# Étude phytochimique antimicrobienne et préliminaire des feuilles et des fleurs de Psydrax subcordata (DC.) bridson (Rubiaceae)

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**RESUME**

**Contexte :** La nécessité d'un agent antimicrobien plus sûr et plus efficace est incontestablement nécessaire, car la résistance aux antibiotiques est un phénomène grave et croissant dans la médecine contemporaine et est devenue l'une des principales préoccupations de santé publique du XXIe siècle.

**Objectifs :** L'étude a été conçue pour étudier l'utilisation folklorique de Psydrax subcordata comme plante antimicrobienne.

**Méthode :** La fraction d'acétate d'éthyle et l'extrait de méthanol des feuilles et des fleurs séchées respectivement ont été examinés pour leurs phyto-constituants à l'aide des méthodes de dépistage phytochimiques standard. Les activités antibactériennes et antifongiques ont été évaluées à l'aide de la méthode de diffusion de puits d'agar tandis que les concentrations inhibitrices minimales et les concentrations bactéricides minimales ont été déterminées à l'aide de la méthode de dilution d'agar.

**Résultats :** Les extraits de P. subcordata contiennent des flavonoïdes, des tanins, de la saponine, des sucres réducteurs et du 2, 6- didésoxy-sucre. Le test antibactérien a montré une activité importante avec des zones d'inhibition pour la fraction de feuilles et l'extrait de fleur contre Escherichia coli ($p>0,05$). La concentration minimale inhibitrice pour les feuilles varie entre 3,2 mg/mL- 12,8 mg/mL, et celle pour la fleur est comprise entre 51,2 mg/mL - 204,8 mg/mL) et la concentration bactéricide minimale est comprise entre 6,4 mg/mL et 25,6 mg/mL pour les feuilles et 51,2 mg / mL - 204,8 mg / mL pour les fleurs. Le test antifongique a montré une activité avec des zones d'inhibition de la fraction et de l'extrait contre Candida albican ($p>0,05$). La sporulation a été inhibée dans Aspergillus niger tandis qu'aucune activité n'a été observée sur Aspergillus flavus par la fraction végétale ou par l'extrait.

**Conclusion :** P. subcordata a montré de bonnes activités antimicrobiennes contre les souches cliniques de Gram positif, Gram négatif et certaines des espèces de champignons utilisées dans cette étude.

**Mots-clés :** Psydrax subcordata, Antibiotiques, Résistance, Dépistage phytochimique, Plante médicinale.

## INTRODUCTION

For thousands of years, medicinal plants have played a significant role in the treatment of a wide range of medical conditions, including infectious diseases. Some naturally occurring chemical compounds serve as models for a large percentage of clinically proven drugs, and many are now being re-assessed as antimicrobial agents. The primary reason for this renaissance is the fact that infectious disease remains a significant cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths in tropical countries and as much as 20% of deaths in the developed world.[1]

Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms and previously unknown disease-causing microbes pose an enormous threat to global public health. These negative health trends call for a global initiative for the development of new strategies for the prevention and treatment of infectious disease, especially, natural products. Literally thousands of plant species have been tested against hundreds of microbial strains in vitro, and many medicinal plants are active against a wide range of gram-positive and gram-negative bacteria, fungi and viruses. [1]

The effective use of an antimicrobial agent is undermined due to the possible tolerance or resistance developed from the very initial time this compound is used. This is true for antimicrobial agents used to treat bacterial, viral, fungal, and parasitic infections. Several physiological and biochemical mechanisms may steer this developing resistance. The intricacy of all the mechanisms associated with the emergence and distribution of the resistance should not be overplayed. Furthermore, lack of elementary data on these specific subjects is a vital concern, which has caused a lack of significant achievements from being made to manage the development of resistance. Worldwide, various institutes and agencies have recognized this serious global public health problem. Many recommendations and resolutions have been proposed, several reports have also been written, but so far little progress has been made. Unfortunately, the increase in antibiotic resistance is a persistent issue.[2]

Antibiotic resistance is one of the biggest threats to global health, food security, and development today. Antibiotic resistance can affect anyone, of any age, in any country. Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process. A growing number of infections - such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis - are becoming harder to treat as the antibiotics used to treat them become less effective. Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality.[3]

The plant *Psydrax subcordata* belongs to the family Rubiaceae and it varies considerably in size according to the area in which it grows. It is a small tree up to 15 metres tall, but in Sierra Leone specimens up to 30 metres tall can be found.[4] The tree is mostly found swamp forests at elevations around 1,500 metre with branches often hollow and swollen, and housing colonies of ants[5], hence popularly known as Ajeeleera among the Yoruba's. *Psydrax subcordata* has been used for the management of haemorrhoids, stomach ulcer, piles, abdominal pains, dyspepsia, enteritis, stomach aches, gastritis, heartburns and intestinal complaints.[6]

The present study was designed to investigate antimicrobial activities of the extract of the flower and ethyl acetate fraction of the leaves of the plant *Psydrax subcordata,* claimed to have antimicrobial activity by folkloric medicine and also to ascertain its phytochemical constituents.

## MATERIALS AND METHOD

### Plant material

The flowers and leaves of P. subcordata were harvested from the woods around the University of Lagos, Akoka campus in March 2017. The plant materials were authenticated by Mr. O.O Oyebanji, at the Department of Botany Herbarium; University of Lagos with the voucher specimen deposited and assigned voucher number LUH 7561.

### Extraction

The plant materials were oven dried at 40$^{O}$C for 3 days and pulverized. The powdered flowers and leaves were extracted exhaustively with methanol, using Soxhlet apparatus for 48hours. The resulting methanol extract was concentrated using rotary evaporator at 40$^{O}$C until it was a semi-solid mass. The concentrated extract was defatted by partitioning with 50mL Hexane thrice with the use of a separating funnel. The methanol extract was then dried to solid using hot air oven at 37$^{O}$C. This was then partitioned with ethyl acetate (for the leaves) to obtain the fraction.

**Phytochemical screening**

The plant extracts were subjected to the different phytochemical screening methods including tests for sugars, alkaloids, anthraquinones, phlobotannins, flavonoids, tannins and saponin, using standard methods adapted from Trease and Evans, 2002; Harborne , 1998 and Sofowora, 2006.[7,8,9]

**Antibacterial assay**

**Preparation of nutrient broth**

Nutrient broth powder (6.5g) was adequately weighed and transferred into a reagent bottle, dissolved in 500ml of distilled water and sterilized by autoclaving at 121$^{O}$C for 15 minutes.[10]

**Preparation of muller hinton agar**

Muller Hinton agar powder (38g) was dispersed in 1 litre of deionized water. Allow to soak for 10 minutes, swirled to mix, then sterilized at 121$^{O}$C for 15 minutes. Cooled to 47$^{O}$C mix well and pour into plates aseptically.[10,11]

**Sub culturing of organism**

The bacteria clinical isolates of *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeroginosa, Escherichia coli, and Salmonella typhi* were inoculated into different prepared plates of Mueller Hinton Agar aseptically using the streak plate method. The inoculated plates were incubated at 37$^{O}$C for 24 hours. Pure isolates of the organisms were then transferred into liquid medium (nutrient broth) and stored in the refrigerator. [10,11]

**Calibration of organism using normal saline infusion**

Five universal bottles were filled with 20ml of normal saline and sterilized by autoclaving at 121$^{O}$C for 15 minutes and thereafter allowed to cool. The stored organisms in liquid media were individually introduced into separate universal bottle containing sterile normal saline until the turbidity matches that of the McFarland standards for turbidity of bacteria suspension which is thus equivalent to 1 x $10^{8}$CFU/ml.[11,12]

**Preparation of McFarland standard**

A 0.5 McFarland standard is prepared by mixing 0.05ml of 1.175% barium chloride dihydrate ($BaCl_2o2H_2O$), with 9.95ml of 1% sulfuric acid ($H_2SO_4$).[12]

**Preparation of standard ciprofloxacin infusion**

The standard ciprofloxacin used was prepared from the stock concentration of intravenous ciprofloxacin infusion of 2000µg/mL. The diluents (distilled water) were measured into universal bottles and autoclaved at 121$^{O}$C for 15 minutes. The standard ciprofloxacin (stock) were added to the diluents as appropriate to give the required concentration as follows;

- 0.25ml of stock (2000µg/ml) + 9.75ml of sterile distilled water to give a concentration of 50µg/ml.
- 3ml of 50µg/ml + 3ml of sterile distilled water to give a concentration of 25µg/ml.
- 3ml of 25µg/ml + 3ml of sterile distilled water to give a concentration of 12.5µg/ml.
- 3ml of 12.5µg/ml + 3ml of sterile distilled water to give a concentration of 6.25µg/ml.

These concentrations obtained were used as the standard working concentration.[11]

**Preparation of samples (extract/fraction)**

The sample used was prepared by weighing 0.5g of the dried leave ethyl acetate fraction which was dissolved in 10 ml of 5% alcohol to obtain a concentration of 50mg/ml. Other concentrations were obtained from this as follows;

- 3ml of stock (50mg/ml) + 3ml of 5% alcohol to give a concentration of 25mg/ml.
- 3ml of 25mg/ml + 3ml of 5% alcohol to give a concentration of 12.5mg/ml.

While 6g of the dried flower extract was dissolved in 10 ml of 5% alcohol to obtain a concentration of 600mg/ml. Other concentrations were obtained from this as follows;

- 3ml of stock (600mg/ml) + 3ml of 5% alcohol to give a concentration of 300mg/ml
- 3ml of 300mg/ml + 3ml of 5% alcohol to give a concentration of 150mg/ml.

The concentrations obtained were then used as the sample working concentrations.

**Preparation of plate and introduction of samples and standard using the agar well diffusion method**

The plant extract and fraction were subjected to antibacterial assay to determine their potency against selected bacteria consisting of both Gram positive and Gram negative bacteria obtained from previously sub-cultured and calibrated pure isolates of various assay organisms. Agar well diffusion method was used with intravenous Ciprofloxacin used as standard.[11,13]

The calibrated organism (1mL) was transferred into a Petri dish aseptically; 25mL of Muller Hinton agar was then added to the organism in the Petri dish and mixed thoroughly to obtain uniform distribution of the bacteria in the agar. The agar was allowed to set and core borer of about 7mm was used to create holes in the agar containing the organism (three holes for the sample and standard). 0.2mL of the extract/fraction was then introduced into each hole starting with the lowest sample working concentration. The procedure was carried out in duplicate for each of the organism and distilled water was used as control. The plates were then placed on the bench for about four hours to give room for diffusion before incubating at 37$^{O}$C for 24 hours.

Similar procedure was carried out using ciprofloxacin at the standard working concentrations prepared.[14]

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

The minimum inhibitory concentrations were determined using twelve working concentrations of the flower methanol extract which ranges from 0.1mg/mL to 204.8mg/mL while that of the leaves ethyl acetate fraction ranges from 0.1mg/mL to 51.2 mg/mL using the agar dilution method.[15]

These concentrations obtained were then used in the determination of the minimum inhibitory concentration by pouring each of the agar on Petri dishes previously labeled to show the name of the five organisms and the point to which the organisms will be inoculated. The agars were allowed to set and with the use of inoculating loop, the organisms were introduced on the noted spot. This was done for the five organisms used in duplicate. The plates were left on the bench for about two hours to give room for diffusion before incubating in the incubator at 37$^{O}$C for 24 hours. Observations were then noted for growth or no growth for the five organisms. The point where growth was not observed was noted as the MIC for the organism.

For the MBC assay, the plates with no growth in the MIC determination were re-cultured in fresh plates of Muller Hinton Agar with no test agent to confirm if the organism were just inhibited or completely killed. The plates were incubated for 24 hours at 37$^{O}$C.[16]

**Antifungal assay**

**Preparation of sabouraud dextrose agar**

Sabouraud Dextrose agar powder (62.12g) was dissolved in 1 liter of distilled water, mixed and heated. The resulting solution was sterilized in an autoclave at 121$^{O}$C for 15 minutes. [10]

**Sub culturing of organism**

The fungi isolates were inoculated into different prepared plates of *Sabouraud Dextrose Agar using the streak plate method aseptically in the laminar hood. The inoculated plates* were then incubated at room temperature for 3 days *(Candida albican, Saccharomyces cerevisiae)* and 7 days for the spore forming fungi *(Aspergillus niger, Aspergillus flavus, Penicillium sp.)* and stored on the bench.[10,11]

**Calibration of organism using 1% Tween 80 and normal saline infusion**

Three universal bottles were filled with 20ml of 1% Tween 80 and two universal bottles filled with normal saline, sterilized by autoclaving at 121$^{O}$C for 15minutes and thereafter allowed to cool. The stored organisms on the bench were then individually introduced by washing the spores into separate universal bottle containing sterile 1% Tween 80 (Aspergillus niger, *Aspergillus flavus, Penicillium sp.)* and normal saline *(Candida albican, Saccharomyces cerevisiae)* until the turbidity is equivalent to 1 x 108 spore forming unit. [11]

**Preparation of standard clotrimazole**

The Clotrimazole (standard) was prepared from the stock concentration of Clotrimazole of 3200microgram/ml. The diluents (15% Alcohol) were measured into universal bottles and autoclaved at 121$^{O}$C for 15 minutes. Then, the Clotrimazole were added to the diluents as appropriate to give the required concentration as follows;

- 0.25ml of stock (3200 µg/ml) + 9.75ml of sterile 15% alcohol to give a concentration of 80µg/ml.
- 3ml of 80µg/ml + 3ml of sterile15% alcohol to give a concentration of 40µg/ml.
- 3ml of 40µg/ml + 3ml of sterile 15% alcohol to give a concentration of 20µg/ml.
- 3ml of 20µg/ml + 3ml of sterile 15% alcohol to give a concentration of 10µg/ml.

The concentrations obtained were then used as the standard working concentration. [11]

**Preparation of plate and introduction of samples and standard using the agar well diffusion method**

Each Petri dish was labeled to indicate the name of the organism and the concentration of the sample/standard

to be introduced. 1mL of the calibrated organism was transferred into a Petri dish aseptically; 25mL of Saboraud dextrose agar was then added to the organism in the Petri dish and mixed properly. The agar was then allowed to set. Core borer of about 7mm was then used to create holes in the agar containing the organism (three holes for the sample and the standard). 0.2mL of the extract was then introduced into each hole starting with the lowest sample working concentration. The procedure was carried out in duplicate and carried out for each of the organism. The plates were then placed on the bench for 3-7days before readings were then taken. Similar procedure was carried out for the standard.[14]

**Statistical analysis**

Statiatical analysis was carried out using Excel spreadsheet Independent T-test (two tails, heteroscedastic) for both the antibacterial and antifungal assay using 0.05 significant values at 95% confidence interval.

**RESULTS**

**Phytochemical screening:** Phytochemical Screening of the methanol extract of the flower and ethyl acetate fraction of *Psydrax subcordata* indicates the presence of carbohydrates, reducing sugars, keto-sugars, steroidal nucleus, 2, 6, di-deoxysugars, flavonoids, glycosides, saponins, tannins and phlobatannins. However, the methanol extract of the flowers and ethyl acetate fraction of *Psydrax subcordata* were negative to all alkaloidal tests indicating the absence of alkaloids and also to Borntrager tests for free and combined hydoxylhydroquinones.

**Antibacterial assay:** Zones of inhibition were observed for the flower, leave and reference standard at different concentrations as expressed in Table 1. The minimum inhibitory concentrations and minimum bactericidal concentrations were also determined (Table 2).

**Table 1: Zone of inhibition (mm) observed with extract/fraction and standard for antibacterial activity**

| Organisms | Ethyl acetate fraction of the leaves (mg/mL) | | | Methanol extract of the flower (mg/mL) | | | Standard (Ciprofloxacin infusion) (µg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50 | 25 | 12.5 | 600 | 300 | 150 | 50 | 25 | 12.5 |
| *Escherichia coli* | 26.5± 0.50 | 23 ± 0.56 | 20 ± 0.58 | 27± 0.50 | 23.0± 1.34 | 16 ± 1.98 | 26±0.58 | 22± 0.00 | 19±1.73 |
| *Staphylococcus aureus* | 18.0 ±1.15 | 15 ± 1.15 | 12±0.87 | 30± 0.98 | 21.0± 0.76 | 14 ± 0.98 | 40±1.73 | 37±0.13 | 34±0.98 |
| *Pseudomonas aeruginosa* | 22.0 ±1.00 | 14 ± 2.31 | 9 ± 1.34 | 21 ± 1.56 | 17.0 ± 0.75 | 14 ± 1.00 | 23±2.52 | 21±1.45 | 16±1.34 |
| *Streptococcus faecalis* | 26.0 ±0.58 | 22 ± 2.08 | 18± 0.53 | 26± 2.34 | 19.5± 0.87 | 17 ± 1.39 | 37±2.52 | 31±2.45 | 26±0.58 |
| *Salmonella typhi* | 20.0 ±0.58 | 18 ± 1.00 | 14 ± 1.73 | 27± 0.50 | 23.0± 0.50 | 14 ± 0.76 | 42.5±0.50 | 42.5±3.24 | 42.5±1.73 |

**Data in mean ±SEM**

**Table 2: Minimum inhibitory concentration and Minimum Bactericidal Concentration (mg/mL)**

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the ethyl acetate fraction of the leaves and methanol fraction of the flower of Psydrax subcordata

| Organism | Ethyl acetate fraction of the leaves | | Methanol extract of the flower | |
|---|---|---|---|---|
| | MIC (mg/mL) | MBC (mg/mL) | MIC(mg/mL) | MBC (mg/mL) |
| *Escherichia coli* | 6.4 | 12.8 | 102.4 | 102.4 |
| *Staphylococcus aureus* | 12.8 | 25.6 | 102.4 | 102.4 |
| *Pseudomonas aeruginosa* | 12.8 | 25.6 | 204.8 | 204.8 |
| *Streptococcus faecalis* | 12.8 | 25.6 | 204.8 | 204.8 |
| *Salmonella typhi* | 3.2 | 6.4 | 51.2 | 51.2 |

**Antifungal assay:** Zones of inhibition (in millimeters) for the antifungal assay were observed for the flower, leave and reference standard at different concentrations as expressed in Table 3.

**Table 3: Zone of inhibition (mm) observed with extracts and standard for antifungal activity**

| Organisms | Ethyl acetate fraction of the leaves (mg/mL) | | | Methanol extract of the flower (mg/mL) | | | Standard (Clotrimaxole Powder) (µg/ml) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 600 | 300 | 150 | 600 | 300 | 150 | 40 | 20 | 10 |
| *Penicillium spp* | 21 ± 1.37 | 17 ± 0.98 | 14 ± 1.35 | 20 ± 2.76 | 16 ± 0.5 | 12 ± 2.76 | 27 ± 0.98 | 22 ± 1.87 | 19 ± 2.76 |
| *Aspergillus flavus* | 0 | 0 | 0 | 0 | 0 | 0 | 28 ± 2.70 | 24 ± 1.34 | 17 ± 1.80 |
| *Aspergillus niger* | 0 | 0 | 0 | 0 | 0 | 0 | 19 ± 1.00 | 16 ± 0.76 | 12 ± 1.76 |
| *Candida albican* | 26 ± 1.34 | 23 ± 1.74 | 18 ± 1.39 | 24 ± 1.98 | 21 ± 1.15 | 18 ± 1.35 | 28 ± 1.15 | 23 ± 2.98 | 20 ± 3.45 |
| *Saccharomyces cerevisiae* | 23 ± 1.73 | 20 ± 1.73 | 16 ± 1.43 | 22 ± 1.76 | 19 ± 1.00 | 12 ± 1.67 | 30 ± 0.54 | 27 ± 2.89 | 20 ± 2.34 |

Data in Mean ±SEM

**Statistical analysis:** Independent T-test was calculated for the extracts for both the antibacterial and antifungal assay as shown in table 4 and 5 respectively.

**Table 4: T-test analysis of antibacterial analysis**

| Organisms | Ethyl acetate fraction of the leaves (mg/mL) | | | Methanol extract of the flower (mg/mL) | | |
|---|---|---|---|---|---|---|
| | **50** | **25** | **12.5** | **600** | **300** | **150** |
| *Escherichia coli* | 0.4961 | 0.2879 | 0.2879 | 0.2879 | 0.2879 | 0.0213 |
| *Staphylococcus aureus* | 0.0000* | 0.0000* | 0.0000* | 0.0003* | 0.0000* | 0.0000* |
| *Pseudomonas aeruginosa* | 0.02131* | 0.0010* | 0.0010* | 0.0705* | 0.0081* | 0.0705* |
| *Streptococcus faecalis* | 0.0002* | 0.0004* | 0.0006* | 0.0002* | 0.0004* | 0.0004* |
| *Salmonella typhi* | 0.0000* | 0.0000* | 0.0000* | 0.0002* | 0.0000* | 0.0000* |

***** = Significantly different from standard at p = 0.05

**Table 5: T-test analysis of antifungal analysis**

| Organisms | Ethyl acetate fraction of the leaves (mg/mL) | | | Methanol extract of the flower (mg/mL) | | |
|---|---|---|---|---|---|---|
| | **600** | **300** | **150** | **600** | **300** | **150** |
| *Penicillium spp* | 0.0010* | 0.0010* | 0.0081* | 0.0006* | 0.0006* | 0.0029* |
| *Aspergillus flavus* | 0.0100* | 0.0006* | 0.0012* | 0.0100* | 0.0006* | 0.0012* |
| *Aspergillus niger* | 0.0009* | 0.0013* | 0.00237* | 0.0009* | 0.0013* | 0.0024* |
| *Candida albican* | 0.0705 | 1.0000 | 0.0705 | 0.0081* | 0.0705 | 0.0705 |
| *Saccharomyces cerevisiae* | 0.0010* | 0.0010* | 0.0081* | 0.0006* | 0.0006* | 0.0029* |

***** = Significantly different from standard at p = 0.05

**DISCUSSION**

The methanol extract of the flower of *Psydrax subcordata* and the ethyl acetate fraction of the leaves were investigated for their antimicrobial activities and screened to confirm the phytochemicals they contain. The phytochemical screening of the extract and fraction of *P. subcordata revealed* the presence of polyphenolic compounds such as flavonoids and tannins. Also present are saponins, carbohydrates, reducing sugars, keto sugar, steroidal nucleus (ring) and glycosides. It however test negative to alkaloidal tests and Borntrager test for anthraquinones, indicating their absence. Compared with earlier work by Oyawaluja *et al*., 2019[11] on the methanol extract of the leaves, the absence of alkaloids and both free and combined anthraquinones were identical. However, the presence of tannins and sugars as confirmed in this study runs contrary to what they reported.

The antimicrobial assay of the flower methanol extract and leaves ethyl acetate fraction showed zones of inhibitions against *Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi* (Tables 1) as against previous work done by Oyawaluja *et al*.,2019[11], where *P. subcordata showed inhibition only against Staphylococcus aureus*. In their research *P. subcordata* had the highest growth inhibition against *Staphylococcus aureus* of all the plants used which in a way is corroborated in this study though the concentrations used differ. In this study Ciprofloxacin was used as the standard used against which the activities of the respective extract/fraction at different concentrations were compared, while in the work by Oyawaluja *et al*., 2019[11], Levofloxacin, another quinolone antibacterial moiety, was used as standard. The minimum inhibitory concentrations were also determined for each of the organisms and the minimum bactericidal concentrations were also determined to ascertain that the organisms were actually killed but not just inhibited (Table 2).

The leaves' ethyl acetate fraction showed good inhibitory activity on *Escherichia coli* at all concentrations which is very comparable to that of the standard (Ciprofloxacin) used and this is further confirmed by the test statistics that showed no statistical difference between the treatment and the control group with p values > 0.05 (Table 4). Similar observations were made with the flower's methanol extract showing no statistical difference against E. coli when compared to the positive control (Table 4).

The antifungal assay of the flower's methanol extract and leaves ethyl acetate fraction also shows some inhibitory activities against *Penicillum species, Candida albican and Saccharomyces cerevisiae* with zones of inhibition (Tables 3), while preventing sporulation in Aspergillus niger with no activity on Aspergillus flavus. The zones observed for the extract and fraction compared with the standard clotrimazole shows no statistical difference only on *C. albicans* (except at 600mg/ml flower extract).

In other antimicrobial activity studies done on the plant *Psydrax subcordata* using the stem bark extract done in Ghana the results also showed a concentration dependent inhibition of the growth of both Gram positive and Gram negative clinically significant human pathogens including *S. aureus, S. pyogenes, E. coli, P. aeruginosa and C. albicans.*[17]

*Agyare* and his colleagues[14] obtained the MIC of *P. subcordata* leaf methanol extract as 12.5 mg/mL against test organisms which include *Bacillus subtilis, Escherichia coli, Streptococcus pyogenes* and *Pseudomonas aeruginosa* while the MIC of the stem bark extract against same test organisms was 25 mg/mL except for P. aeruginosa with MIC of 12.5 mg/mL.[14] The MIC obtained in this study for the leaves ethyl acetate fraction was very much comparable with that of Agyare *et al*.,[18] especially *for Pseudomonas aeruginosa* (12.8mg/mL vs. 12.5mg/mL) while the variation in that of the E. coli was large (6.4mg/mL vs. 12.5mg/mL) which could be attributed to the source since this research made use of clinical strains of all the organisms used.

The leaves ethyl acetate fraction exhibited better antibacterial and antifungal activity than the flower methanol extract as evident in the zones and inhibition for both assays and also reflecting in the lower concentration of the fraction required to elicit the antimicrobial response compared to that of the flower methanol extract . This might be connected to the relative abundance or accumulation of the responsible phytoconstituents in the leaves of the plant than in the flower and partly because of the little purification process done on the leaves via partitioning to obtain the ethyl acetate fraction from the crude methanol extract.

**CONCLUSION**

From this work done, the bio-prospect of the plant *Psydrax subcordata* is not in doubt. The extract and fraction of *Psydrax subcordata possess* some potent antibacterial activities against the test organisms which include *Escherichia coli, Pseudomonas aeruginosa,*

*Staphylococcus aureus, Streptococcus faecalis and Salmonella typhi* and some antifungal activities especially against *Penicillium sp., Candida albican* and *Saccharomyces cerevisiae* but were only able to prevent sporulation in *Aspergillus niger*. The leaves extract showing more activity than the flower extract. Hence, the result obtained validated the local claim that the plant possesses antibacterial and antifungal activity thus can be used to treat infections of susceptible organisms.

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