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Susceptibility Status of Fungal Burden in Thai Traditional Medicine

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Abstract

The use of Thai traditional medicine has sharply increased over the past few years. Concerning health conscious, several safety measures and regulations have been applied to manufacturers and products available in the markets. Therefore, the microbiological quality of Thai traditional medicine should be complied with the microbial limit tests (MLT) indicated in the Thai Pharmacopoeia (TP). The MLT limit the detected number of bacteria and fungi in the sample and require the absence of *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Clostridium* spp. In this study, 87 samples of herbal capsules, honey-pills, powders and tablets procured from 16 provinces showed that 26 samples (29.89%) (with 3 registered samples included) were not complied with the TP. The fungal burden of 42 isolates were representative species of *Aspergillus* (32 isolates) i.e. *A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus* while others were species of *Penicillium* (8 isolates) and *Rhizopus* (2 isolates). All fungal isolates were tested against amphotericin B (AMB), fluconazole (FLZ), itraconazole (ITZ) and ketoconazole (KTZ) using broth dilution assay. The result demonstrated that all test isolates responded at the highest MFC_{90} (90% minimum fungicidal concentration) to FLZ (40.63 µg/ml to 100 µg/ml). The second highest was to KTZ in *A. flavus*, *A. fumigatus* and *Penicillium* and to ITZ in *A. niger* and *Rhizopus*. However, *A. terreus* revealed the similar value of MFC_{90} in responding to all tested drugs. This is the first study of the susceptibility status of fungal burden in traditional medicine and thus indicated the health risk aspect of immunocompromised people.

Key words: antifungal, fungal burden, microbial limit test, pharmacopoeia, susceptibility test, traditional medicine

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INTRODUCTION

Traditional remedies especially herbal medicine has been used by self-medication in many developing countries. Those remedies were emerged from their own traditional wisdoms as the collective knowledge and fundamental practices through conventional beliefs and indigenous experience within cultures. The original treatises are now being under the development process using many measures of modern technologies and scientific data[1]. At the same time, herbal medicines remarkably come into focus in response to the sharp increase of eco-friendly product requirement, to serve self-reliability, to conserve natural resources and to support the agricultural essentials[2-3]. Therefore, those formulations of traditional medicine are now widely accepted and developed as complementary alternative medicine. With an attempt for the traditional medicines to be globally endorsed to modern standard, as described in The Drug Act 1987 (B.E. 2530), Thailand permits chemical additives to be included into the products, and modern technology in manufacturing is allowed under the Ministerial Regulation No. 25 (1994)[4]. In accordance with the Ministry of Public Health Notification on criteria for registration of traditional formulations, the criteria for traditional medicine formulations being registered before distribution to the market should comply with indicated numbers and types of specified organisms according to the announcement[5-6]. However, while regulations of consumer protection are strictly implemented, minor adaptation or revised formulations of herbal products from ancestor's wisdom are widely distributed on the markets, some have been distributed and being sold at the legitimate alleviation.

The exceed number of microbiota found in finished traditional medicines point out the low standard of production processes. Not only the over limit number, but the implication of unknown substances produced from microbial under storage, before and after human consumption, are also of public health concern. The knowledge of exact number of microbial population affecting consumer health and unwanted circumstances or the affecting mechanism that disturbs normal body function are not clearly indicated at the present time.

The disputation on the issue of using natural ingredients from herbs in comparison with the measurable amount of active drugs in modern medicines still exists. The result of this study helps elaborate information whether prohibition or affirmation of using local traditional medicine should be appropriately promoted. The microbiological acceptation of traditional medicine in this study is also based on the pass of regulations to benefit consumer's health.

This study aimed to assess the microbiological qualities of finished herbal products that was complied with the registration criteria of traditional medicines according to the Thai Pharmacopoeia 2000. Further evaluation of susceptibility to antifungal drug of the isolated fungi indicated the health risk of consumers in case of consumption of the contaminated products. The data obtained also disclosed the actual microbiological status of herbal products available to and accessed by the consumers. Apart from the health hazard awareness, the results would help manifest the immediate indigence of more stringent legal control for herbal products.

MATERIALS AND METHODS

Traditional herbal products

Eighty seven samples of Thai traditional herbal products were purchased from Thai local markets form 16 provinces in 2007. The herbal products were 40, 20, 20 and 7 samples in the forms of capsule, honey pill, powder and tablet, respectively.

Microbial limit tests (MLT)

The microbial limit tests were performed according to Thai Pharmacopoeia 1987 in accordance with the Food and Drug Administration's licensing and registration process[6]. This should be conformed to the amendments appeared in the Thai Pharmacopoeia supplement 2005[7]. The assessment of limit number of total viable

aerobic count for bacteria and fungi should not exceed $5x10^4$ and $5x10^2$ per g or ml, respectively, and there should not be more than 10^2 enterobacteria and certain other Gram negative bacteria per g or ml. Another requirement is for the absence of specified organisms i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* per g or ml, and the absence of Clostridium spp. and *Salmonella* spp. each per 10 g or 10 ml.

The isolation of fungal burden

The fungi residing in the samples could be obtained by selecting the isolated colonies appeared after the incubation period of total viable aerobic microbial count for fungi of any appropriate dilution. Subculture was done again to ensure the pure organism on Sabouraud dextrose agar (SDA; Difco, MI, USA.) Full growth on SDA slant was kept at – 20°C and on SDA deep culture covered with sterile liquid paraffin at room temperature.

Fungal identification, especially filamentous fungi, was based on morphology e.g. the macroscopic morphology was the cultural characteristics after the mature growth on solid medium appeared to the naked eyes. The microscopic morphology was the feature of various structures, for instance, hyphae, types, size and shape of conidia, the arrangement obtained from slide culture and the wet mount using lacto-phenol cotton blue. Those morphologies were compared with the identification illustrations of the text to be presumptively identified into species[8].

In vitro Susceptibility test

The susceptibility status of each isolate was tested against the commercial antifungal agents. The antifungal agents used were amphotericin B (AMB) (Bristol-Myers-Squibb, Thailand), Itraconazole (ITZ) (Olic Thailand, Ltd.) and ketoconazole (KTZ) (Karinco, Italy). They were dissolved with 1% dimethyl sulfoxide (DMSO). Fluconazole (FLZ) (Siam Pharmaceutical, Thailand) was dissolved in sterile distilled water. All were stored as aliquots at -20°C and were thawed at room temperature and thoroughly mixed by vortex to ascertain the maximum solubility before used. DMSO at similar concentrations of antifungal agents was tested simultaneously to exclude its effect on fungi.

The susceptibilities of fungal isolates to antifungal agents were determined by a broth dilution assay using modified principle of Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard-Second edition: M38-A2 of the Clinical and Laboratory Standard Institute (CLSI) to obtain MICs and MFCs[9]. The test was carried out against *Aspergillus flavus* BEE 7232 obtained from the Faculty of Public Health, Mahidol University, Thailand, as the fungal control.

The mature cultures on SDA at 25°C were harvested for conidia using Sabouraud dextrose broth (SDB; Difco, MI, USA.). The conidial suspension was adjusted to be equivalent to the density of 0.5 McFarland standard. The suspension was further diluted to 10^4-10^5 conidia/ml. The antifungal agents were tested in duplication. Each drug solution was serially diluted in SDB in sterile 1.5-mL polypropylene microtube (Hycon Plastic Inc., NH, USA.) and individual concentration was inoculated with an equal volume (0.1 ml) of the conidial suspension. A serial dilution set of DMSO at similar concentration residing in the serial test set of antifungal agent was included as the observation of diluent effect. Also, a tube containing medium (medium control), a tube of medium with corresponding drug (drug control), and a tube of medium with fungal inoculum (culture control or drug-free growth control) were used as control system. All tubes were incubated at 25°C and minimum inhibitory concentration value (MIC) was read as the lowest concentration of the drug that inhibited fungal growth at the time of culture control positively appearing. Subculture of concentrations from the tubes before and after the MIC breakpoint onto SDA and being incubated as previously done yielded the minimum fungicidal concentration (MFC).

RESULTS

The traditional herbal products of 87 samples in capsule, honey-pill, powder and tablet forms were categorized by their medicinal properties labeled on the packages. Therefore, grouping did not depend on herbal components since some brands showed the detail of components while some did not, but with description provided by producers (Table 1).

Sample forms were prepared from various herbs as well as their remedies described by producers. However, fungal burden residing in the samples were similar. The mostly found fungi, in order, were the representative species of *Aspergillus* and *Penicillium* of 32 and 8 isolates, respectively. While these two genera were scattered and distributed among capsules, honey-pills and powders; the tablets, with small sample number, was found only aspergilli. On the other hand, only two isolates of the *Rhizopus* spp. were detected (Table 2).

It was found that only 26 out of 87 (or 29.89%) medicinal samples from 16 provinces, which were collected and investigated in this study, did not followed the regulation of product registration. Microbiologically, the limit of microbial contamination in TP supplement 2005 which complied with the registration standard of Thai FDA was considered as the number of the total count for bacteria and for fungi. From 87 samples, the herbal medicine forms of capsule, powder and tablet in total of 34 samples (39.08%) were registered. In accordance with Thai Pharmacopoeia, 26 samples (29.89%) failed this regulation of microbiological assessment while only 3 samples of the registered herbal medicine failed. However, it should be noted that some samples failed for bacteria or fungal count, but some failed both (Table 3).

A brief overview to approach the fungal burden in 4 types of Thai herbal medicine, based on the colonial characteristic and microscopic morphology, the representative fungi obtained by culture were *Aspergillus*, *Penicillium* and *Rhizopus* at 32, 8 and 2 isolates, respectively (Table 4). The specified species of each genus was *Aspergillus flavus, A. fumigatus, A. niger* and *A. terreus.* The others were species of *Penicillium* and *Rhizopus* (Figure1).

Isolates of *Aspergillus* expressed different susceptibilities to the four antifungal agents; AMB, FLZ, ITZ and KTZ. Because the value of many results were recorded with symbol designated to "more than or higher than" (>); therefore the average values of some MFCs were showed with this symbol.

Fungal burden in traditional medicines was identified as representative species of aspergilli (n) i.e. *A. flavus*(11), *A. fumigatus* (3), *A. niger* (12) and *A. terreus* (6). Others were *Penicillium* (8) and *Rhizopus* (2). The susceptibility of all test species to FLZ demonstrated the higher MFC_{90} than those to AMB, KTZ and ITZ. The second highest MFC_{90} were from KTZ against *A. flavus, A. fumigatus, A. terreus, Penicillium* and also against the standard strain of *A. flavus* 7232. While the least value of MFC_{90} were those from AMB against *A. fumigatus, A. niger, A. terreus*, and *Rhizopus* spp. and from ITZ against *A. flavus* and *Penicillium.*

The reference *A. flavus* 7232 expressed different susceptibilities to antifungal drugs used in consideration of MFC_{90}. Susceptibility to FLZ (>50 µg/ml) and KTZ (25.00 µg/ml) was at the highest and the second highest in comparison to AMB (10.00 µg/ml) and ITZ (10.00 µg/ml) which were at the least MFCs value (Table 5).

Table 1. Various forms of traditional herbal products with claims.

Sample type (n)	**Local market (n)**	**Remedies (n)**
Capsule (40)	Bangkok (11) Kanchanaburi (1) Lampang (2) Nakhon Pathom (10) Nontaburi (7) Phetchaburi (3) Phitsanulok (2) Prathum thani (1) Sakon Nakhon (1) Yasothon (2)	Antiasthma (1) Antiflatulence (2) Antihypoglycemic (1) Antilymphatic disorders (1) Antiobesity (5) Antipyretic(1) Antisinus (1) Laxative (2) Relaxant (4) Stomachic (1) Tonic (21)
Honey pill (20)	Bangkok (7) Nakhon Pathom (9) Nakhon Si -Thammarat(2) Ratcha buri (1) Songkhla (1)	Antidiuretic (1) Antiflatulent (2) Antiobesity (1) Antitussive (8) Antisinus (2) Laxative (2) Quit smoking (1) Relaxant (1) Tonic (2)
Powder (20)	Bangkok (13) Nakorn Ratchasima (1) Suphan Buri (3) Uthai thani (3)	Antidiuretic (1) Antiflatulent (3) Antihypertensive (1) Antiobesity (1) Antipyretic (1) Antisyncope (6) Antitussive (3) Emmenagogue (1) Heart tonic (1) Relaxant (1) Tonic (1)
Tablet (7)	Bangkok (7)	Anti-inflamation (7)

Table 2. Fungal genera isolated from traditional herbal products.

Sample (number)	Remedies (no. sample)	Major herbs: Scientific name*	Fungus species
Capsule n=40	Antiflatulence	*Piper nigrum* Linn.	*A. flavus, A.niger*
	Antiobesity	*Garcinia atroviridis* Griff.	*A.flavus*
	Antisinus(2)	unspecified	*A.flavus, A.fumigatus, A.niger, A.terreus*
	Relaxant(4)	*Derris scandens* Benth.*	*Penicillium A.niger, A.terreus Penicillium* 1**, *Penicillium* 2**
	Tonic(3)	*Anamirta cocculus* (L.) Wight & Arn.*	*Rhizopus, A.flavus, A.flavus* 1, *A.flavus*2, *A. niger, A.terreus* 1, *A.terreus* 2, *Penicillium*
Powder n=20	Antiflatulence	*Curcuma longa* Linn.	*A.fumigatus*
	Antipyretic	*Phyllanthus amarus* Schum & Thonn.	*A.flavus*
	Antisyncope(4)	unspecified	*A.niger, Penicillium*
	Antitussive	unspecified	*A.niger, A.flavus*
	Emmenagogue	*Curcuma xanthorrhiza* Roxb.	*A.niger*
	Relaxant	unspecified	*Penicillium*
	Tonic(2)	*Acanthus ebrateatus* Vahl.*	*A.niger, Penicillium*
	Anti-flatulence(2)	unspecified	*A.flavus, A.niger*
	Laxative	*Tamarindus indica* L. *Cassia angustifolia* Vahl.	*A.niger, A.flavus*
Honey-pill n=20	Relaxant	unspecified	*A.flavus*
	Anti-diabetic(2)	*Coscinium fenestratum* (Gaertn.) Colebr.	*A.niger, Penicillium*
	Antipyretic(4)	*Andrographis paniculata* (Burm.f.) Wall.ex Nees *Tinospora crispa* (L.) Miers ex Hook. F & Thoms.	*A.niger, A.terreus, Rhizopus*
Tablet n=7	Anti-inflammation	*Andrographis paniculata* (Burm.f.) Wall.ex Nees	*A.fumigatus, A.terreus*

*specified herbal ingredient/s do not indicated, **1,2=different isolates

Table 3. Microbiological qualification according to Thai Pharmacopoeia 2005.

Types	Capsule n = 40 (%)	Honey-pill n = 20 (%)	Powder n = 20 (%)	Tablet n = 7 (%)	Total n = 87 (%)
Registration (%)	16 (40.00)	0	12 (60.00)	6 (85.71)	34 (39.08)
Fail TP standard*	13** (32.5)	0	12 (60)	1 (14.29)	26 (29.89)
-Bacteria	10** (25.00)	0	11 (55.00)	1	22 (25.29)
-Fungi	6 (15.00)	0	9 (45.00)	0	15 (17.24)

*****one sample can fail for total bacterial count and/or total fungal count
******included 3 registered samples

Table 4. Fungal burden isolated from herbal medicine.

Sample (n)	Number of fungal isolates		
	Aspergillus* spp. (%)	***Penicillium* spp. (%)**	***Rhisopus* spp. (%)**
Capsule (40)	17 (53.13)	4 (50.00)	1 (50.00)
Powder (20)	9 (28.13)	3 (37.50)	0
Honey-pill (20)	4 (12.5)	1 (12.50)	1 (50.00)
Tablet (7)	2 (6.25)	0	0
Total	32 (100.00)	8 (100.00)	2 (100.00)
Total fungal representative isolates = 42 isolates			

*percentage of each species found

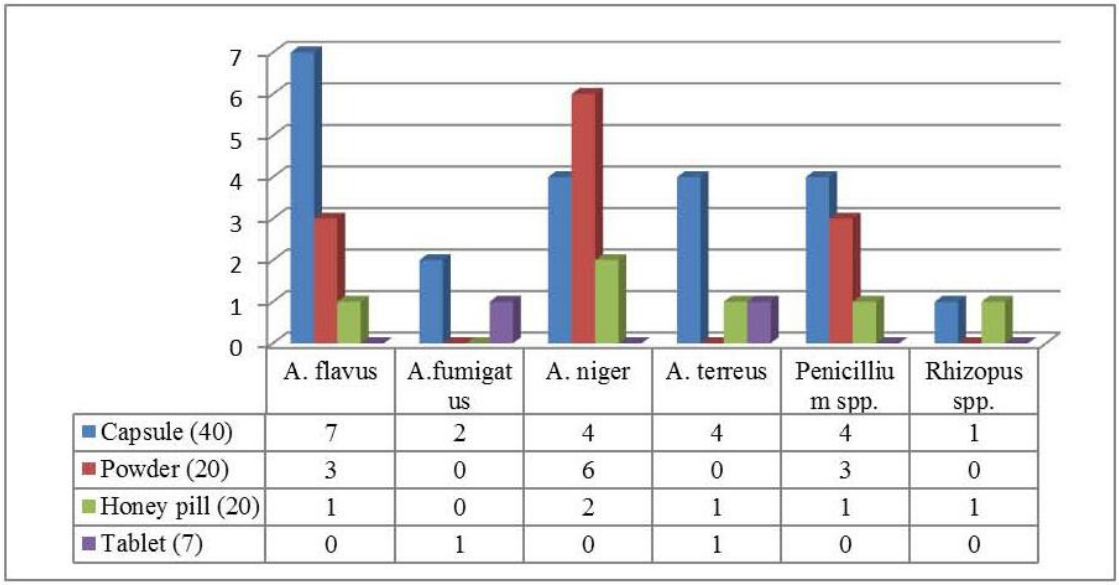

Figure 1. Species of *Aspergillus, Penicillium* spp. And *Rhizopus* spp. Isolated from 87 traditional herbal products.

Table 5. Susceptibility of fungal isolates to antifungal drugs

Fungi (n)	MICs* and MFCs** value; as µg/ml			
	AMB	**FLZ**	**ITZ**	**KTZ**
A. flavus (11)	4.86 (22.29)	18.20 (>77.28)	3.45 (8.25)	7.64 (>49.68)
A. fumigatus (3)	0.08 (0.08)	12.50 (>50.00)	6.88 (6.88)	5.31 (12.81)
A. niger (12)	1.24 (4.38)	46.36 (46.59)	9.56 (30.24)	4.63 (13.19)
A. terreus (6)	5.65 (37.52)	13.13 (40.63)	9.38 (38.13)	7.66 (38.13)
Penicillium (8)	3.17 (>31.34)	>84.38 (>90.62)	8.13 (18.13)	8.20 (>70.52)
Rhizopus (2)	0.08 (0.08)	10.00 (100.00)	12.50 (>50.00)	10.00 (12.50)
A. flavus 7232	2.50 (10.00)	10.00 (>50.00)	10.00 (10.00)	12.50 (25.00)

AMB=amphotericin B, FLZ=fluconazole, ITZ=itraconazole, KTZ=ketoconazole
*regular figure, **figure in the parenthesis

DISCUSSIONS

During 1987 to 2008, the Thai FDA stated that capital spending for local traditional medicine products for human use in Thailand increased from Baht 207.91 to 2,543.15 million while imported products from Baht 45.47 to 330.62 million. Similar trend was found in traditional drugs registered which revealed five-fold increase during 1983 to 2009. It was reported likewise during 1994 to 2001 that the registration of locally produced traditional medicines for humans had increased and the production value increased from Baht 414.86 to 736.91 million[10].

This reflected the popularity of the using or consuming of natural products. By the time of this tremendous proliferation of natural products, herbal usage and traditional medicines business, the regulations were more stringent than that of the past to comply with the international trades. Therefore, products from many local manufacturers failed the regulations. This study found that 26 out of 87 samples (29.89%) failed the criteria of TP. All failed samples were referred to the exceeded number of the total viable aerobic count of either bacteria or fungi or both. However, there was no *Clostridium* detected. Sampling of traditional medicines from 16 provinces indicated that only 39.08% of total 87 samples were registered whereas similar sampling performed in Bangkok showed higher percentage of registration (unpublished data).

Safety assessment of Thai herbal products according to Microbial limit test (MLT)

Fungi are ubiquitously found; therefore, traditional herbal products which comprised of various herbs are subjected to be contaminated with these organisms. Because microbial limit test signifies the limit number of contamination of microbial; therefore, MLT can be considered as safety standard and confidently reliable measure for manufacturers and also for end users. While the clear-cut of hazard level has not been stated to exert the negative effect to health, the over count of contaminants e.g. bacteria and/or fungi, pointed out the under standard of medicinal drug production. Moreover, this also implied to an inappropriate production process of all steps from raw materials, procedures of productions, packaging, storage, transportation to the selling points of these samples under study. Besides, the high count of contaminants

might imply that the production of toxic products i.e. mycotoxin/s or poisonous metabolites even from natural inhabitants and other mycobio-burden was highly possible.

Fungal detection in the present study was isolated from different dilutions of the enumeration stages to achieve microbial count. One genus might come out with many isolates on one or more counting plate/s. For example, two colonies with slight difference in color and texture but gave similar microscopic appearance of Penicillium-like fungi. In this case, these fungi were designated as *Penicillium* 1 and *Penicillium* 2. Therefore, the total representative fungi in this case was counted as 2 isolates which was leading to a total of 42 isolates subjected to evaluation of antifungal drugs susceptibility test in table 5.

While the *aspergilli* isolated from capsule and powder samples at similar amount (17 isolates from 40 samples in capsule and 9 from 20 in powder samples), honeypill showed smaller number of isolates (4 isolates from 20 samples). This should be from the postulation that, raw material of powder drug was bought in small re-packaged items. This bought re-packaging powder drug was again distributed into smaller one and being sold as package of powder form. Again, from the same bought re-packaging powder, capsule form was produced; these procedures increased the chance of fungal contamination more than that of bacteria. On the contrary, for honeypill form, powder of several herb materials was mixed with honey as binding agent and as another useful ingredient. While an important procedure was in simmering honey with or without drug powder, this also contributed to the less microbial contamination. Many manufactureres included the additional step of baking those rounded pills with low heat. These procedures, not only reduced the number of possible contaminated organisms but also hardened the pills and leaded to some difficulty of dissolution in the serial dilution steps. Therefore, it was suggested that grinding the pill before making the dilution was of advantage. The ground pill offered the maximum solubility to this kind of traditional medicine. However, being contaminated at this step as well as the cross contamination among many pill samples were highly cautious.

According to the documents, it was stated that infection by *Aspergillus* spp. could be caused by approximate 19 species. The most frequently found species throughout worldwide infections were from *A. fumigatus, A. flavus, A. niger* and *A. terreus*[11]. The fungal isolates discovered from Thai traditional medicine in this study conformed to the said results i.e. *A. niger, A. flavus, A. terreus,* and *A. fumigatus* were found in 12, 11, 6 and 3 isolates, respectively, which was totally 32 out of 42 isolates or 76.19%.

However, there was no evidence of relevant relationship of the particular isolated fungal species to certain kind or type of herbal components or any specific dosage form. In case of the prevalence of *Aspergilli*, this might be due to the fast growing property especially on the suitable medium like Sabouraud dextrose agar used in serial dilution before the fungi were selected. Beside the appropriate pH, the SDA also contained 4% dextrose which prevented the growth of bacterial contaminants at some level.

The susceptibility testing

One of the testing protocols for susceptibility test is widely quoted and employed in research and is counted as standard methodology. However, many laboratories with facility lacking, modification of testing principles from CLSI's is used instead. However, there are at least 18 methods to test the susceptibility reaction of *Aspergillus*[12]. This implied that the details of the experiments used varied from one laboratory to another. The susceptibility results differed and presented a wide range of interpretations. Therefore, this research stated the ability status of individual representative fungi isolated from medicines in response to antifungal drugs. The comparison with the results from elsewhere should be valid only if the similar step/detail of protocols was followed.

Susceptibility status of fungi isolated from Thai traditional medicine

In the procedures to obtain medicinal form from herbal raw materials, workers are inevitable to be exposed to dust in the storage of dried plant and particles produced in the grinding process etc. These materials would collect and were contaminated with several kinds of microbes, not only the natural residences but also the multiplication of these organisms during storage which enhanced the health risk to the people associated with these procedures. Besides, apart from the exposure or contact with these risky subjects, if there was no adequate protection provided, other negative reactions might occur. As the indirect exposure with these kinds of particle which might carry microbes, the allergies were the effects that should be aware. From these situations, being contacted or exposed to resistant fungi of those immuno-compromised people is considered under health risk conditions. Evaluation of traditional medicine samples in the aspect of susceptibility of natural fungi or any fungal contaminants which emerged from manufacturing procedures partially pointed out the fungal properties associated with their virulence.

The value of MICs/ MFCs accompanied with the sign ">" gave some degrees of ambiguous result. For example, the MFC of FLZ against *A. flavus* 7232 was designated as > 50 µg/ml. However, there was no doubt that the FLZ concentration of more than 50.0 µg/ml was required in order to kill this particular strain of *Aspergillus*.

The non *fumigatus Aspergillus* species expressing the resistant to amphotericin B had more frequently emerged as well as the zygomycetes, another causative agent and one of highly lethal non-*Aspergillus* moulds[13]. This leaded to the risk of being infected by saprophytic species of commonly found fungi. The susceptible status of those species might be considered as indicator of the treatment by antifungals. In this findings, the *in vitro* susceptibility testing revealed the higher concentration level to amphotericin B of *A. terreus* (MIC/MFC=5.65/37.52 µg/ml) than that of *A. flavus*, *A. fumigatus*, and *A. niger* (MIC/MFC=4.86/22.29, 0.08/0.08 and 1.24/4.38 µg/ml, respectively). This pattern of the susceptibility of the Aspergilli in this study was similar to other records[13]. On the other hand, the response of *A. terreus* to itraconazole in this study was not found the best (MIC/MFC=9.38/38.13 µg/ml) as was in other study[4].

At present, there was no relevant relationship demonstrated between the results of susceptibility test and the clinical outcome; however, patient who suffered from fungal infection by strains with high MICs did not respond quite well particularly to amphotericin B[15]. In addition, the susceptibility to antifungal drugs of individual fungal species was achievable; the identification explicit into species of the causative agents in patient with invasive fungal infections was therefore necessary. Although this research had classified Aspergilli into species level, in fact, in most circumstances, the invasive cases of fungal infection by a certain fatal, life threatening strain meant life and death; therefore, proper and immediate treatment and diagnosis were necessary to prevent the patient from such a great risk[16].

Consumers should therefore be aware of safety issues in using of traditional medicine for supporting health conditions, as dietary supplements, to promote immune system or in illness treatment especially in the group of populations at risk, for example, HIV patients[17-19], and people under suppressed immune system drugs, old age or with some exacerbate diseases or chronic infections[20]. Further included are workers exposed to any contaminants of chemicals, toxic products or microbes. To our knowledge, there still was no study demonstrating the indigenous susceptibility of those fungi to antifungal drugs. This is the first report of fungal susceptibility reacting upon antifungal drugs. This brings about alertness to herbal medicine users, patients and risk-laden group of populations and helps increase surveillance for more stringent regulation toward manufacturing GMPs.

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