

In vitro Effectiveness of *Acacia concinna* Extract against Dermatofungal Pathogens

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ABSTRACT

Acacia concinna (Wild.) D.C. is an important medicinal plant in Thailand and throughout Asian countries. Its dried pods are traditionally utilized as herbal medicine to treat many health symptoms e.g. laxative, cough, antidandruff and skin diseases. This investigation was performed in order to demonstrate the antimicrobial potential of different *A. concinna* extracts against the fungal causative agents of ringworm and opportunistic infections of immunocompromised populations. Phytochemical study showed that the crude extract of *A. concinna* pod consisted of alkaloids, flavonoids, saponin and tannin but none of anthraquinone and cyanotic glycosides. The extracts e.g. ethanolic Soxhlet extract and chloroform extract from Soxhlet extractor, and lyophilized extract and macerated ethanolic extract were tested using diffusion method against 35 isolates of dermatophytes and each 20 isolates of *Candida albicans*, *Cryptococcus neoformans* and *Penicillium marneffeii*. The inhibitory effects were observed by considering their average inhibitory zone diameter (IZD) compared to that of ketoconazole's. From the averaged IZDs of all fungal isolates, the antifungal effectiveness of lyophilized, chloroform, ethanolic (Soxhlet) and ethanolic (macerated) extract was at 18.38, 18.34, 16.67 and 14.06 mm, respectively.

Key words: *Candida*, *Cryptococcus*, dermatophytes, traditional medicine, lyophilized extract, soap pod

INTRODUCTION

Dermatophytes are a group of three keratinophilic fungal genera, *Trichophyton*, *Epidermophyton* and *Microsporum*, that cause daily found dermatofungal symptoms called tinea or ringworm on different parts of human body, as well as animals². This contagious mycopathogens may spread among human or from animal or soil to human.^[1] Although dermatofungal diseases do not express any life threatening but their clinical appearances are one of cosmetic problems,^[2-3] mental annoyance and can be regarded as everyday troublesomeness. Most of modern topical drugs available are not always affordable by people who required a long term treatment to clear those disturbing features, these cost a nation high budget for the effective treatment of the daily found mycoses.

Apart from these troubles, *Candida albicans* and *Cryptococcus neoformans* are involved as frequently found mycoses in tropical regions, alongside with acquisition of penicilliosis from *Penicillium marneffeii* which disseminated in patients infected by human immunodeficient virus.^[4-7] The interesting essentials from plant extracts that possess activities against dermatophytes might express similar effects upon these yeast-like fungi and dimorphic fungi.

Fortunately, many of those who faced these difficulties reside in regions where herbs are utilized as traditional medicines to which formularies and recipes have been accustomed. For this reason, using of traditional herbal medicines is an alternative mode in biodiversity rich countries, with the support of Government and by regulations, scientific researches in related area are increasing accordingly. Therefore, the effectiveness of native medicinal plants and their extracts by various methods of extraction can be used as alternative manipulation to treat many common infections.

Acacia concinna (Wild.) D.C. or soap pod, is naturally found and being cultivated throughout South Asian countries. The grind legume fruit powder is good to clean hair for its superior cleansing quality to give lustrous hair, promoting

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hair growth and also reducing dandruff. Besides, other medicinal activities of this pod fruit are for phlegm expellant, laxative, cough syrup, appetizer, skin disease and fever treatment. *A. concinna* is recorded on the use as traditional medicines i.e. the root is to treat fever and intestinal disorder, stem and leaves for laxative, stem bark to expel phlegm and spoilage, flowers to treat ligamentous dysfunction. Therefore, this plant is now being cultivated in large quantities for commercial purpose in India and the Far East because of its versatile properties.^[8-10]

However, various approaches on medicinal plant usages render several varieties of essential ingredients from plant. Because medicinal plants are used on the basis of traditional knowledge in which conventional household consumption is made from fresh plant naturally growth or kitchen garden cultivation. Most of the utilizations are squeezing to obtain juice, boiling with water, maceration in local made alcohol or modifying into preserved part of plant, honey pill, tablet, powder, sachet etc. To fulfill the immediate requirement of traditional medicines and to preserve the over-supply of fresh cultivation of seasonal products, simple expediency to keep raw products for long term storage gives rise to the community the benefit in managerial skill of local raw products. Therefore, instead of freshly used, the specific part of plant can be macerated in a short time and aqueous supernatant is lyophilized, the dried material obtained can be conveniently reconstituted to readily deliver the exact quantitative amount. The activities of this lyophilized extract are compared with those from the use of conventional Soxhlet apparatus.

Biological activity of medicinal plant extracts can be assessed by various techniques, from qualitative diffusion in agar plates, quantitative assay of dilution in broth or in agar, and the commercial E Test which combines those two methods' principles. Research studies reported the congenial results between qualitative diffusion test and quantitative dilution test. Not only for unicellular fungi e.g. *Candida*,^[11-16] or *Geotrichum*,^[17] but also for filamentous fungi e.g. dermatophytes,^[18] *Aspergillus*,^[19-20] opportunistic hyphomycetes i.e. species of *Fusarium*, *Cladosporium*, and ascomycetes i.e. *Chaetomium* spp. as well.^[21] However, these were evaluated for modern drugs activities, different protocols and several conditions were employed and presented in various interesting aspects. These scientific evidences revealed the relationship of disc diffusion test and those of quantitative tests; therefore, the likewise protocol should be effective in the evaluation for bioactivity of medicinal plant extracts to manifest the required substantial properties which lead to further modification of extracts into novelty herbal drugs.

This research is aimed to find out whether the medicinal plants appeared in the records of Thai traditional medicines

demonstrate scientific proof for antifungal activities. The pods of *A. concinna* were macerated in ethanol, maceration in water and further dried out by the use of lyophilizer and the extraction by using Soxhlet apparatus. Therefore, ethanolic extract, aqueous lyophilized extracts and the Soxhlet ethanolic and chloroform extracts, respectively were obtained. All were tested against 35 isolates of dermatophytes and each of 20 isolates of *Candida albicans*, *Cryptococcus neoformans* and *Penicillium marneffeii* by the use of diffusion method.

MATERIALS AND METHODS

Plant materials and plant extracts

Matured and dried fruits of *Acacia concinna* (Wild.) DC. was purchased from local suppliers and identified according to the voucher specimens deposited in Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, and the forest herbarium of the Royal Forest Department, Bangkok, Thailand.

Ethanolic extract and chloroform extract: using Soxhlet extractor

Dried ground powder of *A. concinna* was submitted to sequential extraction with chloroform and methanol using Soxhlet apparatus, the extract obtained was vacuum evaporated.

Ethanolic extract: maceration

A. concinna powder was overnight macerated under aseptic condition in 95% ethanol (1:4, w/v); after filtered through sterile gauze, the filtrate was vacuum evaporated.

Aqueous lyophilized extract: using lyophilizer

All steps in the following procedures were aseptically done. Clean, dried ground fruit of *A. concinna* was macerated in sterile distilled water at 1:4 w/v for 24 h. The supernatant after filtering was quantitatively collected and further processed with lyophilizer (Leybold-Haerareus, Lyovac GT-2, Germany). Aqueous lyophilized extract was kept in bottle with tight closed lid at -20° C.

Antifungal activity test

The antimicrobial activity was determined by agar diffusion assay described as the followings.

Microbial cultures

Clinical strains of 35 isolates of dermatophytes and each of 20 isolates of *Penicillium marneffeii*, *Candida albicans* and

Cryptococcus neoformans were obtained from Department of Microbiology, Faculty of Pharmacy, Mahidol University. All pure isolates were kept as stock strain at -20° C on Sabouraud dextrose agar (SDA) (Pronadisa, S.A. Spain).

Inoculum preparation

Active growth of dermatophytes isolates and *P. marneffei* with the appropriate selected characteristics was suspended in Sabouraud dextrose broth (SDB) (Pronadisa, S.A. Spain), to give the fungal density equivalent to turbidimetric No. 1 McFarland. Culture of *Candida albicans* and *Cryptococcus neoformans* were done likewise but to the density of No. 0.5 McFarland.

Extracts and reference drug preparation

A. concinna extracts were prepared as followings: ethanolic extract and chloroform extract from Soxhlet extractor were diluted 1:1 with their corresponding solvent; aqueous lyophilized extract was treated in similar manner with sterile distilled water. All test extracts were incorporated onto 6 mm in diameter sterile blank disc (Schleicher & Schuell, Germany) at 20 μ l/disc. Ketoconazole (Siemsgluss & Sohn, Germany) was dissolved in methanol to give the net amount of drug at 20 μ g ketoconazole/disc. Test control discs were ethanol and chloroform at 20 μ l/disc each. Test discs were prepared and used immediately after optimally dried out in sterile condition.

Test methods

Suspension of all isolates of dermatophytes and *P. marneffei*, 20 μ l each, was top layer cultivated onto total amount of 20 ml SDA plates whereas suspension of *C. albicans* and *C. neoformans* was aseptically swabbed onto 20 ml SDA plates. After the surfaces of all test plates were properly dried, at least duplicates of prepared discs of each extract and ketoconazole were laid onto the surface of inoculated plates. Inoculated plate of dermatophytes and *P. marneffei* were incubated at room temperature while *C. albicans* and

C. neoformans plates at 37° C. Diameter of each inhibitory zone was 3-time measured at different radial positions and arithmetically averaged. The figures obtained were considered as the activity of test materials. Culture control of each microorganism was included in similar manner to deliver the perfect timing in measuring the inhibitory zones.

RESULTS

Two samples of ethanolic extract were accessed from evaporating macerated sample and from Soxhlet extractor yielded different percentages of extracts. The first mentioned extract gave nearly twice amount compared to the Soxhlet extract whereas aqueous extract gave the highest yield among all (Table 1).

Phytochemical Analysis

Phytochemical properties of *A. concinna* fruit were alkaloids, saponin, tannin, flavonoids and cardiac glycosides but no anthraquinone and cyanotic glycosides (Table2).

However, using equal amount of suspension of each extract incorporated onto each disc, the different amount of ground material of *A. concinna* employed for one single disc could be calculated (Table 3).

Table 1: Percentage yield of *A. concinna* extract from different extraction methods.

Extract	% yield
Ethanolic	14.18
Lyophilized	26.22
Chloroform	10.67
Ethanolic (Soxhlet)	8.73

Table 2: Phytochemical properties of *A. concinna* fruit

Plant	Alkaloids	Saponin	Tannin	Flavonoids
<i>A. concinna</i> fruit	+	+	+	+

Table 3: Antifungal activity of *A. concinna* extracts as inhibitory zone diameter (IZD)

Organisms (n)	Inhibitory Zone Diameter (mean \pm SD, mm)				
	Ethanolic Extract (0.14)*	Lyophilized Extract (0.08)*	Ethanolic (Soxhlet) extract (0.23)*	Chloroform Extract (0.19)*	Ketoconazole 20 μ g/disc**
Dermatophytes (35)	14.88 \pm 0.48	21.66 \pm 0.49	18.50 \pm 0.45	27.69 \pm 0.52	32.22 \pm 0.92
<i>P. marneffei</i> (20)	18.71 \pm 0.55	19.80 \pm 0.66	23.31 \pm 0.66	17.90 \pm 0.85	35.25 \pm 0.59
<i>C. albicans</i> (20)	10.56 \pm 0.71	10.67 \pm 0.83	10.75 \pm 1.05	12.33 \pm 0.67	26.60 \pm 0.48
<i>C. neoformans</i> (20)	12.09 \pm 1.07	21.39 \pm 0.55	14.13 \pm 0.43	15.42 \pm 1.07	31.89 \pm 0.41
Arithmetic mean	14.06	18.38	16.67	18.34	31.49

*mg/disc = calculated mg of raw grinding *A. concinna***approx. eqv. to 1 mg of 2% topical drug

DISCUSSION

Phytochemical ingredients are continuously employed as natural essentials which are being promoted in many regions to preserve and to remunerate local resources. The versatile utilities of *A. concinna* has appeared in diverse traditional records with different stresses in certain property e.g. the use as laxative is accepted and being included as the traditional use in the National List of Essential Drugs and herbal crude drugs in Thai traditional household remedies since 2004.^[22]

This study employed various extraction methods of *A. concinna* fruit and demonstrated the activity of those extracts by a selected test on the basis of return value to support further traditional plant use in public health care. Hence, agar-based method or diffusion test was the method of choice for the fact that this test is simple, less time consuming in performing the test, less labor intensive, low cost, readable result at different times without subculture confirmation, and being reproducible method.

Considering the IZDs of overall isolates, dermatophytes and *P. marneffeii* exhibited the respective highest susceptibility to chloroform extract and ethanolic extract from Soxhlet extractor, respectively. The result from lyophilized extract gave the best result on *C. neoformans*. All extracts exerted less than one half efficacy on *C. albicans* in comparison with those of reference drugs, ketoconazole. Nevertheless, these results exhibited the effectiveness of all *A. concinna* extracts to be selected for further formulation.

On calculation of the raw material used for each disc i.e. calculation of the incorporated extract on disc, extract yield and the first weight of raw material used for extraction, the positive inhibitory reaction revealed that aqueous lyophilized extract was the most economically advantageous (0.08 mg raw powder per disc) compared with other extracts. The second was macerated ethanolic extract (0.14 mg raw powder per disc). This implied the fact that the use of *A. concinna* was so simple and able to verify the mode of utilization of ancient medicine e.g. using herbs with water and/or alcohol.

From various extraction methods, the use of Soxhlet apparatus which is generally employed and basically familiar in pharmacognosy area requires a lot of resources: chemicals, instrument, time consumed and personal expertise. However, while the antifungal activity of *A. concinna* extracts are apparently demonstrated, it is of interest to investigate whether those activities are attributed to any of phytochemical components e.g. alkaloids, saponin, tannin or flavonoids.

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