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(RESEARCH ARTICLE)

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A phytochemical analysis, isolation and anti-fungal investigation of the *Batis maritima* shrub (Crabgrass)

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Abstract

Batis maritima (or Saltwort, Turtle weed and Crabgrass) is halophyte that grows along the coast of many tropical and subtropical regions. It is widely considered a weed as it not widely used. The few documented cases of its uses, however, strongly suggest that it possesses antifungal properties. Crude alcoholic extracts *B. maritima* of varying concentrations were tested against five fungal species: *Aspergillus niger*, *Aspergillus flavus*, *Malassezia sp*., *Pyricularia oryzae*, and *Rhizopus sp*. The Well-diffusion method was used to determine the Zones of Inhibition with respect to the fungi tested. The crude extracts proved especially effective against *P. oryzae*, with an inhibition zone of 26.3 ± 0.95 mm with the 1% solution and maximum inhibition with higher concentrations tested. Conversely, no inhibition zone was observed against *Rhizopus sp*.

The crude alcoholic extract was also subjected to Column Chromatography and six compounds were isolated. Solutions of four of these were tested against the mentioned fungi. Antifungal activity was confirmed in all four of the isolates. A phytochemical screening was also done to identify the active secondary metabolites present in the crude extract. Those confirmed were glycosides, alkaloids, flavonoids, saponins, sterols, tannins and terpenoids.

The aim of this research was therefore to determine the antifungal properties of alcoholic extracts of the *B. maritima* as well as to perform a phytochemical screening on the crude extracts.

Keywords: *Batis maritima*; Saltwort; Turtle weed; Crabgrass; Antifungal activities; Phytochemical screening

1. Introduction

Along the coastal region of Guyana, there exist many species and varieties of plant life, most of which are extremely helpful, both directly and indirectly. One of the most famous of these is the mangrove, which serve as the main form of sea defense in the region. This, along with other flora, protects the coast by breaking the impact of sea water and reducing wave energy, thus preventing flooding.

Moreover, the flora in the region also traps sediments which help to stabilize the shoreline, thus preventing the erosion of soil, along with the loss of soil nutrients. Additionally, these forests also assist in the sequestration of carbon from the atmosphere, by acting as a carbon sink, converting carbon dioxide in the air into a more usable and helpful form, while also reducing the CO_2 in the atmosphere [1].

While most of the plants along the coast are truly useful, especially the mangrove, the focus of this research lies within the study of one of the most abundant of these shrubs which co-exists with the mangrove and may be seen as just as or

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even more helpful. The *Batis maritima* is a shrub which grows abundantly along the Coastal Zone, often among the mangroves. It is commonly referred to as Saltwort, Turtleweed or Crabgrass, and most often grows further inland than the mangroves themselves [2].

B. maritima belongs to the *Bataceae* family, within the order, Batales [3]. This plant also has a sister species, the *B. argillicola*. These two are the only recognized species within *Batis* which is also the only genus within the *Bataceae* family [4].

More plentiful in the more rural locations along the coast, *B. maritima* extends miles inwards, far beyond the mangrove spread. However, despite its abundance, there are currently very few records which relate to the economic potential of this shrub. Regardless, these plants are extremely hardy, resistant to harsh weather [5] and possibly microbial attack. As such, it may be likely that these plants contain phytochemicals which may prove effective against certain microbes.

In the review which follows, the antimicrobial properties of *B. maritima* will be discussed, placing emphasis on both antibacterial and antifungal characteristics. After this, the phytochemical properties will be discussed separately for the different parts of the plant, which will then be linked to the antimicrobial properties which would have been discussed. The results of the actual experimental stage will be discussed and compared to the literature.

B. maritima is halophyte [6], which is a type of plant that grows well under extremely saline conditions [7]. As such, the leaves are capable of storing large quantities of water, making it highly succulent [2]. It also has wind pollinated inflorescences [2], containing pollen receptors that also function to help in seed dispersal via tidal waters [8]. The plant is low lying, about 1 m in height and about 5 cm in diameter at the stem. It is a perennial and dioecious plant which grows in salt marshes and mangrove swamps [2], on the margins of salt pans and wind-tidal flats [5] and, generally, in areas of high wrack [9].

While *B. maritima* grows mainly in mangrove swamps, it is often seen to grow further inland than the mangrove, sometimes reaching approximately 1 to 4 km further inland than the mangrove spread [10]. It is found along tropical and subtropical coastlines, most notably in Guyana, Venezuela, Suriname, Brazil and Columbia in South America, Texas and Florida in the Unites States as well as in most Latin America countries and the Caribbean islands. It is also noted by multiple sources to be an invasive species in the Hawaiian Islands [5] [8].

In Guyana, *B. maritima* is often considered a weed by many communities. This may stem from the fact that not much is known locally about the plant itself and its value, medicinal or otherwise. An indirect aim of this research was therefore to enlighten on the value of *B. maritima*, specifically on its role as an antimicrobial treatment. If more is known about the plant, then it is quite possible that its properties may prove it commercially useful.

As a result of this study, a brief insight as to the potential of the shrub may be achieved. As will be seen from the literature, *B. maritima* possesses some antimicrobial activity. From the study of these characteristics, the fact that the plant has value which may be applied to different sectors of study such as agriculture and medicine, will be highlighted.

Finally, conclusions about the antimicrobial properties of the *B. maritima* will be drawn based on the evidences and discussions made.

2. Material and methods

This study was carried out on samples acquired from three areas along the Lower Corentyne coastline, namely, Rose Hall Town Reef Section, Johns Village and Wellington Park Village. These samples were then transported to the main research site, the University of Guyana Johns Annex. All analyses were done at this location, except the solvent removal from the crude extract by the rotary evaporator, which was done at the University of Guyana Turkeyen Campus.

2.1. Plant and test materials

The present study utilized the plant *B. maritima*. The test materials were the plant fungi *Aspergillus niger*, *Aspergillus flavus*, *Pyricularia oryzae* and *Rhizopus* and the animal pathogenic fungus *Malassezia*.

The overall experiment was carried out following a Completely Randomised Design [11].

2.2. Collection of Samples

B. maritima was collected from three locations. The plant was identified in the Division of Biology at the Johns Science Centre of the University of Guyana Berbice Campus. The specimen collected was used for the purpose of phytochemical screening.

The sampling of the *B. maritima* was done following a Stratified Random method, from the strata of the aforementioned, Rose Hall Reef Section, Johns Village and Wellington Park Village respectively. In each stratum, 10 samples were randomly selected using a 1 m^2 quadrat. The sampling in each stratum was done over a period of 4 weeks.

2.3. Sample Preparation and Extraction

Sample preparation and extraction was carried out following the guidelines stipulated by the World Journal of Science and Research in a review of phytochemical techniques [12], with some modifications. The extraction was carried out using a combination of the Cold Extraction [13] and Rotary Evaporator Extraction Techniques [14].

After collection, the leaves of the samples were stripped and left to dry in an oven, set between 40 to 50 ℃, for 5 days, after which they were ground using a mill. This ground sample was then steeped in 90% alcohol for 1 week. After this, the extract was filtered and the filtrate collected. The residue was then re-steeped, which was done twice with fresh solvent. After repeated steeping and filtering, the filtrates were collected and the solvent was removed via a rotary evaporator.

2.4. Phytochemical Screening

Samples of the crude extract were then subjected to chemical tests for alkaloids, terpenoids, anthocyanins, saponins, tannins, sterols, glycosides, flavonoids, amino acids and reducing sugars. These tests were done following the standard techniques outlined in the International Journal of Advanced Research in Chemical Science (General Techniques Involved in Phytochemical Analysis) [15], along with other sources [16] [17] [18].

2.5. Separation of Components

The separation of the components of the crude extract was done via Thin Layer Chromatography [19] to determine an appropriate solvent mixture, followed by Column Chromatography [20] via a normal phase gradient elution [21]. The gradient elution was achieved using the solvents hexane, ethyl acetate, acetic acid and methanol. The various fractions were collected separately then pooled together based on similarity of retention factor detected in additional TLC carried out, parallel to the column chromatography.

2.6. Antifungal Assay

The antifungal assay was carried out using the Well Diffusion Method [22]. For the preparation of the assay, each of the fungi listed were collected, identified and isolated. Pure cultures of each fungus were then grown and prepared on Potato Dextrose Agar (PDA). The antifungal activities of four different concentrations of the crude extract (1%, 5%, 10% and 50%) were tested against each of the fungi. This test was also done for 1% concentration of each of the separated components obtained from the Chromatography Column. All antifungal tests were done in triplicates.

2.7. Data Analysis

Data obtained was recorded, interpreted and discussed. The results from the phytochemical screening were tabulated and compared to literature. The zones of inhibition from the antifungal assays of crude extracts were recorded and interpreted via descriptive statistics and graphed. The zones of inhibition of the active fractions against each fungus were compared. The zones of inhibition of the crude extract against the animal pathogens and plant pathogens were compared. All comparisons of zones of inhibition were done using t-tests and Analyses of Variance with a confidence level of 95%. All statistics were carried out using SPSS version 23.

3. Results and discussion

3.1. Phytochemical Screening

Based on the phytochemical assay, the crude alcoholic extract of *B. maritima* was found to contain alkaloids, flavonoids, glycosides, saponins, sterols, tannins and terpenoids (Table 1). It was also noted that no traces of amino acids, anthocyanins and reducing sugars were detected.

Table 1 Presence of phytochemicals in crude extract of *B. maritima*

Phytochemicals	Present/Not present
Alkaloids	✓
Amino acids	-
Anthocyanins	-
Flavonoids	✓
Glycosides	✓
Reducing sugars	-
Saponins	✓
Sterols	✓
Tannins	✓
Terpenoids	✓

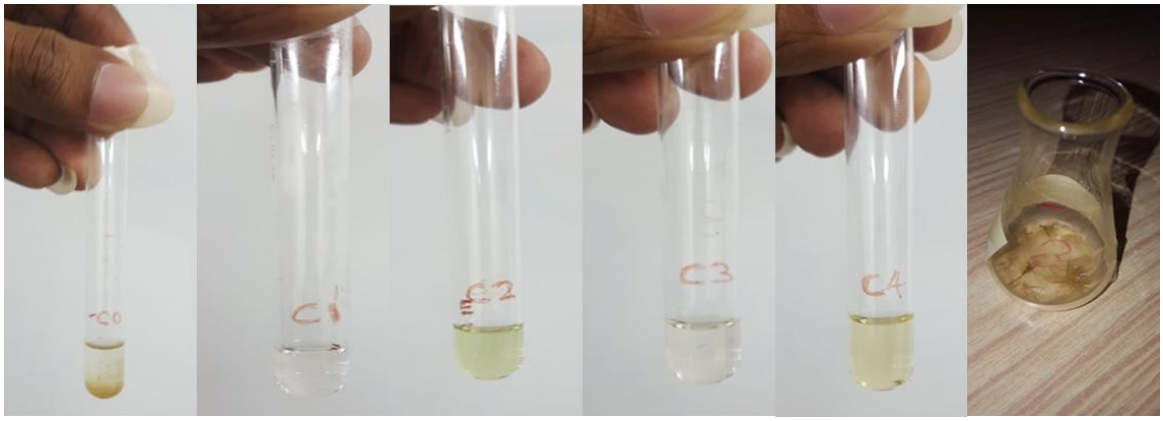

Figure 1 Isolated components (C_0 to C_5) from column chromatography

The extraction and separation of the crude alcoholic extract from *B. maritima* leaves yielded six components which were designated C_0 to C_5. After the removal of the solvent by the rotary evaporator, a compound was precipitated out of solution. This solution was seen to be extremely polar and was denoted C_0. After being subjected to a normal-phase gradient chromatography column, five more compounds were isolated based on polarity. These were denoted as C_1 through C_5 in order of increasing polarity.

After the removal of solvents, however, the masses of compound C_4 and C_5 obtained were too small to be quantifiable with the available equipment. Therefore, standard solutions could only be made from compounds C_0 to C_3 for use in the antifungal assay.

3.2. Antifungal Assay

For the antifungal assay, crude extract solutions were made at concentrations of 50%, 10%, 5% and 1%, designated as X(50), X(10), X(5) and X(1) respectively. Additionally, components C_0 to C_3 were each made up to 1% solutions and also subjected to the antifungal assay.

As may be observed from Figure 2, the crude extract of *B. maritima* proved effective against *A. niger*, *Malassezia sp.*, *P. oryzae* and *A. flavus*. At 50%, the crude extract was effective against *A. niger*, *Malassezia sp.* and *P. oryzae*, with zones of inhibition (ZOI) of 25.9 ± 3.62 mm, 25.3 ± 5.44 mm and 90.0 ± 0.00 mm respectively. However, with 10% concentration of crude extract, *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. Additionally, *A. niger* had an inhibition zone of 16.3

± 2.45 mm with 10% crude extract concentration, while *P. oryzae* maintained complete inhibition with an inhibition zone of 90.0 ± 0.00 mm.

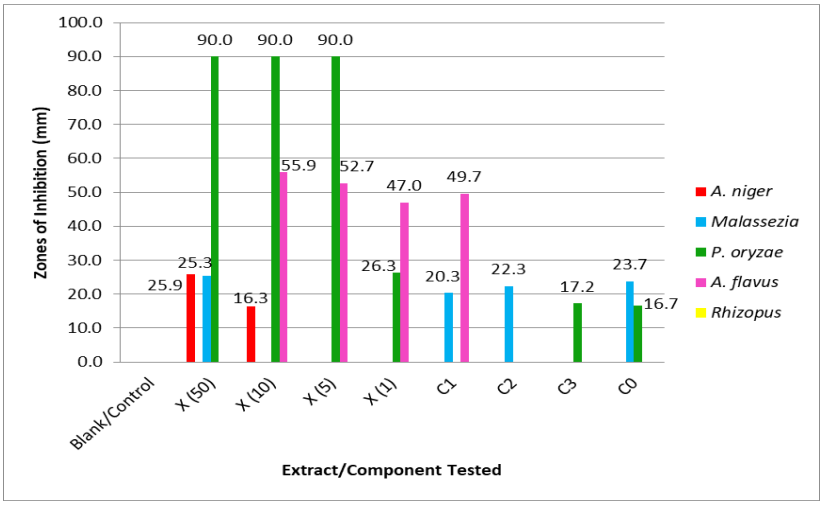

Figure 2 Zone of inhibition of extracts and components

At 5%, the crude extract lost its apparent activity against *A. niger*, with the solution only displaying effectiveness against *A. flavus*, with an inhibition zone of 52.7 ± 1.72 mm, and *P. oryzae* with complete inhibition once again (90.0 ± 0.00 mm). Similarly, at 1%, the crude extract showed the same activities as the 5%, albeit to a lesser degree. At this concentration, the test against *A. flavus* had an inhibition zone of 47.0 ± 3.68 mm, while *P. oryzae* had a ZOI of 26.3 ± 0.95 mm.

In addition to the crude extract, four of the individual components obtained from the chromatographic isolation were also tested for their antifungal activities. These compounds, C_0 to C_3, and their activities may also be seen in Figure 7. The most non-polar component isolated from the chromatography column, C_1, was noted to exhibit antifungal activity against both the *Malassezia sp.*, with an inhibition zone of 20.3 ± 0.91 mm, and *A. flavus* with 49.7 ±1.06 mm. Furthermore, activity of C_2 was displayed only against the *Malassezia sp.*, having an inhibition zone of 22.3 ± 2.84 mm; while activity against a single fungal species was also seen in C_3, in this case *P. oryzae*, with an inhibition zone of 17.2 ± 2.00 mm. Additionally, compound C_0, the most polar component tested, was shown to demonstrate antifungal activity against two of the fungal species tested; viz. *Malassezia sp.*, with a inhibition zone of 23.7 ± 3.02 mm, and *P. oryzae* with 16.7 ± 2.21 mm inhibition zone. Interesting to note is that the activity of C_0 was shown to be very similar to both C_2 and C_3, combined.

A T-test of the ZOI of the 50% crude extract against the affected fungi, i. e. *A. niger*, *P. oryzae* and *Malassezia sp*., was carried out, comparing the ZOI with plant pathogens (*A. niger* and *P. oryzae*) against that of the animal pathogen (*Malassezia sp*.) affected. It was noted from the Levene's test that there was a significant difference between the variances of each group with a significance of 0.000. As such, equal variance was not assumed and the statistic was appropriately adjusted. It was thus seen that the t-test itself yielded a significance of 0.072, which demonstrated no significant differences between the ZOI of the plant and animal pathogens.

Following this, a Post Hoc ANOVA was carried out comparing the ZOI for the 50% crude extract against the three fungi, *A. niger*, *P. oryzae* and *Malassezia sp*. separately. The accompanying Levene's test for homogeneity of variances showed that there was no significant difference among the variances, with a significance of 0.063. Upon comparison of the ZOI, it was seen that there was a significant difference between the ZOI of *A. niger* and *P. oryzae*, with a significance of 0.000. It was also noted that there was also a significant difference between the ZOI of the *Malassezia sp*. and *P. oryzae*, again with a significance of 0.000. However, there was no significant difference between the ZOI of *A. niger* and *Malassezia sp*. as seen with a significance of 0.844. Thus, the ZOI of the *P. oryzae* was significantly higher than the other two.

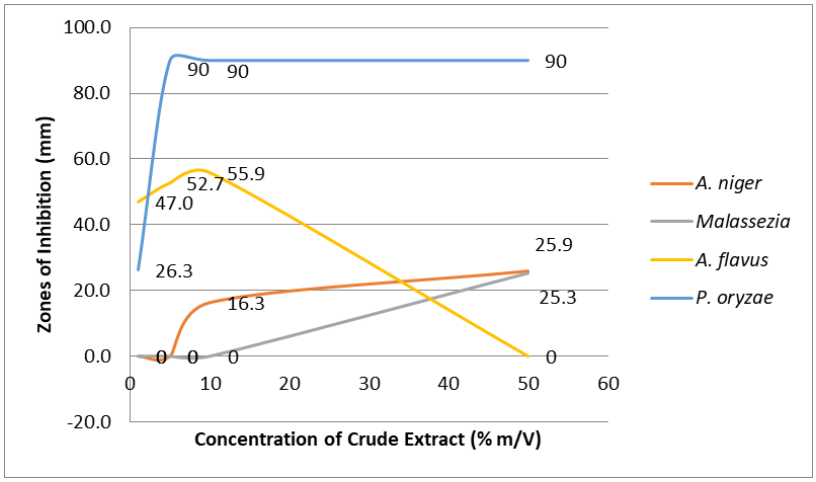

Figure 3a Zone of Inhibition VS. Concentration of Crude Extract

In assessing the antifungal activity of the *B. maritima* crude extract, activities of different concentrations of the extract against each of the susceptible fungal species tested (all but *Rhizopus sp.*) were compared; this may be seen in Figures 3a and 3b. Upon comparison, it could be seen that there was a similar trend with respect to the fungal species (with the exception of *A. flavus*), where a positive relationship was noted, i.e. as the concentration of the crude extract was increased, the inhibition zone of the fungus was also increased.

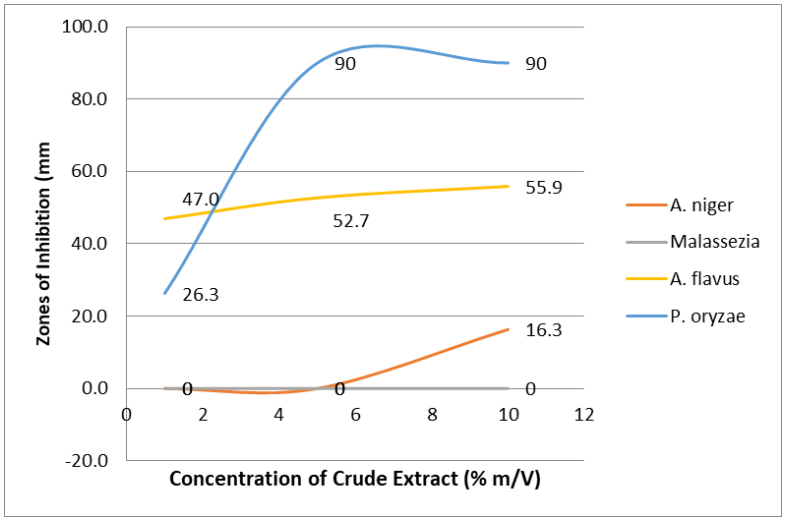

Figure 3b Zones of Inhibition VS. Concentration of Crude Extracts without 50% Concentration

The steepest increase in inhibition zone with respect to concentration change was noted with *P. oryzae*, which experienced full inhibition from 5% (as tested). Furthermore, *A. niger* was affected by 10% and higher solutions, while *Malassezia sp.* was only noted to be affected in any way by 50%. Of the four susceptible fungi, a similar trend is noted with these three species, where increased inhibition zones are seen as the concentration of the *B. maritima* crude extract is increased. However, as shown in Figure 8a, a variation is observed with respect to *A. flavus*, where an initial rise in inhibition is seen from 1% to 10% solution crude extract followed by a steep decrease towards no apparent inhibition at 50%. The initial rise, as displayed more clearly in Figure 8b, is gradual and this rise appears to be the slightest of all the increases observed.

Upon comparison of the range of antifungal activities of the individual components isolated from the crude extract, as shown on Figure 4, it could be seen that both C_1 and C_0, the least and most polar of the isolates, respectively, exhibited positive activity against a wider range of fungal species (two each). Meanwhile, compounds C_2 and C_3, of intermediate polarity only displayed positive activity against one species each.

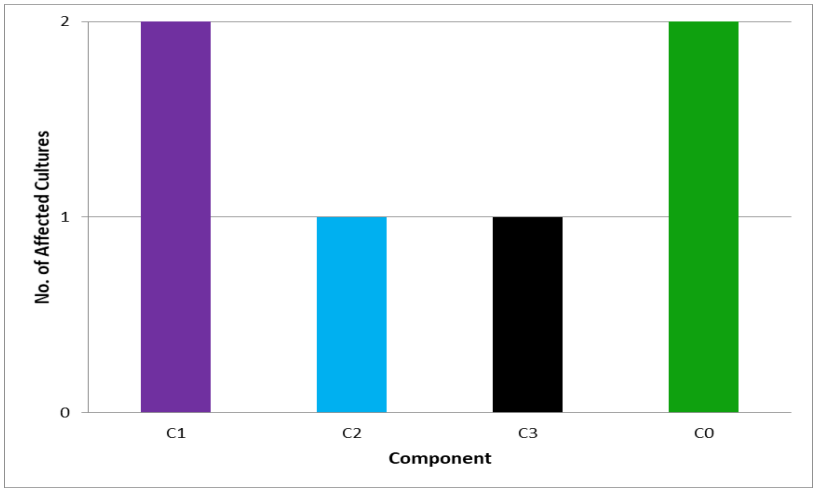

Figure 4 Number of cultures affected by each component

In addition to a comparison of the isolated components and the number of fungal species that were susceptible to each of them, the isolates were also compared based on the average zone of inhibition for all affected species. From Figure 5, it may be seen that C_1 had the highest average inhibition zone of 35.0 mm, far exceeding that of the others. The other components were shown to have very close average inhibition zones, with the second being C_2, effecting a ZOI of 22.3 mm. This was followed by C_0 with an average of 20.2 mm and C_3 with the lowest average inhibition zone of 17.2 mm.

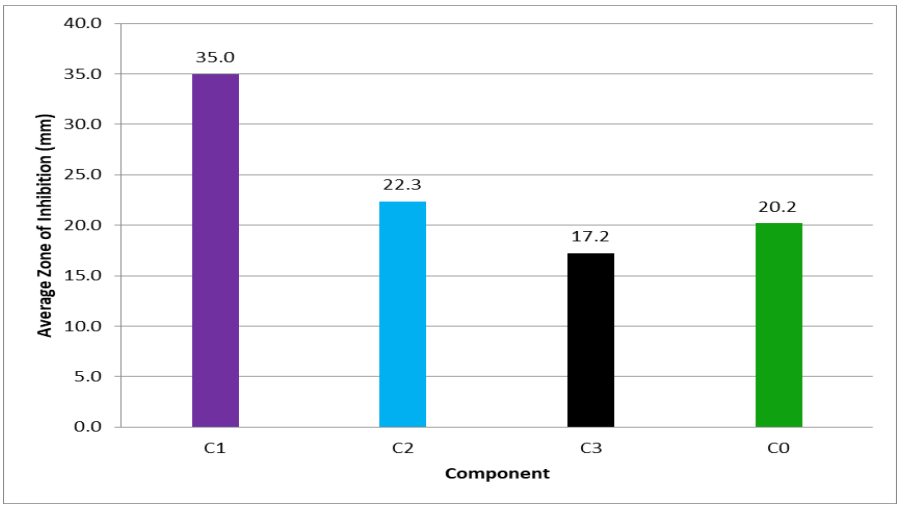

Figure 5 Average zone of inhibition of each component

4. Conclusion

From the study conducted, the following conclusions may be drawn:

Firstly, as noted, the crude extract of *B. maritima* was found to contain several phytochemicals. The phytochemicals that were detected were glycosides, alkaloids, flavonoids, saponins, sterols, tannins and terpenoids.

Secondly, through solvent extraction and normal phase gradient elution column chromatography, six components were isolated from the crude extract. These compounds/components were designated as C_0 to C_5.

From the antifungal assays, the crude extract of *B. maritima* proved ineffective against *Rhizopus sp.*; moderately effective against *Malasezzia sp.*, *A. niger* and *A. flavus*, and especially effective against *P. oryzae*. Further, the four components tested (C_0 to C_3) were all found to be biologically active against one or more of the tested fungi, to some degree. Of these, compound C_1 was found to have the highest mean zone of inhibition of all the tested extracts/isolates. However, there was no significant difference found between the mean ZOI of the crude extracts against animal and plant pathogens.

Compliance with ethical standards

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Disclosure of conflict of interest

We disclosure that we don't have any conflict of interest among authors.

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