J. Bamboo and Rattan, Vol. 4, No. 1, pp. 71–80 (2005)

Also available online - www.vsppub.com

The inhibition of microbial growth by bamboo vinegar

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Abstract—The ability of bamboo vinegar, produced from the pyrolysis of *Gigantochloa scortechinii* Gamble culms from Kedah, Malaysia, to inhibit the growth of micro-organisms was investigated using a laboratory-based assay. The inhibitory effects of cellulose discs treated with bamboo vinegar at 10%, 50% and 100% (no dilution) concentration on the growth of 7 fungal and 3 bacterial species was investigated. The two higher concentrations of bamboo vinegar showed growth-inhibiting effects against *Aureobasidium pullulans* (MBRB1-3), *Chaetomium globosum* (FPRL S70K), all three bacterial species and some effect with the other fungal species except *Coriolus versicolor* (FPRL 28A). The inhibition of growth followed a dose dependent response with the 100% concentration being the most effective. It is concluded that bamboo vinegar contains compounds that are inhibitory to microbial growth although specific evidence for activity at low concentrations, e.g., below 1% total organic compounds, was not obtained.

Key words: Bamboo vinegar; fungi; bacteria; growth inhibition; *Gigantochloa scortechinii*.

INTRODUCTION

Bamboo vinegar is purported to be used widely in Japan and China for applications in agriculture, medicine, healthcare, chemical manufacture and environment protection [1–5]. There are numerous websites referring to such applications [6–18], especially in traditional medicine, and recently bamboo vinegar has become of quite high commercial interest. However, there are very few studies available that present scientific data on the potentially diverse properties of this material.

Bamboo vinegar is obtained during the preliminary stages in the production of bamboo charcoal by smoking bamboo culms at 200°C in the absence of oxygen [19]. The vapour produced from this pyrolysis process is condensed and collected as bamboo vinegar (also known as chikusaku). The charcoaling process is

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normally carried on by smoking and heating without oxygen to higher temperatures of around 800°C for about 4 h [19].

The main constituent in bamboo vinegar is water, which comprises about 80–90% by volume [1]. The composition of organic compounds is about 8%, of which about half is acetic acid. A further 200 or so different chemical compounds have also been found in bamboo vinegar [1] and its chemical composition can be divided into 3 main fractions: (1) the acidic fraction, (2) the neutral fraction and (3) the phenolic fraction [20]. Most of the organic components belong to the acidic and phenolic fractions. Wenbiao *et al.* [21] analysed bamboo vinegar using gas chromatography-mass spectrometry (GC-MS) and concluded that it is an unstable mixture changing over to time and contains 80% water plus other materials such as organic acids, phenols, ketones, alcohols and aldehydes. The disinfection and sterilization properties ascribed to bamboo vinegar are thought to derive from the presence of ingredients such as benzoquinone and acetic acid [1].

The present research was conducted to assess the anti-microbial properties of bamboo vinegar against fungi and bacteria that have been isolated from soil, wood and bamboo materials. It has been suggested that bamboo vinegar may have value as a wood and bamboo preservative and this study provides results from a laboratory assay for its activity against micro-organisms typically found on such materials.

MATERIALS AND METHODS

LB medium (LB) for the bacterial growth assay was prepared using 0.5% sodium chloride, 0.5% yeast extract, 1% tryptone and 1.5% agar. The malt extract medium (MA) for fungal growth assay was prepared with 2% malt extract and 1.5% agar. All media were sterilised by autoclaving at 120°C for 30 min at 15 psi (0.10 N/mm^2)

Table 1.
Effect of bamboo vinegar on bacterial growth after 2 days

Bacteria	Bamboo vinegar concentration (%)	Clearing zone diameter (mm from edge of disc)	Growth on disk
Ralstonia sp.	100	6.39	No
	50	3.78	No
	10	No	Yes
Pseudomonas sp.	100	3.78	No
	50	1.78	No
	10	No	Yes
Alcaligenes sp.	100	3.39	No
	50	0.83	No
	10	No	Yes

Control discs: in all cases no clearing zone was observed and growth occurred on the discs.

and dispensed into 9-cm-diameter vented Petri dishes to solidify. LB medium was used to grow bacteria, while MA was used to grow fungi.

Bamboo vinegar was obtained as a by-product of pyrolysis from *Gigantochloa scortechinii* culms obtained from Kedah, Malaysia. The bamboo vinegar was about 3 years old and had been stored in a plastic container (it is usual practice for bamboo vinegar to be stored for periods up to several years prior to sale). For the experiments, the bamboo vinegar was used at concentrations of 100%, 50% and 10% diluted with sterile distilled water. Aliquots of 50 μl of the different bamboo vinegar concentrations were pipetted onto 15-mm cellulose bio-assay discs (pre-sterilised by γ-irradiation) in a sterile chamber. Bamboo vinegar was used in its original form without sterilization.

Fresh 7–10-days-old cultures of fungi and bacteria (see Tables 1 and 2) grown on MA and LB medium were mixed with 15 ml sodium dioctyl sulfosuccinate. The

Table 2.
Effect of bamboo vinegar on fungal growth after 7 days

Fungus	Type	Bamboo vinegar conc.%	Clearing zone diameter (mm from edge of disc)	Growth on disk*
Gloeophyllum trabeum	Brown rot fungus	100	No	Some
		50	No	Some
		10	No	Yes
Coriolus versicolor	White rot fungus	100	No	Yes
		50	No	Yes
		10	No	Yes
Chaetomium globosom	Soft rot fungus	100	No	No
		50	No	No
		10	No	Yes
Aureobasidium pullulans	Stain fungus	100	2.6 (0.9)	No
		50	0.44 (0.17)	No
		10	No	Yes
Penicillium digitatum	Mould fungus	100	No	Slight
		50	No	Yes
		10	No	Yes
Aspergillus niger	Mould fungus	100	No	Some
		50	No	Yes
		10	No	Yes
Trichoderma viride	Mould fungus	100	No	Slight
		50	No	Slight
		10	No	Yes

Control discs: in all cases no clearing zone was observed and growth occurred on the discs. Values in parentheses denote standard deviation.

surface of the cultures was scraped gently with a glass rod to form a spore/cell suspension that was transferred to a sterilised sprayer. The spore/cell suspension was sprayed directly onto the surface of Petri dishes containing 3 replicate bio-assay discs of a given concentration of bamboo vinegar under aseptic conditions ensuring an even coverage of the medium and bio-assay discs. An exception to the use of spore/cell suspension inoculation was made for *Coriolus versicolor* FPRL 28A due to its particularly leathery mycelium. For this fungus, treated bio-assay discs were placed close to the margin of actively growing colonies. The inoculated plates were incubated at 25°C (bacteria) or 22°C (fungi). The diameter of the 'clearing zone' was measured and recorded at two opposite points around the bio-assay disc after 2 or 7 days of incubation. The clearing zone refers to the distance on the agar surface from the edge of the bio-assay disc that was free of microbial growth. Growth of the micro-organisms on the bio-assay discs themselves was also recorded. Three replicate Petri dishes, each containing 3 bio-assay discs of a single concentration of bamboo vinegar were used for each micro-organism.

RESULTS AND DISCUSSION

In this study, the bio-assay disc method was employed throughout the investigation because it is well established approach to evaluate the effects of antibiotic compounds and micro-biocides. It is possible that bamboo vinegar could also have been evaluated using other methods, such as direct incorporation into the agar media although these sometimes experience difficulties with incorporation, mixing, sterilization temperature and precipitation. Table 1 shows the results for growth of bacteria with bamboo vinegar bio-assay discs and Table 2 the equivalent data for the fungi. The clearing zone represents an area on the agar surface adjacent to the edge of the assay disc where inhibition of microbial growth can be recorded due to diffusion of inhibitory compounds from the disc. Potent, mobile inhibitors will exhibit large clearing zones whilst compounds that are either non-inhibitory or have very low aqueous solubility will exhibit small or no clearing zone. In this case, bamboo vinegar is an aqueous composition and it was expected that its components would diffuse to an extent in the agar medium. Interpretation of the relatively small clearing zones observed, even at high concentrations of bamboo vinegar, suggest that it either does not diffuse very rapidly over the 7-day assay period, or its dilution by diffusion into the medium renders it ineffective at inhibiting growth.

The bamboo vinegar showed a more general inhibitory effect against the 3 species of bacteria examined than against the seven species of fungi. In all cases, bacteria were inhibited from growing in the vicinity of and on the discs at 100% and 50% bamboo vinegar concentrations. These concentrations represent approximately 4 and 2% concentrations of acetic acid and 4 and 2% of other organic components, respectively, based upon literature values [1]. A 10% bamboo vinegar concentration was not inhibitory (i.e., approximately 0.4% acetic acid and 0.4% other organics). The degree of growth inhibition, as detected by the extent of the clearing zone,

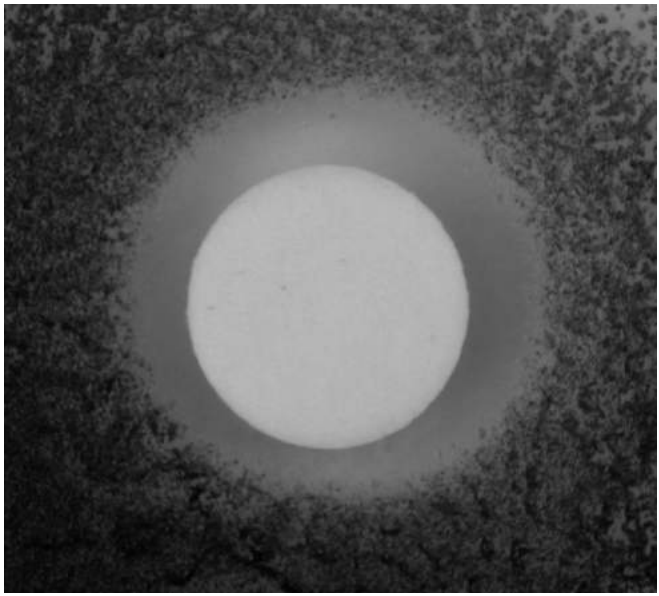

Figure 1. Growth of *A. pullulans* after 7 days on antibiotic assay discs treated with 100% bamboo vinegar and exposed on agar. A distinct clearing zone is visible, indicating inhibition of fungal growth within the zone and on the antibiotic assay disk.

showed a dose-dependant response. *Ralstonia* sp. was the most inhibited of the bacterial species examined.

Only *Aureobasidium pullulans* of all the fungi examined showed a clearing zone around the discs at bamboo vinegar concentrations of 100% and 50% (Table 2, Fig. 1). No clearing zone was noted at a 10% bamboo vinegar concentration with *A. pullulans*. The growth inhibition showed a dose-dependent response with the higher concentrations having progressively more extensive clearing zones. The two higher concentrations of bamboo vinegar also prevented growth by *A. pullulans* and *Chaetomium globosum* directly on the discs (Figs 1 and 2). Some inhibition of growth on the discs was also observed at the higher bamboo vinegar concentrations with *Gloeophyllum trabeum*, *Penicillinum digitatum*, *Aspergillus niger* and *Trichoderma viride* (Figs 3–6). However, with *C. versicolor* no inhibition of growth on the disc or clearing zones was recorded at any concentration.

In general, it is apparent from these results that bamboo vinegar has some inhibitory effects on the growth of several wood and bamboo colonising bacteria and fungi. However, it is also clear that the growth of several important wood/bamboo degrading fungi, and particularly the white rot fungus *C. versicolor*, is inhibited only mildly, if at all. High concentrations of the bamboo vinegar (e.g., 50% or 100%) are also necessary for expression of the inhibitory effects on microbial growth. This is perhaps not surprising as the bamboo vinegar itself is approximately 80 to 90% water and so any active ingredients are present at relatively low concentration even in the ‘pure’, 100% bamboo vinegar. The effective concentrations at reducing

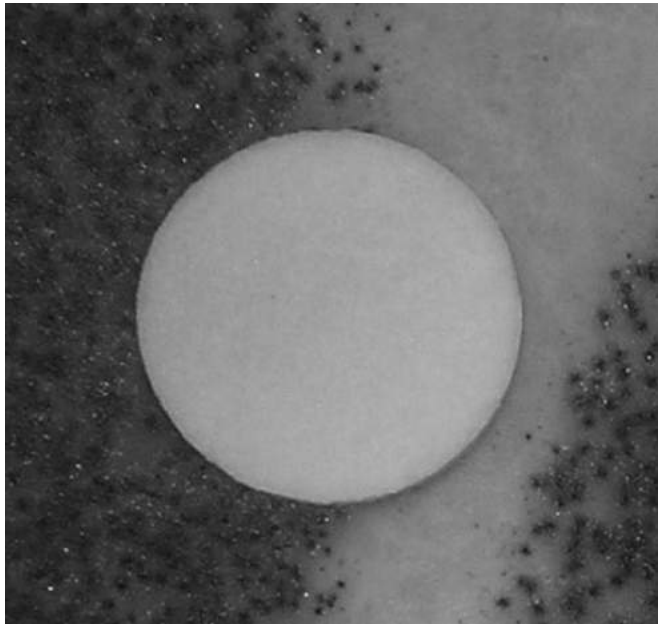

Figure 2. Growth of *C. globosum* after 7 days on antibiotic assay discs treated with 100% bamboo vinegar and exposed on agar. No clearing zone, the antibiotic assay disk is free from fungal growth, indicating inhibition by the bamboo vinegar.

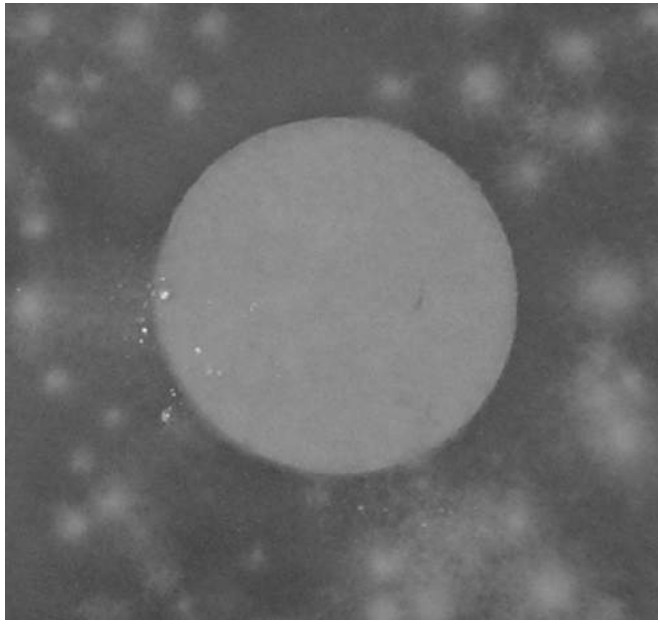

Figure 3. Some reduction of *G. trabeum* growth on the antibiotic assay discs is shown as compared with controls, no clearing zones.

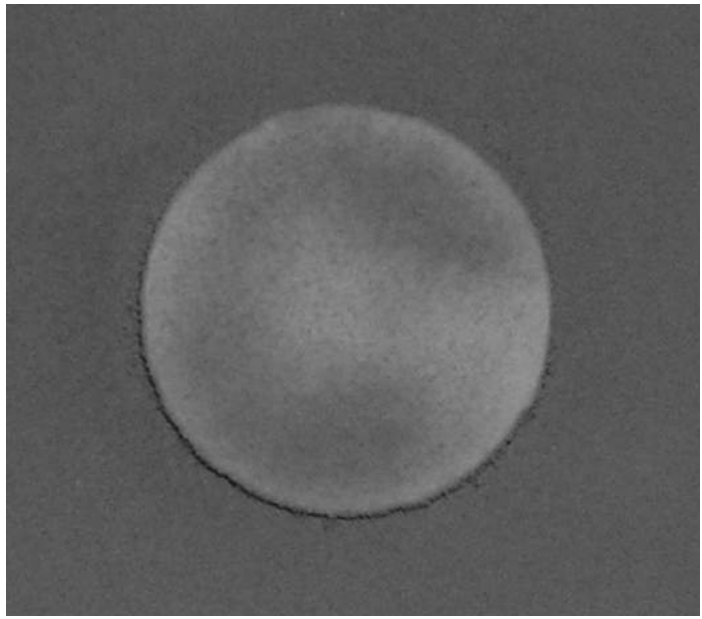

Figure 4. Some reduction of *P. digitatum* growth on the antibiotic assay discs is shown as compared with controls, no clearing zones.

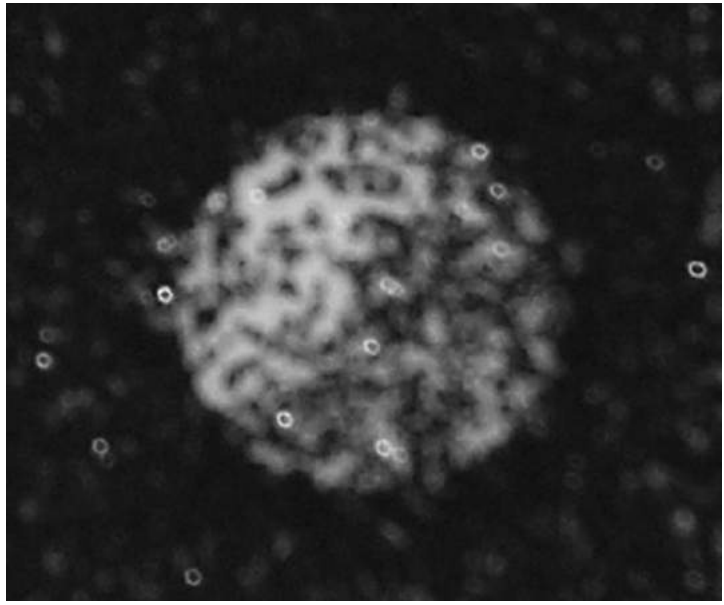

Figure 5. Some reduction of *A. niger* growth on the antibiotic assay discs is shown as compared with controls, no clearing zones.

microbial growth, 100% and 50% bamboo vinegar probably contain respectively about 4% or 2% acetic acid and 4% or 2% other organics (including, e.g., phenols, ketones, alcohols, aldehydes, benzoquinone [1]).

It appears that the components of bamboo vinegar that are inhibitory to bacterial growth are capable of diffusing some distance away from the assay disc (4–6 mm) to give clearing zones, as well as inhibiting growth on the disc itself. This contrasted with the situation with the fungi where a clearing zone was recorded only with *A. pullulans*. These results further support the view that although bamboo vinegar contains aqueous diffusible compounds having some anti-microbial properties, these are not present at high concentrations, as they are not effective at any distance from the discs with six of the seven fungal species examined. It is possible that inhibition of the bacteria by any such compound(s) was enhanced, for example, by the presence of acetic or other organic acids that may lower pH locally and give less favourable general conditions for bacterial growth.

Additionally, it is possible that certain compounds in bamboo vinegar could be substrates for fungal metabolism and thus the activity of any anti-fungal compounds present may be 'masked' by such growth promoting compounds. However, on the assay discs, where concentrations of bamboo vinegar were at their highest, it is clear that the growth of most of the micro-organisms studied was inhibited at least to some

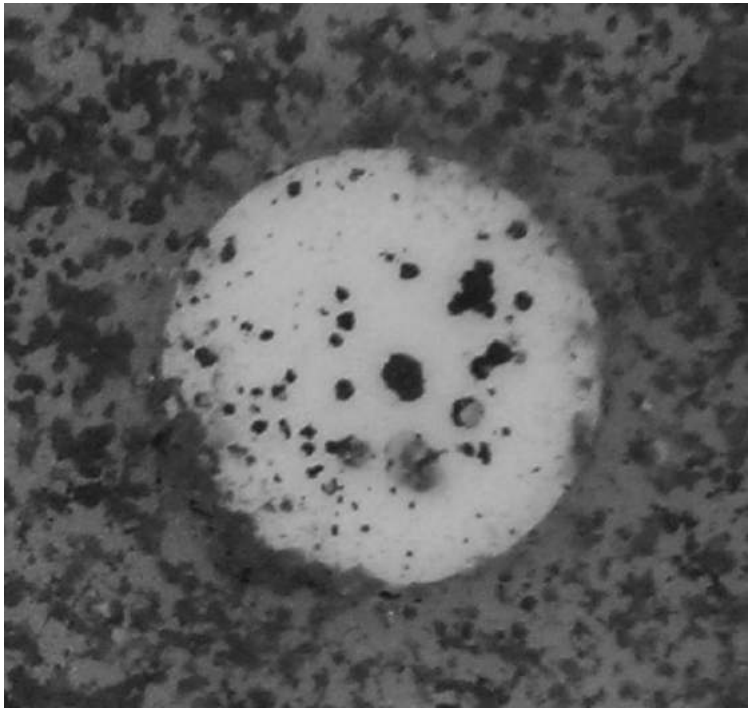

Figure 6. Some reduction of *T. viride* growth on the antibiotic assay discs is shown as compared with controls, no clearing zones.

extent and in some cases completely. The results suggest that dilutions of bamboo vinegar of about 50%, which equates to a content of acetic acid and of other organics of approx. 2% each, can deliver growth inhibitory effects. Furthermore, bamboo vinegar was effective at preventing growth on the discs of the important blue-stain in service (in wood products) fungus *A. pullulans* and of the cellulolytic and soft rot fungus *C. globosum*. A partial inhibition of growth on the discs was also shown with the mould fungi. Stain and mould fungi have negative effects on the appearance and value of bamboo and wood products in high relative humidity environments (such as the tropics where bamboo products are typically manufactured) and there is some scope for further investigation of the potential of bamboo vinegar to prevent such fungal staining and mould growth. The association of the somewhat limited anti-microbial activity observed with whole bamboo vinegar with its specific individual components and with more general aspects of the whole material such as pH, is the subject of ongoing investigation at our laboratories.

CONCLUSIONS

Based on the methods used in this study, concentrations of 100% and 50% bamboo vinegar were found to inhibit the growth of three species of bacteria and, with the exception of *C. versicolor*, showed some inhibition of fungal growth on the assay discs. The growth of *A. pullulans* was also inhibited by diffusion of bamboo vinegar components into the agar medium adjacent to the assay disc. Overall, bamboo vinegar did not exhibit strong anti-microbial effects in these experiments, especially when diluted. Some inhibitory effects on the growth of several of the micro-organisms investigated was observed, however, at the highest concentrations examined.

Acknowledgements

This work was conducted whilst O. S. and R. H. were on sabbatical at Imperial College London and The University Sains Malaysia is thanked for approving their sabbatical study periods. Imperial College London and University Sains Malaysia are gratefully acknowledged for co-sponsoring O. S. R. H. received a Commonwealth Fellowship during the period this work was undertaken and the Association of Commonwealth Universities is gratefully acknowledged for this support. We thank Mr. Zikri, who supplied the bamboo vinegar, David Bacon for the photographic images, and Derek Wallace and Michael Ray for valuable advice and microbial cultures.

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