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). Malhardas et al. (2017) highlighted some compounds with antimicrobial effects to include: protocatechol, a phenolic compound present in onions, avenacin in oat plant, hondatin in barley (Hordeum vulgare) linamarin in cassava (Manihot esculenta) and dihydroyxymethybenzone (DMBZO) 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 batei 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, Canjana cajan and Vernonia amygdalina had great antimicrobial actions against Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger, Trichophyton mentagrophytes and Candida albicans (Doddmina, et al., 2013). The test plants screened were: Vernonaria amygdalina, Azadirachta indica, Persia americana, Citrus aurantifolia, Magnifera indica, Eucalyptus citriodora, Euqatorium odoratum, and Canjana 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.
water bath to evaporate the ethanol content at temperature of 60 °C. 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 °C 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 milliliters 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. The 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 incubated at 37 °C 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 °C 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 °C for 24 to 48 hr. Thereafter, 1 ml of each dilution was pipetted into the 8 SDA media plates, incubated at 37 °C 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       | +           | +                |
| Azadiricta indica         | +           | +                |
| Persea americana          | ++          | −                |
| 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. citrioderia, and C. aurantifolia were further screened to determine the level of clearing zone of test fungal isolates using antymycotic 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. aurantifolia           | 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 mentagrophyte at lowest extract dilution factor, the MIC was reported as followed: E. odoratum: 1:10000; 1:1000, Canjanus cajan: 1:10000; 1:100000, 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: 10000    | 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: 1000            | −           | −                |
| 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           | ++          | +               |
| 1:000000          | ++          | ++              |

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           | –           | ++              |
| 1:1000000         | –           | ++              |

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 dicotyledoned 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 antmycotic activities of E. odoratum (bitter bush) could be due to the endowed Beta cubicine and cadine and useful in the treatment of wounds, burns, skin diseases and applied to decrease cholesterol and blood pressure levels (Hamzah, et al., 2018). The Caujanus 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 diabetes.

Similarly, the E. citriodora extract contained 80 % citrodellal, 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 a protective and therapeutic profile of dermatophyte; Annual Brazilian Dermatophytes, 89(2), 259 - 256, http://doi.org/10.1590/s1806-4841.2014.2569

Barrow, G. L. & Feltham, R. K. A. (2003). Cowan and Steel’s Manual of Medical Microbiology. (3rd ed., pp. 352). Cambridge University Press.

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. Suddenctly put, the extract of C. aurantifolia exhibited MIC at the lowest dilution level (1: 100000) and completely cleared the C. albicans isolate (Table 6). 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 clinical 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 criterion 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, Cajunus cajan and Citrus aurantifolia should be considered as therapeutics in the cure for candidiasis and dermatomycosis diseases caused by the test organisms.

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