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In vitro anticandidal and antioxidant potential of *Mezoneuron benthamianum*

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ABSTRACT

Objective: To investigate the preliminary phytochemical screening and antioxidant properties of the methanolic extract of *Mezoneuron benthamianum* (*M. benthamianum*). **Method:** The anti-*candida*l potential and minimum inhibition concentration (MIC) were evaluated on fresh isolates of *Candida* species using disc diffusion method. While the lipophilic free radical scavenging activity was examined using the spectrophotometric assay on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and compared with standard antioxidant, α-tocopherol. **Result:** The anti-*candida*l activities of *M. benthamianum* root and leaf revealed zones of inhibition (ZI) against *Candida* glabrata as (13.6±1.00) mm and (13.7±1.00) mm respectively while, *Candida* krusei ZI against root (13.7±1.00) mm and leaf (11.8±0.80) mm as the most significant among other *candida* species (*Candida* stellatoidea, *Candida* albicans and *Candida* torulopsis). Phytochemicals present in the plant extracts includes saponin, anthracquinones, flavonoids and tannins. The antioxidant potential of the plant was significant and dose dependent at the concentrations tested. The extract has IC_{50} values of 781 μg/mL while the standard, α-tocopherol has 222.3 μg/mL. **Conclusions:** The result from this research suggests that *M. benthamianum* is a potent anti-*candida* and antioxidant plant and may be a potential anti-*candida* plant for future prospect in drug development.

1. Introduction

Human oral cavity is constantly threatened by opportunistic and pathogenic microbes[1], in which the prominent ones include *candida*. Candidiasis is an infection associated with immunocompromised states such as diabetes, extensive antibiotic usage, malignancies and Human Immunodeficiency Virus (HIV) infection[2]. *C. albicans* is the most common fungus linked to the development of infective endocarditis and associated with a high mortality[3]. *C. albicans* are common flora of the mucous membranes in the gastrointestinal, upper respiratory and female genital tracts[4,5,6]. However, Chander[7], reported that for about two decades back, the incidence of *Candida* species has been significant and non-albicans *Candida* spp continue to replace *C. albicans* at most of the clinical sites like bloodstream infections.

Hence, in severe immunocompromised patients, the oral cavity may harbor *Candida* organism that may disseminate systemically and cause significant morbidity and mortality[8,9]. Odd[8] also reported the carriage rate of *C. albicans* in normal person to be eighteen per cent and that of patients to be forty-one per cent, stating further that fifty per cent of infants from one week to eighteen months of age carry the organism while adults, a mean carriage rate of twenty per cent. There are accumulating evidences that chronic hyperplasia form of *candida* may predispose the oral mucosa of some persons to malignant transformation[10,11].

Nearly ninety species of yeast from at least twenty genera have been isolated from human beings and classified[12], whereas *C. albicans* and some other *candida* species were reported to be common flora of the mucous membranes in the gastrointestinal, upper respiratory and female

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genital tracts, they are even implicated at clinical sites such as blood stream infections[13-16]. When diseased condition is established due to immunocompromise and immunosuppression, there is a need for its treatment with potent antibiotics that the causal organism cannot easily develop resistance to. However the reported challenge of resistant microbial strain development[17], made the continuous search for indigenous plants antimicrobials inevitable. Thus, the pool of medicinal plant which has been serving as a tool of exploiting the natural state for the treatment of different maladies may be employed; this form of medical practice is termed alternative medicine which is widely accepted and used worldwide[18]. *Mezoneuron benthamianum* (Figure 1) is a medicinal plant that belong to the family Caesalpinoideae. The leaves are brightly red when young and dark green when old. The stem is black with reoccurring thorns. The root is brown and hard while the shrubs are branched and its stem is a woody climber of up to 8 meters in height. *M. benthamianum* can be found in waste places of deciduous secondary jungle and savannah forest from Senegal to Nigeria[19]. The plant is locally called 'amuranju' or 'jenifiran' in Yoruba land of Nigeria[20]. It is used in folk medicine for the treatment of dermal infections and wounds in Ghana. The herbalists hold the plant in high premium for its unique medicinal importance. The leaves are considered in Senegal to be antiseptic and used in cleaning and healing of refractory sore. Young leaves are mashed and applied on wounds and swollen parts of the body in Sierra-Leone. The root is reported to be used in Ibadan area, Nigeria as chewing stick[20].

Figure 1. *M. benthamianum*.

The presence of some peptides, unsaturated long chain aldehydes, alkaloidal constituents, essential oils, flavonoids, saponins and tannins have been confirmed in *M. benthamianum*[21.22], therefore this plant is worthy to be studied for its anti-*candida*l and antioxidant properties, since documented studies on the antifungal activities of the plant are scanty to the best of the authors' knowledge. Methyl gallate and gallic acid has been identified as the constituents responsible for antibacterial property found in *M. benthamiamum* where the plant extracts are active against gram positive bacteria which include *Staphylococcus aureus* and *Bacillius substilis* but not active against gram negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) [23]. There are reports of synergistic efficacy of contained phytochemicals in plant crude extracts[24], and this is worth studying in *M. benthamianum* also, treatment of candidiasis cannot be overemphasized, since Candidiasis has been reported to be an emerging drug resistant disease[25-28].

2. Materials and methods

α - Tocopherol, 1,1- diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma- Aldrich (Germany) while the solvents used were analytical grade.

2.1. Isolate collection

Characterized *Candida* species (*Candida* albicans, *Candida* torulopsis, *Candida* krusei, *Candida* glabrata and *Candida* stellatoidea) isolates were collected from Olabisi Onabanjo University Teaching Hospital, Nigeria. Isolates were asceptically plated on Sabouraud Dextrose Agar (SDA) medium fortified with sterilized Streptomycin at a concentration of 100 μg/mL, and incubated at 30 ℃ for 48 h. Isolates were also stored in refrigerator in bijou bottles where they served as stock cultures for further studies.

2.2. Collection and extraction of plant material

Mezoneuron bentamianum whole plant were collected at the Redeemed Christian Church of God (RCCG) camp, Mowe, Ogun State, Nigeria (N 06° 48. 874' E 003° 27. 614'), identified and authenticated at the Department of Applied Plant Science and Zoology (APZ), Olabisi Onabanjo University (OOU), Ago-Iwoye, Ogun State, Nigeria. Sample specimen was deposited at the herbarium of APZ in OOU. (APH 324), and separated into leaves, root and whole plant for the study. Extraction method of Alade and Irobi[29] method was employed where the plant material were thoroughly washed, air-dried at room temperature (29±2) ℃ and grounded to powder. 200 g of the pulverized sample material were soaked in 1 000 mL of absolute ethanol for five days on an intermittent shaker and incubator at 30 ℃. The plant extract were concentrated in the rotary evaporator at 60 ℃ and dried in the oven at 60 ℃ vernight. Concentrated extracts were kept in sample bottles and refrigerated at 4 ℃ prior to testing. Modified dilution method of Abo et al[30] was employed where sample plant extracts were prepared in dimethyl sulphur oxide (10% DMSO solution) at 200 mg/mL for use.

2.3. Phytochemical studies

2.3.1. Test for saponins

Froth test: 0.5 g of the alcoholic extract was dissolved in 10 mL of distilled water contained in a test tube, stoppered, shaken vigorously for about 30 s, allowed to stand in a vertical position and observed over a 30 min period. The appearance of 'honey comb' froth above the surface of liquid indicate positive test.

For confirmation of the test, 500 mg of sample plant extract was introduced into freshly prepared blood agar plate, incubated at 35 ℃ for 6 h. Hemolytic appearance at the sample extract spots indicates positive confirmation.

2.3.2. Test for anthraquinones

Test for free anthraquinones (Borntrager's test): The ethanolic sample plant extract (equivalent to 100 mg) was shaken vigorously with 10 mL of benzene, filtered and 5 mL of 10% ammonia solution added to the filtrate. The presence of a pink, red or violet color in the ammonia (lower) phase indicates the presence of free anthraquinones.

2.3.3. Test for flavonoids

Samples of each ethanolic extracts (0.5 g) was weighed, four drops of concentrated hydrochloric acid added. Thereafter, 0.5 g of magnesium tunings added. Pink or magenta-red color indicates the presence of flavonoids.

2.3.4. Test for tannins

Ferric chloride test: A speck of the alcoholic sample plant extract was dissolved in 2 mL of distilled water, and 0.5 mL of 10% ferric chloride solution was added. A bluish black coloration after the addition indicates positive test.

2.4. Antimicrobial susceptibility test

Method of antibiotic disc diffusion was adopted[31], where Whatman No. 1 filter paper was carefully bored to 6 mm diameter, sterilized and impregnated with 20 μL of 200 mg/mL of the respective plant extracts in six (6) replicates per treatment of *Candida* specie. The discs were asceptically air-dried at 30 ℃ for 8 h thereafter used immediately or store at 4 ℃ until required for use. Nystatin (OXOID, antibiotic susceptibility test disc) was used at the manufacturer's specification as positive control. The discs were placed equidistantly on the agar plates readily seeded with the test *Candida* species (*Candida* albicans, *Candida* torulopsis, *Candida* stellatoidea, *Candida* parapsilosis and *Candida* krusei of 1×10^6 cells) concentration determined with the aid of heamocytometer.

2.5. Determination of minimum inhibition concentration (MIC)

MIC were determined by observing the lowest concentration at which no visible growth of *Candida* species was observed after subjection to 20 different concentrations of ranges 1 mg/mL (a total of twenty sample plant extract concentrations per *Candida* specie). The agar well diffusion method was employed where cork borer was used to bore 6 mm diameter holes aseptically on sterile Mueller Hinton agar plate, after a 2 mL of 1×10^6 cell per mL of *candida* test organism were inoculated on each plate by spread plate method, air-dried in a laminar airflow. Different concentrations were aseptically introduced into the bored holes on the agar plates and incubated at 30 ℃ after 48 h.

2.6. Assay of DPPH radical scavenging activity

The antioxidant activity was measured using DPPH assay. This spectrophotometric assay uses the stable radical 1,1-diphenyl-1-picrylhydrazyl (DPPH) as a reagent[29]. The DPPH free radical was prepared at a 0.1 mM concentration (25 mg/L) in methanol, following the procedure described by Sa´nchez-Moreno et al[28] and Atolani et al[34] Stock solution of the extract (1 mg/mL) was diluted to final concentrations of 500, 250, 200, and 100 μg/mL in methanol. 1 mL of 0.1 mM DPPH methanol solution was added to solutions of the extract or standard, α - tocopherol and absorbance taken after 30 min of incubation in the dark. The experiment was duplicated. The percentage antioxidant capacity (AOC%) was obtained using the Arnao,expression[35].

AOC % = 100 x (Acontrol - Asample)/Acontrol (DPPH in methanol (0.1 mM) served as a control)

Absorbance was recorded to check the stability of the radical throughout the time of analysis. The extract in methanol served as blank while the machine was constantly stabilized using 98% methanol. The IC_{50}, (the concentration that inhibited 50% of the free DPPH radicals) was determined.

2.7. Statistical analysis

MIC data were collected and subjected to analysis of variance (ANOVA) and Duncan multiple Range test with the aid of SPSS statistical software package.

Data on antioxidant group mean ± S.E.M. was calculated for each analyte. The IC_{50} was calculated from dose-response-inhibition curves on graphpad prism 3 software using nonlinear regression analysis. The results represent the mean ± standard error of the mean values of duplicate experiments. IC_{50} values were approximated with statistical significance $P\leqslant0.01$ and with high regression coefficients.

3. Results

M. benthamianum ethanolic extract were positive for saponin, anthraquinones, flavonoids and tannins as presented in Table 1.

Table 1
The phytochemical constituents of *M. benthamianum*.

Phytochemical test	*M. benthamianum*
Saponins	+
Anthraquinones	+
Flavonoids	+
Tannins	+

Key: ++ means strongly present; + means moderately present; – means not present.

Table 2
IC_{50} values of DPPH assay on *M. benthamianum* extract and α – tocopherol.

Samples	IC_{50} μg/mL	R^2
M. benthamianum extract	781.50 ± 1.11	0.982 7
α – tocopherol	222.30 ± 4.22	0.984 2

IC_{50}: 50% inhibition concentration. Results represent mean ± standard error of mean; *n*=3.

Table 3 showed inhibition zone from root extract of *Mezoneuron benthamianum* as *C. albicans* (10.2 ± 1.00) mm, *C. torulopsis* (10.7 ± 1.00) mm, *C. stellatoidea* (9.4 ± 0.50) mm, *C. glabrata* (13.8 ± 1.00) mm and *C. krusei* (13.7 ± 1.00) mm.

The ethanolic leaf extract's response to *M. benthamianum* by *Candida* species (Table 3) recorded the inhibition zone of *C. albicans* (7.2 ± 1.00) mm, *C. torulopsis* (6.2 ± 0.40) mm, *C. stellatoidea* (7.2 ± 0.70) mm, *C. glabrata* (13.7 ± 1.00) mm and *C. krusei* (11.8 ± 0.80) mm respectively.

The inhibition zone of the whole plant extract for, *C. albicans, C. torulopsis, C. stellatoidea, C. glabrata* and *C. krusei* are (8.7 ± 0.24) mm, (8.5 ± 0.36) mm, (8.0 ± 0.47) mm, (7.8 ± 0.62) mm and (12.8 ± 0.18) mm respectively.

The ethanolic extract of *M. benthamianum*, in this study was found to contain saponins, anthraquinones, flavonoids and tannins of which may be responsible for the anti*candida*l activity observed on *Candida* species (Table 3) in this research.

M. benthamianum (Table 1) antimicrobial activity against *C. glabrata* for root (13.8 ± 1.00), leaf (13.7 ± 1.00) and whole plant (7.80 ± 0.63) indicate that the combination of these two different plant parts (root and leaf) extracts reduces the activity of the plant when combined.

3.1. Minimum inhibition concentration (MIC)

Candida glabrata is the most susceptible *Candida* among the studied *Candida* species to *M. benthamianum* ethanolic extract with an MIC of 5 mg/mL (Table 4),

3.2. In vitro antioxidant study

The in vitro free radical scavenging activity of the extract (Figure 2) is high and dose-dependent at all concentrations. The activity of the extract is higher than that of α – tocopherol at lower concentrations and lower at higher concentrations of 500 and 1 000 μg/mL. The extract has IC_{50} values of 781 μg/mL while the standard, α –tocopherol has 222.3 μg/mL.

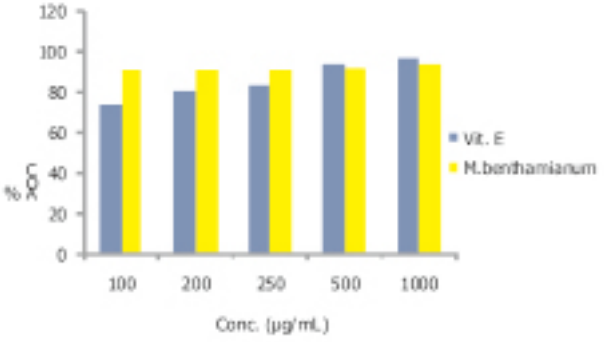

Figure 2. AOC of the *M. benthamianum* extract and α – tocopherol.

Table 3
Zone of inhibition (ZI) of ethanolic extracts of *M. benthamianum* and controls on isolated five *Candida* spp from buccal cavity.

Candida species	*M. benthamianum* Zone of inhibition (ZI)			Control Zone of inhibition (ZI)	
	Root extract (mm)	Leaf extract (mm)	Whole plant (mm)	DMSO (in mm)	Nystatin (in mm)
C. albicans	10.20bc ± 1.00	7.20c ± 1.00	8.70b ±0.24	6.00a ± 0.00	17.00a ± 0.90
C. torulopsis	10.70b ± 1.00	6.20d ± 0.40	8.50b ±0.36	6.00a ± 0.00	16.00a ± 0.90
C.stellatoidea	9.40c ± 0.50	7.20c ± 0.70	8.00b ±0.47	6.00a ± 0.00	17.00a ± 0.90
C. glabrata	13.80a ± 1.00	13.70a ± 1.00	7.80c ±0.62	6.00a ± 0.00	12.00b ± 0.90
C. krusei	13.70a ± 1.00	11.80b ± 0.80	12.80a ±0.18	6.00a ± 0.00	17.00a ± 0.90
F value	31.016	100.707	150.436	–	35.250

Note: Means with the same letter in a column are not statistically different.

Table 4
Minimum Inhibition of *M. benthamianum* on *Candida* species.

	C. albicans	*C. torulopsis*	*C.stellatoidea*	*C. glabrata*	*C. krusei*
M. benthamianum (20 μL of 200 mg/mL crude extract inhibition zone)	9.00±0.60	7.00±1.00	8.00±1.20	8.00±0.60	6.00±0.60
MIC (mg/mL)	8	6	8	5	15
DMSO (negative control) 20 μL	na	na	na	na	na
Nystatin (positive control) 20 μL of 10 μg/mL	17.00±0.90	16.00±0.90	17.00±0.90	12.00±0.90	17.00±0.90

Key: MIC = Minimum Inhibition Concentration; na = not applicable.

The study showed that the extracts have the electron-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants in the plant. The ethanolic extract showed a high lipophilic antioxidant power for all the concentrations tested. Hence, the ethanolic extract with high antioxidant potentials could be a viable source of antioxidant compounds which may compliment the anti-*candidal* activity of the plant.

4. Discussion

The results as recorded in Table 3 revealed that *C. glabrata* (13.80 ± 1.00) mm and *C. krusei* (13.70 ± 1.00) mm indicated the highest susceptibility to root ethanolic extracts of *M. benthamianum* among other *Candida* species. Considering C. glabrata that is frequently implicated as fast replacing *C. albicans* in cases of drug resistance in the treatment of candidiasis[14,18,21], Table 3 also indicates that ethanolic root extract of *M. benthamianum* showed a comparable antimicrobial activity when compared with the commercially available standard drug (Nystatin). Inhibition zones of (13.80 ± 1.00) mm recorded against *C. glabrata* and Nystatin (12.00 ± 0.90) may indicate that purified extracts of *M. benthamianum* root may favorably compare with commercially available Nystatin drugs in treating *C. glabrata*.

The recorded value of (6.20 ± 0.40) mm exhibited by *C. torulopsis* may be expressed as being resistant to leaf extracts of *M. benthamianum* while, the results obtained for the ethanolic leaf extract (Table 3) of *M. benthamianum* against C. glabrata (13.70 ± 1.00) mm maybe an indication that the antimicrobial activity may compare favorably with the commercially available drug Nystatin of (12.00 ± 0.90) mm.

Comparing the zone of inhibition results obtained from extracts of whole plant, roots and leaf of *M. benthamianum*, for instance *C. albicans* and *C. glabrata* showed records of (8.70 ± 0.24) mm, (7.20 ± 1.00) mm, (10.20 ± 1.00) mm and (7.80 ± 0.62) mm, (13.70 ± 1.00) mm, (13.80 ± 1.00) mm for extracts of whole plant, roots and leaf respectively, these results for whole plant extract indicate the low zone of inhibition when compared with that of leaf and root thus, there may be antagonistic effects on the combination of the different phytochemicals contained in the crude extracts of *M. benthamianum*. And this will be confirmed in the further studies whereby the phytochemicals at these different portions of the plant is separated and their antimicrobial activity investigated against *Candida* spp.

The antimicrobial activity recorded against *C. albicans* by leaf and whole plant ethanolic extracts of *M. benthamianum* was low when compared with that of root extract thus, when compared with the work done by Binutu and Cordell[16], where reports that gallate and gallic acid were responsible for the antimicrobial activity of *M. benthamianum* were stated, it further emphasized that *M. benthamianum* is not active against *C. albicans* despite its activity against bacteria therefore, a suggestion of further studies in order to determine the active ingredient(s) responsible for the antimicrobial activity against *C. albicans* in the root of *M. benthamianum* is suggested by this research. The data obtained in this research supports the studies of Seher et al[31] in their treatment of microorganisms with crude medicinal plants extracts from whole plant as well as other parts and organ of linseed.

The lowest MIC of *M. benthamianum* crude extracts exhibited by *C. glabrata* is worth studying further since *C. glabrata* is one of the most prominent *Candida* spp reported to be replacing *C. albicans* which is the most pathogenic among the genus but also consistently developing resistance to commercially available drugs. Purification of the extracts of this plant is therefore necessary in order to ascertain the level of potency of this medicinal plant. Isolated from human diseased sites, it is also to.

The effect of antioxidants on DPPH is thought to be due to their electron donating ability of the extract[41]. Antioxidant-rich plant extracts serves as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases. An effort has been made to explore the antioxidant properties of this herbal plant.

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Conflict of interest statement

We declare that we have no conflict of interest.

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