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Comparison of Antimicrobial Effects of *Mezoneuron benthamianum*, *Heliotropium indicum* and *Flabellaria paniculata* on *Candida species*

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Abstract Alternative medicine, also called unconventional medicine, is therapeutic practices, techniques, and beliefs that are outside the realm of mainstream Western healthcare. Antimicrobial activities, minimum inhibition concentrations (MIC) and phytochemical tests of *Mezoneuron benthamianum*, *Heliotropium indicum* and *Flabellaria paniculata* ethanolic plant extracts were investigated against characterized *Candida albicans*, *Candida torulopsis*, *Candida krusei*, *Candida glabrata* and *Candida stellatoidea* isolated from human buccal cavity. The zones of inhibition for the whole plant extract of *F. paniculata* range from 12.8 ± 0.30 mm against *C. krusei* to 14.5 ± 0.50 mm observed against *C. albicans* while that of *H. indicum* range between 8.6 ± 0.50mm against *C. torulopsis* to 13.4 ± 0.50 mm observed against *C. glabrata*, and *M. benthamiamun* was from 7.8 ± 0.60 mm against *C. glabrata* to 12.8 ± 0.20 mm against *C. krusei*. Phytochemical tests revealed saponnins, alkaloids, anthraquinones, flavonoids and tannins in *F. paniculata* extracts, and *M. benthamianum* extracts is positive for saponins, anthraquinones, flavonoids and tannins. But, *H. indicum* contained saponins and tannins only. Between 5mg/ml and 8mg/ml was recorded as MIC for *Candida species* against *F. paniculata*. *M. benthamianum* recorded 6-15mg/ml while, *H. indicum* indicated 6-8mg/ml. The highest zone of inhibition in this study was obtained in *F. paniculata* followed by *H. indicum* and *M. benthamianum* consecutively. The conclusion was that there is the need to further investigate and characterize individual phytochemical compounds in these plants and their anti-candidal effect determined.

Keywords *Heliotropium indicum*, *Mezoneuron benthamianum*, *Flabellaria paniculata*, *Candida species*

1. Introduction

Generally, diseased condition may result to irritation, discomfort, malfunction of organ or the overall breakdown of the body system of which may lead to permanent disability or death of the host. The wealth of any nation is hinged on the health of such therefore, medically sick people in any nation needs treatment. Alternative medicine is therapeutic practices, techniques, and beliefs that are outside the realm of mainstream Western healthcare. Alternative medicine emphasizes therapies that improve quality of life, prevent disease, and address conditions that conventional medicine has limited success in curing, such as chronic back pain and certain cancers[1]. Diseases treated by alternative medical therapies include candidiasis among others.

Candidiasis is usually an opportunistic infection that is associated with immunocompromised states such as diabetes, extensive antibiotic usage, malignancies and Human Immunodeficiency Virus (HIV) infection[2]. However, for over two decades now the incidence of *Candida species* has been significant and non-albicans *Candida species* continue to replace *Candida albicans* at most of the clinical sites like bloodstream infections[3].

The healing potential of certain plants have been discovered long before the conception of microbes that characterize them as antimicrobials, which are still in use for the treatment of various maladies to date[4]. Many studies revealed that plants possess substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenol, flavonoids, saponins and tannins which may be soluble in water, ethanol, chloroform, methanol, butanol and other solvents. The characteristics of such contained compounds may then emerge them as a potentially significant therapeutic agent against human pathogens, including bacteria, fungi and virus[5-7]. However, *M. benthamianum, H. indicum* and *F. paniculata*[8] are candidate plants investigated for the treatment of candidiasis in this research.

M. benthamianum is a member of the family *Caesalpinoideae*. The leaves are brightly red when young and dark green when old. The stem is black with reoccurred thorn. The root is brown and hard. *M. benthamianum* has branched shrubs and is a woody climber of up to 8 metres in height. It can be found in waste places of deciduous secondary jungle

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and savannah forest from Senegal to Nigeria[9]. The plant is locally called 'amuranju' or 'jenifiran' in Yoruba land[10]. *M. benthamianum* is used in folk medicine for the treatment of dermal infections and wounds in Ghana. The herbalists show a respectful regard for this plant. The leaves are considered in Senegal to be antiseptic and used in cleaning, 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[10].

H. indicum is a member of the family *Boraginaceae*. It is an annual, erect branched hairsute plant that can reach 15-50cm in height. Its leaves can be opposite or alternate; base decurrent along the petiole is 3-8cm long. Flowers are white with a green calyx, five stamens borne on a corolla tube; a terminal style and a four lobed ovary[8]. It is a common weed in waste places and settled areas; flowering all year round. It is found in Australia, thought to originate from South America and seen as a significant weed in South-East Asia. Also a crop weeds in Asia, Africa and the Caribbean. It is called the cock's comb in Gambia; the French call it herbe a verrues (i.e. plant with warts). In West Africa Ghana it is called Akan-Ashante or Akomfem Atiko. In Guinean-Bissau it is called Manding –Maninka. Datokoro Kombo in Ivory Coast and in Nigeria the efik call it edisimmon, igbo call it ililo isi mwa-eku. The Yorubas call it agogo igun or ogbe akuko. The plant has been locally used to treat inflammatory tumours, leaf-powders applied to dermatoses, especially eczema and impetigo in children in West Africa[11]. In Nigeria and Ghana, the leaf infusion is applied topically to sores, stings, pimples and the sap to gumboils, clean ulcers, to the eyes for ophthalmia, and mixed with castor oil to stings and poisonous bites[12]. Nigerian uses the plant locally to treat umbilical hernia[13].

F. paniculata belongs to the family malpigbiaceae. It is a climbing shrub of 3-15 meters high; the leaves are slivery under surface with white pale pink flowers[14]. It is an herb indigenous to the tropical Africa. The plant is found in wooded savannah riverine desert or forest subjected to flooding distributed across the region from Senegal West Cameroon and Fernanda Po, and across the Congo Basin to Uganda and Tangayika. It is known by Yoruba as 'ajidere'[13]. And also 'ewe aran' in different part of Yoruba land in Nigeria. Commonly called 'manding maninka Conombo' in Senegal, 'lokohebe' in Serria Leone, 'baule in Ivory Coast and in Ghana it is called 'adangme'. Leaves are used on wounds and sores in Ghana[15], and in Nigeria it is used for the treatment of diarrhea and dysentery as well as the treatment of sores and wounds. In Ivory Coast the leaf sap is frequently used as amenorrhea and sometimes in ancebolic[16]. The lainous stem serves as ties in some Ghana hut buildings[17], and the root serve as part of the ingredients used for snake immunization[18].

The objective of this study is therefore to test the antimicrobial effects of *M. benthamianum, H. indicum* and *F. paniculata* on *Candida* species.

2. Materials and Methods

2.1. Sample Collection and Isolation

A total of fifty students (twenty-five males and twenty-five females 400L students of Olabisi Onabanjo University, Nigeria) were randomly sampled from the University in this research. Epstein *et al.*[19] method was employed for *Candida* species isolation by plating on Sabouraud Dextrose Agar (SDA) medium. Plant were collected at Ajibode (behind University of Ibadan, Akinyele Local Government, Ibadan, Oyo state, Nigeria. Authentication was carried out at APZ Department Herbarium at the Faculty of Science, Olabisi Onabanjo University, Ago-Iwoye, Nigeria

2.2. Characterization of Isolate

Physiological and morphological characteristics were employed for the characterization of the Candida organisms. These include germ tube, fermentation utilization of carbon compounds[20], assimilation of organic compound as carbon source[21], growth at 50% or 60% D-glucose, Diazolium blue B (DBB) [20], Urease[21], production of extracellular starch like compounds, production of pseudomycelium formation[22] and starch hydrolysis tests for targets of *Candida* species.

2.3. Extraction and Determination of Phytochemical Constituents of M. Bentamianum, H. Indicum and F. Paniculata

Alade and Irobi[23] method of extraction was employed, plant extract were then placed in the rotary evaporator at 60^{o}C and active ingredients concentrated. Thereafter extracts then kept in sample bottles and refrigerated at 4^{o}C prior to testing. Modified Abo *et al*.[14] method of dilution was employed where sample plant extracts were individually prepared in dimethyl sulphur oxide (DMSO) at 200mg/ml for use.

The following phytochemical tests were carried out on the plant extracts: flavones; tannins with Trease and Evans,[24] method; anthraquinones to indicate the presence of free hydroxyl-anthraquinones and or contains anthraquinones derivatives (combined anthraquinones); phenols by employing Trease and Evans[24]; saponins using Walls *et al.*[25,26] method.

2.4. Antimicrobial Sensitivity Testing of the Plant Extract

Taylor *et al.*[27] method of antibiotic disc diffusion method was employed. Whatman No. 1 filter paper was carefully bored into 6 mm diameter, sterilized and impregnated with 20µl of 200mg per ml of the respective plant extract. Nystatin (OXOID, antibiotic susceptibility test disc) was used at the manufacturer's specification as a positive control. The discs were then placed equidistantly on the agar plates readily seeded with the test *Candida* species (*C. albicans, C. torulopsis, C. stellatoidea, C. parapsilosis and C. krusei)* of 1x10^{6} concentration determined by heamocytometer.

2.5. Determination of Minimum Inhibition Concentration

Minimum inhibitory concentration (MIC) was determined by incorporating various amounts (1–256 mg/ml) of reconstituted plant extract into the medium (Jennifer, 2001). The plates were incubated at 37℃ for 24hrs. The antimicrobial activity was determined by measurement of the inhibition zone. The experiment had three replicates, while the mean values were presented. The MIC was interpreted as the lowest concentration of the extracts that did not permit any visible growth when compared with that of the control. The Negative control was 20µl DMSO solvent. The result of inhibition of the extract was compared with standard antibiotics Nystatin (10 µg)[28].

2.6. Statistical Analysis

Collected data were subjected to analysis using the Statistical Analytical System (SAS) Software version 8 and Microsoft excel 2007.

Table 1. The phytochemical constituents of F. paniculata, M. benthamianum and H. indicum

Phytochemical test	*Flabellaria paniculata*	*Mezoneuron benthamianum*	*Heliotropium indicum*
Saponins	++	+	+
Anthraquinones	+	+	-
Flavonoids	+	+	-
Tannins	+	+	+
Phenols	-	-	-

Key: ++ means strongly present
+ means moderately present
- means not present

3. Results and Discussion

3.1. Phytochemical Constituents

Table 1 shows that *F. paniculata*, *M. benthamianum* and *H. indicum* contain saponin but was strongly present in *F.paniculata* while moderate presence was recorded for *M. benthaminum* and *H. indicum*. Anthraquinones was moderately present in both *F. paniculata* and *M. benthamianum* but, absent in *H. indicum*. Flavonoids also recorded moderately present in *F .paniculata* and *M. benthamianum* but was absent in *H. indicum* (table 1). Tannins were found to be present in the extracts of the three plants in moderate quantities (table1). Phenols were conspicuously absent in the plant extracts (table1)

Effects of M. benthamianum, H. indicum and F. paniculata on Candida species

Flabellaria paniculata: Response of *Candida species* to extracts of *F. paniculata* in table 2 demonstrates inhibition zone of *C. albicans*, *C. torulopsis, C. stellatoidea, C. glabrata and C. krusei* as 14.5 ± 0.50 mm, 13.4 ± 0.50 mm, 13.0 ± 0.90 mm, 14.1 ± 0.40 mm and 12.8 ± 0.30 mm respectively. This result revealed that C. *albicans* has the highest susceptiblity to ethanolic extracts of *F. paniculata.* The results as shown in table 2 demonstrate antimicrobial activity of of *F. paniculata* extract when compared with that of the standard and commercially available drug. The inhibition zone of 14.1 ± 0.40 mm recorded against *C. glabrata* by *F. paniculata* further demonstrate this activity especially when compared with 12.0 ± 0.90 recorded for the standard drug Nystatin.

Heliotropium indicum: Responses of C. albicans, C. *torulopsis, C. stellatoidea, C. glabrata and C. krusei* records 9.3 ± 0.50 mm, 8.6 ± 0.50 mm, 11.3 ± 0.20 mm, 13.4 ± 0.50 mm and 12.4 ± 0.50 mm respectively. This indicates that extracts of *H. indicum* may not possess the strength of antifungal property displayed by *F. paniculata* as indicated in table 2. The results also showed the extracts antimicrobial activity of the extract of *H. indicum* when compared with that of the standard and commercially available drug. The inhibition zone of 13.4 ± 0.50 mm recorded against *C. glabrata* by *H. indicum* further demonstrate this activity especially when compared with 12.0 ± 0.90 recorded for the standard drug Nystatin**.**

3.2. Minimum Inhibitory Concentration

The results obtained by the minimum inhibition concentration (MIC) studies of *F. paniculata* on *Candida species* records 5-8 mg/ml. Thus MIC indicated on *C. albicans* and *C. glabrata* records 5 mg/ml, *C. stellatoidea* and *C. torulopsis* records 8 mg/ml, and *C. krusei* records 7 mg/ml.

MIC studies on *M. benthamianum* revealed 5-15 mg/ml. This study indicates 8mg/ml for *C. albicans and C. stellatoidea,* 15 mg/ml for *C. krusei,* 6 mg/ml for *C. torulopsis* and 5 mg/ml for *C. glabrata. H. indicum* records 6-8mg/ml MIC against the studies *Candida species.* Thus, *C. stellatoidea* and *C. glabrata* indicated 8mg/ml while *C. albicans* and *C. torulopsis* records 6mg/ml and *C. krusei* records 7mg/ml.

Table 2. Effects of *M. benthamianum*, *H. indicum* and *F. paniculata* on *Candida species*

Candida species	Inhibition Zones (mm)				
	Flabellaria paniculata (200mg/ml)	*Heliotropium indicum.* (200mg/ml)	*Mezoneuron benthamianum* (200mg/ml)	Control (DMSO)	Control (Nystatin) (10 µg/ml)
C.albicans	14.5 ± 0.50	9.3 ± 0.50	8.7 ± 0.20	6.0 ± 0.00	17.0 ± 0.90
C.torulopsis	13.4 ± 0.50	8.6 ± 0.50	8.5 ± 0.40	6.0 ± 0.00	16.0 ± 0.90
C.Stellatoidea	13.0 ± 0.90	11.3 ± 0.20	8.3 ± 0.50	6.0 ± 0.00	17.0 ± 0.90
C.glabrata	14.1 ± 0.40	13.4 ± 0.50	7.8 ± 0.60	6.0 ± 0.00	12.0 ± 0.90
C. krusei	12.8 ± 0.30	12.4 ± 0.50	12.8 ± 0.20	6.0 ± 0.00	17.0 ± 0.90

The presence of saponin and tannins in the ethanolic extracts of *Heliotropium indicum* supports the earlier report of Akinlolu *et al*.[29]. However, observation in this study further revealed the presence of alkaloids in the aqueous extract of *Heliotropium indicum*. The antimicrobial activity observed may be attributed to these phytochemicals. This is fully corroborated by Adelaja *et al*.[30] who reported the histo-gastroprotective property of this plant extract on laboratory winster rats and linked the observed results to the presence of saponins, tannis and alkaloids in *Heliotropium indicum*. Also the antimicrobial activity of *M. benthaminum* was found to be high in this study, which was similar to the report made by Dickson *et al*.[31] who reported that the antimicrobial activity of the ethanol extract of *M. benthaminum* was better than that of the petroleum spirit extract and chloroform extract of the plant.

The anticandidal properties of *M. benthamianum* may be due to the presence of saponins, anthracquinones, flavonoids and tannins and the best anticandidal performance displayed by *F. paniculata* extracts among the three plant extracts may be due to the strong saponins indicated in this study. Hence, in *H. indicum* phytochemical analysis, anthracquinones and flavonoids are absent.

The antimicrobial activity of ethanolic extracts of *M. benthamianum* is more effective in this study than that of petroleum spirit and chloroform extracts reported by Dickson *et al*.[31] and in line with this assertion, the ethanolic extract contained saponins, anthraquinones, flavonoids and tannins of which may be responsible for the anticandidal activity observed in the plant.

The minimum inhibition concentration values obtained for the *F. paniculata* plants extract indicates that there is a need for further purification when compared to the values obtained from similar work done by Akinlolu *et al.*[29] where he obtained for *S. aureus* (2 mg/ml) and *P. aeruginosa* (1.75 mg/mI) from a purified extract.

The broad spectrum nature of *F. paniculata* anticandida activity on *Candida species* is in this study may be attributed to the strong presence of saponins in addition to other phytochemicals contained in the ethanolic extracts of the plant. This is also found in Abo and Olugbuyiro[14] studies on bacterial isolates treatment of the plant.

4. Discussion

The presence of saponin and tannins in the ethanolic extracts of *Heliotropium indicum* supports the earlier report of Akinlolu *et al*. (2008). However, observation in this study further revealed the presence of alkaloids in the aqueous extract of *Heliotropium indicum*. The antimicrobial activity observed may be attributed to these phytochemicals. This is fully corroborated by Adelaja *et al*. (2008) who reported the histo-gastroprotective property of this plant extract on laboratory winster rats and linked the observed results to the presence of saponins, tannis and alkaloids in *Heliotropium indicum*. Also the antimicrobial activity of *M. benthaminum* was found to be high in this study, which was similar to the report made by Dickson *et al*. (2006) who reported that the antimicrobial activity of the ethanol extract of *M. benthaminum* was better than that of the petroleum spirit extract and chloroform extract of the plant. The anticandidal properties of *M. benthamianum* may be due to the presence of saponins, anthracquinones, flavonoids and tannins and the best anticandidal performance displayed by *F. paniculata* extracts among the three plant extracts may be due to the strong saponins indicated in this study. Hence, in *H. indicum* phytochemical analysis, anthracquinones and flavonoids are absent.

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Table 3. Minimum Inhibition of *M. benthamianum, H. indicum* and *F. paniculata* on *Candida* species

	C.albicans	*C.torulopsis*	*C.stellatoidea*	*C.glabrata*	*C. krusei*
F. paniculata (20µl of crude extract inhibition zone)	9.0±1.00	6.0±0.5	7.0±0.00	7.0±1.2	7.0±1.8
MIC (mg/ml)	5	8	8	5	7
M.benthamianum (20µl of crude extract inhibition zone)	9.0±0.60	7.0±1.00	8.0±1.20	8.0±0.60	6.0±0.60
MIC (mg/ml)	8	6	8	5	15
H. indicum (20µl of crude extract inhibition zone)	8.0±1.5	7.0±0.60	7.0±1.00	9.0±0.60	7.0±0.60
MIC (mg/ml)	6	6	8	8	7
DMSO (control) 20µl	na	na	na	na	na
Nystatin (control) 20µl of 10µg/ml	17±0.90	16±0.90	17±0.90	12±0.90	17±0.90

Key: MIC = Minimum Inhibition Concentration
na = not applicable

5. Conclusions

In this study, although all the three screened plants exhibits anticandidal activity but, *F. paniculata* appears to have broad anticandidal spectrum than *M. benthamianum and H. indicum*. There is need for more work to be done on the extraction of each phytochemical candidate and their respective antimicrobial or anticandidal studies on the studied plants.

The authors hereby declare that there is no conflict of interest between the authors of this research paper.

Ethical approval is not required for this research work.

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