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REGULAR ARTICLE

ANTIMICROBIAL EFFECTS OF SOME DICOTYLEDONOUS PLANTS ON FUNGAL ISOLATES OF *CANDIDA albicans* AND *TRICHOPHYTON mentagrophyte*

Enaigbe, A.A., **Akpoka, O.A.*, *Okwu, M.U.*, *Izevbuwa, O.E.*, *Ufuah, E.A.*

Address (es): Obhioze Augustine Akpoka,
Department of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada (IUO) Edo State, Nigeria.

*Corresponding author : ausbones@gmail.com; ausbones@iuokada.edu.ng

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ABSTRACT

The preliminary phytochemical screening revealed that, antimicrobial properties of the leaf extracts were due to secondary metabolites such as amino acids, essential oils, flavonoids and saponins contained. The antimicrobial activities of alcoholic extracts were tested against pathogenic fungal isolates of *Candida albicans* and *Trichophyton mentagrophyte*. This was performed by inoculating the isolates into the pure extract, spread onto petri plates containing Sabouraud dextrose agar (SDA) media, observed for growth at stipulated standards. The sensitivity test was done by the disk diffusion method to test the effectiveness of an antimycotic (Griseofulvin) applied on the specific isolates. The minimum inhibitory concentration (MIC) was determined to ascertain the lowest drug concentrations that inhibited the fungal growths. The antimicrobial test revealed that, the leaf extracts of *Eupatorium odoratum* and *Canjanus cajan* inhibited the growths of the organisms while extracts of *Citrus aurantifolia* and *Eucalyptus citriodora* only prevented the growth of *Candida albicans*. The sensitivity test recorded the inhibition zone to range from 11 mm to 32 mm, with the lowest cleared area reported in the extract of *E. citriodora* and the highest in *E, odoratum*. Consequently, the MIC values of extracts at dilution levels were; *E. odoratum*: 1: 10000; 1: 1000, *C. cajan*: 1: 1000; 1: 10000, E. *citriodora*: 1:1000; 1:100 and *C. aurantifolia*: 1: 100000; 1: 100 respectively. This work has confirmed the progressive utilization of plants as antimicrobials for the benefit of mankind, to have originated from microbial sources.

Keywords: Antimicrobial, fungal isolates, leaf extracts Lam, minimum inhibitory concentration and sensitivity test

INTRODUCTION

Antimicrobial plants are described as those with therapeutic active principles or inhibitory chemical substances known to cure ailments or stop the growth and proliferation of microorganisms, by the administration of extracts from whole, parts, juices and exudes of plants. This act of medicinal plants as nature's remedies dates back to creation and first awakening of man when he sought to fight, control and treat infectious diseases and pains. In this concept, herbs form interface between two realms of nature; when humanity and plants meet, a synergistic energy can be created and exchanged (**Lam, 2007**).

The antimicrobial properties are conferred in plants in part by the compounds synthesized by secondary metabolism of plants that might act individually, additively, or in synergy (**Akpoka *et al*., 2019**). **Malhadas *et al*. (2017)** highlighted some compounds with antimicrobial effects to include: protocathechol, a phenolic compound present in onions, avenacin in oat plant, hondatin in barley (*Hordium vulgare)*) linamarin in cassava (*Manihot esculenta*) and dihydroxymethoxybenzonone (DIMBZO) in wheat (*Triticum aestivum)*. Other compounds that have established therapeutic actions are alkaloids, glycosides, tannin, essential oils, saponins, anthraquinones and polyphenols.

Globally, important drugs elaborated from crude plant's extracts are being applied to treat diseases through various mechanisms such as osmotic pressure, disruptions of cell membrane and cell wall by hydrolysis of glycosidic bond, inhibition of protein synthesis by non-transcription of the mRNA. The other modes are, inhibition of nucleic acid synthesis and formation of curling effect of terminal hyphae of fungi, leading to growth retardation and death (**Lam, 2007; Jia *et al*., 2016**).

Consequently, it has been reported that, aqueous alcoholic extracts of *Diospyros bateri* and *Ziziphus abyssinica* showed strong antimicrobial prowess against *Aspergillus niger* and *Candida albicans*. Additionally, aqueous petroleum ether and dichloromethane extracts of bark and leaves of *Citrus aurantifolia*, *Canjanus cajan* and *Vernonia amygdalina* had great antimicrobial actions against *Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger, Trichophyton mentagrophytes* and *Candida albicans* (**Doddmna, *et al*., 2013**).

The value for medicinal plants is gaining more attention today due to huge plant's based active principles, increasing cost of orthodox medicines, ability to respond to specific organ or system of the body (**Mishra, *et al*., 2016**). More so, other needs that promote herbal use include; resistance of certain organisms to drugs, herbal preparations retaining life-giving nutrients such as vitamins and minerals contained in the original plant's composition. These valuable substances cannot be supplied by a single drug, rather one must resort to combined drug intake with its toxicological potential hazards.

In determining the high potency of extracts, sterile water was chosen instead of (or used along) the antimycotic as a negative control. However, such inclusion showed that, any reported antimicrobial activities are a direct product of the extract and not a constituent within the plate and disc. This indicated that, if the test plant used in this study were unavailable, other plants known to possess high antimicrobial active principles could conceivably be considered. This study was aimed to validate the claims by herbal medicine practitioners in their folklores, as to the efficacy of plants' materials in the treatment of various infectious diseases. To formally bring to public domain, the need for people around the globe without access or opportunity to orthodox or foreign drugs that, they could reliably apply herbal extracts as an option. The outcome of this study was to compare the growth inhibitory effects and the lowest concentration of extract dilutions and known antimycotic, on test organisms.

MATERIALS METHODS

Sample Collections

The Test Plants

The test plants screened were: *Vernonia amygdalina, Azadirachta indica, Persia americana, Citrus aurantifolia, Magnifera indica, Eucalyptus citriodora, Eupatorium odoratum,* and *Canjanus cajan*. The leaves used in this investigation were collected from the University of Benin botanical garden and identified in the Department of Pharmacognosy, University of Benin, Nigeria

The Test Cultures

Pure cultures of the test organisms; *Candida albicans* and *Trichophyton mentagrophyte* were obtained from Medical Laboratory Science Department, University of Benin Teaching Hospital (UBTH) Nigeria. The sub-culturing of pure culture was carried out aseptically by transferring a loopful of each of the test organisms into sterile test tubes containing Sabouraud dextrose agar (SDA) and stored at 4 ^{o}C.

Extract Preparation

Fifty grams (50 g) of fresh leaves of each collected plants were washed in distilled water to remove dust particles and insect larvae, dried in an oven at temperature of 40 ^{o}C for 8 hrs. The dried leaves were made to powdered form by using mortar and pestle. Ten Milliliter (10 ml) of 75 % ethanol was added to each of the test powdered dried leaves in each test tube and were placed in hot

water bath to evaporate the ethanol content at temperature of 60 ºC. This process eventually left paste-like substance at the bottom of the test tube. This substance was then diluted with moderate amount of distilled water, shaken thoroughly and finally filtered with Whatman No. 1 filter paper to obtain the complete pure extract into sterile McCarthy bottles and stored at 4 ºC for subsequent use (**Nayan & Shukla, 2011**).

Phytochemical Testing

The extracts were subjected to preliminary testing to detect for the presence of different chemical groups of compounds. Air dried and powdered plant materials were screened for the presence of amino acids, phenols, saponins, glycosides, anthraquinone, tannins and isoflavones as described by (**Jia, *et al.*, 2016**) and modified according to (**AOAC, 2019**).

Antimicrobial Test Properties

The antimicrobial tests were conducted using the test tube technique: Five milliliter of each leaf extract was measured into 2 test tubes, 1 ml each of the 2 test organisms was used to inoculate each of the test tubes and left for 24 hr. Sterile SDA was poured onto pre -sterilized petri plates and allowed to set. These agar plates were then seeded with 0.2 ml of the test organism which was inoculated into the pure extract in the test tubes and spread evenly with a flamed, but cool glass spreader to derive effective growth of a smooth fungal lawn. Finally, the plates were then incubated at 37 ºC for 24 to 48 hr. However, control plates were prepared using distilled water and 2.5 % phenol as negative and positive controls respectively (**Malhades, *et al.*, 2007**).

Sensitivity Test

The extracts with high spectra of activities against the test organisms obtained from the test tube approach of plants' screening were further confirmed for their extent of inhibiting the growth of prevailing organisms by employing the Disc diffusion method. This was conducted by sterilizing the filter paper soaked in an appropriate extract before each was inserted at middle of the plates previously flooded with test organisms. The plates were incubated at 37 ºC for 24 to 48 hrs and the cleared zones of inhibition produced around the epicenter of the plates were observed and measured in diameter (**Cassini *et al.*, 2016; Hans, *et al.*, 2017**).

Minimum Inhibitory Concentration (Mic)

Five milliliters (5 ml) of varying dilutions of the extract was prepared using peptone water as diluents; the serial dilution made ranged from 10^{-1} to 10^{-5} (1: 10 to 1: 100000). In preparing the dilution, 8 test tubes containing antibiotic concentration was prepared and inoculated with standard quantity of each extract. These dilutions were aseptically inoculated with the test organisms and incubated at 37 ºC for 24 to 48 hr. Thereafter, 1 ml of each dilution was pipetted into the 8 SDA media plates, incubated at 37 ºC for 24 to 48 hrs (**Barrow & Felham, 2003**).

RESULTS

The results of the phytochemical screening showed that, the alcoholic extracts of the test plants contained saponins, tannins, glycosides and isoflavones. The antimicrobial activities of the extracts of the test plants were studied against pathogenic fungal strains of *Candida. albicans* and *Trichophyton mentagrophyte.* The leaf extracts of *Eupatorium odoratum* and *Canjanus cajan* inhibited fungal growth (-) or no colonies were observed, while the extracts of *Citrus aurantifolia and Eucalyptus citriodora* inhibited the growth of *Candida albicans only,* with mild growth recorded for *Trichophyton mentagrophyte.* High fungal growth was reported in the extract of *Magnifera indica* against the test isolates (++) (Table 1).

Table 1 Effects of plant leaf extracts on growth rate of fungal isolates

Extracts	*C. albicans*	*T. mentagrophyte*
Vernonia amygdalina	+	+
Azadaricta indica	+	+
Persea americana	++	–s
Citrus aurantifolia	–	+
Magnifera indica	++	++
Eucalyptus citriodora	–	+
Eupatorium odoratum	–	–
Canjanus cajan	–	–
Sterile water	++	++
2.5 % Phenol	–	–

Key: No fungal growth (-) Slight growth (+) High growth (++)

The plant's leaf extracts that indicated high potential of antimicrobial properties; *E. odoratum, C. cajan, E. citriodera,* and *C. aurantifolia* were further screened to determine the level of clearing zone of test fungal isolates using antimycotic drug (griseofulvin) and measured in millimeters (mm).

Table 2 Inhibition zones (mm) of the potential leaf extracts on test fungal isolates

Extracts	*C. albicans*	*T. mentagrophyte*
C. aurantifolia	22	16
E. citriodora	16	11
E. odoratum	32	28
C. cajan	26	28
Distilled water	2.0	1.5
2.5 % Phenol	40	43

The minimum inhibitory concentration (MIC) of a leaf extract is the lowest concentration of the therapeutic agent or chemical substance present in plant, that prevents visible growth of an organism. In the determination of MIC for leaf extracts on the test fungal isolates; *Candida albicans* and *Trichophyton metagrophyte* at lowest extract dilution factor, the MIC was reported as followed: *E. odoratum*: 1:10000; 1:1000, *Cajanus cajan*: 1:10000; 1:10000, *E. citriodora*: 1: 1000; 1: 100 and *C aurantifolia*: 1:100000; 1:100 respectively (Tables 3 - 6).

Table 3 Minimum inhibitory concentration (MIC) of leaf extract (*E. odoratum*) for test fungal isolates

Extracts dilutions	*C. albicans*	*T. mentagrophyte*
1: 10	–	–
1: 100	–	–
1: 1000	–	–
1: 10000	++	–
1: 100000	++	+
MIC	1: 0000	1: 000

Key: No fungal growth (–) Slight growth (+) High growth (++)

Table 4 Minimum inhibitory concentration (MIC) of leaf extract (*C. cajan*) for test fungal isolates

Extract dilutions	*C. albicans*	*T. mentagrophyte*
1: 10	–	–
1: 100	–	–
1: 000	–	–
1: 10000	–	–
1: 100000	+	++
MIC	1: 10000	1: 10000

Key: No fungal growth (–) Slight growth (+) High growth (++)

Table 5 Minimum inhibitory concentration (MIC) of leaf extract (*E. citriodora*) for test fungal isolates

Extract dilutions	*C. albicans*	*T. mentagrophyte*
1: 10	–	–
1: 00	–	–
1: 1000	–	+
1:10000	++	+
1: 00000	++	++
MIC	1: 1000	1: 100

Key: No fungal growth (–) Slight growth (+) High growth (++)

Table 6 Minimum inhibitory concentration (MIC) of leaf extract (*C. aurantifolia*) for test fungal isolates

Extract dilutions	*C. albicans*	*T. mentagrophyte*
1: 10	–	–
1; 100	–	–
1:1000	–	+
1: 10000	–	++
1: 100000	–	++
MIC	1: 100000	1: 100

Key: No fungal growth (–) Slight growth (+) High growth (++)

DISCUSSION

The plants around man's surrounding attracted its attention with the various parts; bark, flowers, fruits, leaves and roots in the long history of human civilization and became known for their nutritional and therapeutic properties, hence formed the basis of medicine. The present study justified the claimed uses of leaves in the traditional approach to curb various ailments (**Akpoka, 2019**).

The results obtained from the phytochemical screening showed that, alcoholic extracts of leaves contained amino acids, essential oils, flavonoids, glycosides and saponins. This confirmed the previous report that, presence of various phytochemicals with active biological principles can be of imperative therapeutic index (**AOAC, 2019**).

In this study, eight dicotyledonous plants were screened against fungal isolates of *Candida albicans* and *Trichophyton mentagrophyte*. The results (Table 1) showed that, the leaf extracts of *Eupatorium odoratum* and *Canjanus cajan* completely inhibited the growths of tested organisms; *C. albicans* and *Trichophyton mentagrophyte*, indicating their effectiveness in preventing the test organisms related infections.

However, the leaf extracts of *Citrus aurantifolia* and *Eucalyptus citriodora* prevented their growths, while slight or moderate growths were noticed for tested organisms. Meanwhile, high growth rate recorded in the extract of *Magnifera indica*, demonstrated the resistance of the fungal isolates to the tested extract. This indicated that, the extract cannot be applied as preventive or treatment measure against pathogenic effects of the test organisms.

The high antimycotic activities of *E. odoratum* (bitter bush) could be due to the endowed Beta cuberine and cadinene and useful in the treatment of wounds, burns, skin diseases and applied to decrease cholesterol and blood pressure levels (**Hamzah, *et al*., 2018**). The *Cajanus cajan* has also been reported to contain dry matter, crude protein and minerals and used in the cure of cough, bronchitis, sore throat infections and diabetics.

Similarly, the *E. citriodora* extract contained 80 % citriodellal, an aldehyde, responsible for its antimicrobial properties, essential oil, a valuable constituent in the treatment of athletic foot disease. The *C. aurantifolia* (Key lime) serves as anticancer, antifungal and antioxidant, protects the liver and heart, prevents urinary tract infections (UTIs) and neutralizes odours (**Nayan & Shukla, 2011; Jia, *et al*., 2016**). Consequently, the *C. albicans* is responsible for vulvovaginal infections, invasive and oropharyngeal candidiasis, while the T. mentagrophyte causes dermatophytosis, impetigo and athletic foot disease (*Tinea pedis*) (**Doddmna, *et al*., 2013**).

Avelar-Pires, *et al*. (2004) had previously indicated the ability of hydroalcoholic extract of the whole plant of *Xanthoriza simplissima* to exhibit good activity against the acquired immune deficiency disease syndrome (AIDS) related opportunistic pathogens; *Candida albicans, Mycobacterium intracellularae* and *Crystococcus neoformatis*. However, the bioassay fractionation of the extract led to the isolation of the known alkaloid as the major active component.

The growth inhibition zone measured ranged from 11 to 32 mm for the test organisms. However, the lowest cleared area on the plate was recorded in the extract of *E. Citriodora* (11 mm) and the highest approximated killed zones were in the extracts of *E. odoratum* and *C. cajan* (32 mm and 28 mm respectively (Table 2). This illustrated the rich potent chemical principles contained in extracts of these leaves, as the most active agents in curbing the spread and proliferation of the tested isolates amongst the surveyed extracts. Nevertheless, the low and high inhibition levels obtained from the controls: sterile water (negative control) and 2.5 % Phenol (positive control) relatively confirmed the presence of compounds to be responsible for antimicrobial activities.

The minimum inhibitory concentration (MIC) described as the lowest concentration of chemical (drug) that prevents visible growth of an organism is considered to indicate that; lower MIC values meant that, less drug is required to inhibit microbial growth. Therefore, drugs with lower MIC scores are more effective antimicrobial agents (**Lam, 2007; Mishra., 2016).**

The findings revealed that, *E. odoratum* and *Canjanus cajan* were considered extracts, with high potential antimicrobial properties against *C. albicans* and *T. mentagrophyte* (1:1000; 1:10000) extract dilutions respectively) (Tables 3 and 4). Consequently, the MIC for *E. citriodora* against test organisms were 1:1000; 1:00 (Table 5). This showed that, this extract concentration is more potent on *C. albicans* (lower value) than the *T. mentagrophyte*. Succinctly put, the extract of *C. aurantifolia* exhibited MIC at the lowest dilution level (1: 100000) and completely cleared the *C. albicans* isolate (Table6). This showed that, low concentration of this extract is very effective and therefore can be considered for the treatment of infectious diseases caused by this organism.

CONCLUSION

The alcoholic extracts of *E. odoratum* and *C. cajan* could be administered to cure the clinically pathogenic effects of the isolates, while the extract of *C. aurantifolia* is recommended for the treatment of candidiasis associated diseases. Hence, this study has validated the claimed importance of plants in the herbal system of health care services to humanity. It will also form the primary criterium for selection of plant species in further analysis, for the potential need of recent natural bioactive components. It is recommended that the use of plant extracts from *Eupatorium odoratum, Cajanus cajan* and *Citrus aurantifolia* should be considered as therapeutics in the cure for candidiasis and dermatophytosis diseases caused by the test organisms.

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