



Phytochemicals Screening and Antifungal Activity of *Balanites aegyptiaca* Seed and Callus Extract against *Candida albicans*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AKA and GAI designed the study and wrote the first draft of the manuscript. Authors AH and BPA wrote the protocol, performed the statistical analysis and managed the analyses of the study. Authors AKA, GAI and AH managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2020/v4i430091

Editor(s):

(1) Dr. Vassya Bankova, Bulgarian Academy of Sciences, Bulgaria.

Reviewers:

(1) Sandeep Onkar Waghulde, University of Mumbai, India.

(2) Jayath P. Kirthisinghe, University of Peradeniya, Sri Lanka.

(3) A. Doss, RVS College of Arts and Science, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/56951>

Original Research Article

Received 22 March 2020

Accepted 28 May 2020

Published 04 June 2020

ABSTRACT

Balanites aegyptiaca is a medicinal plant that serves as a source of phytochemicals with antimicrobial effect. This work aimed at screening for phytochemical constituents and investigating the antifungal activity of *B. aegyptiaca* seed and callus extract against *Candida albicans*. Callus induction from *B. aegyptiaca* seed kernel explant was done on MS basal nutrient medium supplemented with 0.5 BAP + 1.0 2, 4-D + 1.0 NAA. Cultures were kept under a controlled temperature and light conditions for five weeks. Plant materials were extracted using solvent extraction. 50 ml of the solvents: methanol and n-hexane were mixed with five grams (5 g) each of

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the grounded plant materials (1:10) w/v. Determination of antimicrobial activity was done using disc diffusion assay. Diffusion discs of approximately 6 mm diameter were prepared from Whatman No. 1 filter paper, then sterilized and autoclaved before drying in an oven. 10 μ l of 50 and 100 mg/ml concentration of each crude extracts was impregnated on separate sterile disc using sterile micropipette tips. The diameter of zone of inhibition at 100 and 50 mg/ml showed the methanolic extract of callus had the highest zone of inhibition with 17 ± 0.69 mm and 11 ± 0.94 mm. The lowest diameter of zone inhibition of callus extracts was recorded by n-hexane extract at 100 (15 ± 0.46 mm) and 50 mg/ml (09 ± 0.57 mm) respectively. Also, the MIC ranged between 6.25 and 12.50 mg/ml and MFC recorded value of 12.50 mg/ml. Seed methanolic extract had the highest zones of inhibition of 15 ± 0.34 mm and 10 ± 0.62 mm at 100 and 50 mg/ml respectively, while the lowest value (12 ± 0.51 mm and 09 ± 0.23) was recorded in n-hexane at 100 and 50 mg/ml. From the results, both the MIC and MFC of seed extracts ranged from 12.50 to 25.00 mg/ml. Callus extracts showed stronger antifungal activities compared to the seed extracts. Therefore, from the result, Callus extract from *B. aegyptiaca* can serve as a good source of therapeutic compounds for fungal related disease.

Keywords: Antifungal; callus extract; *Candida albicans*; phytochemicals and seed extract.

1. INTRODUCTION

Balanites aegyptiaca (L) is a spiny, xerophytic tree found in the tropics, adapted to diverse agro climatic conditions specified by arid and semi-arid climatic features [1]. The fruit is the most important part of the tree and it is commonly referred to as desert date and contains four layers [2,3]. The four layers can be exploited for various industrial and pharmaceutical products. The outer skin termed the epicarp, the fleshy pulp: mesocarp, the woody shell: endocarp and the inner seed called kernel [4]. *B. aegyptiaca* is a versatile plant use in folk medicine, as pesticides, fodder, and charcoal. The plant has antifungal, antibacterial, antidiabetic, antihelminthic and antiviral activities primarily as a result of the production of different types of secondary metabolites such as alkaloids, steroids and flavonoids. Several authors [5,6] reported antifungal activity of *B. aegyptiaca* against *Candida albicans*. Recently, the growing development of microorganism resistance pattern to most presently used antimicrobial drugs has increased scientific interest in these metabolites for the search of new antimicrobial agents such as using callus [7,8].

Candida albicans belongs to the family Candidaceae and is an encapsulated polymorphic fungus which exist as yeast or pseudohyphal forms base on nutrients, temperature, and pH [9]. *C. albicans* is an opportunistic pathogen that occurs as harmless commensal and member of the microbiota in the genitourinary and gastrointestinal tracts of humans. It is estimated that about 75% of females suffer from candidiasis (candida

infection) at least once in their lifetime [10,11,12]. *C. albicans* caused over 70% of human Candida infections the rest by *C. parapsilosis*, *C. tropicalis*, *C. guilliermondii*, *C. kruzei*, and a few other rare Candida species [13]. Presently, there have been increase in number of yeast resistant to antifungal drugs worldwide. Candida species have the ability to form drug resistant biofilms which is a key factor in causing human disease. *C. albicans* biofilms sessile cells are less susceptible to antimicrobial agents than planktonic cells [14].

This work aimed at screening for phytochemical constituents and investigating the antifungal activity of *B. aegyptiaca* seed and callus extract against *C. albicans*.

2. MATERIALS AND METHODS

2.1 Callus Induction

Induction of callus from *B. aegyptiaca* seed kernel explant was done on MS basal nutrient medium [15] supplemented with 0.5 BAP + 1.0 2, 4-D + 1.0 NAA. Cultures were kept under a controlled temperature and light conditions for five weeks.

2.2 Preparation and Extraction of the Extracts

Seeds and callus of *B. aegyptiaca* were dried at 40-45°C in an oven. Mortar and pestle were used to ground the plant materials into powdered form and stored at room temperature for further use. 50 ml of the solvents: methanol (polar) and n-hexane (non-polar) were mixed with five grams

(5 g) each of the grounded powder (1:10) w/v. The mixtures were agitated vigorously and kept on an orbital shaker at 150 rpm for 24 hrs, then filtered according to [16,17]. Stock solution of 100 mg/ml of the extracts was prepared by adding 1 g of the powdered extract to 10 ml of 5% Dimethyl sulfoxide (DMSO), and stored at -4°C until further use [18].

2.3 Phytochemicals Analysis

Phytochemical analysis of the seed and callus extract was carried out to investigate the presence of the several secondary metabolites that are responsible for their various antimicrobial properties. Phytochemical analysis of plant materials was determined for constituents such as saponins, tannins, glycosides, oxalate, flavonoids, anthraquinones, and steroids [19].

2.4 Test Organism

The test fungi: *C. albicans* was obtained from the Specialist Hospital, Yola, Adamawa State, Nigeria. The microbial culture was maintained on Sabouraud dextrose agar.

2.5 Antimicrobial Assay

Disc diffusion assay was used, crude extracts of callus and seed were obtained using solvent extraction. Methanol and n-hexane were used as extraction solvents and were prepared to concentrations of 50 and 100 mg/ml. Diffusion discs of approximately 6mm diameter were prepared from Whatman No. 1 filter paper then sterilized by autoclaving before oven drying. 10 µl of each concentration of crude extracts was impregnated on separate sterile disc using sterile micropipette tips and stored in separate sterile containers. Then, disc diffusion assay was carried out according to [20].

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

Varying concentrations of the extract were prepared by diluting the stock with agar. The working concentrations were 1.56, 3.125, 6.25, 12.50, 25, 50, 100 mg/ml. The MIC and MBC was determined according to [21]. Plates were observed for fungal growth and the concentrations at which there was no visible fungal growth was recorded. The concentration at which the fungi were completely dead and not

just inactive was recorded as the minimum fungicidal concentration.

2.7 Statistical Analysis

All the experiments were performed in triplicate and the results were expressed as mean ± standard errors. Data on zones of inhibition by extracts were pooled and analyzed using one-way ANOVA (Software SAS version 9.4) and means were separated using least significant difference at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Callus Induction

Callus was successfully induced from *B. aegyptiaca* seed explant (Fig. 1). The figure shows callus formation on MS basal nutrient medium supplemented with 0.5 BAP + 1.0 2, 4-D + 1.0 NAA after five weeks.

3.2 Phytochemical Analysis

Phytochemical analysis of plant seed and callus extracts was determined for constituents such as saponins, tannins, glycosides, oxalate, flavonoids, anthraquinones, steroids, (Tables 1 and 2). Saponins, tannins, glycosides, oxalate, steroids were found in callus extract of *B. aegyptiaca*. Similarly, Tannins, oxalate, steroids were present in seed kernel extract of *B. aegyptiaca*. On the other hand, steroids, tannins and oxalate were found both in seed and callus extracts of *B. aegyptiaca*.

3.3 Antifungal Activities of Callus Extract

From Table 3 and Fig. 2, the diameter of zone of inhibition at 100 and 50 mg/ml showed the methanolic extract of callus had the highest zone of inhibition. The highest zone of inhibition was 17 ± 0.69 mm and 11 ± 0.94 mm at 100 and 50 mg/ml. The lowest diameter of zone inhibition was recorded by n-Hexane extract at 100 (15 ± 0.46 mm) and 50 mg/ml (09 ± 0.57 mm) respectively. Table 3 also shows the MIC ranged between 6.25 and 12.50 mg/ml and MFC recorded value of 12.50 mg/ml.

3.4 Antifungal Activities of Seed Extract

The diameter of zone of inhibition recorded by seed extract at 100 and 50 mg/ml reveals methanolic extracts of seed kernel had the

highest zone of inhibition than the n-hexane extracts (Table 4 and Fig. 3). The highest zones of inhibition was 15 ± 0.34 mm and 10 ± 0.62 mm by methanolic extract of seed kernel at 100 and 50 mg/ml respectively, while the lowest value (12 ± 0.51 mm and 09 ± 0.23) was recorded in n-hexane at 100 and 50 mg/ml. From the result in Table 4, both the MIC and MFC values ranged from 12.50 to 25.00 mg/ml.

The results on callus culture showed that low concentration of auxin and cytokinin were essential for the production of callus from *B. aegyptiaca* seed kernel. Sharma and Sen [22,23] reported that BAP in combination with NAA and

2, 4-D gave higher formation of callus on MS media which is in line with report of this studies. The phytochemical analysis of the plant extracts indicates the presence of saponins, tannins, glycosides, oxalate, and steroids in the test sample. Tannin; a class of polyphenol compound present in these plant materials is an inhibitor of microbial growth and also, with an astringent property [24]. Steroids have strongest antimicrobial property and are essential compound for synthesis of sex hormones. Some of these phytochemicals tends to inhibit the binding of microbes on host cell surface membranes and act as potential antiadhesive agents [25].



Fig. 1. Callus culture of *B. aegyptiaca* at 0.5 BAP + 1.0 2, 4-D + 1.0 NAA

Table 1. Qualitative and quantitative analysis of *B. aegyptiaca* callus extract phytochemicals constituents

Parameters	Observation	QTY per 0.5 g Extracts	Percentage
Saponins	++	5 mg	1.0
Tannins	+	1.1 mg	0.22
Glycosides	+	0.9 mg	0.18
Oxalate	++	2.82 mg	0.56
Flavonoid	-	NA	NA
Anthraquinones	-	NA	NA
Steroids	++	3.4 mg	0.68

(++) Moderate; (+) Present; (-) Absent

Table 2. Qualitative and quantitative analysis of *B. aegyptiaca* seed extract phytochemicals constituents

Parameters	Observation	QTY per 0.5 g Extracts	Percentage
Saponins	-	NA	NA
Tannins	+	0.82mg	0.16
Glycosides	-	NA	NA
Oxalate	++	2.82mg	0.56
Flavonoid	-	NA	NA
Anthraquinones	-	NA	NA
Steroids	+	2.2mg	0.44

(++) Moderate; (+) Present; (-) Absent

Table 3. Mean diameter of zone of inhibition (mm) of test organism treated with 50 and 100 mg/ml of methanol and n-hexane extract of *B. aegyptiaca* callus extract after 24 hours of incubation, Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *B. aegyptiaca* callus Extract in mg/ml

Test organism	Diameter of zone of inhibition (mm)				MIC and MFC (mg/ml)	
	Solvent	100 mg/ml	50 mg/ml	Control (5% DMSO)	MIC mg/ml	MFC mg/ml
<i>C. albicans</i>	Methanol	17 ± 0.69	11 ± 0.94	0	6.25	12.50
	n-hexane	15 ± 0.46	09 ± 0.57	0	12.50	12.50

Key: Mean of 3 replications ± SEM, diameter of zones inhibition excluding diameter of 6 mm disc

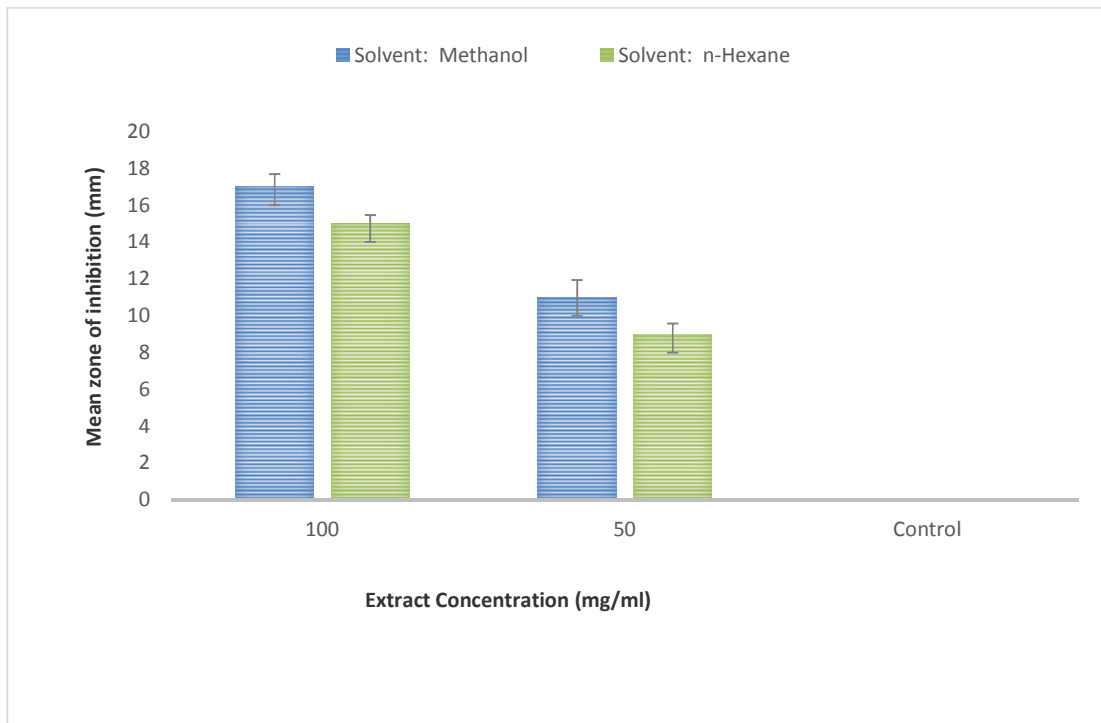


Fig. 2. Antifungal activity of 100 and 50 mg/ml of methanol and hexane of *B. aegyptiaca* callus extract

Table 4. Mean diameter of zone of inhibition (mm) of test organism treated with 50 and 100 mg/ml of methanol and n-hexane extract of *B. aegyptiaca* seed extract after 24 hours of incubation, Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *B. aegyptiaca* seed kernel extract in mg/ml

Test organism	Diameter of zone of inhibition (mm)				MIC and MFC (mg/ml)	
	Solvent	100 mg/ml	50 mg/ml	Control (5% DMSO)	MIC mg/ml	MFC mg/ml
<i>C. albicans</i>	Methanol	15 ± 0.34	10 ± 0.62	0	12.50	12.50
	n-hexane	12 ± 0.51	09 ± 0.23	0	12.50	25.00

Key: Mean of 3 replications ± SEM, diameter of zones inhibition excluding diameter of 6 mm disc

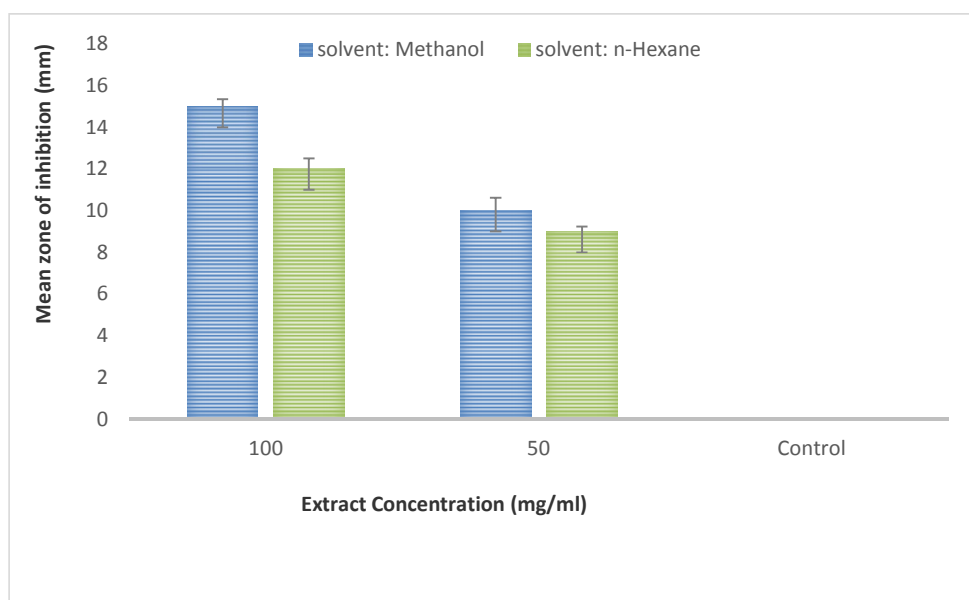


Fig. 3. Antifungal activity of 100 and 50 mg/ml of methanol and hexane of *B. aegyptiaca* seed extract

When the two results (callus and kernel antifungal activities) were pooled and compared, the study revealed presence of highly significant difference ($P < 0.001$) between the performance of Callus and seed kernel extract, with callus extract having the highest zone of inhibition at both levels of concentrations. The strong antifungal activities exhibited by the methanolic and n-hexane extracts used in this study are in accordance to the reports of Srinivasan et al. [26] who stated that solvents of varying polarity possess different extraction ability and have different solubility for the phytochemicals components. Similarly, this study reveals the high antifungal activity of *B. aegyptiaca* callus extract than seed extracts against *C. albicans*. Zone of inhibition equal or above 14 mm is considered to have high antimicrobial property [27,8]. Surprisingly, results from this study reveal zones of inhibition by callus methanolic and n-hexane extract at 100 mg/ml has diameter of mean inhibitory zones equal or above 15 mm. Low MIC and MBC implies high antimicrobial activity of extracts [28]. From this study, MIC and MBC in callus extracts had smaller values with the seed extracts. This suggests that *B. aegyptiaca* callus extracts possess high antifungal activity but generally, the callus and seed extracts both antifungal property against the fungal pathogen *C. albicans* [29]. Medicinal plants such as *B. aegyptiaca* provide prospects for new drugs discovery as a result of their high

possession of phytochemical compounds known for various antimicrobial activities [24,30,31,32].

4. CONCLUSION

The result from this study showed that all the extracts were effective in inhibiting the microbial growth of the *C. albicans*. The callus extracts however showed stronger antifungal activities compared to the seed extracts. Therefore, Callus from *B. aegyptiaca* can serve as a good source of therapeutic compounds treatment of *C. albicans* and other fungi fungal related disease. Callus culture provides tool for harnessing Plant-derived compounds and the in vitro production of natural bioactive compounds.

ACKNOWLEDGEMENTS

Authors are grateful to Prof Usman, Abdullahi Wurochekke, the Dean School of Life Sciences (former coordinator of Chevron Biotechnology Center, MAUTECH) for improving the state of the plant tissue culture laboratory and Mr. Abdulmumini A. Kadang of Bliss Diagnostics Services, Yola for assistance during the antifungal assay.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:
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