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Antifungal activity of alcoholic extracts of *Batis maritima* against animal pathogens

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Abstract

The increase in resistance of pathogenic fungi towards normal antifungals has led to a decrease in their effectiveness in the treatment of fungal diseases. Therefore, the need for newer forms of treatment is urgent, leading to studies into traditional medicines, specifically those of herbal natures. *Batis maritima* is one such plant, which has historically been used in herbal remedies for the treatment of a wide range of diseases, many of which are results of fungal pathogens. The focus of this study was therefore to determine the effectiveness of alcoholic extract of leaves of *B. maritima* as an antifungal agent against two animal pathogens.

Crude alcoholic extracts of leaves of *B. maritima* were prepared in concentrations of 50%, 10% 5% and 1%. These were then subjected to antifungal assays against *Aspergillus flavus* and *Malassezia sp*., using the Well Diffusion method.

The results of the tests showed Zones of Inhibition of 55.9 ± 3.40 mm, 52.7 ± 1.72 mm and 47.0 ± 3.68 mm, respectively, for the 10% 5% and 1% extracts against *A. flavus*; no inhibition was seen with the 50% extract concentration. With respect to *Malassezia sp*., inhibition of 25.3 ± 5.44 mm was observed at 50% extract concentration, with no inhibition at lower concentrations.

It may therefore be seen from these findings that alcoholic leaf extracts of *B. maritima* prove to be effective against *A. flavus* while lesser effective against *Malassezia sp*.

Keywords: *Batis Maritima*; Antifungal; *Aspergillus Flavus; Malassezia Sp.*

1. Introduction

Over recent years it has been recognized that there is an increase in the resistance of microorganisms towards normal antibiotics [1]. This was noted to be as a result of poor administrative practices with respect to these antibiotics [2]. Nevertheless, this has led to an escalating concern as to the effectiveness of these traditional antibiotics [1], as it is seen that there is a reduction in their successful use as treatment against pathogenic diseases [2]. Thus, more work is now being invested in seeking antimicrobial treatment from newer sources, such as plant extracts and their metabolites [1]. Mangrove and the associated plants within such forests usually contain biologically active antibacterial, antifungal and antiviral compounds [2]. Therefore, it is logical to suggest that *Batis maritima*, being one of these mangrove associates may contain some amount of antimicrobial activity.

Although the literature suggests that not much experimental work has been carried out on the *B. maritima* shrub, what little has been done, along with the hardy nature and traditional uses of the plant and its extracts, indicate that not only does the plant possess antimicrobial properties, but may do so over a wide range of pathogens.

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Aqueous and alcoholic extracts of *B. maritima* were shown to be significantly effective against both animal and human pathogenic bacteria. Antibacterial assays of these extracts were carried out against *Pseudomonas aeruginosa* and *Streptococcus mutans* (human pathogens) as well as *Vibrio harveyi* and *Vibrio parahaemolyticus* (animal pathogens). Of these, the alcoholic extract was shown to be effective against both sets of pathogens [2]. Zones of Inhibition of the alcoholic extracts may be seen in Table 1.

Table 1 Zones of Inhibition of Alcoholic Extracts of *B. maritima* Against Four Bacteria **[2]**

Bacteria		**Zones of Inhibition**	
		B. maritima	**Positive control**
Animal Pathogens	*V. parahaemolyticus*	12 ± 2	23 ± 2
	V. harveyi	10 ± 2	21 ± 2
Human Pathogens	*P. aeruginosa*	8.33 ± 1.52	29 ± 2
	S. mutans	6.66 ± 2.51	8 ± 2

It may be observed that the alcoholic extract was proven to be most effective against *S. mutans*, as this was closest to its corresponding positive control [2].

In addition, there are multiple references of *B. maritima* being used in traditional medicines and food additives, which suggest antimicrobial activity in the plant extracts [3] [4]. It has been noted that extracts of the plant have been used in the Yucatan peninsula of Mexico to treat against cutaneous infections [3]. This further reinforces the results obtained from the alcoholic extracts of the plant in the antibacterial screening noted previously. This is due to the fact that one of the main causes of cutaneous infections is the *P. aeruginosa* [5], which *B. maritima* has proven effective against [2].

Further, *B. maritima* is also noted to have been used in herbal medicine in Puerto Rico to treat against eczema [3]. As one of the major causes of eczema is the bacterium *Staphylococcus aureus* [6], it may therefore be inferred that there is a high possibility of the plant being effective against this bacterium, further reinforcing its antibacterial capabilities. Further, eczema has also been noted to be treated by antifungals and antivirals [6]. It may therefore be inferred that *B. maritima* may display antifungal and/or antiviral capabilities.

From the literature, it should be noted that, although the potential for *B. maritima* to display antifungal and antiviral properties exists, no specific research that covers this has been documented. Additionally, it may be possible that the plant displays much more antibacterial potential that have been examined; as only four bacteria have been specifically tested against it [2]. Upon consideration of the number of medicinal uses noted from various sources, a test of only four bacteria seems insignificant compared to the vast scope of information that may be gathered if the extracts from *B. maritima* were to be subjected to a broader array of screening from different types and classes of pathogens: fungal, bacterial and viral. In the current study an attempt was made to determine if alcoholic leaf extracts of *B. maritima* displayed antifungal activity against two animal pathogenic fungi: *Aspergillus. flavus* and *Malassezia sp*.

2. Methodology

2.1. Plant Material and Test Fungi

For this study, the leaves of the *B. maritima* were used.

Additionally, for the test fungi, *A. flavus* and *Malassezia sp*. were obtained from sources within the university campus. These were then cultured in Potato Dextrose Agar (PDA).

2.2. Collection of Samples

Samples for the study were obtained along the Corentyne Coast, at various locations. These were then taken to the University of Guyana Johns Science Centre, where the analyses were carried out.

2.3. Sample Preparation and Extraction

For the preparation and extraction of the samples, the guidelines outlined by Sivanandham [7] were followed, with appropriate substitutions.

Leaves of the *B. maritima* shrub were dried in an oven, between 40 to 50 ^{o}C for 5 days. These were then mill ground to a fine powder. The ground leaves were then added to 90 % ethanol and left for 1 week, for cold extraction. The residue was then re-extracted twice, after which the filtrates were concentrated using a rotavapor. Through serial dilution, the extract was then made up to 50%, 10%, 5% and 1% concentrations, using acetone as the solvent.

2.4. Antifungal Assay

For the antifungal assay, the *A. flavus* and *Malassezia sp*. that were previously obtained, were isolated and pure cultured on PDA slants. These were then inoculated onto petri plates of PDA. The various concentrations of extracts prepared were then subjected to triplicate antifungal tests, using the Well Diffusion Method [8]

3. Results

Table 2 Zones of Inhibition of Extracts

Test Fungus	Zones of Inhibition for each Concentration (mm)				
	50 %	10 %	5 %	1 %	Control
A. flavus	0.0 ± 0.00	55.9 ± 3.40	52.7 ± 1.72	47.0 ± 3.68	0.0 ± 0.00
Malassezia sp.	25.3 ± 5.44	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

As may be observed from Table 2, the crude extract of *B. maritima* proved effective against *A. flavus* and somewhat effective against *Malassezia sp*. The extract was only effective against the *Malassezia sp.* at 50%, with a zone of inhibition (ZOI) of 25.3 ± 5.44 mm. However, with 10% concentration of crude extract and below, *Malassezia sp.* was unaffected, while at this concentration, *A. flavus* was seen to be affected (contrasting what was seen at 50%), with a zone of inhibition of 55.9 ± 3.40 mm. At 5%, the extract displayed an effectiveness against *A. flavus*, with an inhibition zone of 52.7 ± 1.72 mm, while at 1%, similar activities were seen, albeit to a lesser degree. At this concentration, the test against *A. flavus* had an inhibition zone of 47.0 ± 3.68 mm. For both fungi the control yielded a ZOI of 0.0 ± 0.00.

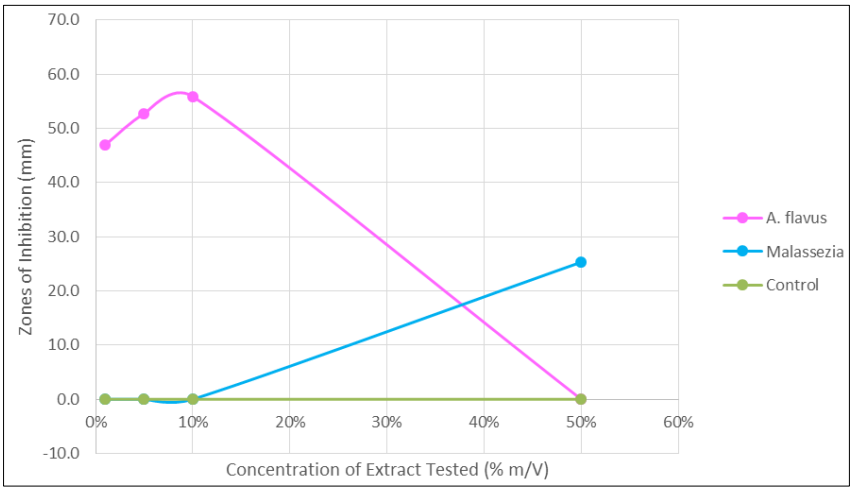

Figure 1 Zones of Inhibition VS. Concentration of Crude Extract

In assessing the antifungal activity of the *B. maritima* crude extract with respect to the extract concentrations, it may be seen that, while there seems to be a positive correlation between the activities and concentrations of the extract against the *Malassezia sp*., there appears to be a 'maximum effective concentration' against *A. flavus*. From Figure 1, this point appears to be around 10 % concentration of crude extract.

4. Discussion

Batis maritima is a halophytic shrub found along the coastal areas of various tropical and sub-tropical regions. It has been recorded to have been used in a variety of ways, but the most prominent being as a herbal remedy against medical conditions, particularly by the natives of Puerto Rico and Mexico [3]. Many of the medical conditions that were treated with this herbal remedy were fungal in nature [9]. This is further mirrored by additional literature in which it was shown that halophytes have been widely used, traditionally, for medicinal purposes. Living in harsh environments has led to an adaptation of halophytes in the development of several metabolites, many of which are bioactive compounds. These phytochemicals have been known, historically, to possess antioxidant, anti-inflammatory and antimicrobial properties which counteract certain medical conditions [10]. Due to this, it was surmised that the plant extracts of *B. maritima*, a halophyte itself, possess active phytochemicals with antimicrobial, specifically antifungal, properties.

In this study, an alcoholic extract of the leaves of *B. maritima* was produced and subjected to the antifungal analyses. Ethanol of 90% (v/v) concentration was used in the cold extraction process to draw the active metabolites from the macerated leaf tissues into solution.

The antifungal assay was carried out on the crude extract, made up to solutions of concentrations 50%, 10%, 5% and 1% (m/V), using acetone as the solvent. Acetone was used as a replacement for the ethanol as the solvent, since ethanol would interfere with the antifungal assay. This is due to the fact that ethanol itself displays high antifungal activity and fungal spore inhibition, even in its vapor form [11]. This interference would have been even more severe considering the high concentration of ethanol utilized (90%).

As may be noted from the results, two animal pathogens (*A. flavus* and *Malassezia sp*.) were used in the analyses of the antifungal properties of the extract. The *Malassezia sp.* is noted to be the fungus associated with rash and other skin related disorders, [12] similar to the conditions which were historically treated with concoctions of *B. maritima* [3]. It was therefore predicted that, since ailments related to *Malassezia sp.* were successfully treated with the plant extracts, *B. maritima* would be shown to display some antifungal effects against the fungus. *A. flavus*, similarly, is another human (and animal pathogen) which has been proven to be extremely hazardous. It has been noted as one of the most dangerous species in the of the *Aspergillus* genus, known to cause pulmonary and systemic infections. It has also shown to cause lung and liver failure in lab mice [13]. It was therefore surmised that if the *B. maritima* had been used in traditional medicine to treat fungal causing infections, then it may prove effective against other animal/human pathogens.

From the results displayed in Table 1 and Figure 1, it could be seen that the crude extracts of *B. maritima* showed some amount of antifungal activity against both of the fungi tested.

With respect to *A. flavus*, positive results were noted with most of the concentrations of crude extracts. The Zones of Inhibition for the 10%, 5% and 1% crude extracts were found to be 55.9 ± 3.54 mm, 52.7 ± 1.72 mm and 47.0 ± 3.68 mm, respectively. For the most part, a typical concentration-effect relationship was noted where the inhibition zone increased as concentration increased. This may be explained by the increased frequency of contact of the molecules and the fungal spores as the distribution of the molecules became denser. This is analogous to the collision theory of reactions where, as concentration is increased, the rate and number of effective interactions occurring would also be increased [14]; with this case being the interaction of the crude extract molecules and the fungal spores. However, it is interesting to note that, the 50% concentration crude extract displayed no apparent antifungal activity against the *A. flavus*, contrary to the initial trend of the graph, as seen in Figure 1. This phenomenon may be due to an antagonistic effect between certain compounds within the extract. Antagonism is the masking or negating of effects of an active compound by another compound [15]. However, as may be seen from the results, there was a general increase in the antifungal activity of the crude extract with respect to *A. flavus*, up to the 10% concentration. The most likely inference is that the masking compound was in too low of a concentration to affect the active compound. This therefore meant that the critical concentration for the masking effect lay somewhere between 10% and 50% crude extract concentration.

Next, with respect to the *Malassezia sp*., this genus of fungi is responsible for various forms of dermatitis and associated rashes [12]. This was the main reason behind this inclusion in the antifungal assay, as it is one of the identified causes of the ailment that *B. maritima* was reported to have been used to treat. While no apparent zones of inhibition were noted with the lower concentrations of crude extracts against this fungus, a zone of inhibition of 25.3 ± 5.44 mm was seen with the 50% crude extract solution. This would suggest that the minimum inhibition of *Malassezia sp.* by this solution occurs between concentration levels of 10% to 50%. This appears to be too high for *B. maritima* extract to be considered an effective antifungal against *Malassezia sp*.

Nonetheless, this somewhat positive activity against the *Malassezia sp.* may explain why *B. maritima* has been documented to have been used in herbal medicine to treat certain skin disorders and rashes [16], as this fungus is responsible for a wide range of rashes [12]. If the herb were to be ground, made into a paste and applied to the rash, as was usually the method of application of various traditional herbal treatments, employed by different cultures throughout the ages, against dermatologic conditions [17], then this may have resulted in a high enough concentration that would have yielded results analogous to the 50% concentration of the extract in this study, which would, therefore, have led to an appropriate concentration for an effective herbal treatment.

5. Conclusion

From the study conducted, it may be seen that the crude alcoholic extract of leaves of *B. maritima* proved somewhat effective against *Malasezzia sp*., and effective against *A. flavus*. This is a viable explanation of the traditional use of *B. maritima* to treat certain disorders which are caused by fungal pathogens.

Compliance with ethical standards

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Disclosure of conflict of interest

We hereby disclose there to be no conflict of interest between authors in this work.

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