



RESEARCH ARTICLE

Antimycotic potential of Kawayang tinik against pathogenic fungal species

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ABSTRACT

The importance of discovering and obtaining new, natural and sustainable sources of potential drugs have been the focus of scientific communities due to the emergence of increasing cases of microbial resistance, one of the biggest health threats in our society today. This study aimed to determine the antimycotic potential of *Bambusa blumeana* (kawayang tinik) specifically its leaf, rhizome, root, inner culm and outer culm extracts using the agar well diffusion assay. Results of the study revealed that all kawayang tinik extracts produced statistically equal size zone of inhibition (ZOI) against *Aspergillus niger* at 1 mg/ml concentration while the ethanolic root and leaf extracts showed larger ZOI against *Penicillium chrysogenum* compared to other kawayang tinik extracts. Furthermore, the results of the antifungal assay showed comparable activity of kawayang tinik extracts to Fluconazole, a pharmaceutically approved antifungal drug, at 1 mg/ml concentration. Phytochemical studies further revealed the presence of alkaloids, tannins, phenols, sterols, triterpenes and flavonoids in its different parts which may support its potential antimycotic properties.

Introduction

The Center of Disease and Prevention (CDC) and World Health Organization (WHO) considers antibiotic resistance, in both bacterial and fungal species, as one of the biggest public health threats today (1, 2). Accordingly, widespread occurrences of antibiotic resistance were recorded among 500000 people across several countries including Asia. It is due to this fact that scientific communities are gearing towards drug discovery especially those coming from natural, highly available and sustainable resources.

Plants have been known for ages to contain bioactive compounds that possessed disease - fighting and ailment - preventing capabilities. In fact, the use of plants as ethnobotanical – based remedies and medication for various illnesses have been a long-dated tradition not only in the Philippines, but in other countries as well (3-5). Moreover, most of the pharmaceutical products being sold in the market today were obtained and patterned from bioactive compounds found in plants and plant products.

The bamboo plant was traditionally utilized to make home furniture and its shoots were usually obtained for food. However, secondary to technological advances in phytochemical screening and testing, several bamboo species are now being studied and observed as potential sources of new pharmacotherapeutic products. Leaves and shoot extracts from bamboo species such as *Bambusa balacooa*, *Bambusa bambos*, *Dendrocalamus hamiltonii*, *Bambusa vulgaris* and *Bambusa vulgaris* exhibited potential antimicrobial properties against several pathogenic microorganisms, bacterial and fungal alike (6-14). Leaf extracts of *Dendrocalamus strictus* were also cited to show antimycotic potentials against pathogenic fungal strains such as *Aspergillus niger*, *Penicillium chrysogenum*, *Aspergillus flavus* and *Fusarium moniliforme* (15). It was further observed that the antimicrobial activities in bamboo plants can be detected in the entirety of the plant, specifically in the branches, roots, knots, inner culms and rhizomes and not only on the leaf and shoot parts which are the most common plant parts used in antimicrobial studies involving bamboo plants (16).

The *Bambusa blumeana*, also known as spiny bamboo or thorny bamboo, is a species of bamboo occurring in tropical Asia and is abundant in the Philippines. This bamboo species is known locally as 'kawayang tinik' and ethnobotanically known as 'kawayan siitan' for Ilocanos and 'kawayang batakan' for Bisayans. Although widely available in the community and also tagged as the top economically important bamboo species in the Philippines (17), very few research studies and literatures, both local and international, have been published providing information on its biological activities especially with regard to its potential antimycotic properties.

With various evidences citing the potential biological activity in several bamboo plants against pathogenic fungal species, the present study determined the innate antimycotic potentials of *B. blumeana* (kawayang tinik). Specifically, the study explored the following:

1. Presence of potential antifungal activity in *Bambusa blumeana* (kawayang tinik) against *Aspergillus niger* and *Penicillium chrysogenum*.
2. Differences in the antifungal properties of the ethanolic and aqueous extracts of *Bambusa blumeana* (kawayang tinik) in terms of its different plant parts including the leaf, rhizomes, roots and inner and outer culms.
3. Identified different phytochemicals in various *B. blumeana* (kawayang tinik) extracts.

Materials and Methods

Selection, Gathering and Preparation of Plant Extracts

Plant parts such as leaves, rhizomes, roots, inner and outer culms were identified and gathered from locally – grown *B. blumeana* (kawayang tinik) plants found at Tarlac Agricultural University (TAU) Bamboo Forest Park located in Sitio Calao, Mayantoc, Tarlac. Collected parts were initially washed and cleaned with tap water to remove majority of the dirt and debris while distilled water was used for the final washing. Clean plant specimens were afterwards air dried and powdered separately using electric blender and corn miller. Individual powdered plant parts were placed in clean, zip – locked containers, labelled and stored in the refrigerator with temperature regulation at 4 °C until further use (14, 18, 19).

Powdered plant parts for ethanolic extraction were then mixed and macerated for 48 hrs (12, 16, 20) at room temperature using 95% ethanol utilizing 1: 4 extract to solvent ratio to produce ethanolic extracts. As for the preparation of aqueous extracts, 400 ml of distilled water was added with 100 gm of individual powdered dry plant parts (leaves, roots, rhizomes, inner and outer culms). Afterwards the individual aliquots were boiled at 80 °C for at least 15 min. cooled and allowed to macerate for 24 hrs (16, 21, 22).

Frequent agitation of the solutions was observed during the course of the maceration period to further facilitate the extraction of potential bioactive

compounds (20). After maceration, the aqueous and ethanolic solutions were filtered using Whatman filter paper No. 1. The filtrates were exposed thereafter to rotary evaporation to remove the solvents and concentrate the extracts.

Stock solutions were then formulated to 1mg/ml concentration and sterility- proofed through filtration of individual plant extracts using sterile millipore filter syringe (Whatman® at 0.22 millipore size) connected to a sterile syringe and test tube (23). The filtered sterile extracts contained in sterile tubes are then stored in the refrigerator with temperature regulation at 4 °C until needed for antimicrobial assay (24).

Antifungal Assay

Pure cultures of pathogenic fungi (*Aspergillus niger* and *Penicillium chrysogenum*) from the College of Arts and Sciences of Benguet State University located La Trinidad, Benguet were used to test the antifungal property of different kawayang tinik extracts.

Agar well diffusion method was utilized to determine the biological activity of *B. blumeana* (kawayang tinik) against *A. niger* and *P. chrysogenum*. In this method, a 3 mm diameter fungal or mycelium disk was placed at the center of the petri dish while various kawayang tinik extracts were placed in wells distributed in even distances around the fungal disk culture (Fig. 1). Fluconazole 1 mg/ml was used as positive control while sterile water and 95% ethanol were used as negative controls. After 72 hrs of room incubation, antifungal activity was determined by measuring the inhibition zones formed between the extracts and the mycelium disk. The zone of inhibition (ZOI) is measured by obtaining the distance of mycelium growth from the disk towards the individual kawayang tinik extracts as indicated by the blue line (Fig. 1). As shown by various studies, mycelium or fungal species grown on



Fig. 1. Antifungal assay using agar-well diffusion method.

laboratory agar tend to deviate from extracts containing antimycotic compounds, thus, minimal growth between the disk and the wells containing the individual extracts may possibly correlate to presence of bioactive compounds responsible for antifungal mechanisms (25-28). Additionally, five

replications were made per treatment per fungal species.

Phytochemical testing

Individual kawayang tinik plant extracts are subjected to qualitative phytochemical screening to determine the presence or absence of various phytochemicals using prescribed techniques and procedures (10, 29, 30). The presence of alkaloids was tested using different reagents (Mayer, Wagner, Bouchardat and Valser) while sterols and triterpenes were tested using the Lieberman's Buchard Test. The presence of flavonoids were otherwise confirmed using the Salkowski Test and Bate-Smith Metcalf test while the presence of cardiac glycosides were tested using the Keller-Killiani Test. Froth test was utilized to observed for the presence of saponin, gelatin test and ferric chloride test for the presence of tannins and phenolic compounds, Borntrager Test for the presence of antraquinone and Guignard Test for the possible presence of cyanogenic glycosides. The results of the phytochemical testing is presented in Table 3.

Table 3. Phytochemical analysis of different *Bambusa blumeana* (kawayang tinik) extracts

Bioactive Compounds	Plant Extracts									
	Aqueous					Ethanollic				
	Leaf	Rhizome	Roots	Inner Culm	Outer Culm	Leaf	Rhizome	Roots	Inner Culm	Outer Culm
Alkaloid	+	+	+	+	+	+	+	+	+	+
Sterol and Triterpenes	+	-	-	+	+	+	-	-	+	+
Flavonoid	+	-	-	+	+	+	-	-	+	-
Cardiac glycoside	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-
Tannins and Phenols	+	+	+	+	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-
Cyanogenic Glycoside	-	-	-	-	-	-	-	-	-	-

Results and Discussion

One-way Analysis of Variance (ANOVA) was used to determine the variation in the antifungal property of the various plant extracts derived from the different parts of *B. blumeana* (kawayang tinik).

Variations in the Antifungal Property of *Bambusa blumeana* (kawayang tinik) against *Aspergillus niger*

Table 1 presents the variations in the antimycotic potentials of *B. blumeana* (kawayang tinik) against *A. niger*. Results showed that there are highly significant variations among the *B. blumeana* (kawayang tinik) extracts against *A. niger* in terms of plant parts and the extraction solvents used since the F-computed value (3.74) is higher than the F-critical value (1.94) and the probability is less than 0.05.

The table further shows that in terms of the specific extracts derived from *B. blumeana*, the ethanollic root extract exhibited the highest comparable mean zone of inhibition (ZOI) at 1.13 mm against *A. niger*. This is followed by the extracts of ethanollic inner culm (1.04 mm), ethanollic leaves (0.98 mm), ethanollic outer culm (0.75 mm), aqueous outer culm (0.47 mm) and aqueous inner culm (0.41 mm). On the other hand, the ethanollic rhizome (0.20 mm), aqueous root (0.10 mm) and aqueous leaves

Table 1. Variations in the antifungal property of *Bambusa blumeana* (kawayang tinik) against *Aspergillus niger*

Extracts (1 mg/ml)	Zone of Inhibition (mm)
Ethanollic Roots	1.13a
Ethanollic Inner culm	1.04ab
Ethanollic Leaves	0.98ab
Ethanollic Outer culm	0.75ab
Fluconazole	0.55ab
Aqueous Outer culm	0.47ab
Aqueous Inner culm	0.41ab
Aqueous Rhizomes	0.22ab
Ethanollic Rhizomes	0.20b
Aqueous Roots	0.10b
Aqueous Leaves	0.09b
Water	0c
Fluconazole	0c

F-computed value = 3.74, F-critical value = 1.94, Probability = .0004

(0.09 mm) extracts of *B. blumeana* (kawayang tinik) also showed lesser statistically comparable inhibitory activities against *A. niger*. It was further observed that the above-mentioned extracts are statistically comparable to Fluconazole, a pharmaceutically-approved medication and antifungal agent, at 1

mg/ml concentration. This would imply that the extracts of *B. blumeana* have the same inhibitory effect as the standard antifungal drug Fluconazole at 1 mg/ml drug concentration (Fig. 2).

Various phytochemicals were observed to be

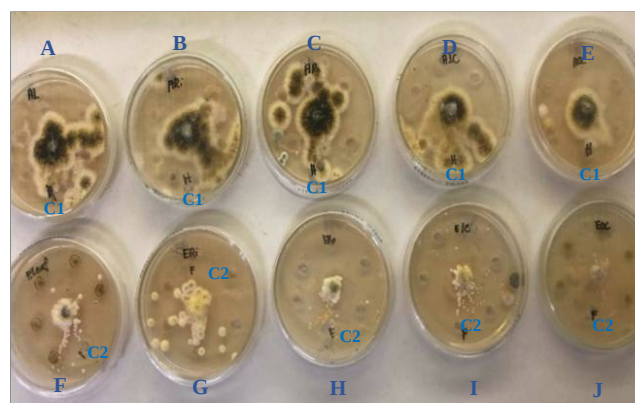


Fig. 2. *Aspergillus niger* growth on plates planted with various kawayang tinik extracts: (A) aqueous leaf, (B) aqueous rhizome (C) aqueous root, (D) aqueous inner culm, (E) aqueous outer culm, (F) ethanollic leaf, (G) ethanollic rhizome (H) ethanollic root, (I) ethanollic inner culm, (J) ethanollic outer culm, (C1) water and (C2) Fluconazole at 1 mg/ml.

present in different species of bamboo which could possibly explain the ZOI formed by the extracts

against *A. niger*. For instance, apigenin, luteolin and p- coumaric acid were discovered in the different parts of *P. pubescence*, specifically in its leaf, root, rhizome and culms (16). These phytochemicals were noted to have the same antimicrobial mechanisms as that of the fluconazole, a pharmaceutically – approved antifungal drug. Fluconazole as an antifungal agent exhibits its action by increasing fungal cell wall permeability and disrupting the uptake of essential nutrients needed by the cell leading to eventual death (28). Through the application of chemotaxonomy principles which states that plants that are taxonomically related contains the same biochemical compositions (31, 32), we could consider the possibility that kawayang tinik extracts may contain the same phytochemicals hence, justifying its potential antifungal property and the ZOI produced by its individual extracts against *A. niger*. The antimycotic potentials of bamboo species could further be claimed through the identification of another antifungal protein known called Dendrocin (33). Its mechanism of action includes the formation of membrane channels, degradation of polymers in fungal cell wall or disruption of cellular ribosomes (34).

In terms of extraction solutions used in the study, it was observed that majority of ethanolic *B. blumeana* extracts exhibited better antifungal potential compared to aqueous extracts although statistical comparability is noted among these extracts at 1 mg/ml concentration. This could be associated with the polarity of the solvents used to extract the potential phytochemicals present in the different plant parts of *B. blumeana*. Water is a pure polar solvent thus, can only attract or extract polar compounds while ethanol is less polar than water hence, it can possibly attract both polar and non-polar compounds (35). The greater variety of phytochemicals which may be extracted via ethanolic extraction may provide explanation on the better inhibitory performance of ethanolic extracts compared to the aqueous extracts of kawayang tinik. Moreover, when plant sources are homogenized or extracted using the same protocols, the polarity of the solvent is the main factor to be considered (36, 37).

Variations in the Antifungal Property of *Bambusa blumeana* (kawayang tinik) against *Penicillium chrysogenum*

Table 2 presents the variations in the antifungal potential of *B. blumeana* (kawayang tinik) against *P. chrysogenum*. Results of the analysis revealed high significant variations in the antifungal potential of different *B. blumeana* (kawayang tinik) extracts against *P. chrysogenum* since the computed F- value (20.21) is higher than the F – critical value (1.94) and the probability is lower than 0.05.

Statistical analysis further revealed that the ethanolic root and leaf extracts of *B. blumeana* exhibited the highest statistically comparable antifungal potential against *P. chrysogenum* both with mean ZOI of 1.59 mm. These were followed by kawayang tinik extracts of ethanolic inner culm (0.48 mm), ethanolic rhizome (0.41 mm), ethanolic outer culm (0.12 mm), aqueous inner culm (0.06 mm) and

Table 2. Variations in the antifungal property of *Bambusa blumeana* (kawayang tinik) against *Penicillium chrysogenum*

Extracts (1 mg/ml)	Zone of Inhibition (mm)
Ethanolic Roots	1.59 a
Ethanolic Leaves	1.59 a
Ethanolic Inner culm	0.48 b
Ethanolic Rhizomes	0.41 b
Ethanolic Outer culm	0.12 b
Aqueous Inner culm	0.06 b
Aqueous Rhizomes	0.03 b
Aqueous Leaves	0 c
Aqueous Roots	0 c
Aqueous Outer culm	0 c
Water	0 c
Fluconazole	0 c

F-computed value = 20.21, F-critical value = 1.94.
Probability = 1.75E-17=.0000

Note: Means followed by the same letter/s are not significantly different at 5% level

aqueous rhizome (0.03 mm) which are comparable or having equal antifungal effects against *P. chrysogenum* at 1 mg/ml concentration. On the contrary, the aqueous extracts of leaves, roots and outer culm did not exhibit any effects against *P. chrysogenum* exhibiting mean ZOI of 0.00 mm (Fig. 3).

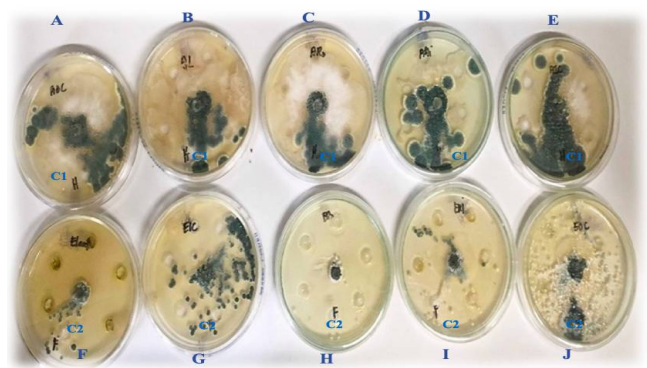


Fig. 3. *Penicillium chrysogenum* growth on plates planted with various kawayang tinik extracts: (A) aqueous outer culm, (B) aqueous leaf (C) aqueous root, (D) aqueous rhizome, (E) aqueous inner culm, (F) ethanolic leaf, (G) ethanolic inner culm (H) ethanolic root, (I) ethanolic rhizome, (J) ethanolic outer culm, (C1) water and (C2) Fluconazole at 1 mg/ml.

As observed in the study, ethanolic extracts produced higher mean ZOI compared to the aqueous extracts of inner culm, rhizome, leaves, roots and outer culms that exhibited mean ZOI of 0.06 mm, 0.03 mm and 0.00 mm respectively. This result could still be associated to the polarity of extraction solutions used in the study whereby ethanol being less polar could harness both polar and non- polar compounds while water, a pure polar solvent, could only harness polar phytochemicals (37). Since ethanolic extraction could harness a wider variety of phytochemicals, better inhibitory potentials could be observed compared to aqueous extracts. Moreover, the better inhibitory performance of the ethanolic extracts could also indicate the possibility of greater proportions or amount of non- polar phytochemicals residing in the different plant parts of kawayang tinik especially that aqueous counterparts does not exhibit any inhibitory effects against *P. chrysogenum* (i.e. aqueous roots, leaves and outer culms producing mean ZOI of 0.00 mm).

The findings mentioned may also be correlated a previous study result wherein it was observed that phytochemicals from the leaf extracts of bamboo species *Phyllostachys* and *Moso* bamboo are not water soluble (38). However, the presence of polar phytochemicals in the different parts of *B. blumeana* could not be totally eliminated from the current study since aqueous extracts also exhibited zones of inhibitions against the previously discussed fungal species. It could be a possibility that the either *P. chrysogenum* is resistant to the polar compounds present in the different extracts of kawayang tinik or the concentration of extracts which is 1 mg/ml is inadequate to show inhibitory action against the said fungal species. Therefore, additional and more advanced studies should be conducted to fully determine the various phytochemicals present in the different parts of *B. blumeana* and how these phytochemicals relates to the antifungal potentials of kawayang tinik as well as devise further experimentations catering to different concentrations of extracts against fungal species.

The results of the study also coincide with previous findings citing that various phytochemicals were present in the different parts of the bamboo species *P. pubescence* (16). Specifically, apigenin and tricetin derivatives were noted to be abundant in the root and leaf part of the said bamboo species. Antifungal mechanisms of these identified phytochemicals were noted to be fungal membrane and metabolic disruption (38, 39) the same inhibitory action of fluconazole, a standard antifungal drug (28).

Currently, there are no standard medical treatment of choice for the management of penicilliosis, however, antibiotics like amphotericin B, itraconazole, or fluconazole were reported to be effective against the said organism. The various ranges of inhibition zones exhibited by the ethanolic extracts of *B. blumeana* may indicate potential effectiveness of the said bamboo species in inhibiting the fungi *P. chrysogenum*, especially that the ethanolic root and leaf extracts exhibited more significant results compared to the standard antibiotic treatment Fluconazole at 1 mg/ml concentration. Moreover, other *B. blumeana* plant parts such as rhizomes and culms also exhibited potential antifungal activities as evidenced by the inhibition zones formed which further highlights the potential of the said bamboo species as source of potential antifungal drug against *P. chrysogenum*.

Phytochemical Testing

Table 3 shows the results of the phytochemical test involving different extracts of kawayang tinik. As shown, it is evident that all of the extracts contain bioactive compounds such as alkaloid, tannins and phenols while majority of the extracts also contains sterols and flavonoids. On the other hand, all of the extracts were screened negative for the presence of cardiac glycoside, saponins, anthraquinone and cyanogenic glycoside.

The presence of various phytochemicals in the ethanolic and aqueous extracts and their innate abilities to promote microbial inhibition may possibly verify the antimycotic potentials of

kawayang tinik extracts. Alkaloid as a phytochemical exerts its antimicrobial mechanism via multitarget action such as outer membrane or cytoplasmic membrane disruption, cellular respiratory inhibition and nucleic acid synthesis or cell division inhibition (39, 40). Flavonoid, on the other hand, is able to promote inhibition of nucleic acid synthesis and disrupt the energy metabolism in pathogenic microorganisms (39). Tannins are also able to induce inhibition of bacterial and fungal enzymatic activity via direct interference on the microorganism's metabolism while triterpenes and sterols work by increasing the tolerance and resistance of plants to pathogen attacks, although the exact pathways and mechanisms are not yet known (41, 42).

Conclusion

In line with the objectives of the study, analysis and results, the following conclusions and implications were made:

1. *B. blumeana* (kawayang tinik) extracts showed varying ranges of antimycotic activity against *A. niger* and *P. chrysogenum*. Thus, kawayang tinik can be a potential source of natural pharmacotherapeutic products against these pathogenic fungal species.
2. Ethanolic extracts of roots and inner culm exhibited the highest mean zone of inhibition (ZOI) against *A. niger* while ethanolic root and leaf extracts exhibited better inhibitory potentials against *P. chrysogenum*.
3. *B. blumeana* (kawayang tinik) extracts contain phytochemicals such as alkaloid, sterols, flavonoids, tannins and phenol. Hence, further studies are needed to specifically harness the compounds producing antifungal effects.

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Conflict of interests

Author do not have any conflict of interests to declare.

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