Pharmacognostic Evaluation, Phytochemical Screening and Anti-Candidal Activities of Leaves of Some Medicinal Plants against *Candida albicans*

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Abstract

Three Nigerian medicinal plants *Psidium guajava* Linn, *Senna siamea* Lam, and *Senna obtusifolia* L were investigated to determine the pharmacognostic characteristics, phytochemical contents and their activities against *Candida albicans*. The fresh leaves of each plant were examined for their microscopic characters and then processed. The dried ground leaves were extracted using water and ethanol. The extracts were subjected to phytochemical screening and their anticandida activities were determined using paper disc diffusion method at concentrations of 25mg/ml; 50mg/ml; 100mg/ml; 200mg/ml and 400mg/ml with Ketoconazole as control. The study indicated that the plant extracts contained alkaloids, anthraquinones, flavonoids, saponins, steroids, tannins and terpenoids. Glycoside was found to be absent. The extracts of the plants showed varying mean zones of inhibition (MZI) against *C. albicans* ranging from 6.00±0.00 to 16.67±1.53mm. The Minimum Inhibitory Concentration (MIC) of the extracts ranged from 10mg/ml to 20mg/ml while Minimum Fungicidal Concentration (MFC) ranged from 20mg/ml to >80mg/ml with *P. guajava* having the least MIC and MFC of 10mg/ml and 20mg/ml respectively. The results indicated that MZI of the aqueous and ethanol extracts of *P. guajava* at concentration of 400mg/ml had no significant differences at p ≤ 0.05. Further research is recommended for possible combinations of lower doses of the extracts to study their synergistic effects on *C. albicans* and to explore other potentials of the plants against other pathogens.

**Keywords:** Antimicrobial, Inhibition, Pharmacognostic, Phytochemical, *Psidium guajava*, *Senna siamea*, *Senna obtusifolia*.

Introduction

Medicinal plants are rich sources of antimicrobial agents. Plants are the primary source of basic materials utilised for curing a wide range of human illnesses and disorders, as such people are turning to natural drugs, which are mostly found in the form of medicinal plants (Nakar et al., 2017). The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientists worldwide. Olivera et al. (2024) also stated that plants have been used successively as antioxidant, anti-inflammatory, anticancer, and possess antimicrobial properties. Many investigations are
being conducted on medicinal plants based on information supplied by the local populations to find out phytochemical constituents for application in the prevention and treatment of infectious diseases and other diseases of non-microbial aetiology. Some higher plant products have attracted the attention of microbiologists to search for some phytochemicals for their exploitation as antimicrobials such plant products would be biodegradable and safe for human health (Wang et al., 2010; Reddy et al., 2022). Despite vast improved health and longevity in the United States and Europe, millions of their people are turning back to traditional herbal medicine to prevent or treat many illnesses (World Health Organization [WHO], 2023) and to circumvent resistance of many human pathogens to conventional drugs (antibiotics), some of which have side effects like hypersensitivity and immune-suppression.

The incidence of fungal infections has increased significantly in the last 20 years (Alam, 2009). Yeast infections are among the ailments treated. The most common yeast infection encountered in Nigeria is vaginal candidiasis. The yeasts commonly encountered are Candida albicans, Candida tropicalis, Candida glabrata, and Candida krusei (Xiao et al., 2015). The incidence of Candida albicans is higher than those of other Candida species. The majority of clinically used anti-fungal drugs have various drawbacks in terms of toxicity, efficacy, and cost, and their frequent use has led to the emergence of resistant strains. The challenge has been to develop effective strategies for the treatment of candidiasis and other fungal diseases. Candidiasis is an infection whose causative agent Candida albicans is the most important human pathogenic fungus. Unfortunately, the limited numbers of antifungal agents available in the market are toxic and expensive and Whaley et al. (2017) pointed out that C. albicans has developed resistance to commonly used antifungals. Plants generally are known not to have side effects when used by man even when used at high doses.

Psidium guajava (Plate I) is a spreading tree of the Myrtaceae family, commonly called guava, today is considered minor in terms of commercial world trade but is widely grown in the tropics, enriching the diet of hundreds of millions of people in the tropics of the world (Patel et al., 2021). Senna siamea Lam. (Plate II) also called Cassia siamea Lam. is a member of the family Fabaceae or Caesalpiniaceae. Senna is an Arabian name, and the herb was first brought into use by the Arabian physicians Serapion and Mesue (Bernard, 2006), and was introduced to Africa from tropical Asia. It is widely grown throughout tropical Africa. Senna siamea Lam is a non-nitrogen-fixing leguminous tree, the plant is commonly called Thailand shower, minjiri, or kassod and has many regional names (F/FRED, 1994). This plant is a medium-sized shrub, 10-12 m in height, occasionally reaching 20m. The bole is short; crown dense and rounded at first, later becoming irregular and spreading. Chinsembu (2015) indicated that Leaves and bark of medicinal plants were reported to be used locally as antimalarial medications. Studies noted that S. siamea could be useful in candidosis and against the growth of fungi in agricultural products. The flowers and young fruits were used as curries (Kiepe, 2001).
Senna obtusifolia also called Cassia obtusifolia L. (Plate III) is of the family Fabaceae (alt. Leguminosae, subfamily Caesalpinioideae). The stems are erect, branched, lack hairs (glabrous) and can reach heights of 1 - 6 feet. Sicklepod has alternate leaves comprised of 4 - 6 leaflets that are egg-shaped and arranged pinnately compound, which means that the leaflets are opposite to one another. The leaf decoction is used for the treatment of scorpion stings, gingivitis, urinary tract infections, dysentery and diarrhoea in Nigeria, as traditional fever remedies in Zimbabwe and for the treatment of cough in Tzeital Maya (David, 2002). In Nigeria, the seeds, leaves, and roots of S. obtusifolia possess' laxative effects. However, the weedy nature and the toxic properties require caution (Irwin and Barneby, 1982).

Candidiasis is a fungal infection that can affect areas such as the skin, genitals, throat, mouth, and blood. It is caused by the overgrowth of a type of yeast called Candida, usually Candida albicans. This yeast is normally found in small amounts in the human body. The most common type of candidiasis is a superficial infection of the mouth, vagina, or skin that causes white or red patches and itching, irritation, or both. Kashem and Kaplan (2016), also found that superficial infections of skin and mucosal membrane by Candida causing local inflammation and discomfort are common. This study was aimed at evaluating the pharmacognostics characteristics through investigating phytochemical constituents and establishing the antifungal activities of the leaves of Psidium guajava, Senna siamea, and Senna obtusifolia against Candida albicans.

Materials and Methods
Collection of Plant Materials
The fresh leaves of the medicinal plants used in this study (Psidium guajava, Senna siamea, and Senna obtusifolia) were collected in clean polyethylene bags from Abubakar Tafawa Balewa University (ATBU) and environment at Yelwa area in Bauchi Local Government of Bauchi state, Nigeria. The location of the university and environment within latitude N0°16’45.8” and longitude E9°47’38.6” was determined using Etrex Garmin GPS (Model No: 16Q720250). The collected samples were taken to the Department of Biological Sciences herbarium of ATBU and were identified with the help of relevant keys (Dalziel, 1937) and personal communication with an expert (Gani, 2013) to be herbarium specimens with voucher numbers Psidium guajava (96/2466), Senna siamea (89/2097), Senna obtusifolia (89/2277).

Pharmacognostic Studies
Macroscopic observation of the fresh fully expanded leaves of the plants was done by observing the external characters with the help of a magnifying hand lens (Kokate et al., 2013). The colour, shape and surface characters were examined with the aid of features described by Gill (1988), the size was determined by measuring the length and breadth with a ruler. Microscopic studies were carried out from transverse sections of fresh leaflets. The leaves were cleaned and dried. A leaf was laid out on the working surface and about 2.5cm section was cut crosswise out of the centre, slicing across a section of the vein, using a razor
blade. Starting at one of the short ends of the strip (the edges that was not cut), the leaf section was tightly rolled and carefully several thin slices were made off one end of the roll with a razor blade. Each of the slices is a cross-section. A very thin slice was picked and placed on a plain slide with the inner part of the leaf section facing for the inner cells to be visible. A wet mount was made by adding a drop of water over the leaf section, then covered with a coverslip and observed under a microscope for the identification of various tissues and their arrangement. The cells surrounding the central vein of the leaf were viewed. The microphotographs of sections were taken (Kokate et al., 2013).

**Processing and Preparation of Ethanol and Aqueous Extract from the Leaves of the Plants**

The fresh leaves were thoroughly washed with tap water, air-dried under room temperature of 25°C for 5 days, and pulverized using mortar and pestle into fine powder. All powdered samples were sieved with No.10 sieve and stored at room temperature. The method described by Gbadamosi and Egunyomi (2010) was used for aqueous extraction. The air-dried powdered plant samples - 10.0 g of each were soaked in 100 ml of distilled water in 500ml sterile conical flasks. The flasks were covered with cotton wool plugs, wrapped with aluminium foil, and shaken vigorously at intervals for 48 hours at room temperature. The crude extract was then filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness or concentrated with a rotary evaporator at 40°C. Further concentration was done on a water bath at 40°C after which extracts were transferred into an air-tight sample bottle pending further analysis. About 500 g of each powdered sample was extracted in 1.5 litre of ethanol (95 % w/v) for 24 hours using Soxhlet apparatus. The extracts were transferred into the sample holder of the rotary vacuum evaporator, where the extracts were concentrated to dryness at 40°C and then air-dried to constant weight. The extracts were refrigerated at 4°C before use. The extracts were subjected to various preliminary phytochemical tests to determine the active constituents present in the crude extracts, following standard procedures (Sofowora, 1993; Trease and Evans, 2009).

**Test for alkaloids:** To 5mL of 1% aqueous hydrochloric acid, 0.5 g of each extract was added and stirred on a steam bath. About 1ml of the filtrate was treated with a few drops of the Dragendorff’s reagent. An orange or red precipitate with this reagent indicated the presence of alkaloids.

**Test for Anthraquinones:** 0.5 g of each plant extract was boiled with 1 ml of 10% sulphuric acid and filtered while it was hot; 2.5 ml of benzene was added to the filtrate and shaken. The benzene layer separated and half its volume (1.25ml) of 10% ammonia solution was added. The presence of a pink, red, or violet colour in the lower ammonia phase indicated the presence of anthraquinones.

**Test for Flavonoids:** To 0.5 g of each extract, a few pieces of magnesium strips were added and mixed with 2ml of concentrated HCl. A faint orange or brown colour of the effervescence solution indicated the presence of flavonoids.
Test for Glycoside (Keller-kiliani test): 0.5g of each extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then underlaid with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of digitalis glycoside.

Test for Saponins: To 0.5 g of each plant extract, 5ml of distilled water was added and shaken in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

Test for Steroids: About 1ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for Tannins: To 0.5 g of the plant extract, 1ml of distilled water was added and stirred, filtered and ferric chloride reagent was added to the filtrate. A blue-black or blue-green precipitate was taken as evidence for the presence of tannins.

Test for Terpenoids (Salkowski test): 10 mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Concentrated H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

Collection and Confirmation of Test Organism
Isolates of Candida albicans with stock number 828 were collected from the Department of Microbiology of Abubakar Tafawa Balewa University Teaching Hospital, Bauchi. The isolates were confirmed by putting about 3 drops of serum into a small glass tube. Using a sterile wire loop, a colony of yeast was picked and gently inoculated (emulsified) in the serum. It was incubated at 37°C for 2 to 3 hours but not longer. A drop of the serum was placed onto a slide and covered using an over slip then it was examined microscopically using x 40 objective (Haley and Callaway, 2001). The isolates were maintained on Sabouraud dextrose agar slants prepared according to the manufacturer's specification at 4°C prior to use.

Preparation of Stock Solution of Extracts and Inocula
Stock solutions were prepared from the plant extract by weighing 0.4g of the extract and dissolved in 5.0ml of Dimethyl sulfoxide (DMSO), making a solution of 80mg/ml from where serial dilutions of different concentrations by dissolving 0.5ml of the 80mg/ml in 0.5ml of DMSO to get 40mg/ml. Appropriate dilutions were made to get 20mg/ml, 5mg/ml, and 25mg/ml. This was done for each of the plant extract.

Antifungal Screening of the Extracts
Anti-candida activities of the aqueous and ethanol extracts of the plant sample were evaluated by the paper disc diffusion method (Aida et al., 2001). Candida albicans was first reactivated by inoculating into freshly prepared peptone water and incubated for 18 – 24 hours at 37°C. Cultures of C. albicans in a sterile solution of 0.9% normal saline already
adjusted to 0.5% MacFarland standard were inoculated onto Sabouraud Dextrose Agar plates. Sterilized filter paper discs (Whatman No. 1; 6 mm in diameter) soaked in the different test tubes containing the dissolved extracts of different concentrations was taken out with sterilized forceps, air-dried, and placed on plates with the organisms. The plates were incubated for 24 hours at 37°C. The susceptibility of the fungi to the standard drug was tested by placing discs seeded with ketoconazole on each plate of different concentrations; this was used as positive control while sterilized paper discs with DMSO were used as negative controls for all the plates. The experiment was performed in triplicates. Anticandidal activity were determined by measurement of zone of inhibition around each paper discs and results for that of the antifungal agent was compared with those of the plant extract (Dhiman et al., 2011). The results were reported as mean of zones of inhibition for each plant extract.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Plants’ Extracts on Candida albicans
Minimum inhibitory concentration was determined on susceptible isolates. Broth dilution method was used for the determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) (Cheesbrough, 2000). The extracts were serially diluted to give concentrations of 80, 40, 20, 10 and 5 mg/ml in test tubes. To 1ml of varying concentrations of the extracts, 4ml of nutrient broth was added and then the tubes were inoculated with 0.5ml of candida suspension previously diluted to10^8 cfu/ml in the saline solution. The procedure was repeated on the test organisms using the standard antifungal drug (ketoconazole). A tube containing nutrient broth only was seeded with the test organisms as described above to serve as a positive control. Tubes containing fungal cultures were incubated for 24 hours at room temperature (35±2°C). After incubation, the tubes were then examined for microbial growth by observing for turbidity. The test tube with the least concentration of extracts which showed no turbidity indicates the MIC.

Determination of Activity Index
The activity index was used to compare the inhibitory effect of the extracts and standard antifungal drugs. This was calculated as the mean zones of inhibition for the crude (test) extracts divided by the mean inhibition zones for standard drugs (Singh et al., 2002, Adegoke and Adebayo-Tayo, 2009).

\[
\text{Activity index (AI)} = \frac{\text{Mean zone of inhibition of test sample}}{\text{Mean inhibition zone for standard drug}}
\]

Results
The pharmacognostic evaluation of the fresh leaves showed different macroscopic features presented in Table 1 and the morphological features are shown in Plates I to III while the microscopic features of the leaves were presented in Plates IV and V. The phytochemical screening of the plant extracts showed the presence of some chemical components, both
in the aqueous and ethanol extracts (Table 2). The phytochemicals found vary among the plant extracts while some were similar.

**Table 1: Macroscopic Characteristic of the Three Fresh Leaves**

<table>
<thead>
<tr>
<th>Parameter</th>
<th><strong>Psidium guajava</strong></th>
<th><strong>Senna siamea</strong></th>
<th><strong>Senna obtusifolia</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Yellow green</td>
<td>Dull green</td>
<td>Yellow green</td>
</tr>
<tr>
<td>Shape</td>
<td>Ovate to oblong elliptic</td>
<td>Oblong</td>
<td>Obovate</td>
</tr>
<tr>
<td>Length(mm)</td>
<td>117 – 132</td>
<td>74 – 76</td>
<td>43 – 51</td>
</tr>
<tr>
<td>Breadth(mm)</td>
<td>55 – 64</td>
<td>22 – 25</td>
<td>27 – 29</td>
</tr>
<tr>
<td>Apex</td>
<td>Obtuse to acuminate</td>
<td>Emarginate</td>
<td>Obtuse</td>
</tr>
<tr>
<td>Veins</td>
<td>Reticulate</td>
<td>Reticulate</td>
<td>Reticulate</td>
</tr>
<tr>
<td>Margin</td>
<td>Entire</td>
<td>Entire</td>
<td>Entire</td>
</tr>
<tr>
<td>Base</td>
<td>Rounded to Subcuneate</td>
<td>Acute</td>
<td>Oblique</td>
</tr>
</tbody>
</table>

**Plate I:** The morphological features of the fresh leaf of *Psidum guajava*
Plate II: The morphological features of the fresh leaf of *Senna siamea*

Plate III: The morphological features of the fresh leaf of *Senna obtusifoli*

Plate IV: Microscopic features of midrib of leaf of *Psidium guajava* (T. S of the midrib)
Table 2: Phytochemical Contents of the Leaves’ Extracts of the Sample Plants

<table>
<thead>
<tr>
<th>Plants</th>
<th>Alkaloids</th>
<th>Anthraquinones</th>
<th>Flavonoids</th>
<th>Glycosides</th>
<th>Saponins</th>
<th>Steroids</th>
<th>Tannins</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psidium guajava</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(Aqueous extract)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td><em>Psidium guajava</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(Ethanol extract)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Senna siamea</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(Aqueous extract)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Senna siamea</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(Ethanol extract)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Senna obtusifolia</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(Aqueous extract)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Senna obtusifolia</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(Ethanol extract)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

KEY: + Present; - Absent

Anti-Candida Activities of the Aqueous and Ethanol Extracts of the Plants on the Test Organism

The findings of the anti-candida activities of the aqueous and ethanol extracts of the plants are presented in Figures 1 to 3 and Table 3. The plants’ extracts were shown to exhibit varying inhibitory profiles on *Candida albicans*. Out of the six extracts, the aqueous extracts of *Psidium guajava* exhibited the highest anti-candida activity with mean zones of inhibition ranging from 7.00mm to 17.00mm followed by the ethanol extracts of *Psidium guajava* with mean zones of inhibition ranging from 8.00mm to 16.00mm then aqueous extracts of *Senna siamea*, then ethanol extract of *Senna siamea*. *Senna obtusifolia* showed the least mean zones of inhibitions both for its aqueous and ethanol extracts.
Figure 1: Antifungal effects of the aqueous extract of three plants on *Candida albicans*

Figure 2: Antifungal effects of the ethanol extracts of three plants on *Candida albicans*
Figure 3: Comparative effects of the plant extracts on *Candida albicans*

Table 3: Anticandidal activity of different concentrations of leaf extracts of *Psidium guajava*, *Senna siamea* and *Senna obtusifolia*

<table>
<thead>
<tr>
<th>Concentration in mg/ml</th>
<th>Mean zones of inhibition in mm of Aqueous extract</th>
<th>Mean zones of inhibition in mm of Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. guajava</em></td>
<td><em>S. siamea</em></td>
</tr>
<tr>
<td>25</td>
<td>7.33±1.15&lt;sup&gt;npq&lt;/sup&gt;</td>
<td>7.00±1.00&lt;sup&gt;npq&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>8.67±0.58&lt;sup&gt;klmnp&lt;/sup&gt;</td>
<td>8.00±0.00&lt;sup&gt;mnpp&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>10.33±0.58&lt;sup&gt;ghdl&lt;/sup&gt;</td>
<td>12.00±1.00&lt;sup&gt;defg&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>14.33±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.67±0.58&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>400</td>
<td>16.67±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.33±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are means ± Standard Deviation (SD) of three replicates. Values with different superscripts along row and column are statistically different (p < 0.05). Means with same alphabets as superscripts are similar.
At low concentration of the extracts *S. siamea* showed very low inhibition zone while *S. obtusifolia* at low concentration showed no measurable zone of inhibition except for the size of the disc which is 6mm. The control (ketoconazole) had MZI of 21.33mm. The anti-candida activities of the plant samples were observed to be concentration and plant-species-dependent.

**Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Leaves’ Extracts of the Plant Samples**

The Minimum Inhibitory concentration (MIC) and Minimum Fungicidal Concentration (MFC) were shown in Table 4. The MIC values varied from 40mg/ml to 10mg/ml.

**Table 4:** Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Plants’ Extracts against *Candida albicans*

<table>
<thead>
<tr>
<th>Plants</th>
<th>MIC (mg/ml)</th>
<th>MFC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psidium guajava</em> (aq)</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><em>Psidium guajava</em> (eth)</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td><em>Senna siamea</em> (aq)</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td><em>Senna siamea</em> (eth)</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td><em>Senna obtusifolia</em> (aq)</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td><em>Senna obtusifolia</em> (eth)</td>
<td>40</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Control (Ketoconazole)</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

**Activity Indices of the Plants’ Extracts**

The activity index of the extracts against the standard antifungal agent is presented in Table 5. Using ketoconazole as standard, all the plant extracts had activity indices less than unity (AI<1) on *Candida albicans*.

**Table 5:** Activity Indices of the Extracts against Commercial Antifungal Agent, ketoconazole

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th><em>P. guajava</em> (Aqueous)</th>
<th><em>S. siamea</em> (Aqueous)</th>
<th><em>S. obtusifolia</em> (Aqueous)</th>
<th><em>P. guajava</em> (Ethanol)</th>
<th><em>S. siamea</em> (Ethanol)</th>
<th><em>S. obtusifolia</em> (Ethanol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.34</td>
<td>0.33</td>
<td>0.28</td>
<td>0.38</td>
<td>0.31</td>
<td>0.28</td>
</tr>
<tr>
<td>10</td>
<td>0.41</td>
<td>0.38</td>
<td>0.38</td>
<td>0.48</td>
<td>0.36</td>
<td>0.31</td>
</tr>
<tr>
<td>20</td>
<td>0.48</td>
<td>0.56</td>
<td>0.41</td>
<td>0.50</td>
<td>0.50</td>
<td>0.42</td>
</tr>
<tr>
<td>40</td>
<td>0.67</td>
<td>0.64</td>
<td>0.53</td>
<td>0.59</td>
<td>0.61</td>
<td>0.47</td>
</tr>
<tr>
<td>80</td>
<td>0.78</td>
<td>0.67</td>
<td>0.61</td>
<td>0.73</td>
<td>0.64</td>
<td>0.59</td>
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**Discussions**

The fresh leaves of the three plants were different based on their sizes, shapes, apexes as presented in Table 1. The macroscopic and microscopic description of a medicinal plant is
the first step towards establishing its identity and serve as a tool for developing standards for identification, quality and purity (WHO, 2002).

The results of the phytochemical screening of the plant extracts (Table 2) have revealed the presence of saponin, steroids, tannins, terpenoids, flavonoid, alkaloid and anthraquinones. These active phytochemicals are known for their medicinal activity as well as physiological actions; as such they confer the therapeutic potentials of all medicinal plants. The leaves of guava (*Psidium guajava*) are rich in flavonoids; much of guava's therapeutic activity is attributed to these flavonoids (Naseer et al., 2018).

Antibacterial and antifungal compounds isolated from leaves of *P. guajava* showed that in the polar extract (alcohol), flavonoids such as quercetin and its glycosides derivates are responsible for the strong antibacterial activity, including against *C. albicans* (Arima and Danno, 2002; Metwally et al., 2010). Phytochemical screening of the aqueous extract of *Senna siamea* revealed the presence of alkaloids, tannins, saponins, glycosides, steroids, phenols, and flavonoids as reported by Dahiru et al. (2013). The observed results were also consistent with the findings by Naseer et al. (2018) who also confirmed the presence of tannins and steroids.

The toxic principles of the plant include anthraquinones, emodin glycosides, toxalbumins, and alkaloids (Majorie, 1999). The results of the phytochemical screening of *Senna obtusifolia* indicated the presence of alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids. This is similar to the work of Sudi et al., (2011), who carried out a study on the preliminary qualitative phytochemical screening of *Senna obtusifolia*. The results revealed that all the plant extracts had varying degrees of inhibition against *Candida albicans*. The aqueous extracts gave a higher inhibition than the ethanol extracts. The plants were found to possess significant antifungal properties (Minimum Zones of Inhibition) at p≤0.05 against *Candida albicans* at averagely 20mg/ml (Table 3). The highest activities were exhibited by aqueous extract of *Psidium guajava* and the least by *Senna obtusifolia*. These results corroborated with that reported by Fugaban-Hizon (2016) that *Psidium guajava* has also demonstrated antifungal, anti-yeast (*Candida*), anti-amoebic, and antimalarial actions. *Senna siamea* have exhibited anticandidial activity at 100 mg/ml (Prabhakar et al., 2008) and the MIC of the plants ranged from 10mg/ml to 40mg/ml (Table 4). The aqueous and ethanol extracts of *Psidium guajava* were found to be the best at 400mg/ml with 16.67±1.53mm and 15.67±0.58mm Zones of Inhibition respectively when compared with others statistically at p≤0.05 (Table 3). All the plant extracts had activity indices less than unity (AI<1) though most were more than half (Table 5).

This work is significant since it assessed the pharmacognostic characteristics of *Senna siamea*, *Senna obtusifolia*, and *Psidium guajava* leaves to facilitate simple plant identification. The phytochemical study of these leaves also demonstrated potent anti-*Candida albicans* activity.
Conclusion
The leaves of *Psidium guajava*, *Senna siamea*, and *Senna obtusifolia* have been known to be medically important in many ways. Their pharmacognostic features are standards which are useful in identification and authentication of these plants as drugs. The photochemical screening of the leaves extracts of these plants have revealed the presence of alkaloids, anthraquinone, flavonoids, saponins, steroids, tannins, terpenoids. Plants are sources of secondary metabolites and leaves extracts of *Psidium guajava*, *Senna siamea* and *Senna obtusifolia* showed antifungal activities against *Candida albicans*. The antifungal activities are due to the presence of the above mentioned secondary metabolites. Among the 6 plant extracts, the aqueous extract of *Psidium guajava* was the most potent irrespective of the solvent used for extraction thus the extracts still possess antifungal properties. However, it is important to point out that the extracts of *Psidium guajava*, *Senna siamea* and *Senna obtusifolia* are potential agents against pathogenic yeast cells like *Candida albicans*.

Recommendations
1. There is need for further works to carry out research into possible combinations of plant extracts, that would give a better and more exploitative effects by synergistic or drug additive combinations in a single therapeutic formulation. This will give an advantage for a better, low dosage, and effective natural product than synthetic drugs
2. There is a need for further studies on these plants to assess other potential effects like anti-cancer, anti-helminthes and so forth. Also, the various combinations in which these plants can be used against *C. albicans* and other infectious pathogens.
3. Further studies are necessary for the formulation of herbal nutritional supplements from these plants. As these can help to improve the immune system such that the body can resist infections.
4. There is a need for further studies to establish the potentials of these plants against other fungal species or isolates since only *Candida albicans* were tested in this study.
5. The development of a better exploitable way to use aqueous extract of plants in the production of herbal drugs is necessary. Water is affordable and readily available compared to other extracts media like ethanol and methanol.
6. Further studies are necessary to test these plants against *C. albicans in-vivo* and also to assess the toxicity and safety of these plants’ extracts.
7. Since the leaves of these plants have shown pharmacognostic properties, like the root, stem, and seed. It is important to use the leaves for research and drug preparation instead of other parts of the plants. This will reduce deforestation and likelihood of over exploitation of the plants.
8. Also, tincture, ointment, cream and soap could be prepared from the extracts of the plants for treatment of candidiasis and fungal infections of the skin.
References
Gani, I. (Personal Communication, 6th May, 2013.)


