PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.1451

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RESEARCH ARTICLE

Potentials of kawayang tinik (*Bambusa blumeana*) as new source antimicrobial agents

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OPEN ACCESS

ARTICLE HISTORY

Received: 25 August 2021
Accepted: 14 March 2022

Available online
Version 1.0 (Early Access): 17 April 2022

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, etc. See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Saducos A G. Potentials of kawayang tinik (*Bambusa blumeana*) as new source antimicrobial agents. Plant Science Today (Early Access). https://doi.org/10.14719/pst.1451

Abstract

In this time where health is priority and surges of microbial resistance is highly observed within the society, discovering new, effective and sustainable sources of potential pharmacologic products is highly significant. The study explored the antimicrobial potentials of the different parts of *Bambusa blumeana* (*kawayang tinik*), a common Philippine bamboo species, against selected pathogenic bacterial and fungal species utilizing minimum inhibitory concentration via agar well diffusion method. Results of the study showed that extracts of *B. blumeana*, specifically the leaf, rhizome, roots, inner and outer culms, are capable of inhibiting microbial growth at 0.06 to 0.98 mg/ml concentrations. Specifically, the aqueous outer culm extract of *B. blumeana* proved to be most effective in inhibiting the growth of *Pasteurella multocida* at 0.24 mg/ml while *Staphylococcus aureus* and *Escherichia coli* were most susceptible to ethanolic outer culm extracts at 0.06 mg/ml and 0.12 mg/ml respectively. *Bacillus subtilis*, on the other hand, was observed to be the most sensitive to ethanolic root extracts at 0.06 mg/ml. Furthermore, *Aspergillus niger* was observed to be susceptible to ethanolic rhizome extract (0.24 mg/ml) while the ethanolic leaf, roots, inner and outer culms were equally effective in inhibiting *Penicillium chrysogenum* at 0.98 mg/ml extract concentration. Phytochemical testing further revealed the presence of phenols and flavonoids in the different parts of the bamboo species which further support its potential as a new source of pharmaceutical biocompounds.

Keywords

Antimicrobial, *Bambusa blumeana*, bamboo

Introduction

Rising cases of antibiotic resistance urges scientific communities to look for new and sustainable sources of drugs. Plants, on the other hand, are known for their ecological as well pharmaceutical potentials and was proven by sustained and effective use of ethnobotanical remedies around the world. Moreover, approved pharmaceutical products are mostly obtained from phytocompounds found in plants and plant products.

One common bamboo species found in the Philippines, particularly in Central Luzon, is *Bambusa blumeana* (*kawayang tinik*). It is frequently utilized for making furniture, native homes and is also consumed as food although, some studies have mentioned its use for the treatment of respiratory symptoms, kidney stones and Dengue fever by some ethnic communities in Luzon, Philippines (1).

Despite the commonality of the said bamboo species, limited studies, both local and international, have been conducted detailing its potential as a pharmaceutical source. On the other hand, other bamboo species thriving in other Southeast Asian countries have been established to contain phytocompounds with potential antimicrobial properties (2-4). Hence, this study was done to determine the potential of *B. blumeana* as source antimicrobial biocompounds. Specifically, the study aimed at exploring the presence and differences in antimicrobial property of the different parts of *B. blumeana* against *Penicillium multocida*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger* and *Penicillium chrysogenum*. Moreover, identification of total phenolic and flavonoid contents in various *B. blumeana* extracts were also explored and correlated.

Materials and Methods

Plant Material Preparation

Plant parts of *B. blumeana* (*kawayang tinik*) were first identified and collected from the Bamboo Forest Park of Tarlac Agricultural University in Tarlac, Philippines. Plant parts including the leaves, roots, rhizomes, inner culms and outer culms were used and utilized in the study. Selected plant parts are afterwards cleaned, air dried, powderized, stored in sterile, dark containers and kept in the refrigerator with temperature regulation at 4 °C until use (5 -7). Extraction, purification and concentration of plant materials are done thereafter. Ethanolic extraction was done by macerating the individual powderized plant materials with 95% ethanol using 1:4 plant material to solvent ratio for 48 hrs under room temperature (2, 8, 9). Aqueous extraction, on the other hand, was made by mixing 100 g of plant materials in 400 ml of distilled water, boiled for about 15 min at 80 °C then cooled and macerated for 24 hrs under room temperature (5, 8). The aliquots are then strained via Whatman filter paper No. 1 and the filtrates are subjected to rotary evaporation. In making the stock solutions for antimicrobial testing, individual plant extracts are reformulated to 1 mg/ml and are sterilized with sterile millipore filter syringe (Whatman® at 0.22 millipore size) (10). The sterile plant extracts are then stored in sterile, dark containers and kept in refrigerator with temperature regulation of 4 °C (11).

Minimum Inhibitory Concentration Determination

The MIC value was performed by doing combination of the classic two- fold dilution method and agar well diffusion method (12). The same methodology was employed by various studies which validated the efficiency and effectivity of the method employed in the study (13-15). Bacterial MIC determination was facilitated by preparing sterile Himedia® nutrient agar swabbed aseptically with 100 µl of selected bacterial species initially maintained at Himedia® Nutrient Broth (NB) and further adjusted to 0.5 McFarland standards at (1.0 x 106) CFU/ml (16, 17). After drying the plates for 15 min, wells are created by punching the previously swabbed agar plates with sterile cork borer. 100 µl individual plant extracts with various concentrations were then aseptically poured in the wells. Afterwards, the prepared plates are incubated for 24 hrs at 37 °C.

Fungal MIC, on the other hand, was done by placing a 3- mm mycelium disk culture (*A. niger* and *P. chrysogenum*) at the center of a sterile, solidified Sabouraud® agar plate wherein various kawayang tinik extracts were placed on even distances around the fungal disk. The seeded plates were afterwards incubated at 72 to 96 hrs at room temperature. The interpretation of MIC for bacterial and fungi species were based on the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI). These standards rely on the formation of optically clear inhibition zones thus, *B. blumeana* extracts which displayed optically clear zones of inhibition, both on the initial and validation testing, were included in the results of the study and those that display hazy or unclear zones are tagged at not determined. Furthermore, the extracts with the least concentration level that exhibited optically clear ZOI was treated as the MIC (18).

Qualitative Phytochemical Testing

The various *B. blumeana* ethanolic extracts was brought to Saint Louis University Natural Science Research Institute (NSRI) in Baguio City and the University of Sto. Tomas Analytical Services Laboratory in Manila, Philippines for total phenolic and flavonoid determination. The total phenolic content of ethanolic extracts was determined by diluting 100 µl of the plant extract with 3 ml of analytical grade water then mix with 0.5 ml of Folin – Ciocalteau reagent. After 3 min, 2 ml of 20% Na2CO3 (w/v) solution was added and were mixed thoroughly. The absorbance was measured at 750 nm against the blank using a spectrometer. A standard solution was prepared using gallic acid monohydrate at 15.63 µg/ml to 500 µg/ml (r2= 0.99), and the linear regression equation was obtained to determine the total phenolic content of the plant extract (19).

Flavonoid content determination in ethanolic extracts, on the other hand, were taken by mixing 500 µl of the plant extract with 2.5 ml of analytical grade water and 150 µl of 5% $NaNO_2$ solution. After 6 min, 300 µl of 10% AlCl3 ·6H2O solution was added. One (1) ml of 1M NaOH solution was added after 5 min and the mixture was brought to 5 ml using analytical grade water and mixed well. The absorbance was measured immediately at 510 nm. A standard solution was prepared using quercetin (r2= 0.99), and the linear regression equation was obtained to determine the total flavonoid content of the plant extracts (20).

Total phenol contents in aqueous extracts were evaluated by treating the aliquots with Folin-Ciocalteau reagent and Na_2CO_3 with increasing concentrations of Gallic acid used as standard. The aliquots were allowed to stand at room temperature for 90 min and the absorbance was measured at 750 nm wavelength utilizing a microplate reader. Total flavonoid of aqueous extracts, on the other hand, were calculated through the addition of equal volumes of extracts and 2% $AlCl_3$ in wells of a 96-well plat whereby absorbance was measured at 420nm using a microplate reader after 1hr at room temperature (21).

Results and Discussion

Minimum Inhibitory Concentration (MIC) of Different Bambusa blumeana (Kawayang tinik) Extracts

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of antimicrobial agent that will inhibit the visible growth of a microorganism after an incubation period (22). Lower MIC values can further be interpreted as lower concentration of drug or extract needed to inhibit a certain bacterial or fungal strain. Hence, lower concentrations or MIC values mean greater bacterial susceptibility or sensitivity against the antibacterial agent being tested. The minimum inhibitory concentration (MIC) values of the different extracts of *B. blumeana* tested against several microbial species were reflected in Table 1. The variations in MIC values reveals varying susceptibility of the selected bacterial and fungal species against the ethanolic and aqueous extracts of *B. blumeana*.

Table 1. Minimum inhibitory concentration (MIC) of different *B. blumeana* extracts against several microbial species

PLANT EXTRACTS (mg/ml)	BACTERIAL SPECIES				FUNGAL SPECIES	
	P. multocida	*S. aureus*	*E. coli*	*B. subtilis*	*A. niger*	*P. chrysogenum*
AQUEOUS						
Leaf	0.49	62.5	1.95	3.91	3.91	15.63
Rhizome	nd	nd	nd	nd	1.95	3.91
Roots	0.98	3.91	7.81	nd	nd	1.95
Inner Culm	0.98	nd	3.91	15.63	nd	1.95
Outer Culm	0.24	nd	nd	nd	0.47	7.81
ETHANOLIC						
Leaf	nd	0.98	0.98	0.24	0.98	0.98
Rhizome	3.91	0.98	0.98	3.91	0.24	1.95
Roots	7.81	3.91	3.91	0.06	0.98	0.98
Inner Culm	0.49	0.49	0.49	0.98	0.98	0.98
Outer Culm	0.98	0.06	0.06	0.12	0.98	0.98

It is also revealed in Table 1 that the aqueous outer culms extract of *B. blumeana* exhibited the lowest MIC value against *P. multocida* (0.24 mg/ml) while *S. aureus* and *E. coli* were mostly susceptible to ethanolic outer culm extracts (0.06 mg/ml and 0.12 mg/ml respectively). *B. subtilis*, on the other hand, was observed to be most sensitive to ethanolic root extract at 0.06 mg/ml.

The table further revealed that selected fungal species are also susceptible to most *B. blumeana* extracts. *A. niger* and *P. chrysogenum* were also found to be more susceptible to ethanolic extracts compared to their aqueous counterparts such that ethanolic rhizome extract exhibited lower MIC value (0.24 mg/ml) against *A. niger* while ethanolic leaf, roots, inner and outer culm extract were equally effective in inhibiting *P. chrysogenum* at 0.98 mg/ml.

The antimicrobial potential of bamboo species could be attributed to various phytocompounds previously observed in the different parts of the plant. For instance, the compound apigenin, luteolin and p - coumaric acid are found in the leaf, inner and outer culms and root part of *P. pubescence* (8). Apigenin works by inhibiting cellular metabolism (23) while luteolin has the ability to destroy bacterial cell membrane (24). P- coumaric acid, on the other hand, inhibits bacterial DNA function and also disrupts bacterial cell membrane (25). Other studies also observed the presence of tannins, cardiac glycoside, terpenoids, saponins and steroids in *B. blumeana* and other bamboo species (5, 26, 27). These phytochemicals are known for having innate antimicrobial effects via various mechanisms.

Several studies also document the potentials of other bamboo species as antimicrobials. For instance, it was found out that different plant parts of *P. pubescence*, a Japanese bamboo, were effective against *S. aureus* and its ethanolic outer culm extract can express 98 – 100% inhibition rate and has a MIC value of 0.4 to 1.6 mg/ml (8). The outer culms of bamboo species from *Gramine* family were also noted to display antimicrobial potentials when tested against several microorganisms using water extraction and agar diffusion method. Accordingly, its MIC value against *S. aureus*, *E. coli*, *B. subtilis* and *A. niger* were 4.9 mg/ml, 5.3 mg/ml, 6.4 mg/ml and 4.9 mg/ml respectively (28). Additionally, ethanolic leaf extracts of *D. strictus* were also noted to be effective against *E. coli*, *S. aureus* and *B. subtilis* at 0.5 to 1.0 mg/ml (29). N- hexane, chloroform and ethyl acetate leaf extracts of *B. vulgaris*, on the other hand, was observed to inhibit *E. coli* and *S. aureus* at less than 2.5 mg/ ml MIC concentration (30).

Bamboo plants were also noted to be effective in inhibiting the growth of some fungal species. N- hexane, chloroform and ethyl acetate leaf extracts of *B. vulgaris* was observed to inhibit *A. niger* and *V. alboatrum* at less than 2.5 mg/ ml (30) while *D. strictus* ethanolic leaf extract was noted to be effective against *A niger*, *P. chrysogenum*, *F. moneliforme* and A. *flavus* at 0.5 to 1.0 mg/ml (29). Aqueous leaf extracts of *P. pubescence* were also observed to effectively inhibit *P. grisea*, causative agent for rice blast disease, at 0. 5 – 1.0 mg/ml concentration (25) while aqueous shavings of bamboo coming from the *Gramine* family also manifested effective inhibition against *A. niger* at 4.9 (+/-0.2) mg/ ml MIC concentration (28).

It can be observed that the MIC values exhibited by the *B. blumeana* extracts were comparable and even lower compared to the studies previously mentioned. This may further validate the antimicrobial potentials of *B. blumeana* (*kawayang tinik*) plant extracts. Moreover, it can also be observed that lower MIC values were obtained from bamboo plant parts that are not usually being studied such as the rhizome, root, outer culm and inner culm. These results may further support the evidences of the potential presence of antimicrobial activity on *B. blumeana* (*kawayang tinik*) not only on the leaf part but all other bamboo plant parts as well.

Determination of the Quantitative Total Phenolics and Flavonoid Content of Bambusa blumeana (Kawayang tinik) Extracts

Presented in Table 2 are the results of the total phenolic and flavonoid content determination of plant extracts derived from the different parts of *B. blumeana* (*kawayang tinik*). It was observed that all extracts are positive for the

presence of phenols and flavonoids at varying levels of concentration. Phenols and flavonoids possess innate antimicrobial properties through various mechanism such as inhibition of nucleic acid synthesis and cytoplasmic membrane function, disruption of metabolism and inhibition of oxidative phosphorylation cycle (31 - 34).

Table 2. Total phenolic (TPC) and total flavonoid content (TFC) of different *Bambusa blumeana* extracts

EXTRACTS	TOTAL PHENOLIC CONTENT (TP) ug/ml GAE	TOTAL FLAVONOID CONTENT (TF) ugQE/ml
ETHANOLIC		
Leaves	141.00	247.60
Rhizome	154. 57	440.00
Roots	140.00	156.00
Inner Culm	57.85	176.80
Outer Culm	131.29	48.80
AQUEOUS		
Leaves	1509.10	667.10
Rhizome	216.82	169.40
Roots	894.41	259.80
Inner Culm	488.67	177.30
Outer Culm	510.71	218.30

For phenolic content determination in ethanolic extracts, rhizome exhibited the highest phenol concentration (154.57 ug/ml GAE) followed by leaf (141 ug/ml GAE), root (140 ug/ml GAE) and outer culm (131. 29 ug/ml GAE) extracts. The ethanolic inner culm extracts on the contrary, shown the least amount of phenolic compound (57. 85 ug/ml GAE). Aqueous plant extracts such as the aqueous leaf extracts obtained the highest phenol concentration at 1509.10 ug/ml GAE followed by the aqueous root extracts (894.41 ug/ml GAE), outer culm (510. 71 ug/ml GAE), inner culm (488.67 ug/ml GAE) and rhizome extracts (216.82ug/ml GAE) respectively.

Flavonoid content in ethanolic extracts were also evaluated and results shows that rhizome extracts exhibited the highest flavonoid concentration (440 ug/ml GAE) followed by the ethanolic extracts of leaves (247.60 ug/ml GAE), inner culm extracts (176.80 ug/ml GAE) and root (156.00 ug/ml GAE). The ethanolic outer culm extracts exhibited the lowest total flavonoid content at 48. 80 ug/ml GAE, on the contrary. In terms of the aqueous extracts, results showed that leaf extracts contain the highest total flavonoid content at 677. 10 ug/ml GAE followed by the root extracts (259. 80 ug/ml GAE), outer culm extracts (218. 30 ug/ml GAE), inner culm (177. 30 ug/ml GAE) and aqueous rhizome extracts (169. 40 ug/ml GAE).

It is evident in the results of the TPC and TFC that majority of the aqueous extracts exhibited higher total phenolics and flavonoid concentration except for rhizome extracts which elicited higher TPC and TFC in ethanolic extraction and inner culm extracts for total flavonoid content. These observations seem to contradict the previous MIC results obtained wherein ethanolic extracts exhibited lower inhibitory values compared to aqueous extracts. This could be explained by the fact that plants are natural sources of abundant numbers of phytochemicals and bionutrients (33) and contain more than 5000 classes of phytochemical that were already discovered but not yet fully studied (34). Since only phenolics and flavonoids were measured in the study, the better performance of ethanolic extracts in the previous MIC determination could be associated with the presence of other phytochemicals of *B. blumeana*. Local studies have also documented the presence various phytocompounds in *B. blumeana* such as alkaloids, sterols, triterpenes and tannins (26, 27) which also has innate antimicrobial properties. Therefore, the antimicrobial potential of *B. blumeana* extracts could not only be associated with the amounts of flavonoids and phenolic contents alone and but rather with the totality of phytochemicals present in the extracts. This further implies the need for additional studies to fully harness and understand the antimicrobial potential of *B. blumeana*.

Differences in assay results and phytochemical testing may further be attributed to the endointeractions of individual phytochemicals (35) such that individual biomolecules in plants may either potentiate or interfere with the biological activities of phytocompounds and works by either interfering with the bioavailability of other phytocompounds, interloping with cellular transport processes, activation of pro-drugs or deactivation of active compounds to inactive metabolites, multi-target effects or inhibition of binding to target proteins (36). Thus, recommendations to further purify extracts to determine the active components responsible for the antimicrobial potentials especially in *B. blumeana* (*kawayang tinik*) is highly suggested.

Furthermore, looking at the solvents used in the study, phenolic compounds and flavonoids are better extracted in polar solvents (37). Therefore, given that water is more polar than ethanol, phenolic compounds as well as flavonoid could be better extracted using aqueous rather than ethanolic extraction. This and the above- mentioned conditions and rationale provide justification on the higher TPC and TFC values of the aqueous extracts than ethanolic extracts.

Conclusion

All findings of the study suggest *B. blumeana* as a potential source of phytocompounds for drug development. Its extracts manifested varying antimicrobial activities against selected microorganisms and its minimum inhibitory concentration is noted to range from 0.06 to 0.98 mg/ml which is comparable or even lower compared to previously studied bamboo species. Moreover, *B. blumeana* extracts also showed to contain phenols and flavonoids which are phytocompounds with known innate antimicrobial potentials.

Acknowledgements

The researcher would like to extend her gratitude to Tarlac Agriculture University, Commission on Higher Education Kto12 Scholarship Program, Benguet State University, Virgen Milagrosa University Foundation, Saint Louis University, University of Sto. Tomas, family, mentors specially Dr. Marie Sandoval, Dr. Denisa Domondon, Dr. Romeo Gomez

and Dr. Joanna Alafag, her friends and other supporters for their outmost kindness and encouragement.

Authors contributions

AS coordinated and conducted the experiment. She also sent the specimens in accredited laboratories, interpreted results and wrote the final article to communicate pertinent results of the study.

Compliance with ethical standards

Conflict of interest: Author do not have any conflict of interests to declare.

Ethical issues: None

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