Department of Nursing, Mackay Junior College of Medicine, Nursing, and Management, Taiwan
[2]Department of Food Science, Yuanpei University of Medical Technology, Taiwan
[3]Department of Center for General Education, St. Mary's Junior College of Medicine, Nursing and Management, Taiwan
[4]Sunshin Area Farmer's Association, Taiwan
Email: *s354@eip.mkc.edu.tw

Received 8 May 2016; accepted 12 June 2016; published 15 June 2016

Abstract

Antifungal activities of the commercial rice wine extracts of *Allium fistulosum* were analyzed. Antifungal activities were tested against 7 pathogenic fungi by using agar disc diffusion and tube dilution tests. The results show that the commercial rice wine extracts of *Allium fistulosum* have strong antifungal activity against *Aspergillus brasiliensis* ATCC 16404, *Candida albicans* ATCC 10231, *Microsporumcanis* ATCC 36299, *M. gypseum* ATCC 24102, *Trichophyton mentagrophytes* ATCC 9533, *T. rubrum* ATCC 28188, and *T. tonsurans* ATCC 28942. The commercial rice wine extracts of different *A. fistulosum* parts were found to exhibit significant antifungal activities with the minimal inhibitory concentration (MIC) in the range of 0.2 - 1.0 mg/mL. The antifungal activity of the extracts of different *A. fistulosum* parts was in the order of AFS (stem) > AFI (plant body) > AFL (leaf) > AFR (root).

Keywords

***Allium fistulosum*, Allicin, Antifungal Activity, Pathogenic Fungi, Minimal Inhibitory Concentration (MIC)**

1. Introduction

Pathogenic fungi often cause nosocomial infection and invade the keratinized tissues of humans and animals

*Corresponding author.

How to cite this paper: Chang, T.-C., Jang, H.-D., Lin, W.-D. and Duan, P.-F. (2016) Antifungal Activities of Commercial Rice Wine Extracts of Taiwanese *Allium fistulosum*. *Advances in Microbiology*, **6**, 471-478.
http://dx.doi.org/10.4236/aim.2016.67046

causing several diseases. Opportunistic fungal infections are difficult to treat in immunocompromised patients, such as transplant patients, AIDS patients, cancer patients, and other immunocompromised hosts; moreover, approximately 40% of systemic infections result in serious consequences, such as death [1] [2]. Dermatophytes that grow on skin, mucous membranes, hair, nails, feathers, and other body surfaces cause ringworm and related diseases. A variety of pathogenic fungi, such as *Aspergillus* sp. and *Candida albicans*, secrete mycotoxins and cause allergic reactions and localized or systemic infection [1]-[3]. Only a limited number of antifungal agents (such as polyenes and azoles) are currently available for the treatment of life-threatening fungal infections in modern medicine; however, very few antifungal agents from natural products could effectively suppressed of pathogenic fungi.

Allium fistulosum (Welsh onion), a member of the *Allium* family, is rich in fiber and can facilitate digestion, prevent constipation and colon diseases, possesses antioxidant and antimicrobial properties, and exert other effects [4]-[8]. In addition, allicin, or chemically known as diallyl thiosulfinate, is the bioactive compound derived from the *Allium* family and can exert antioxidant and antibacterial activities [9] [10]. Allicin has been reported to possess good antifungal properties [1] [11] [12]. *A. fistulosum* plant extract had a MIC of 140 μl/ml against *Fusarium oxysporum* f. sp. *tulipae*, a fungus that lives in soil and on plant debris, compared to that of allicin (160 μl/ml) and fluconazole (100 μl/ml) [13]. However, the studies of Sohn *et al.* [14] showed that fistulosides, the dominant compound from *A. fistulosum* extract, exhibited comparatively lower MIC of 3.1 - 6.2 μg/ml against *Candida albicans* ATCC10231. Taiwanese *A. fistulosum* extracts obtained using rice wine have been shown to exhibit strong antioxidant and antibacterial activities in our recent study [10]. To broaden the inhibitory spectrum the antimicrobial activities against fungal pathogens that commonly caused infection in human were examined, and the MIC and minimum fungicidal concentration (MFC), and allicin content of the *A. fistulosum* wine extracts was studied in this work.

Rice wine is commonly used in Taiwanese cooking to make food more delicious. We developed a rapid method for obtaining the active constituents of *A. fistulosum* by using commercial rice wine. Welsh onion was used as a raw material and commercial rice wine Michiu Tou (MT) (34% alcohol) was used for preparing extracts of different plant parts of Welsh onion. The potential use of *A. fistulosum* extracts as natural antifungals was evaluated by determining their antifungal activities.

2. Materials and Methods

2.1. Test Materials and Chemicals

A. fistulosum cultivar, Lanyang No.1, grown in Sunshin, Yilan County, Taiwan, was used as the test material. The planting to harvesting duration was approximately 84 - 90 d; hence, the *A. fistulosum* plant (AFI) was planted in April 2013 and harvested in July 2013. The characteristics of the *A. fistulosum* parts were shown in **Table 1**. The *A. fistulosum* root (AFR) length was 3.6 - 12.8 cm, *A. fistulosum* stem (AFS) length was 14.7 - 19.2 cm, and *A. fistulosum* leaf (AFL) length was 38.5 - 51.8 cm. These materials were placed in a drying oven at 50˚C. After drying, they were placed in MT wine for 7 d. Whatman filter paper no. 1 was used to filter impurities from the extracts. The filtrate was concentrated under reduced pressure at 50˚C, and the *A. fistulosum* extracts were dried for 24 h in a vacuum oven. All extracts were evaporated to dryness under nitrogen and used within 24 h for experiments.

Table 1. The characteristics of different *A. fistulosum* parts.

Plant parts*	Length (cm)	Moisture (%)	Extraction yields (%)
AFI	62.3 - 79.7	$91.6^{a} \pm 1.3^{**}$	$42.3^{a} \pm 3.8^{**}$
AFS	14.7 - 19.2	$93.2^{a} \pm 1.5$	$43.6^{a} \pm 4.7$
AFL	38.5 - 51.8	$93.6^{a} \pm 1.6$	$42.4^{a} \pm 4.3$
AFR	3.6 - 12.8	$85.5^{b} \pm 0.5$	$15.5^{b} \pm 1.2$

*AFI, Whole *A. fistulosum* plant body; AFS, *A. fistulosum* stem; AFL, *A. fistulosum* leaf; and AFR, *A. fistulosum* root; **Each test was performed in triplicate, and data are presented as the mean ± standard deviation (SD). Data with different superscript lowercase letters in the individual column are significantly different at $p < 0.05$, according to the Scheffe's test.

All solvents and reagents were purchased from Sigma Chemical Co. Potato dextrose agar (PDA), potato dextrose broth (PDB), and Mueller Hinton agar (MHA) media were purchased from Difco Chemical Co., USA.

2.2. Antifungal Activity of Taiwanese *A. fistulosum*

Fungal strains used were *Aspergillus brasiliensis* ATCC 16404, *Candida albicans* ATCC 10231, *Microsporum canis* ATCC 36299, *M. gypseum* ATCC 24102, *Trichophyton mentagrophytes* ATCC 9533, *T. rubrum* ATCC 28188, and *T. tonsurans* ATCC 28942. These strains were purchased from the Bioresource Collection and Research Center of the Food Industry Research Institute in Hsinchu City, Taiwan. A tube dilution test [15] and an agar disc diffusion test [16] [17] were performed to study the antifungal activity of *A. fistulosum* extracts against the aforementioned seven fungal strains. Fifty microliters of the *A. fistulosum* extract at 1 mg/ml was applied to an ethanol-sterilized paper disc (8 mm in diameter) and placed onto the PDA agar plates. After incubation at 30˚C for 24 h, the inhibition zone around the disc was measured [16] [17]. Nystatin at a concentration of 50 μg/ml was used as the control in the antifungal assay. Inhibition zones of the extract-coated discs and the control-coated discs were compared. In addition, the minimal inhibitory concentration (MIC) of the samples was determined using the broth dilution method [3] by employing serially diluted *A. fistulosum* extracts. Subsequently, fungal cultures were prepared in the PDB and incubated at 30˚C for 24 h. The media containing various *A. fistulosum* extracts were diluted with distilled water to obtain concentrations in the range of 2 to 0.05 mg/ml. The mixture was incubated at 30˚C for 24 h to determine the minimal concentration at which fungal cell growth was fully inhibited. The MIC and minimum fungicidal concentration (MFC), the lowest concentration of *A. fistulosum* extracts required to inhibit microbial growth and kill them were determined. MFC was defined as the concentration of antifungal agents at which the number of colony forming units was zero [5].

2.3. HPLC Assay of Allicin

HPLC analysis of allicin was performed using the Agilent 1100 HPLC UV-VIS (DAD) detector (Heisenburg), Finnigan LCQ-DECA (CURIE) spectrometer, and the Phenomenex Luna C18(2) HPLC assay column (dimensions: 150 mm × 4.6 mm; particle size: 5 μm). The mobile phase was acetonitrile and distilled water in a 30:70 ratio, and the flow rate was 1.0 mL/min. Samples were analyzed using UV detection at 195 nm. The injection volume was 1 mL, and the column temperature was maintained at 25˚C. All samples were filtered through a 0.45 μm filter before HPLC analysis. The eluate was detected using a UV detector at 25˚C. A standard solution containing authentic allicin was used for calibrating the retention time and standard curve.

2.4. Statistical Analysis

Data from triplicate experiments were subjected to analysis of variance for a completely random design by using SAS. The data are presented as the mean ± standard deviation of triplicate determinations. Means were compared using the Scheffe's test, and differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Plant Material Characteristics

The moisture content of AFI, AFR, AFS, and AFL was 91.6%, 85.5%, 93.2%, and 93.6% (**Table 1**), respectively. The moisture content of these test *A. fistulosum* was similar to that of other Taiwanese Welsh onions, which is up to 92% approximately. The results show that the extraction yields of AFI, AFS, and AFL was 42.3%, 43.6%, 42.4%, respectively, which are higher than those of AFR (15.5%). Furthermore, the moisture content and extraction yields of these *A. fistulosum* parts were also similar to that obtained in our previous study [9] [10].

3.2. Antifungal Activity of Taiwanese *A. fistulosum*

Growth inhibition caused by compounds in *A. fistulosum* extracts was apparent as a clear zone around the paper disk where no fungi could be recovered. A larger zone of inhibition around the control-disc indicates that the fungi are more sensitive to Nystatin. As expected the blank disc (34% (v/v) ethanol) shows no clear zone at all. AFS extracts had the highest activity against *T. rubrum* and *T. tonsurans*, with an inhibition zone diameter of 24.0 ± 1.1 mm and 20.3 ± 1.3 mm, respectively, for an MIC of 0.2 mg/mL and MFC of 0.4 mg/mL for both

(**Table 2**). For *A. brasiliensis*, the inhibition zone diameter, MIC, and MFC of the AFI extracts were 13.3 ± 0.7 mm, 0.4 mg/mL, and 0.8 mg/mL, respectively. The MIC range for *A. brasiliensis* was 0.4 - 0.8 mg/mL, whereas the MFC range was 0.8 - 1.0 mg/mL. AFS extracts had the weakest antifungal activity against *C. albicans* and *M. canis*, with an inhibition zone diameter of 12.0 ± 0.3 mm and 12.3 ± 0.6 mm, respectively, for a MIC of 0.8 mg/mL and MFC of 1.0 mg/mL. The MIC was 0.4 mg/mL whereas the MFC was 0.8 mg/mL for both AFI and AFS extracts against *M. gypseum* and *T. mentagrophytes*. In general, the antifungal activities of Taiwanese *A. fistulosum* extracts against the seven pathogenic fungi were in the order of AFS > AFI > AFL > AFR.

Yamada and Azuma [3] used agar dilution and broth dilution methods for *in vitro* evaluation of antifungal activity of allicin against *Candida*, *Trichophyton*, and *Microsporum* species and found that the MIC ranged from 1.57 to 6.25 μg/ml. Sohn *et al.* [14] reported antifungal activity of the prominent compound, fistulosides from *A. fistulosum*, with the MIC ranged from 3.1 to 6.2 μg/ml and the MFC ranged from 3.1 to 6.2 μg/ml. Khodavandi *et al.* [12] used allicin to demonstrate its intrinsic antifungal activity, and the MIC of allicin against six *Candida* species ranged from 0.05 to 25 μg/ml. Aala *et al.* [11] evaluated the *in vitro* efficacy of pure allicin alone against six dermatophyte isolates, and the MIC ranged from 0.098 to 25.0 μg/ml. Kim *et al.* [1] studied the antifungal activity of allicin alone and its synergistic effects with the antifungal agents. They proposed that allicin had antifungal activity but an extremely high MIC against pathogenic fungi and could reduce the MIC of amphotericin B while retaining its efficacy. Commercial rice wine extracts of different *A. fistulosum* parts in our study exhibited different antifungal activities against *A. brasiliensis*, *C. albicans*, *M. canis*, *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, and *T. tonsurans*, probably because the amount of allicin differed in each extract. In general, the antifungal activities of Taiwanese *A. fistulosum* extracts against the seven pathogenic fungi were in the order of AFS > AFI > AFL > AFR. Most chemical antifungal drugs were prone to cause side effects. Our study demonstrated that the commercial rice wine extracts of *Allium fistulosum* had the antifungal activities. We can use to enhance the extraction and purification technology to the development of the natural antifungal agent in future. Therefore, HPLC analysis was performed to determine the allicin content of the extracts and its inhibitory activity.

Table 2. Antifungal activities of extracts of different *A. fistulosum* parts extracts Obtained using commercial MT wine.

Organisms	Antifungal activities	AFI	AFS	AFL	AFR	Nystatin*	Blank**
A. brasiliensis	In. zone***	$13.3^{b} \pm 0.7$***	$15.8^{b} \pm 0.5$	$11.3^{c} \pm 0.4$	$10.7^{bc} \pm 0.3$	$36.6^{b} \pm 2.5$	-****
	MIC/MFC	0.4/0.8	0.4/0.8	0.8/1.0	0.8/1.0		
C. albicans	In. zone	$10.7^{c} \pm 0.7$	$12.0^{c} \pm 0.3$	$9.3^{c} \pm 1.3$	$8.3^{c} \pm 0.3$	$11.5^{c} \pm 0.5$	-
	MIC/MFC	0.8/1.0	0.8/1.0	1.0/2.0	1.0/2.0		
M. canis	In. zone	$10.0^{c} \pm 0.5$	$12.3^{c} \pm 0.6$	$9.7^{c} \pm 0.5$	$8.7^{c} \pm 0.2$	$20.8^{bc} \pm 1.6$	-
	MIC/MFC	1.0/1.6	0.8/1.0	1.0/1.6	1.0/2.0		
M. gypseum	In. zone	$13.0^{b} \pm 1.0$	$13.3^{c} \pm 1.2$	$10.7^{c} \pm 0.7$	$9.3^{c} \pm 0.6$	$26.3^{bc} \pm 1.8$	-
	MIC/MFC	0.4/0.8	0.4/0.8	1.0/1.6	1.0/2.0		
T. mentagrophytes	In. zone	$14.0^{b} \pm 0.5$	$16.7^{b} \pm 0.6$	$13.3^{bc} \pm 0.5$	$10.7^{bc} \pm 0.2$	$34.0^{b} \pm 1.5$	-
	MIC/MFC	0.4/0.8	0.4/0.8	0.4/0.8	0.8/1.0		
T. rubrum	In. zone	$20.7^{a} \pm 0.8$	$24.0^{a} \pm 1.1$	$20.6^{a} \pm 0.6$	$19.3^{a} \pm 0.7$	$43.5^{a} \pm 2.3$	-
	MIC/MFC	0.2/0.4	0.2/0.4	0.2/0.4	0.4/0.8		
T. tonsurans	In. zone	$18.7^{a} \pm 1.6$	$20.3^{ab} \pm 1.3$	$15.3^{b} \pm 1.0$	$12.7^{b} \pm 0.8$	$32.6^{b} \pm 2.2$	-
	MIC/MFC	0.4/0.8	0.2/0.4	0.4/0.8	0.4/0.8		

AFI, Whole *A. fistulosum* plant; AFS, *A. fistulosum* stem; AFL, *A. fistulosum* leave; AFR, *A. fistulosum* root; and MT wine, MichiuTou wine. MIC, minimal inhibitory concentration (mg/mL); MFC, minimum fungicidal concentration (mg/mL) was defined as the concentration of the antifungal agent at which the number of colony forming units was zero. *Nystatin was used as control. The concentration was 50 μg/mL. **Blank was 34% ethanol of the commercial MT wine. ***In. zone represents the inhibition zone diameter (mm) of extracts. Each test was performed in triplicate, and data are presented as the mean ± standard deviation (SD). Data with different superscript lowercase letters in the individual column are significantly different at $p < 0.05$, according to the Scheffe's test. ****Not detected.

3.3. Correlation of Antifungal Activity and Allicin Content in Wine Extracts of Different *A. fistulosum* Parts

For the commercial MT rice wine extracts from different *A. fistulosum* parts, the allicin content ranged from 89.6 to 95.9 μg/mL. The allicin content was the highest in the AFS extract (95.9 ± 2.5 μg/mL). The allicin content of the AFI and AFL extracts were 93.5 ± 2.1 μg/mL and 94.5 ± 2.7 μg/mL, respectively. The allicin content was the lowest in the AFR extract (89.6 ± 2.5 μg/mL). As shown in **Figure 1**, the correlation between the inhibition zone diameter and allicin content in different *A. fistulosum* part extracts obtained using MT wines was determined. The r^2 values between the inhibition zone diameter and allicin content for *A. brasiliensis*, *C. albicans*, *M. canis*, *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, and *T. tonsurans* were 0.55, 0.67, 0.71, 0.61, 0.86, 0.64, and 0.68, respectively. The calibration results are more or less acceptable and show a positive correlation when these r^2 values are equal and bigger than 0.6.

Samuel *et al.* [18] evaluated the antifungal activity of *Allium sativum* bulb extract against *T. rubrum*, and a positive correlation was observed between the inhibitory zone diameter and the allicin content. In previous studies [17] [19], allicin extracts were obtained from different plants of the genus *Allium*, including garlic and *A. fistulosum*, by using hot water and alcohol. In these *Allium* plant extracts, the 20% - 30% alcoholic extracts had the highest allicin content. Our study had found similar results and indicated that different *A. fistulosum* parts extracted using commercial MT wine have different allicin contents. The antifungal activity depends on the allicin content of the *A. fistulosum* part extracts. The r^2 values between the inhibition zone diameter and allicin content for these test strains have indicated a positive correlation.

3.4. Relationship between Allicin Content and Antifungal Activity

To determine the inhibitory effect of allicin towards the test fungi, commercial pure allicin (99%) were prepared at concentrations of 10^{-5} mg/mL to 10^{-1} mg/mL, and the correlation between the inhibition zone diameter (mm) and allicin content (mg/mL) was determined (**Figure 2**). The r^2 values of the correlation between the inhibition zone diameter and allicin content for *A. brasiliensis*, *C. albicans*, *M. canis*, *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, and *T. tonsurans* were 0.94, 0.60, 0.82, 0.81, 0.93, 0.93, and 0.95, respectively, thus indicating a strong positive correlation. The results suggested that allicin exhibited good fungicidal activity against all the test fungi except *C. albicans*.

Table 3 showed the results of antifungal activity of allicin determined using the MIC and MFC in the broth

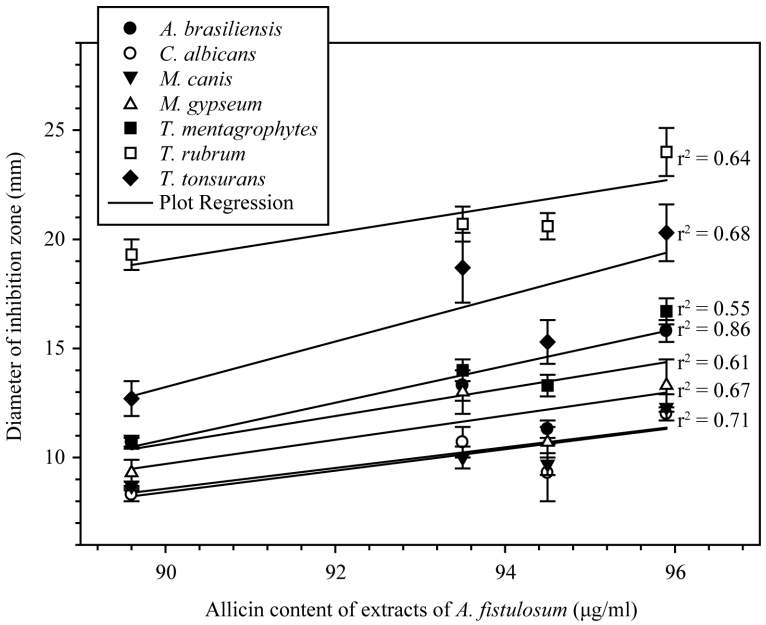

Figure 1. Correlation between antifungal inhibition zone and allicin content of extracts of different *A. fistulosum* parts.

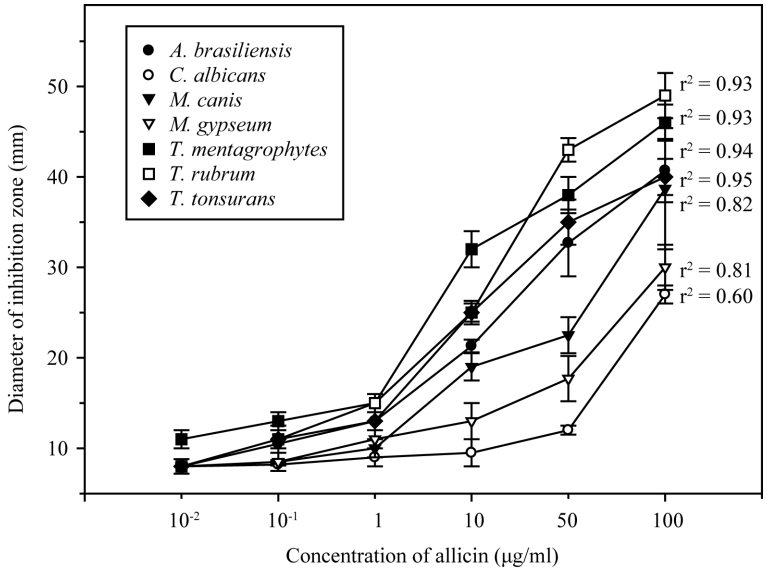

Figure 2. Correlation between antifungal inhibition zone and allicin content.

Table 3. Antifungal activities of allicin content according to MIC and MFC.

Organisms Allicin conc.	*A. brasiliensis*	*C. albicans*	*M. canis*	*M. gypseum*	*T. mentagrophytes*	*T. rubrum*	*T. tonsurans*
	(μg/mL)						
MIC*	10^{-1}*	10	10	1	10^{-1}	1	1
MFC**	1**	100	50	10	1	10	10

*MIC (μg/mL): Minimal inhibitory concentration; **MFC (μg/mL): Minimal fungicidal concentration.

dilution method. The commercial allicin had the highest antifungal activity against *A. brasiliensis* and *T. mentagrophytes*, with the MIC of 0.1 μg/mL and MFC of 1.0 μg/mL. The MIC and MFC for *M. gypseum*, *T. rubrum*, and *T. tonsurans* were 1.0 μg/mL and 10.0 μg/mL, respectively. The MIC for *M. canis* was 10.0 μg/mL, whereas the MFC was 50.0 μg/mL. Among the test fungi, allicin had the weakest inhibitory activity towards *C. albicans*, which the MIC and MFC were 10.0 μg/mL and 100.0 μg/mL, respectively.

Yamada and Azuma [3] studied the antifungal activity of allicin by using the agar dilution methods in Sabouraud glucose medium, in which the MIC of allicin against *Candida*, *Cryptococcus*, *Ttichophyton*, *Epidermophyton*, and *Microsporum* ranged from 3.13 to 25.0 μg/ml. Khodavandi *et al.* [12] investigated the antifungal activity of allicin against *Candida* species, and the MIC of allicin alone against six *Candida* species within the range of 0.05 - 25 μg/ml. Aala *et al.* [11] evaluated the *in vitro* efficacy of pure allicin alone against six dermatophytes, and the MIC of allicin ranged from 0.098 to 25.0 μg/ml. **Figure 2** showed the r^2 values of the correlation between the inhibition zone diameter and allicin content for these test strains have a strong positive correlation. The results suggested that allicin exhibited good fungicidal activity against all the test fungi except *C. albicans*. The commercial allicin had the highest antifungal activity against *A. brasiliensis* and *T. mentagrophytes*, and had the weakest inhibitory activity towards *C. albicans*. The present study used extracts of different *A. fistulosum* parts and obtained results consistent with those of the aforementioned studies.

4. Conclusion

The commercial MT wine extracts of Taiwanese *A. fistulosum* exhibited antifungal activities, and the MIC and MFC of allicin were within the range of 0.2 - 1.0 and 0.4 - 2.0 mg/mL, respectively. This study also evaluated the *in vitro* efficacy of pure allicin used alone against seven dermatophytes, and the MIC and MFC of the allicin content were 0.1 - 10 and 1 - 100 μg/mL, respectively. A strong positive correlation was observed between the

antifungicidal activity and allicin content of the *A. fistulosum* extracts for seven dermatophytes. Therefore, we suggest that *A. fistulosum* not only increases food flavor but also acts as a natural fungicide used in human health care.

References

[1] Kim, Y.S., Kim, K.S., Han, I., Kim, M.H., Jung, M.H. and Park, H.K. (2012) Quantitative and Qualitative Analysis of the Antifungal Activity of Allicin Alone and in Combination with Antifungal Drugs. *Plos One*, **7**, 1-8. http://dx.doi.org/10.1371/journal.pone.0038242

[2] Serge, A. and David, M. (1999) Antimicrobial Properties of Allicin from Garlic. *Microbes and Infection*, **2**, 125-129.

[3] Yamada, Y. and Azuma, K. (1977) Evaluation of the *in Vitro* Antifungal Activity of Allicin. *Antimicrobial Agents and Chemotherapy*, **11**, 743-749. http://dx.doi.org/10.1128/AAC.11.4.743

[4] Aoyama, S. and Yamamoto, Y. (2007) Antioxidant Activity and Flavonoid Content of Onion (*Allium fistulosum*) and the Effect of Thermal Treatment. *Food Science and Technology Research*, **13**, 67-72. http://dx.doi.org/10.3136/fstr.13.67

[5] Bagiu, R.V., Vlaicu, B. and Butnariu, M. (2012) Chemical Composition and *in Vitro* Antifungal Activity Screening of the *Allium ursinum* L. (Liliaceae). *International Journal of Molecular Sciences*, **13**, 1426-1436. http://dx.doi.org/10.3390/ijms13021426

[6] Marta, C.M., Nieves, C. and Mar, V. (2007) Biological Properties of Onions and Garlic. *Trends in Food Science & Technology*, **18**, 609-625. http://dx.doi.org/10.1016/j.tifs.2007.07.011

[7] Stajner, D., Igic, R., Popovic, B.M. and Malencic, D.J. (2008) Comparative Study of Antioxidant Properties of Wild Growing and Cultivated *Allium* Species. *Phytotherapy Research*, **22**, 113-117. http://dx.doi.org/10.1002/ptr.2278

[8] Stajner, D., Milic, N., Canadanovic-Brunet, J., Kapor, A., Stajner, M. and Popovic, B.M. (2006) Exploring *Allium* Species as a Source of Potential Medicinal Agents. *Phytotherapy Research*, **20**, 581-584. http://dx.doi.org/10.1002/ptr.1917

[9] Chang, T.C., Chang, H.T., Chang, S.T., Lin, S.F., Chang, Y.H. and Jang, H.D. (2013) A Comparative Study on the Total Antioxidant and Antimicrobial Potentials of Ethanolic Extracts from Various Organ Tissues of *Alliums* sp. *Food and Nutrition Sciences*, **4**, 182-190. http://dx.doi.org/10.4236/fns.2013.48A022

[10] Chang, T.C., Jang, H.D., Lin, W.D. and Duan, P.F. (2016) Antioxidant and Antimicrobial Activities of Commercial Rice Wine Extracts of Taiwanese *Alliums fistulosum*. *Food Chemistry*, **190**, 724-729. http://dx.doi.org/10.1016/j.foodchem.2015.06.019

[11] Aala, F., Yusuf, U.K., Jamal, F. and Khodavandi, A. (2010) *In Vitro* Antifungal Activity of Allicin Alone and in Combination with Two Medications against *Trichophyton rubrum*. *World Journal of Microbiology and Biotechnology*, **26**, 2193-2198. http://dx.doi.org/10.1007/s11274-010-0404-9

[12] Khodavandi, A., Alizadeh, F., Aala, F., Sekawi, Z. and Chong, P.P. (2010) *In Vitro* Investigation of Antifungal Activity of Allicin Alone and in Combination with Azoles against *Candida* Species. *Mycopathologia*, **169**, 287-295. http://dx.doi.org/10.1007/s11046-009-9251-3

[13] Pârvu, M., Barbu-Tudoran, L., Roşca-Casian, O., Vlase, L. and Tripon, S. (2010) Ultrastructural Changes in *Fusarium oxysporum* f. sp. *tulipae* Hyphae Treated *in Vitro* with *Allium fistulosum* Plant Extract. *Annals of the Romanian Society for Cell Biology*, **15**, 65-71.

[14] Sohn, H.Y., Kum, E.J., Ryu, H.Y., Jeon, S.J., Kim, N.S. and Son, K.H. (2006) Antifungal Activity of Fistulosides, Steroidal Saponins, from *Allium fistulosum* L. *Journal of Life Science*, **16**, 310-314. http://dx.doi.org/10.5352/JLS.2006.16.2.310

[15] Jones, R.N., Barry, A.L., Gavan, T.L. and Washaington, J.A. (1985) Susceptibility Tests: Microdilution and Macrodilution Broth Procedures in Manual of Clinical Microbiology. 4th Edition, *American Society for Microbiology*, Washington DC, 972-977.

[16] Boussaada, O., Chriaa, J., Nabli, R., Ammar, S., Saidana, D., Mahjoub, M.A., Chraeif, I., Helal, A.N. and Mighri, Z. (2008) Antimicrobial and Antioxidant Activities of Methanol Extracts of *Evaxpygmaea* (Asteraceae) Growing Wild in Tunisia. *World Journal of Microbiology and Biotechnology*, **24**, 1289-1296. http://dx.doi.org/10.1007/s11274-007-9600-7

[17] Fujisawa, H., Suma, K., Origuchi, K., Kumagai, H., Seki, T. and Ariga, T. (2008) Biological and Chemical Stability of Garlic-Derived Allicin. *Journal of Agricultural and Food Chemistry*, **56**, 4229-4235. http://dx.doi.org/10.1021/jf8000907

[18] Samuel, J.K., Andrews, B. and Jebashree, H.S. (2000) *In Vitro* Evaluation of the Antifungal Activity of *Allium sativum*

Bulb Extract against *Trichophyton rubrum*, a Human Skin Pathogen. *World Journal of Microbiology and Biotechnology*, **16**, 617-620. http://dx.doi.org/10.1023/A:1008972016316

[19] Hunter, R., Caira, M. and Stellemboom, N. (2005) Thiosulfinate Allicin from Garlic-Inspiration for a New Antimicrobial Agent. *Annals of the New York Academy of Sciences*, **1056**, 234-241. http://dx.doi.org/10.1196/annals.1352.011